Description of cell mass growth

Qualitative

Substrates + Cells $\rightarrow$ (extracellular Products) + (more Cells)

Quantitative

$$\sum S_i + X \rightarrow \sum P_i + nX$$

Stoichiometry (example, aerobic)

$$\text{CH}_n\text{O}_m + a \text{O}_2 + b \text{NH}_3 \rightarrow c \text{CH}_i\text{O}_j\text{N}_k + d \text{H}_2\text{O} + e \text{CO}_2$$

Substrate

Biomass
Material Balance – Batch Reactor

Cell Balances:

\[ V_e \frac{dX}{dt} = V_e \mu_{e} X - V_e k_p X = V_e \mu_{net} X \]

\[ \mu_{net} = \frac{1}{X} \frac{dX}{dt} \]

Substrate Consumption & Product Growth:

\[ q_S = -\frac{1}{X} \frac{dS}{dt} \]

\[ q_P = +\frac{1}{X} \frac{dP}{dt} \]

If \( \mu_{net} \) is constant then get exponential growth phase

\[ \frac{dX}{dt} = \mu_{net} X \]

\[ \frac{dX}{X} = \mu_{net} dt \]

\[ \ln \left( \frac{X}{X_0} \right) = \mu_{net} t \Rightarrow X = X_0 \exp(\mu_{net} t) \]

Followed by deceleration growth (unbalanced growth) & stationary (growth equal to death) phases
Material Balance – Batch Reactor

Death phase is 1st order in cell concentration & gives exponential decay

\[
\frac{dX}{dt} = -k_d X \\
\ln \left( \frac{X}{X_{SO}} \right) = -k_d t \quad \Rightarrow \quad X = X_{SO} \exp(\mu_m t)
\]

Some Growth Models

Substrate-Limited Growth (Moser equation, Monod for \( n=1 \))

\[
\mu_g = \frac{\mu_m S}{K_s + S}
\]

Substrate-Limited Growth (Contois equation)

\[
\mu_g = \frac{\mu_m S}{K_s X + S}
\]

Noncompetitive Substrate Inhibition

\[
\mu_g = \frac{\mu_m}{1 + \frac{K_s + S}{K_s}}
\]

Competitive Substrate Inhibition

\[
\mu_g = \frac{\mu_m}{K_s \left( 1 + \frac{S}{K_s} + S \right)}
\]

Noncompetitive Product Inhibition

\[
\mu_g = \frac{\mu_m}{\left( 1 + \frac{K_s}{S} \right) \left( 1 + \frac{P}{K_p} \right)}
\]

Competitive Product Inhibition

\[
\mu_g = \frac{\mu_m}{K_s \left( 1 + \frac{P}{K_p} \right) + S}
\]
Monod Growth Model

Substrate-Limited Growth

\[
\frac{\mu}{\mu_m} = \frac{S}{K_S + S} \Rightarrow \frac{\mu}{\mu_m} = \frac{S / K_S}{1 + (S / K_S)}
\]

- Limits:
  - Constant growth rate at large substrate concentrations
  - Proportional to substrate concentration at low concentrations

Also:

\[
\frac{\mu}{\mu_m} = \frac{S}{K_S + S} \Rightarrow S = \frac{\mu g K_S}{\mu_m - \mu g}
\]
Material Balances – Ideal Chemostat (CSTR)

Cell balance:

\[ \frac{dX}{dt} = V_e \frac{dX}{dt} - FX + \left( V_e \mu_e X - V_e k_d X \right) \]

where: \( D = \frac{F}{V_R} \)

Usually feed is cell mass & product free

\[ \frac{dX}{dt} = \left( \mu_e - k_d - D \right) X = (\mu_{net} - D) X \]

At steady state & negligible death rate

\[ 0 = \left( \mu_e - D \right) X \quad \Rightarrow \quad \mu_e = D \]

Growth rate can be controlled by changing the dilution rate!

- However, if the dilution rate is too large then the cell mass is “washed out” — the culture cannot reproduce fast enough to grow before it is removed
Material Balances – Ideal Chemostat (CSTR)

Substrate balance
\[
\frac{dS}{dt} = FS_0 - FS + \nu \left( \frac{\mu_s X}{Y_{X/S}} - \frac{q_r X}{Y_{P/S}} - m X \right)
\]

At steady state
\[
0 = D(S_0 - S) - \left( \frac{\mu_s}{Y_{X/S}} + \frac{q_r}{Y_{P/S}} + m \right) X \quad \Rightarrow \quad \frac{D(S_0 - S)}{X} = \frac{\mu_s}{Y_{X/S}} + \frac{q_r}{Y_{P/S}} + m
\]

- Linear equation of substrate consumption
  - Grow cell mass
  - Create product
  - Provide energy to the cell mass

Material Balances – Ideal Chemostat (CSTR)

If negligible product formation & maintenance, then:

\[
\frac{D(S_0 - S)}{X} = \frac{\mu_s}{Y_{X/S}} \Rightarrow X = Y_{X/S} \frac{D}{\mu_s} (S_0 - S) = \frac{Y_{X/S}}{\mu_s} (S_0 - S)
\]

Substrate (for Monod eqn):

\[
\mu_s = \frac{\mu_\infty S}{K_s + S} \quad \Rightarrow \quad S = \frac{K_s \mu_\infty}{\mu_\infty - \mu_s}
\]

\[
X = Y_{X/S} \left( S_0 - \frac{K_s \mu_\infty}{\mu_\infty - \mu_s} \right) = Y_{X/S} \left( S_0 - \frac{K_s D}{\mu_\infty - \mu_s} \right)
\]
Material Balances – Ideal Chemostat (CSTR)

Product formation – steady state with introduction of cell mass (but no net growth):

- From cell balance:
  \[ 0 = D X_0 + \left( \mu_{\text{max}} - D \right) X \Rightarrow X = X_0 \]

- From substrate balance:
  \[ 0 = D (S_0 - S) - \left( \frac{\mu_g}{Y_{X/S}^M} + \frac{q_p + m_i}{Y_{P/S}} \right) X \Rightarrow S = S_0 - \frac{1}{D} \frac{q_p X}{Y_{P/S}} \]

- From product yield definition:
  \[ P - P_0 = Y_{P/S} (S - S_0) \]

Other Configurations – Chemostat with Recycle

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Other Configurations – Multi-Stage Chemostat

Other Configurations – Fed Batch
Use of Batch Data in Flow Reactors

For a batch reactor

\[ \frac{dX}{dt} = \mu_{\text{net}} X \]

For a CSTR it makes sense that the outlet concentration is related to the batch reactor’s results such that:

\[ \mu_{\text{net}} (X - X_0) = \left( \frac{dX}{dt} \right)_{\text{batch}} |_{t = \text{extent}} \]

where \( t_{\text{extent}} \) is some characteristic batch time that represents the extent of reaction.
Use of Batch Data in Flow Reactors

For a chemostat the dilution factor $D$ controls the growth factor $\mu_{\text{net}}$

You can relate the two systems & show performance by

- Plot $dX/dt$ vs $X$ for the batch data
- Plot a straight line through $X_0$ on the horizontal axis with a slope of $D$
- The intersection of the batch results curve & the chemostat performance line will give the value of $X$ within the chemostat. The original batch $X$ vs. $t$ data will then give the corresponding $t_{\text{extent}}$

Product composition can be determined either by:

- Find the corresponding $P$ at $t_{\text{extent}}$, or
- Do a similar $D/P/dt$ vs. $P$ analysis

Use of Batch Data in Flow Reactors

Using data from Example 6.2, ethanol from glucose using *S. cerevisiae*

- Time derivatives estimated from central differences

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<th>Time (h)</th>
<th>Glucose (g/L)</th>
<th>Biomass (g/L)</th>
<th>Ethanol (g/L)</th>
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</tbody>
</table>
Use of Batch Data in Flow Reactors

For a chemostat, $D=0.05 \, \text{h}^{-1}$

Use of Batch Data in Flow Reactors

For a batch reactor “productivity” is the time-derivative increase in concentration vs. time.

For a CSTR the analogous term is the dilution factor times the concentration, e.g., $D \times P$
Details for Other Bioreactor Configurations

Other Configurations – Chemostat with Recycle

- Medium: $F, X_0 (1+a)$, $F, X_1$
- $\alpha F$
- $S_0$
- $F, X_2$
- Cell Separator
- $C X_1$
Material Balances – Chemostat with Recycle

Cell balance:

\[ \frac{dX_1}{dt} = V\frac{dX_0}{dt} = FX_0 + \alpha F(CX_1) - (1 + \alpha)FX_1 + V\mu_{\text{ref}}X_1 \]
\[ \frac{dX_i}{dt} = D\left[X_0 + \alpha(CX_i)\right] - (1 + \alpha)DX_1 + \mu_{\text{ref}}X_1 \]

\[ \alpha = \text{Ratio recycle flowrate to fresh feed rate} \]
\[ C = \text{Concentration factor in Cell Separation} \]

At steady state with \( X_0 = 0 \)

\[ 0 = D[\alpha(CX_i) - (1 + \alpha)DX_1 + \mu_{\text{ref}}X_1] \]
\[ \mu_{\text{ref}} = D[1 + \alpha(1 - C)] \]

Material Balances – Chemostat with Recycle

Cell balance around Cell Separator @ steady state:

\[ (1 + \alpha)FX_1 = \alpha F(CX_1) + FX_2 \]
\[ X_2 = \left[1 + \alpha(1 - C)\right]X_1 \]

Since \( C > 1 \) then \( X_2 < X_1 \)
Material Balances – Chemostat with Recycle

Substrate balance

\[ V_\ell \frac{dS}{dt} = F S_0 + \alpha F S - (1 + \alpha) F S + V_k \left( \frac{\mu X_i}{Y_{X/S}} - \frac{q X_i}{Y_{P/S}} - m X_i \right) \]

\[ \frac{dS}{dt} = D (S_0 - S) - \left( \frac{\mu_i X_i}{Y_{X/S}} + \frac{q_i X_i}{Y_{P/S}} + m_i X_i \right) \]

At steady state & growth limited

\[ 0 = D (S_0 - S) - \left( \frac{\mu_i X_i}{Y_{X/S}} + \frac{q_i X_i}{Y_{P/S}} + m_i X_i \right) \Rightarrow \]

\[ X_i = \frac{D}{\mu_i} Y_{X/S} (S_0 - S) \Rightarrow X_i = \frac{Y_{X/S}^m (S_0 - S)}{1 + \alpha(1 - C)} \]

Other Configurations – Multi-Stage Chemostat
Material Balances – Multi-Stage Chemostat

Cell balance – 2 reactors in series

\[ V_1 \frac{dX_1}{dt} = FX_0 - FX_1 + \mu_{\text{net},1} X_1 V_1 \]
\[ V_2 \frac{dX_2}{dt} = FX_1 + F'X_0 - (F + F')X_2 + \mu_{\text{net},2} X_2 V_2 \]

1st reactor looks like a single reactor. Focus on the downstream reactor(s)

At steady state with \( X_0 = 0 \)

- Now growth rate dependent on cell mass compositions

\[ 0 = FX_1 - (F + F')X_2 + \mu_{\text{net},2} X_2 V_2 \quad \Rightarrow \quad \mu_{\text{net},2} = \frac{F + F'}{V_2} - \frac{F}{V_2} \frac{X_1}{X_2} = D_2 - \frac{F}{V_2} \frac{X_1}{X_2} \]

Material Balances – Multi-Stage Chemostat

Substrate balance – focus on 2nd reactor

\[ V_2 \frac{dS_2}{dt} = FS_1 + F'S_0 - (F + F')S_2 \]
\[ + V_2 \left( \frac{\mu_{\text{g},2}}{Y_{X/S}} - \frac{S_2}{Y_{P/S}} - m_s \right) X_2 \]

At steady state with only cell mass growth:

\[ 0 = FS_1 + F'S_0 - (F + F')S_2 - \frac{\mu_{g,2} X_2}{Y_{X/S}} V_2 \quad \Rightarrow \]
\[ S_2 = \frac{FS_1 + F'S_0}{F + F'} - \frac{\mu_{g,2} X_2}{Y_{X/S}} \frac{V_2}{F + F'} = \frac{FS_1 + F'S_0}{F + F'} - \frac{\mu_{g,2} X_2}{D_2 Y_{X/S}} \]
Material Balances – Multi-Stage Chemostat

Must simultaneously solve the 3 equations for cell mass & substrate concentrations as well as growth rate

For Monod eqn:

\[
\mu_{p,2} = \frac{F \cdot X_1}{V_2 \cdot X_2}
\]

\[
S_2 = \frac{F \cdot S_i + F' \cdot S_0}{F + F'} - \frac{\mu_{p,2} X_2}{D_2 \cdot Y_{X/S}}
\]

\[
\mu_{p,2} = \frac{\mu_m S_2}{K_s + S_2}
\]