“Washout” for CSTR

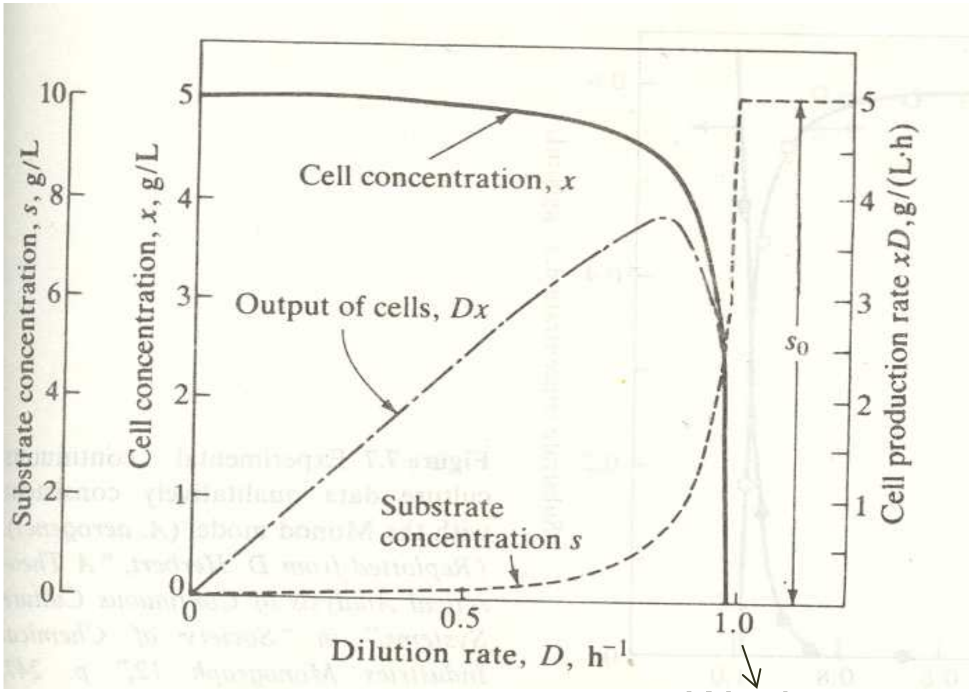
$$X = Y_{x/s}(S_o - S)$$

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

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

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

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



Wash out

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

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

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

$$D = \mu - k_d \quad \rightarrow \quad \mu = D + k_d$$

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

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

$\rightarrow$ 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 $\left(\frac{g\ P}{g\ cells \bullet hr}\right)$

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

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

Cell concentration in CSTR

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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.

AP: apparent (when $k_d \neq 0$)

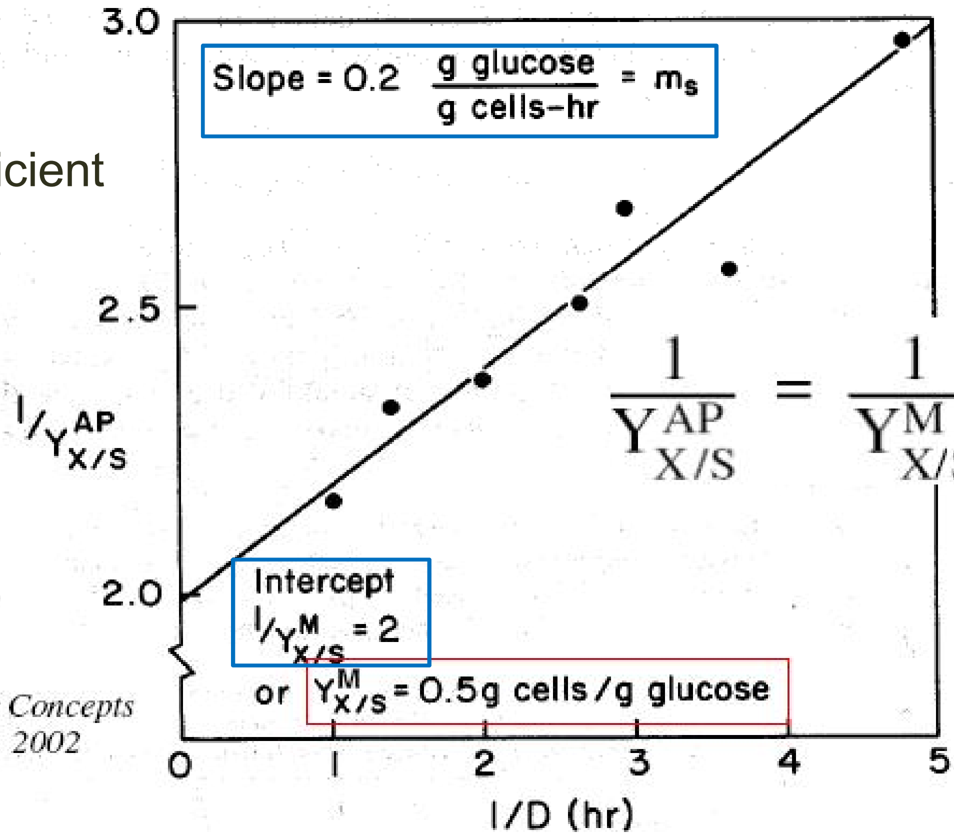
Measurement of maximum cell yield and maintenance coeff using a chemostat

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

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

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



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

"Bioprocess Engineering: Basic Concepts Shuler and Kargi, Prentice Hall, 2002

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),

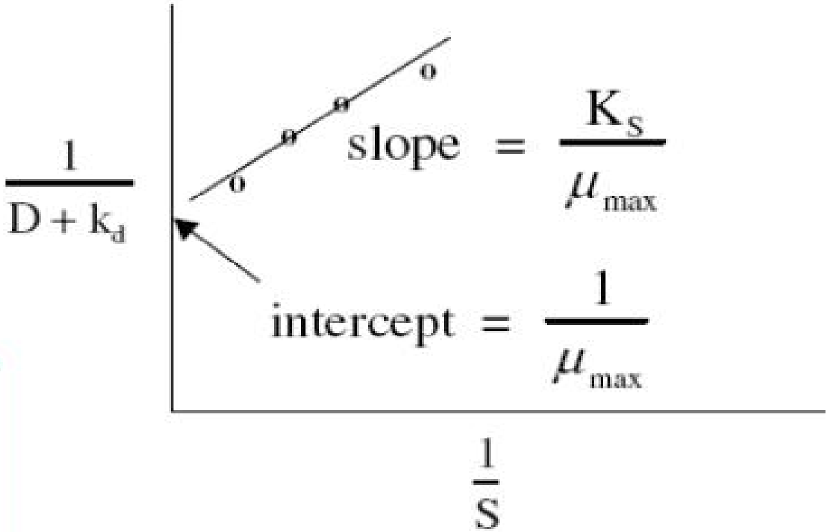
Recall that,

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

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

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 dDX/dD = 0 and solving for D ($D_{optimum}$).

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

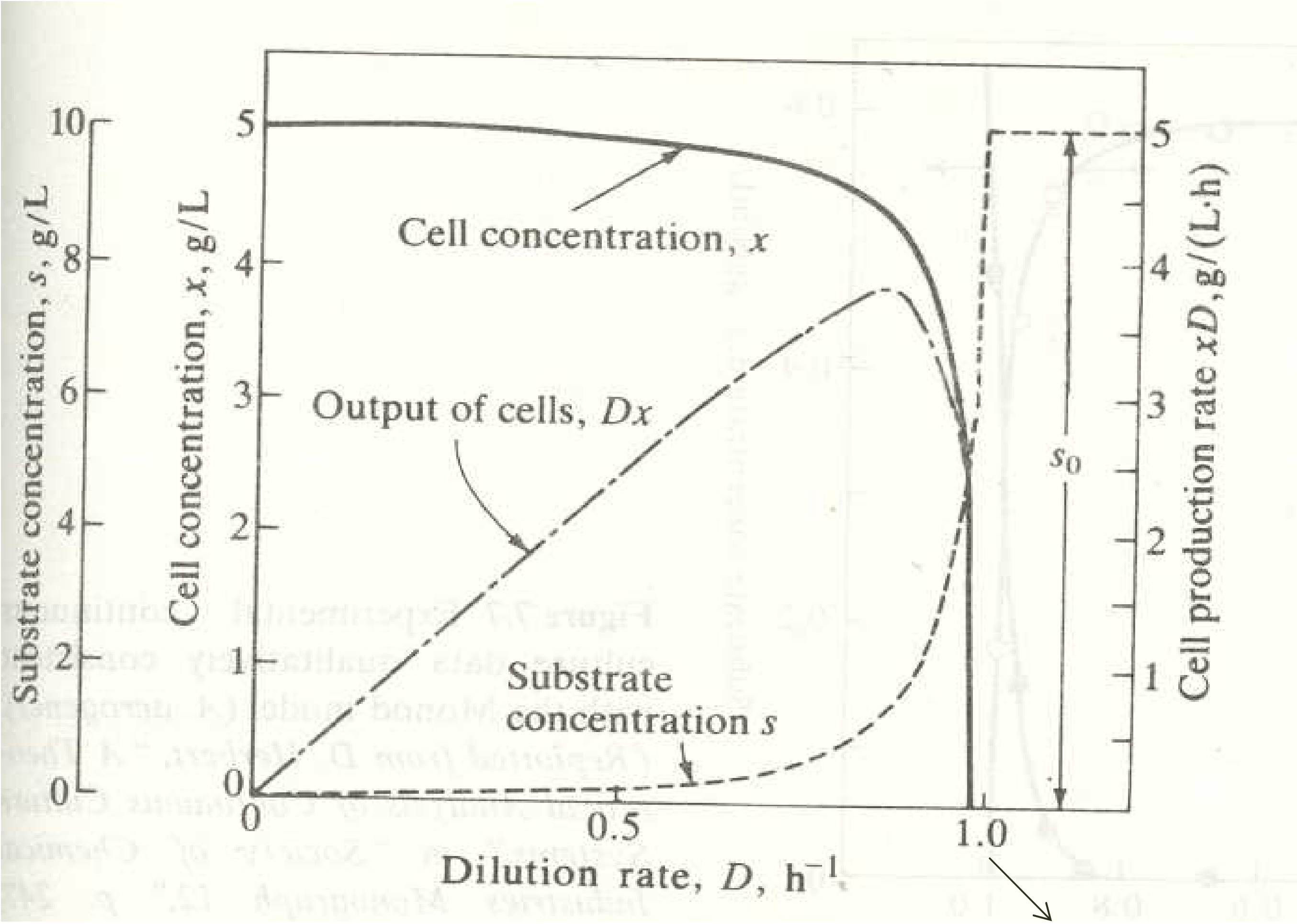
$$X = Y^{M}_{X/S}\left(S_o - \frac{K_S D}{\mu_{max} - D}\right) \quad \Rightarrow \quad DX = Y^{M}_{X/S} D\left(S_o - \frac{K_S D}{\mu_{max} - D}\right)$$

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

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

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

Chemstat response to D



Wash out

$$D = \frac{\mu_{max} S_o}{K_s + S_o}$$

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

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

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

to determine D for optimum P formation,

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

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,

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

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})

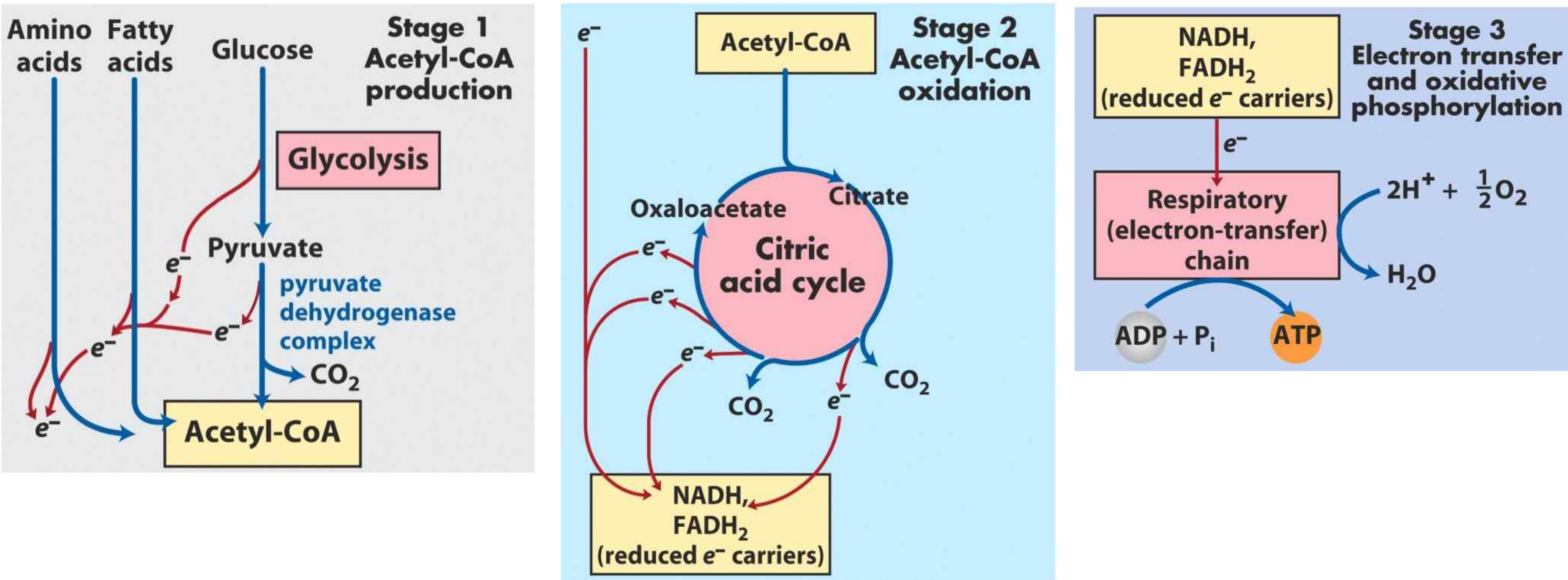
4. Determine the optimum values for bioreactor operating parameters.

Chapter 7

Stoichiometry of Microbial Growth and Product Formation

Some Definitions of Yield Coefficients

- Respiration



- RQ (Respiratory Quotient)
  - moles of CO_2 produced per mole of O_2 consumed

Elemental Balances

- **Cell composition**
  - $CH_{\alpha}O_{\beta}N_{\delta}$

- **1 mole of biological materials**
  - the amount containing 1 gram atom of carbon, such as $CH_{\alpha}O_{\beta}N_{\delta}$
  - gram atom = mole

Elemental Balances

- **Simple biological conversion**

$$CH_mO_n + a\ O_2 + b\ NH_3 \rightarrow c\ CH_\alpha O_\beta N_\delta + d\ H_2O + e\ CO_2$$

(carbohydrate) (cell)

where CH_mO_n : 1 mole of carbohydrate
$CH_\alpha O_\beta N_\delta$: 1 mole of cellular material

Elemental Balances

C:	$1 = c + e$
H:	$m + 3b = c\alpha + 2d$
O:	$n + 2a = c\beta + d + 2e$
N:	$b = c\delta$

RQ = e/a

five equations, five unknowns (a, b, c, d, and e)
→ Stoichiometric coefficients (a, b, c, d, and e) can be determined

Degree of Reduction

- **Elemental balances provide no insight into the energetics of a reaction.**
  - The concept of degree of reduction has been developed and used for proton-electron balances in bioreactions.

- **Degree of reduction (γ) for organic compounds**
  - The number of equivalents of available electrons per 1 gram atom (= mole) C

- **Available electrons**
  - Electrons transferred upon oxidation of a compound

Degree of Reduction

- **Degree of reduction for some key elements**
  - C=4, H=1, N =-3, O =-2, P=5, S=6
  - Valance of the element (원자가)

- **Degree of reduction (γ) of substrate**
  - Methane (CH_4): 1x4 + 4x1 = 8

    γ = 8/1 = 8

  - Glucose ($C_6H_{12}O_6$): 6x4 + 12x1 + 6x(-2)=24

    γ = 24/6 = 4

  - Ethanol (C_2H_5OH): 2x4 + 6x1 + 1x(-2) = 12

    γ = 12/2 =6