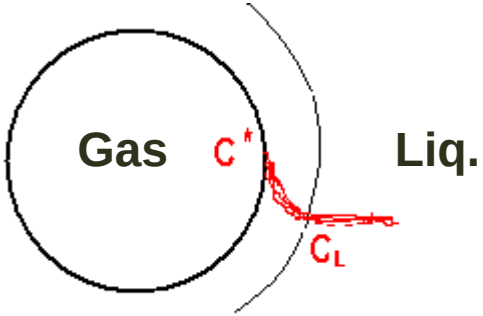
Oxygen transfer rate (OTR)

- **Oxygen transfer from gas to liquid**



- **OTR = k_L a (C^* - C_L)**
  - OTR [mg O_2 / L / h]
  - k_L : oxygen transfer coefficient (cm/h)
  - a : gas-liquid interfacial area per unit vol. (cm^2/cm^3)
  - k_L a : volumetric oxygen transfer coefficient (1/h)
  - C^* : saturated DO concentration (mg/L)
  - C_L : DO concentration in the broth (mg/L)

Oxygen uptake rate (OUR)

- **OUR from liquid to cell**

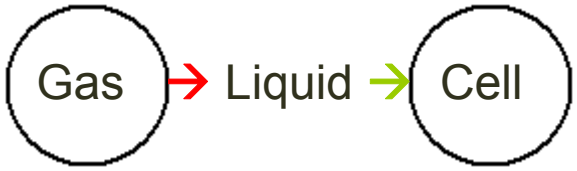
$$OUR = q_{O2} X = (\mu X) / Y_{X/O2}$$

  - OUR [mg O_2 / L / h]
  - q_{O2} : specific rate of oxygen consumption (mg O_2/g cell/h)
  - $Y_{X/O2}$: oxygen yield coefficient (g cell/g O_2)

- **When oxygen transfer is the rate-limiting step,**

$$OTR (\rightarrow) = OUR (\rightarrow)$$

$$k_L a (C^* - C_L) = (\mu X) / Y_{X/O2}$$



$$Y_{X/O2} k_L a (C^* - C_L) = dX / dt$$

$$Y_{X/O2} \bullet OTR = \text{cell growth rate}$$

Modeling cell growth ; Monod equation

Similar to Michaelis-Menten Kinetics

Assumes that a single enzyme system is responsible for the uptake of substrate (S), and that S is limited (growth-dependent variable). This is the most common kinetic model for cell growth.

$$\mu = \frac{\mu_m S}{K_S + S}$$

μ = specific cell growth rate (hr^{-1})

μ_m = maximum specific cell growth rate (hr^{-1})

S = substrate concentration (g/L)

K_S = Saturation constant (g/L) = S when $\mu = 1/2\ \mu_m$.

Batch culture growth model

X(t) = ?

$$\mu = \frac{1}{X}\frac{dX}{dt} = \frac{\mu_m S}{K_S + S} \quad \cdots\cdots (1)$$

We relate changes in S to changes in X through $Y_{X/S}$

$$X - X_o = Y_{X/S}(S_o - S), \text{ or}$$

$$S = S_o + X_o/Y_{X/S} - X/Y_{X/S} \quad \cdots\cdots (2)$$

$Y_{X/S}$ = cell mass yield (g dcw/g S consumed)

X_o, S_o = initial concentrations of cells and substrate

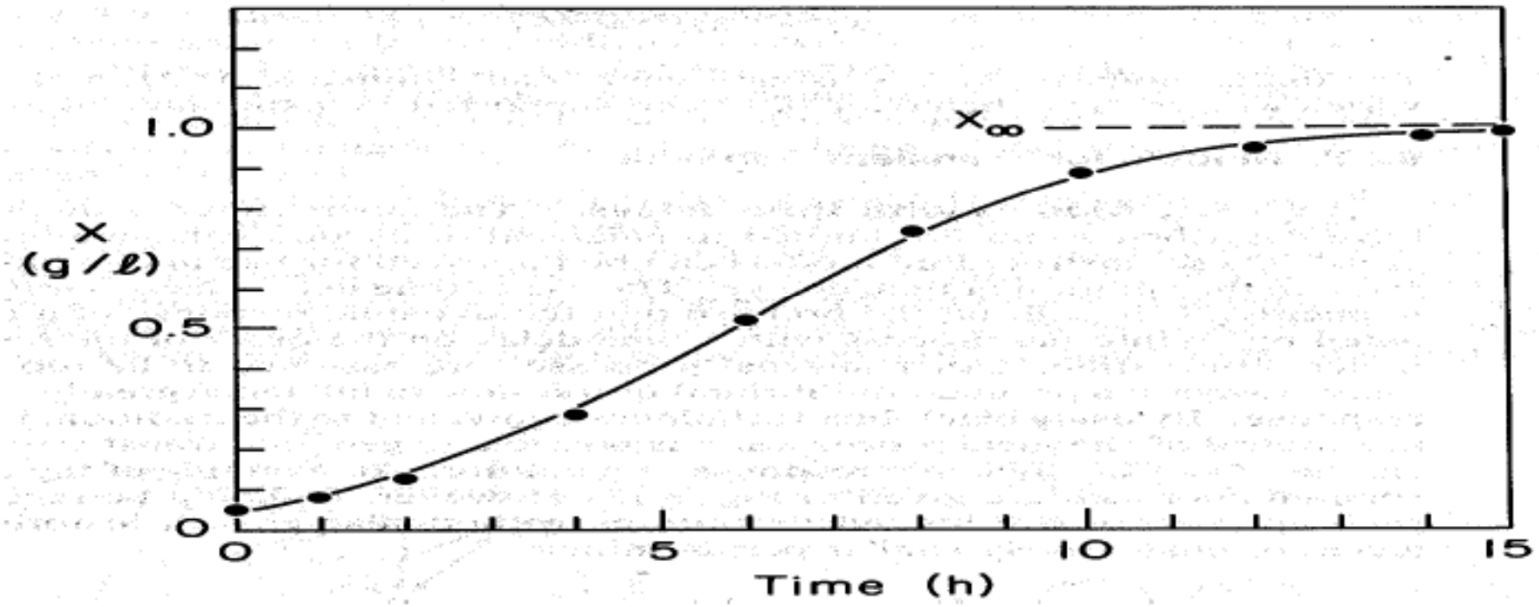
Combine (1) and (2), and rearrange

$$\frac{dX}{dt} = \frac{\mu_m (S_o Y_{X/S} + X_o - X)}{(K_S Y_{X/S} + S_o Y_{X/S} + X_o - X)} X \quad ; \quad \text{at } t = 0,\ X = X_o$$

Batch culture growth model (cont.)

Logistic Equation

$$\frac{(K_S Y_{X/S} + S_o Y_{X/S} + X_o)}{(S_o Y_{X/S} + X_o)} \ln\left(\frac{X}{X_o}\right) - \frac{K_S Y_{X/S}}{(S_o Y_{X/S} + X_o)} \ln\{(S_o Y_{X/S} + X_o - X) S_o Y_{X/S}\} = \mu_m t$$



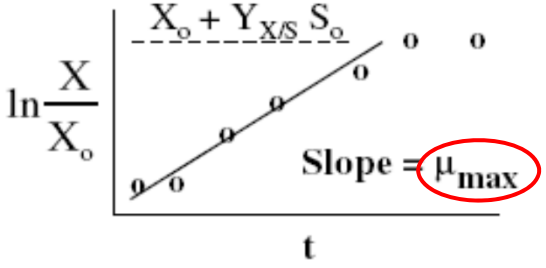
How to determine Monod parameters, K_s and μ_{max}

$$\mu = \frac{1}{X}\frac{dX}{dt}$$

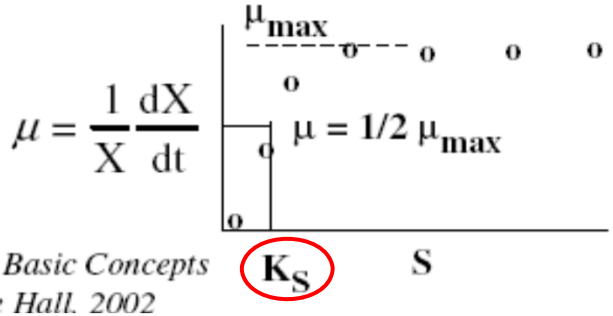
$$\mu\, t = \ln\left(\frac{X}{X_o}\right)$$

K_S is determined differently.

K_S is equal to S when $\mu = 1/2\ \mu_{max}$



$\mu = 1/X\ dX/dt$ needs to be determined from available data, especially data at low S concentrations.



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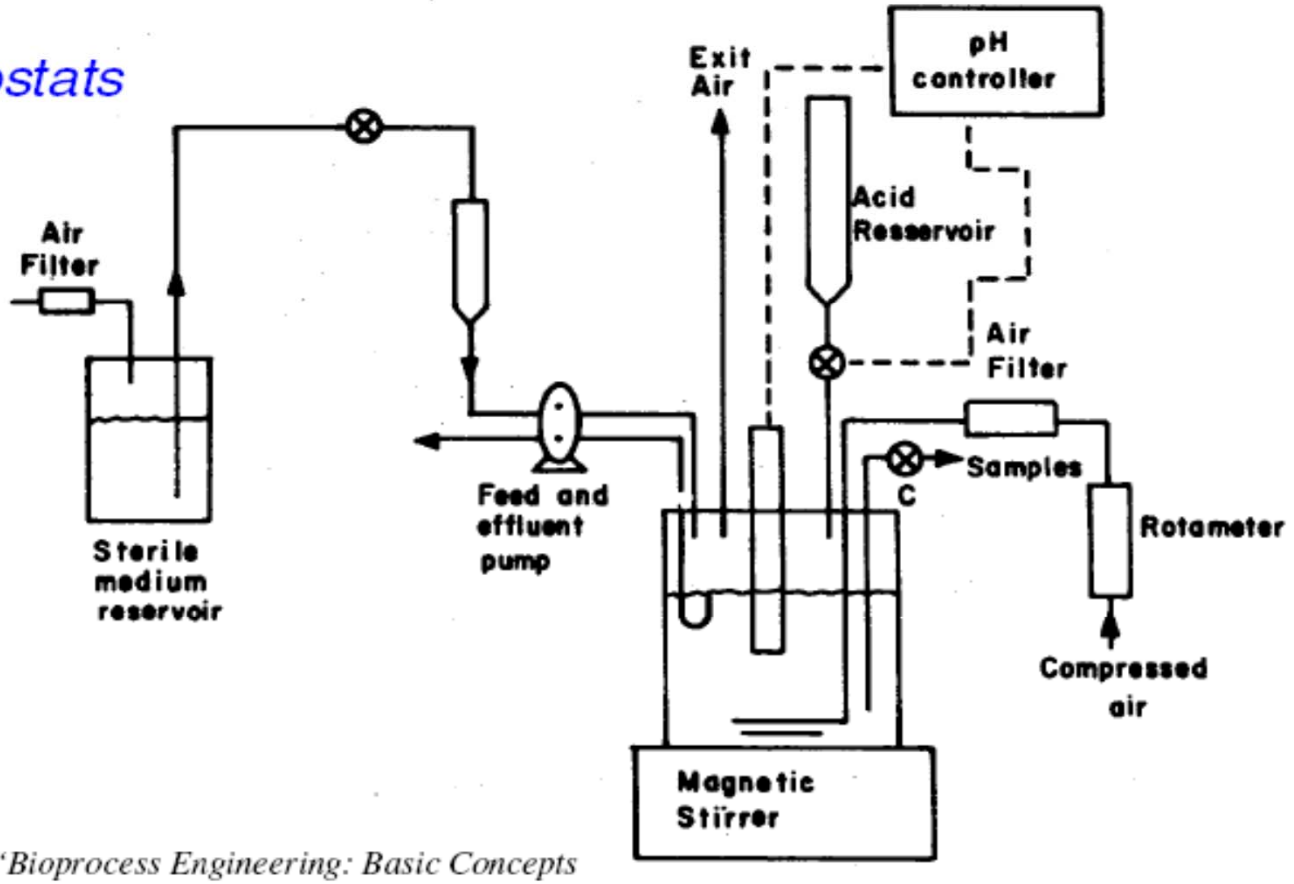
Cell growth in continuous culture

Automated Chemostats

→ control of pH, temp. agitation, dissolved oxygen

→ sterilization required



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Chemostat as a tool

→ evaluate K_S, μ_{max}, $Y_{X/S}$ and other system parameters

→ study changes in environment and effects on cell physiology

→ select for cells with desired metabolic capabilities (e.g. selection for cells capable of degrading a toxic compound)

Chemostat mass balance

Why derive mass balance equation?

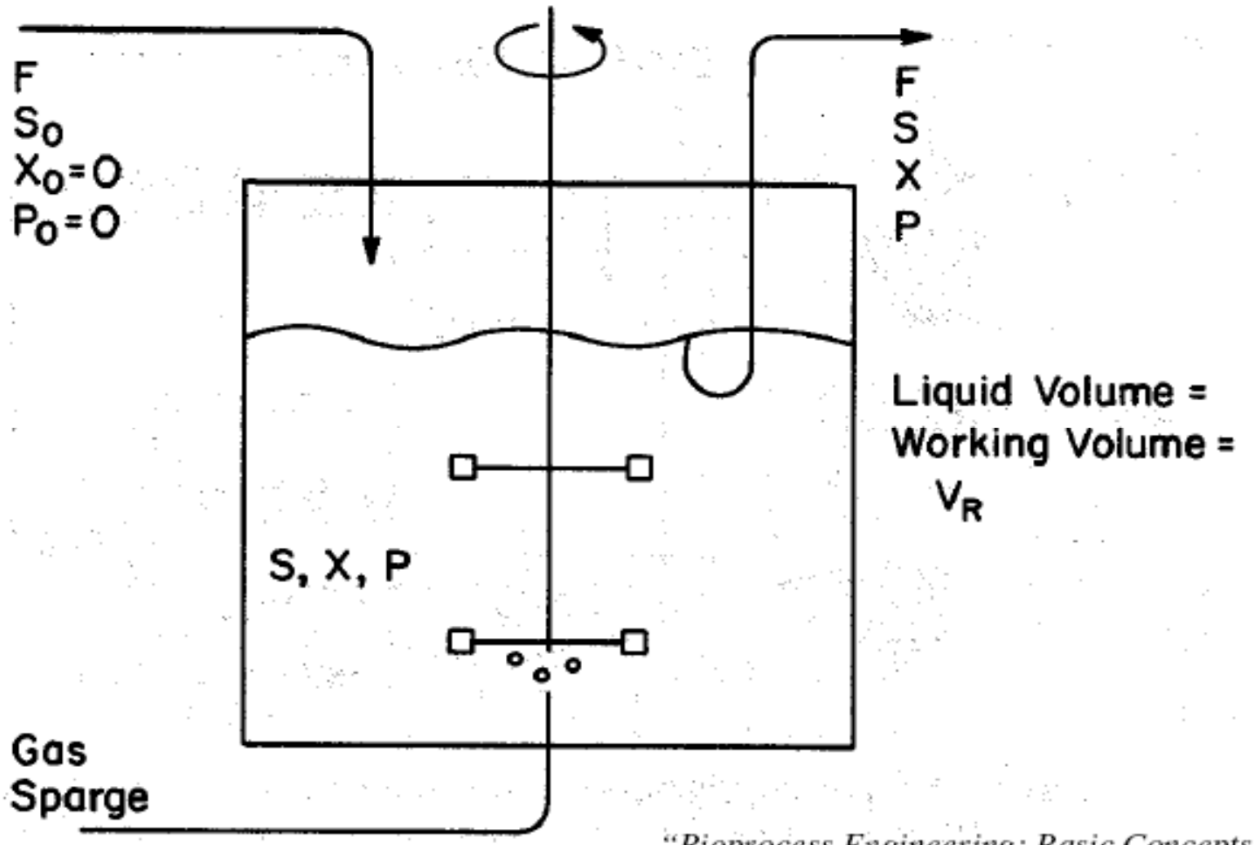
1. Describe dynamics of cell growth, substrate utilization, and product formation.

2. Useful for control of bioreactors.

3. Evaluate kinetic and yield parameters. ($Y_{x/s}$, K_s, μ_{max})

4. Determine the optimum values for bioreactor operating parameters.

Continuous-stirred tank reactor (CSTR), chemostat



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Cell mass balance in CSTR to get S

[mass rate of cells into bioreactor] - [mass rate of cells out of bioreactor] + [mass rate of cell growth without endogenous metabolism] - [mass rate of cell loss by endogenous metabolism] = [mass rate of cells accumulation in bioreactor]

or

$$FX_o - FX + V_R\mu X - V_R k_d X = V_R \frac{dX}{dt}$$

F = in and out volumetric flow rate (L / hr)

X = bioreactor and outlet cell mass concentration (g/L)

X_o = inlet cell mass concentration (g/L) = 0

μ = specific cell growth rate neglecting endogenous metabolism (hr^{-1})

k_d = endogenous cell loss rate constant (hr^{-1})

Steady state and sterile feed

Chemostats are normally operated at steady-state, d/X/dt = 0. Assume a sterile feed ($X_o = 0$), and k_d is so small that is neglected, $k_d = 0$.

The cell mass balance equations becomes,

$$\begin{bmatrix}\text{mass rate}\\ \text{of cells out}\\ \text{of bioreactor}\end{bmatrix} = \begin{bmatrix}\text{mass rate of cell}\\ \text{growth without}\\ \text{endogenous}\\ \text{metabolism}\end{bmatrix}$$

or

$$FX = V_R \mu X$$

$$\frac{F}{V_R} = \mu \quad \text{or} \quad \boxed{D = \mu}$$

where $\frac{F}{V_R} = D$, dilution rate

D [sec^{-1}] ; how many times of rxtor vol. flow per second

Substrate concentration in CSTR when $k_d = 0$

Using the Monod Equation, we can predict the bioreactor and outlet stream concentration of Substrate.

$$\mu = \frac{\mu_{max} S}{K_S + S} = D$$

rearranging, $$\boxed{S = \frac{K_S D}{\mu_{max} - D}}$$

"Washout" for CSTR

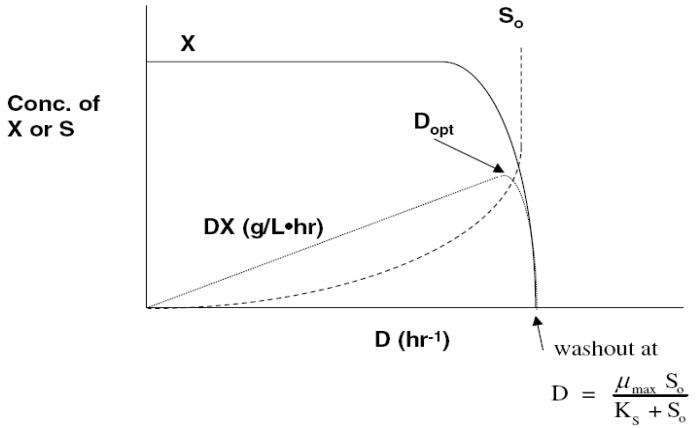
$$X = Y_{x/s}(S_o - S)$$

$$= Y_{x/s}\left(S_o - \frac{DK_s}{\mu_{max} - D}\right)$$

$$@\ D = D_{w.o.}, \quad X = 0$$

$$0 = S_o - \frac{D_{w.o.} K_s}{\mu_{max} - D_{w.o.}}$$

$$D_{w.o.} = \frac{\mu_{max} S_o}{K_s + S_o}$$



There is an upper limit on D, or the cells will washed out of the bioreactor.

$$D \leq \frac{\mu_{max} S_o}{K_S + S_o}$$

Substrate concentration in CSTR when $k_d \neq 0$

From cell mass balance

$$F\,X = V_R\,\mu\,X - V_R\,k_d\,X$$

$$F = V_R\,(\mu - k_d)$$

$$D = \mu - k_d$$

$$\mu = \frac{\mu_{max}\,S}{K_s + S} = D + k_d$$

$$S = \frac{K_s(D + k_d)}{\mu_{max} - D - k_d}$$

$\rightarrow$ S is higher than the case when $k_d = 0$

Substrate mass balance in CSTR to get X

How is X affected by D? A similar mass balance equation for S *in the absence* of endogenous metabolism is written to answer this question.

$$FS_o - FS - V_R \mu X \frac{1}{Y^M_{X/S}} - V_R q_p X \frac{1}{Y_{P/S}} = V_R \frac{dS}{dt}$$

S = bioreactor and outlet substrate concentration (g/L)

S_o = inlet substrate concentration (g/L)

$Y^M_{X/S}$ = maximum cell yield coefficient (g cells / g substrate)

$Y_{P/S}$ = product yield coefficient (g product / g substrate)

q_p = specific rate of extracellular product formation $\left(\frac{\text{g P}}{\text{g cells} \bullet \text{hr}}\right)$

Cell concentration in CSTR

For the simple case of no product formation ($q_p=0$), steady-state ($dS/dt=0$), and no endogenous metabolism, $k_d=0$.

$$D(S_o - S) = \frac{\mu X}{Y^M_{X/S}}$$

at steady-state, $\mu = D$, and solving for X,

$$X = Y^M_{X/S}(S_o - S)$$

or

$$X = Y^M_{X/S}\left(S_o - \frac{K_S D}{\mu_{max} - D}\right)$$

when $k_d \neq 0$

Thus far, the substrate balance eqn. Has been written assuming that $Y_{X/S}$ is a constant at $Y^M_{X/S}$.

With endogenous metabolism,

$\mu = D + k_d$

and with no extracellular product formation, the substrate mass balance is at steady-state,

$$D\frac{(S_o - S)}{X} - \frac{(D+k_d)}{Y^M_{X/S}} = 0$$

rearranging,

$$D\frac{(S_o - S)}{X} - \frac{D}{Y^M_{X/S}} - \frac{k_d}{Y^M_{X/S}} = 0$$

and

$$\frac{D}{Y^{AP}_{X/S}} - \frac{D}{Y^M_{X/S}} - \frac{k_d}{Y^M_{X/S}} = 0$$

$$\frac{1}{Y^{AP}_{X/S}} = \frac{1}{Y^M_{X/S}} + \frac{m_S}{D} = 0$$

where $m_S = \frac{k_d}{Y^M_{X/S}}$

maintenance coefficient based on S.

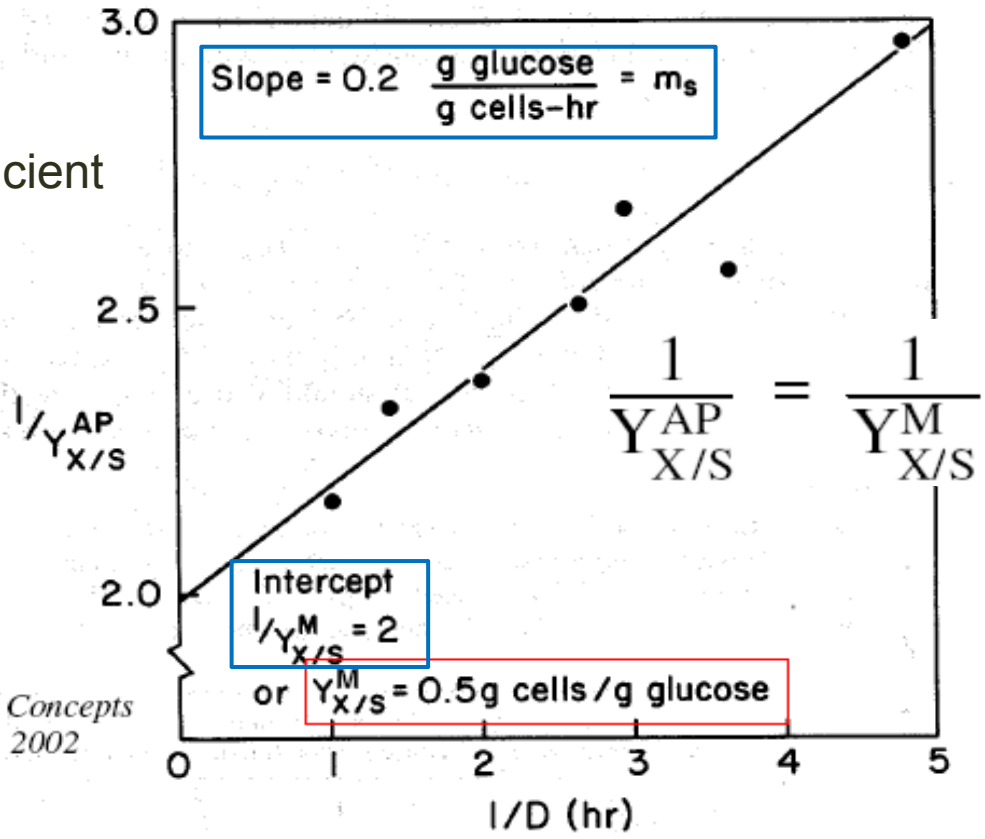
Measurement of maximum cell yield and maintenance using a chemostat

From measurements of X, S, S_o, and D in a chemostat experiment at different D values, a double reciprocal plot can be made.

Maintenance coefficient

Slope ; $m_S = \frac{k_d}{Y^M_{X/S}}$

$k_d = m_S Y^M_{X/S}$



$$\frac{1}{Y^{AP}_{X/S}} = \frac{1}{Y^M_{X/S}} + \frac{m_S}{D} = 0$$

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Determination of μ_{max} and K_s using a chemostat

From data collected using a chemostat, we can obtain the Monod Equation kinetic parameters.

Data include S at several Dilution Rates (D),

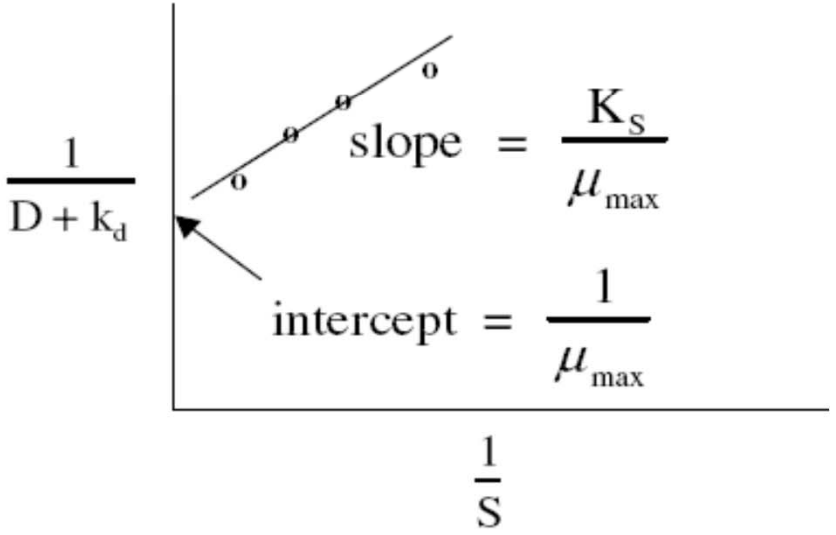
Recall that,

$$D = \mu - k_d \quad \text{(when } k_d \neq 0\text{)}$$

$$D = \frac{\mu_{max} S}{K_S + S} - k_d$$

rearranging

$$\frac{1}{D + k_d} = \frac{1}{\mu_{max}} + \frac{K_S}{\mu_{max}} \frac{1}{S}$$



Productivity of a chemostat

Cell production rate in CSTR [g/h] = FX

Pr_X = productivity for cell production = DX = FX / V

Pr_P = productivity for product formation = DP

The dilution rate (D) which maximizes productivity is found by taking dPr/dD = 0 and solving for D ($D_{optimum}$).

For example, $D_{optimum}$ for X with $k_d = 0$ and $q_P = 0$

$$X = Y^{M}_{X/S}\left(S_o - \frac{K_S\, D}{\mu_{max} - D}\right) \quad \Rightarrow \quad DX = Y^{M}_{X/S} D\left(S_o - \frac{K_S\, D}{\mu_{max} - D}\right)$$

take $\frac{d(DX)}{dD} = 0$ and solve for D (D_{opt})

$$D_{opt} = \mu_{max}\left(1 - \sqrt{\frac{K_S}{K_S + S_o}}\right)$$

K_S is usually << S
so $D_{opt} \sim \mu_{max}$ (washout point)

Product mass balance

$$FP_o - FP + V_R q_P X = V_R \frac{dP}{dt}$$

at steady - state, $dP / dt = 0$ and for $P_o = 0$

$$DP = q_P X \text{ or } \boxed{P = \frac{q_P X}{D}}$$

for $k_d = 0$, no endogenous metabolism

$$S = \frac{K_S D}{\mu_{max} - D} \text{ from X mass balance}$$

$$X = Y^M_{X/S}(S_o - S)\frac{D}{(D + q_P \frac{Y^M_{X/S}}{Y_{P/S}})} \text{ from S mass balance}$$

Product mass balance

for $k_d \neq 0$, with endogenous metabolism

$$S = \frac{K_S (D + k_d)}{(\mu_{max} - D - k_d)} \quad \text{from X mass balance}$$

$$X = Y^M_{X/S}(S_o - S)\frac{D}{(D + k_d + q_P \frac{Y^M_{X/S}}{Y_{P/S}})} \quad \text{from S mass balance}$$

to determine D for optimum P formation,

$$\frac{d(DP)}{dD} = 0 \quad \Rightarrow \quad \text{solve for } D_{opt}$$

Chemstat response to D