Downstream Processing (Textbook Chapter 11 & Supplemental Chapter 11)
Topics

Downstream processing needed to separate cell mass & other byproducts from the water solvent

Major categories

- Separation of solids & liquids
  - Filtration
  - Centrifugation

- Drying of solids
  - Dryness limited by the dryness of air & adhesion of water to solid

- Separation of soluble components
  - Adsorption – chromatography as special case
Typical process to manufacture enzymes
Need for downstream processing

Fermentation products are formed in dilute solutions

- Remove water without harming the products formed
- Byproducts?

Biological products are labile (i.e., sensitive to temperature, solvents, etc.)

Fermentation broths are susceptible to contamination
General processing schemes

1. Cell removal
   - If the cells are the product then little additional processing may be required
   - Removal of the cells may make the separation of liquid species easier
   - Options..
     - Filtration
     - Microfiltration
     - Centrifugation

2. Cell disruption & cell debris removal
   - Required if product is within the cells
     - Cell debris must then be removed
   - Option
     - High pressure homogenization
General processing schemes (cont.)

3. Primary isolation
   ▪ Remove components that are very different from the product
     • May not be selective but overall help the efficiency of other separation
   ▪ Options…
     • Solvent extraction
     • Two-phase liquid extraction
     • Adsorption
     • Precipitation
     • Ultrafiltration

4. Product enrichment
   ▪ Separation of product for high concentrations
   ▪ Option…
     • Chromatography

5. Final isolation
   ▪ Depends on product, phase, & final purity
   ▪ Options…
     • Ultrafiltration (liquid)
     • Crystallization followed by centrifugation, filtration, and drying (solids)
Generalized downstream processing schemes
Water Content & Drying
Drying

Remove relatively small amounts of residual water and/or solvent

- May be necessary to minimize chemical or physical degradation

Relative terms – “dry” may range form 0 to 20 wt% water

Energy intensive operation
Water Content of Air

Absolute humidity defined as the ratio of the mass of water in dry air

\[
\text{Humidity} = \frac{m_w}{m_{\text{air}}} \quad \Rightarrow \quad \mathcal{W}_w = \frac{m_w}{m_w + m_{\text{air}}}
\]

Relative humidity is the actual amount of water compared to the maximum amount at the same pressure & temperature

\[
\text{Relative humidity} = \frac{p_w}{p_{w,\text{sat}}} \times 100\% \quad \text{where} \quad p_w = \gamma_w P
\]

May also discuss properties of “wet bulb” temperature, …
Water Content of Air

Example: What is the wet bulb temperature for air @ 80°F & 60% relative humidity?

Answer: 70°F

Ref: GPSA Data Book, 13th ed.
Water Content of Solids

FIGURE 11.56  Equilibrium moisture content isotherms.

Bioprocess Engineering Principles, 2nd ed
Pauline Doran, Elsevier Science & Technology
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Mechanisms of Drying

Moisture transport in solids
- Molecular diffusion of liquid water
- Capillary flow of liquid water within porous solids
- Molecular diffusion of vapor evaporated within the solid
- Convective transport of vapor evaporated within the solid

When the drying rate is controlled by the vaporization of water from the surface then the heat transfer & mass transfer effects are balanced

\[ \dot{N}_c = \frac{h_s A_h (T_a - T)}{\Delta H_{\text{vap}}} = (k_c a)(X - X_a) \]

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Drying Time

Can define the rate of drying to the water content of the solids

\[ \dot{N} = -m_s \frac{dX}{dt} \quad \text{where} \quad X = \frac{m_w}{m_s} \]

\[ \dot{n}_A = -\frac{m_s}{A} \frac{dX}{dt} \]

Over the period of constant rate of drying

\[ \dot{N}_c = -m_s \frac{\Delta X}{\Delta t} \quad \Rightarrow \quad \Delta t = \frac{m_s}{\dot{N}_c} (X_0 - X_1) \]
Drying Example – Humidity of Air

Dry sodium benzyl penicillin to a moisture content of 20 wt% (wet basis). Use air at 25°C & 1 atm pressure in a fluidized bed drier. Assume equilibrium (open circles)

Target dry basis water content:

\[ X_0 = \frac{20}{80} = 0.25 \Rightarrow 25 \frac{\text{kg water}}{100 \text{ kg dry mass}} \]

Required relative humidity – 65% (from chart)

Mole fraction water – water’s vapor pressure @ 25°C is 3.17 kPa

\[ y_w = \frac{p_w}{P} = \frac{0.65 \text{ } p_{\text{sat}}}{P} = \frac{0.65 \times 3.17}{101.28} = 0.020 \]
Drying Time Example

Want to dry 10 kg washed filter cake (wet basis) from 15% to 8% water (both wet bases) under constant drying conditions

- Drying area 1.2 m²
- Air temperature 35°C & surface temperature of solids 28°C
- Heat transfer coefficient 25 J/m²·sec·°C
- Drying time?

From initial conditions:

\[
\frac{m_{w,0}}{m_{w,0} + m_s} = \frac{m_{w,0}}{100} = 0.15 \quad \Rightarrow \quad m_{w,0} = 1.5 \quad \text{and} \quad m_s = 8.5
\]

\[
\Rightarrow \quad \chi_0 = \frac{1.5}{8.5} = 0.176
\]
Drying Time Example (cont.)

Final conditions

\[
\frac{m_{w,1}}{m_{w,1} + 8.5} = 0.08 \quad \Rightarrow \quad m_{w,1} = \frac{0.08(8.5)}{1 - 0.08} = 0.74
\]

\[
\Rightarrow \quad \chi_1 = \frac{0.74}{8.5} = 0.087
\]

Rate of drying

\[
\dot{N}_c = \frac{h_s A_h (T_a - T)}{\Delta H_{vap}} = \frac{(25)(1.2)(35 - 28)}{2435.4 \cdot 10^3} = 8.62 \cdot 10^{-5} \text{ kg/sec}
\]

Drying time

\[
\Delta t = \frac{8.5}{8.62 \cdot 10^{-5} (0.176 - 0.087)} = 8770 \text{ sec} \quad \Rightarrow \quad 2.4 \text{ hr}
\]
Freeze Drying

Wet solids are frozen then exposed to vacuum – water directly sublimes from solid to vapor state

Steps

- Freezing
- Primary drying (sublimation)
- Secondary drying (desorption)
Cell Removal
Filtration

Solid particles removed from a fluid suspension by forcing the fluid through a filter media which holds back the solid particles

Ease of filtration depends on the properties of the solid & fluid

- Crystalline incompressible solids fairly easy to filter, …
- Fermentation broths not so much
  - Small particle size & gelatinous nature

May use filter aids to improve efficiency of filtration
Filtration Equipment

Plate & frame filter press
- Batch operation
- Accumulates solids. Must be opened & cleaned
- https://www.youtube.com/watch?v=M4wBd1_CvNw
- https://www.youtube.com/watch?v=aOQPr7Eomek

Rotary drum filters
- Can be operated continuously
- Can be operated at pressure with controlled atmosphere
- https://www.youtube.com/watch?v=zSflxzE0nlo

Horizontal belt filters
- Continuous operation
- Typically pull a vacuum below the filter
- https://www.youtube.com/watch?v=6voXE1HxYsY
Filtration Theory

Rate of filtration depends on the pressure drop across the cake and the filter media

\[
\frac{1}{A} \frac{dV_f}{dt} = \mu_f \left[ \frac{\Delta P}{\alpha \left( \frac{m_{solids}}{A} \right) + R_m} \right] \approx \frac{\Delta P}{\mu_f \alpha \left( \frac{cV_f}{A} \right)}
\]

- \( R_m \) is the resistance due to the filter media & is typically negligible compared to the resistance due to the cake
- Various correlations to relate \( \alpha \) (the specific cake resistance) to the cake properties
  - Compressible cake
    \[
    \alpha = \alpha' \left( \Delta P \right)^s
    \]
  - Rigid particles
    \[
    \alpha = \frac{K_v \alpha^2 (1 - \varepsilon)}{\varepsilon^3 \rho_p}
    \]
Filtration Theory

Can integrate the reciprocal form...

\[
\frac{1}{A} \frac{dV_f}{dt} = \frac{\Delta P}{\mu_f \left[ \alpha \left( \frac{cV_f}{A} \right) + R_m \right]}
\]

\[
\Rightarrow A \frac{dt}{dV_f} = \mu_f \alpha c \left( \frac{V_f}{A \Delta P} \right) + \frac{\mu_f R_m}{\Delta P}
\]

\[
A \int dt = \int_0^{V_f} \mu_f \alpha c \left( \frac{V_f}{A \Delta P} \right) dV_f + \int_0^{V_f} \frac{\mu_f R_m}{\Delta P} dV_f
\]

\[
At = \frac{\mu_f \alpha c}{2} \left( \frac{V_f^2}{A \Delta P} \right) + \frac{\mu_f R_m}{\Delta P} V_f
\]

Linearized form

\[
\frac{t}{V_f} = \frac{\mu_f \alpha c}{2A^2 (\Delta P)} V_f + \frac{\mu_f R_m}{A (\Delta P)}
\]
Filtration Example

Can filter 30 mL broth from penicillin fermentation on 3 cm² filter in 4.5 min using 5 psi pressure drop. Neglect media resistance & use s=0.5:

\[ \alpha = \alpha' (\Delta P)^s = \alpha' (\Delta P)^{0.5} \]

Lab conditions to “tune” filter equation

\[ \frac{t}{V_f} = \frac{\mu_f \alpha' (\Delta P)^{0.5} c}{2A^2 (\Delta P)} \quad \Rightarrow \quad \mu_f \alpha' c = \frac{2A^2 t (\Delta P)^{0.5}}{V_f^2} = \frac{2(3)^2 (4.5)(5)^{0.5}}{(30)^2} = 0.201 \text{ psi}^{-0.5} \text{ min cm}^{-2} \]

Filter 500 L broth in 1 hour using 10 psi pressure drop

\[ \frac{t}{V_f} = \frac{\mu_f \alpha' (\Delta P)^{0.5} c}{2A^2 (\Delta P)} \quad \Rightarrow \quad A = V_f \sqrt{\frac{(\mu_f \alpha' c)}{2t (\Delta P)^{0.5}}} = 11500 \text{ cm}^2 \quad \Rightarrow \quad 1.15 \text{ m}^2 \]
Filtration Example (cont.)

What if the cake is much less compressible? Let’s resize with $s=0.1$:

$$\alpha = \alpha' (\Delta P)^s = \alpha' (\Delta P)^{0.1}$$

Lab conditions to “tune” filter equation

$$\frac{t}{V_f} = \frac{\mu_f \alpha' (\Delta P)^{0.5}}{2A^2 (\Delta P)} c V_f$$

$$\Rightarrow \quad \mu_f \alpha' c = \frac{2A^2 t (\Delta P)^{0.1}}{V_f^2} = \frac{2(3)^2 (4.5)(5)^{0.9}}{(30)^2} = 0.383 \text{ psi}^{0.9} \text{ min} \frac{\text{cm}^2}{\text{psi}}$$

Filter 500 L broth in 1 hour using 10 psi pressure drop

$$\frac{t}{V_f} = \frac{\mu_f \alpha' (\Delta P)^{0.1}}{2A^2 (\Delta P)} c V_f$$

$$\Rightarrow \quad A = V_f \sqrt{\frac{(\mu_f \alpha' c)}{2t (\Delta P)^{0.9}}} = 10000 \text{ cm}^2 \Rightarrow 1.0 \text{ m}^2$$

Compressible cake will require more surface area
Centrifugation

Increase the “gravitational” field experienced by particles to promote faster settling times

Stoke’s law:

\[ u_g = \frac{\rho_p - \rho_l}{18\mu_l} d_p^2 g \implies u_C = \frac{\rho_p - \rho_l}{18\mu_l} d_p^2 \omega^2 r \]

\( Z \) is the ratio of the acceleration due to the centrifuge relative to the acceleration due to gravity (the g-force), \( \omega^2 r / g \)

- Industrial centrifuges can achieve 16,000, small lab centrifuges up to 500,000
Equipment – Tubular Bowl Centrifuge

http://slideplayer.es/slide/7225569/24/images/11/EQUIPOS+DE+CENTRIFUGACI%C3%93N.jpg
Equipment – Disc Stack Bowl Centrifuge

Updated: November 27, 2017
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Equipment – Disc Stack Bowl Centrifuge

Can be run in batch or continuous modes

- Batch mode – equipment stopped, opening up, & solids removed
- Continuous – either side discharge or movement of solids out the top
  - Disadvantage – the solids must remain wet to allow them to move

Video: Alfa Laval PX Disc Stack Centrifuge

- [https://www.youtube.com/watch?v=ZmTMz4VkAwI](https://www.youtube.com/watch?v=ZmTMz4VkAwI)

Separator design type SX

- Unseparated liquid
- Clean liquid
- Concentrate

Equipment – Decanter Separator

Andritz 2-phase decanter with CIP (clean in place)

- Video: [https://www.youtube.com/watch?v=OqEODWcJwnY](https://www.youtube.com/watch?v=OqEODWcJwnY)

Centrifuge Performance

Relate the performance based on the centrifuge’s cross-sectional area to that of a gravity separator

\[ \Sigma = \frac{\dot{Q}}{2u_g} \]

Two centrifuges that operate with equal effectiveness

\[ \frac{\dot{Q}_1}{\Sigma_1} = \frac{\dot{Q}_2}{\Sigma_2} \]

Disc stack bowl centrifuge

\[ \Sigma = \frac{2\pi\omega^2(N-1)}{3g \tan(\theta)} (r_2^3 - r_1^3) \]

Tubular bowl centrifuge

\[ \Sigma = \frac{\pi\omega^2 b}{2g} \left(3r_2^2 + r_1^2\right) \approx \frac{2\pi\omega^2 br^2}{g} \]
Centrifuge Example

Use continuous disc stack centrifuge @ 5000 rpm to recover 50% baker’s yeast cells at 60 L/min.

- Found that at constant rpm the solids recovery factor is inversely proportional to flow rate

At 5000 rpm what flow rate will give 90% solids recovery?

\[ Y \propto \frac{1}{Q} \implies \dot{Q}_2 = \dot{Q}_1 \frac{Y_1}{Y_2} = (60) \frac{0.5}{0.9} = 33.3 \text{ L/min} \]

What rpm necessary to get 90% recovery @ 60 L/min?

- At equal effectiveness by adjusting flowrate:

\[ \frac{\dot{Q}_1}{\Sigma_1} = \frac{\dot{Q}_2}{\Sigma_2} \implies \frac{\Sigma_2}{\Sigma_1} = \frac{\dot{Q}_2}{\dot{Q}_1} = \frac{33.3}{60} = 0.556 \]

- By adjusting rotational speed:

\[ \frac{\Sigma_2}{\Sigma_1} = 0.556 = \frac{\omega_2^2}{\omega_1^2} \implies \omega_2 = \frac{\omega_1}{\sqrt{0.556}} = 6710 \text{ rpm} \]
Separation of Soluble Components
Separation of Soluble Components

Liquid-liquid extraction

Precipitation
  - Followed by solid/liquid separation & possibly drying

Adsorption
  - Chromatography a special case

Membrane separations
  - Dialysis (separation by selective membrane)
  - Reverse Osmosis – transport of water from low to higher concentration
  - Ultrafiltration & microfiltration
  - Electrodialysis

Crystallization
Absorption vs Adsorption

Absorption

Adsorption

From Prof. Art Kidnay
Adsorption

Chemical species cling to the surface of the adsorbent allowing species that don’t adsorb to pass on by

Equilibrium relationship between components in liquid & to surface

\[ C_s^* = K_F \left( C_L^* \right)^{1/n} \] (Freundlich)

\[ C_s^* = \frac{C_{L_{sm}} K_L C_L^*}{1 + K_L C_L^*} \] (Langmuir)

Differential material balance in a packed bed column

\[ u \frac{\partial C_L}{\partial z} + \varepsilon \frac{\partial C_L}{\partial t} = -\left(1 - \varepsilon\right) \frac{\partial C_s}{\partial t} \]

Including overall mass transfer coefficient

\[ \frac{dC_s}{dt} = (K_L \alpha)(C_L - C_L^*) \Rightarrow u \frac{\partial C_L}{\partial z} + \varepsilon \frac{\partial C_L}{\partial t} = -\left(1 - \varepsilon\right)(K_L \alpha)(C_L - C_L^*) \]
Adsorption (cont.)

Approximate steady flow through fixed-bed adsorption column as a steady-state moving-bed operation

\[ u \frac{dC_L}{dz} = -(1 - \varepsilon)(K_L \alpha)(C_L - C_L^*) \]

Can integrate to determine the height of a column for degree of separation

\[ H = \int_0^H dz = -\frac{u}{(1 - \varepsilon)(K_L \alpha)} \int_{C_L}^{C_L^0} \frac{dC_L}{C_L - C_L^*} \]

Need the equilibrium relationship to fully integrate
Adsorption (cont.)

Mass balance considerations on the adsorbent provides an operating line & relates the concentrations in the liquid & on the solid

\[ F (C_{L0} - 0) = B (C_{S0}^* - 0) \]

\[ \frac{B}{F} = \frac{C_L}{C_S} = \frac{C_{L0}}{C_{S0}^*} \]

F is the liquid volumetric flow (uA) & B is the resin’s volumetric flow
Adsorption (cont.)

Fixed beds can be thought to have three zones:

- Saturated zone
- Adsorption zone – also called the mass transfer zone
- Virgin zone

We would stop flow before the adsorption zone gets to the exit (i.e., before the virgin zone goes completely to zero)

If we let the zones travel through the column then we would get a typical breakthrough curve
Adsorption (cont.)

Figure 11.17. Movement of adsorption zone through a fixed-bed downflow adsorber and the corresponding breakthrough curve. (With permission, from D. W. Sundstrom and H. E. Klei, Wastewater Treatment, Pearson Education, Upper Saddle River, NJ, 1979, p. 281.)
Adsorption Example

Separate cephalosporin on to an ion-exchange resin

- Bed is 4 cm diameter
- Resin density is 1.3 g/cm³ & packed to 0.8 cm³ resin per cm³ bed
- Feed solution $C_{L0} = 5$ g/L
- Broth superficial velocity $u = 1.5$ m/h & broth to resin feed ratio $F/B = 10$
- Equilibrium relationship $C_S = 25\left(C_L^*ight)^{1/2}$ & mass transfer $K_La = 15$ h⁻¹
- What is the height necessary to get $C_L = 0.2$ g/L?

\[
\frac{B}{F} = \frac{C_L}{C_S} = \frac{C_{L0}}{C_{S0}} = \frac{1}{10} \quad \Rightarrow \quad C_S = 10C_L \quad \text{and} \quad C_{S0} = 10C_{L0}
\]

\[
C_S^* = 25\left(C_L^*ight)^{1/2} \quad \Rightarrow \quad C_L^* = \frac{1}{625}C_S^* = \frac{1}{625}\left(10C_L^2\right) = 0.16C_L^2
\]
Adsorption Example (cont.)

Substitute into integral equation for the height

\[ H = -\frac{u}{(1 - \varepsilon)(K_a)} \int _{C_L}^{C_{L_0}} \frac{dC_L}{C_L - C_L^*} \]

\[ = -\frac{1.5}{(0.8)(15)} \int _{0.2}^{5} \frac{dC_L}{C_L - 0.16 C_L^2} \]

\[ = -\frac{1.5}{(0.8)(15)} \ln \left( \frac{C_L}{1 - 0.16 C_L} \right) \bigg|_{0.2}^{5} \]

\[ = -0.6 \text{ m} \]

Amount of resin needed?

\[ m_{\text{resin}} = \rho_{\text{resin}} \frac{\pi D^2}{4} H = (1.3) \frac{\pi (4)^2}{4} (60) = 980 \text{ g} \]
Chromatography

Form of adsorption where chemical species adsorb & desorb at different rates

- Those species that interact most strongly with the stationary phase will elute later than those species that weakly interact
- Separation of a “pulse” of mixture
  - Followed up by more solvent
  - Recovered material separated from the other species of the original mixture but diluted with more solvent
  - Can obtain multiple products from each pulse

We’ll focus on chromatography as a batch process

- Continuous & semi-continuous options
  - Moving Bed Chromatography
  - Simulated Moving Bed Chromatography
Chromatography

Concentrations in the separation of mixture with 3 solutes

Species that adsorb onto the stationary phase the least will elute the earliest

Chromatogram

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Chromatography Methods

Adsorption chromatography (ADC)
- Controlled by weak van der Waals forces & steric interactions

Liquid-liquid partition chromatography (LLC)

Ion-exchange chromatography (IEC)
- Adsorption of ions on resin particles by electrostatic forces
- Widely used, especially for protein recovery

Gel Permeation Chromatography – Size Exclusion Chromatography
- Smaller molecules wander into the pores of the solid

Affinity chromatography (AFC)
- Based on specific chemical interactions between solute molecules & ligands
- Possible to get very high resolution (degree of separation) but expensive sorbent

Hydrophobic chromatography (HC)

High-pressure liquid chromatography (HPLC)
Chromatography Separation of Peaks

The species will move through the chromatography column slower than the rest of the solvent – *differential migration*.

Given enough column length the species will elute from column with little to no overlap.

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Chromatography Zone Spreading

Elution bands spread out so each solute takes time to pass across the end of the column

Factors that lead to zone spreading

- Axial diffusion
- Eddy diffusion
- Local nonequilibrium effects

Minimize zone spreading by improving mass transfer more closely approach equilibrium between the phases

- Good: increasing the particle surface area per unit volume
- Bad: increasing liquid flow rate
Chromatography Separation of Peaks

Descriptors

- $V_e$ – volume of eluting solvent needed to carry the solute through column
- $V_o$ – void volume in the column ($\varepsilon \cdot V_{empty}$)
  - In gel chromatography (size exclusion) there is also a volume term for the interstitial volume only accessible by the smaller molecules
- $k_i$ – capacity factor for species “$i$”

$$k_i = \frac{V_{e,i} - V_o}{V_o}$$

- $\delta$ – selectivity between components “1” & “2”

$$\delta = \frac{k_1}{k_2}$$
Chromatography Separation of Peaks

Descriptors based on solute peaks as normal distributions

- $y_{\text{max},i}$ – Maximum concentration of eluting peak
- $t_{\text{max},i}$ – Time at which peak elutes
- $\sigma$ – Standard deviation of peak shape

\[ y_i = y_{\text{max},i} \exp \left[ -\frac{(t - t_{\text{max},i})^2}{2(\sigma t_{\text{max},i})^2} \right] \]
Chromatography Separation of Peaks

Descriptors based on solute peaks as normal distributions

- $Y_i$ — Yield of solute (ratio amount recovered to total in original mixture & recoverable)

$$Y_{t,i} = \frac{\int_0^t \dot{V}y_i \, dt}{\int_0^\infty \dot{V}y_i \, dt}$$

- Cumulative yield for normal distribution

$$Y_{t,i} = \frac{1}{2} \left[ 1 + \text{erf} \left( \frac{t - t_{\text{max},i}}{\sqrt{2} \sigma_{t_{\text{max},i}}} \right) \right]$$

- Incremental yield the difference between the cumulative yields @ two times
Chromatography Separation of Peaks

Descriptors based on solute peaks as normal distributions

- Excel has functions for normal Gaussian distributions – scaled so that the cumulative amount is 1

  Cumulative:  \( \text{NORM.DIST}( x, \text{mean}, \text{standard\_dev}, \text{TRUE} ) \)

  Differential:  \( \text{NORM.DIST}( x, \text{mean}, \text{standard\_dev}, \text{FALSE} ) \)
Chromatography Separation of Peaks

Descriptors based on solute peaks as normal distributions

- $\sigma^2$ is essentially a *Peclet number* — ratio of flowrate to reaction rate

\[
\sigma^2 = \frac{u}{k_a L} = \frac{\dot{V}}{A}
\]

- Value depends on the rate controlling step

\[
\sigma^2 \propto \frac{ud^2}{L} \quad \text{Internal diffusion control}
\]

\[
\sigma^2 \propto \frac{u^{1/2}}{d^{3/2}} \quad \text{External film control}
\]

\[
\sigma^2 \propto \frac{ud^2}{DL} \quad \text{Taylor diffusion}
\]
Chromatography Separation of Peaks

Descriptors based on HETP concept

- \( N \) – Number of theoretical stages (theoretical plates)

\[
N = 16 \left( \frac{V_e}{w} \right)^2
\]

- HETP – Height of a theoretical plate (\( L \) = length of column)

\[
H = \frac{L}{N}
\]

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Chromatography Separation of Peaks

Resolution – ratio distance of solute to solvent

- Resolution between two adjacent peaks

\[ R_S = \frac{t_{\text{max},i} - t_{\text{max},i}}{\frac{1}{2}(t_{w,i} + t_{w,i})} \]

\[ = \frac{t_{\text{max},i} - t_{\text{max},i}}{\frac{1}{2}\left[(4\sigma_i t_{\text{max},i}) + (4\sigma_i 4t_{\text{max},i})\right]} = \frac{2(V_{e,2} - V_{e,1})}{w_2 + w_1} \]

- Assume the spreading is about the same \((w2 = w1)\) & use concept of number of theoretical stages...

\[ R_S = \frac{V_{e,2} - V_{e,1}}{w} = \frac{1}{4}\sqrt{N}\frac{V_{e,2} - V_{e,1}}{V_{e,2}} = \frac{1}{4}\sqrt{N}\left(\frac{\delta - 1}{\delta}\right)\left(\frac{k_2}{k_2 + 1}\right) \]
Chromatography Material Balance

Assume downward flow through a fixed-bed chromatography column – adsorbed species come to very quick equilibrium with the adsorbent.

Following a small pulse (moving reference system)

\[
\frac{\partial C'_L}{\partial x} + A \left( \varepsilon \frac{\partial C'_L}{\partial V} + \frac{\partial C'_{S}}{\partial V} \right) = 0
\]

Integrated form...

\[
\Delta x = \frac{\Delta V}{A \left[ \varepsilon + M \, f'(C'_L) \right]}
\]

Uses equilibrium relationship...

\[
C'_{S} = M \, f(C'_L) \quad \text{and} \quad f'(C'_L) \equiv df'/dC'_L
\]
Chromatography Material Balance Example #1

Chromatography column 10 cm long with cross sectional. Packed with adsorbent, \( \varepsilon=0.35 \) & \( M=50 \) mg adsorbent per mL column volume. Adsorption isotherm given by:

\[
f(C_L) = 0.2 C_L^3\]

Determine position of the solute band & solvent front when \( \Delta V=250 \) mL & \( C_L=0.05 \) mg/mL

- **Solvent front:**
  \[
  \Delta x = \frac{\Delta V}{A \varepsilon} = \frac{250 \text{ cm}^3}{\left(10 \text{ cm}^2\right)(0.35)} = 71.4 \text{ cm}
  \]

- **Solute:**
  \[
  f'(C_L) = 0.6 C_L^2 \quad \Rightarrow \quad f'(C_L) = 0.6 \left(0.05\right)^2 = 0.0015
  \]
  \[
  \Delta x = \frac{\Delta V}{A \left[ \varepsilon + M \cdot f'(C_L) \right]} = \frac{250}{10 \left[ 0.35 + 50(0.0015) \right]} = 58.8 \text{ cm}
  \]
Chromatography Material Balance Example #2

Ion-exchange chromatography to purify 20 g of Protein A. Operate column @ 20 cm/hr. Peak exits at 80 min ($t_{\text{max}}$) with 10 min standard deviation ($\sigma t_{\text{max}}$).

How long must the column be run to get 95% yield?

$$Y_{t,i} = \frac{1}{2} \left\{ \text{erf} \left[ \frac{(t - t_{\text{max},i})}{\sqrt{2} \sigma t_{\text{max},i}} \right] \right\} + 1$$

$$= \frac{1}{2} \left\{ \text{erf} \left[ \frac{(t - 80)}{\sqrt{2} (10)} \right] \right\} + 1 = 0.95$$

- By iterative solution $t = 96.4$ min
Chromatography Material Balance Example #3

Let’s double the flowrate to 40 cm/hr. Assume the peak spreading depends on Taylor diffusion.

How long must the column be run to get 95% yield?

- Adjust the $t_{max} \& \sigma$ parameters

$$
\frac{t_{max,2}}{t_{max,1}} = \frac{u_1}{u_2} = \frac{20 \text{ cm/hr}}{40 \text{ cm/hr}} = 0.5 \quad \Rightarrow \quad t_{max,2} = 40 \text{ min}
$$

$$
\frac{\sigma_2^2}{\sigma_1^2} = \frac{u_2}{u_1} = 2 \quad \Rightarrow \quad \sigma_2 = \sigma_1 \sqrt{2} = \left(\frac{10}{80}\right) \sqrt{2} = 0.177
$$

- By iterative solution $t = 51.6$ min

$$
Y_{t,i} = \frac{1}{2} \left\{ \text{erf} \left[ \frac{(t - 40)}{\sqrt{2} (0.177 \cdot 40)} \right] + 1 \right\} = 0.95
$$
Material Balance Examples #2 & #3

Example #3 requires relatively more time because of the change in peak shape at the two velocities.
Summary

Downstream processing needed to separate cell mass & other byproducts from the water solvent

Major categories

- Separation of solids & liquids
  - Bulk separation – chemical species remain in their respective phases
  - Types
    - Filtration
    - Centrifugation
- Drying of solids
  - Dryness limited by the dryness of air & adhesion of water to solid
- Separation of soluble components
  - Adsorption – chromatography as special case