LECTURE SEVEN: Heat Effects in reversible exothermic reactions.

This lecture covers: theory of the chemostat, fed batch or semi-continuous fermenter operations, Significance, typical values and diffusion limit, approach to equilibrium, and multi-valency Significance, typical values and diffusion limit, approach to equilibrium, and multi-valency

1. **Biological Reactors- Chemostats**

Biological Reactors (Chemostat)

Concentration/Combustion constant
Biological CSTR

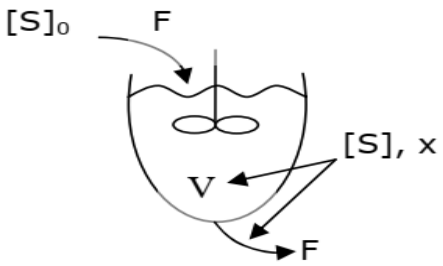

Figure 1. Diagram of a chemostat.

F = Volumetric flow rate

$$x = \frac{\text{biomass}}{\text{volume}}$$

$[S]_o$ = Concentration of growth limiting substrate. (for growing cells)

At steady-state, biomass balance

In – Out + Prod = Acc

Sterile feed: In=0
Steady state: Acc=0

$-Fx + r_x \mathrm{V} = 0$ at steady-state

Cell growth kinetics $r_x = \mu x$

$-Fx + \mu x \mathrm{V} = 0$

Solve $\mu = \frac{F}{\mathrm{V}}$

D=Dilution rate $\equiv \frac{F}{\mathrm{V}} = \frac{1}{\tau}$

$\mu = D$

Biological Mechanical

When at steady-state, can control cell mass.

Allows precisely reproducible cell states.

Not easy to run at steady-state.

Material balance on [S] (sugar concentration)

In – Out + Prod = Acc

0 at steady-state

$$F[S]_0 - F[S] - \frac{1}{Y_{\frac{x}{s}}}\mu x \mathrm{V} = 0$$

Yield coefficient $\dfrac{\text{mass biomass created}}{\text{mass substrate consumed}}$

Divide by V

$$D\underbrace{([S]_0 - [S])}_{\substack{\text{change in sugar} \\ \text{concentration}}} = \frac{\mu x}{Y_{\frac{x}{s}}}$$

At steady-state $\mu = D$

$$x = Y_{\frac{x}{s}}([S]_0 - [S])$$

What is the value of $[S]$? What more information do we need?

$\mu = f([S])$ ← must choose a growth model to connect μ and $[S]$

Monod growth model:

$\mu = \dfrac{\mu_{\max}[S]}{K_s + [S]}$ → at steady-state → $D = \dfrac{\mu_{\max}[S]}{K_s + [S]}$

$$\boxed{[S] = \frac{K_s D}{\mu_{\max} - D}}$$ ← substitute in x equation

$$x = Y_{\frac{x}{s}}\left([S]_0 - \frac{K_s D}{\mu_{\max} - D}\right)$$

Specifying $\mu_{\max}$, K_s, $Y_{\frac{x}{s}}$, D, $[S]_0$, can predict x, $[S]$.

$x < 0$ is non-physical but formally in solution

$\mu_{\max} - D$ can go to 0. If you turn knobs incorrectly: if D is too high, the cells cannot grow fast enough to reach steady-state. Washout will occur.

so use $x = 0$ to find $D_{\max}$

$$D_{\max} = \frac{\mu_{\max}[S]_0}{K_s + [S]_0}$$

For $D > D_{\max}$ "washout", no steady-state.

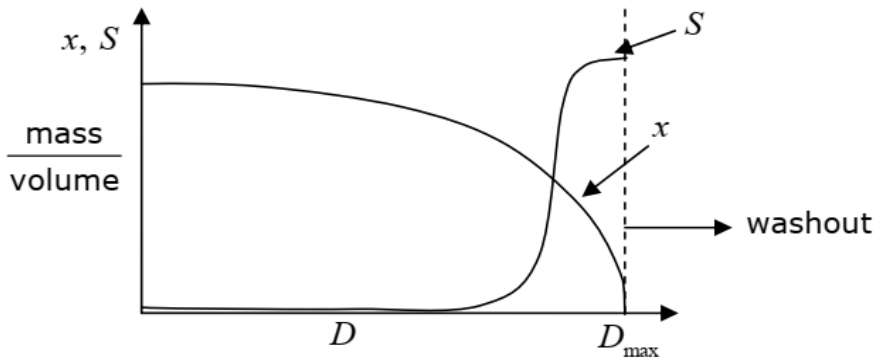

Figure 2. Biomass/volume versus dilution rate. Beyond the maximum dilution rate, washout occurs.
For real systems $K_s << [S]_0$. Most cell growth systems reach maximum at fairly low concentrations; hence x is flat, then drops off sharply.

If biomass is the product, is there a best operating condition?

What should we consider?

$\frac{dx}{dD}$ optimize x with respect to D? D=0 (no, because this would be batch reactor)

Define productivity as $\frac{\text{biomass}}{(\text{reactor volume})(\text{time})} = xD$

$\frac{d(xD)}{dD} = 0$ for optimum. (is a maximum)

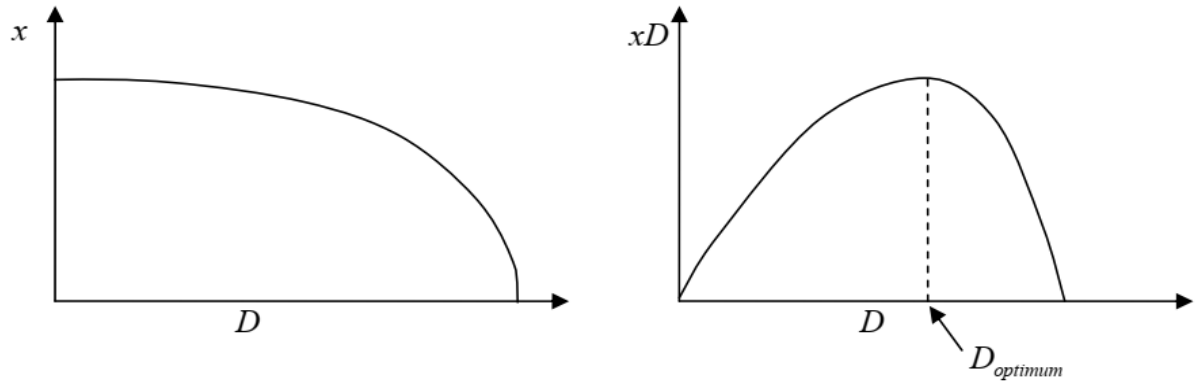

Figure 3. Left: Biomass/volume versus dilution rate. Right: Productivity versus dilution rate.

$$D_{optimum} = \mu_{\max}\left(1 - \sqrt{\frac{K_s}{K_s + [S]_0}}\right)$$

$K_s << [S]_0$

$$D_{optimum} \approx \mu_{\max}$$
$$\approx D_{\max}$$

Close to washout conditions.

Operability would be difficult. We would not want to run too close to washout conditions.

Fed-batch fermentor (microbes or mammalian cells)
-used to achieve very high cell densities (e.g. hundreds of grams cell dry weight (c.d.w)/liter)

If you want $x_{final} = \frac{100\ g}{L}$

If $Y_{\frac{x}{s}} \approx 0.5$, $[S]_0 = \frac{200\ g}{L} \approx 20\% \frac{wt}{volume}$ ← Toxic, sugar content cells will die

Why do we not feed all at once? Cells will die.

Calculate medium feed rate in order to hold μ constant.

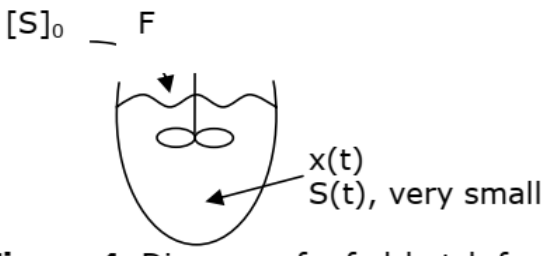

Figure 4. Diagram of a fed-batch fermentor.

If μ is constant, biomass = biomass$\big|_{t=0} e^{\mu t}$

There is a dilution term, because as we feed in fresh medium, volume will change. Volume often doubles.

$$x\,\mathrm{V} = x_0\,\mathrm{V}_0\,e^{\mu t}$$

$$\underbrace{\text{Feed } F[S]_0}_{\text{sugar feed}} = \underbrace{\frac{\mu x_0\,\mathrm{V}_0\,e^{\mu t}}{Y_{\frac{x}{s}}}}_{\text{sugar consumed}}$$

Assume <u>all</u> converted into biomass.

$$F = \frac{x_0\,\mathrm{V}_0}{[S]_0 Y_{\frac{x}{s}}}\,\mu e^{\mu t}$$

Exponential flow rate. Typically μ specified as "small."

Dilution:

$$\frac{d\,\mathrm{V}}{dt} = F$$

$$\mathrm{V}(t) = \mathrm{V}_0\left(1 + \frac{x_0}{[S]_0 Y_{\frac{x}{s}}}\left(e^{\mu t} - 1\right)\right)$$

$$x = \frac{\text{biomass}}{\mathrm{V}} = \frac{x_0 e^{\mu t}}{1 + \frac{x_0}{Y_{\frac{x}{s}}[S]_0}\left(e^{\mu t} - 1\right)}$$

$$= \frac{x_0\,\mathrm{V}_0\,e^{\mu t}}{\mathrm{V}}$$

"Logistic equation"

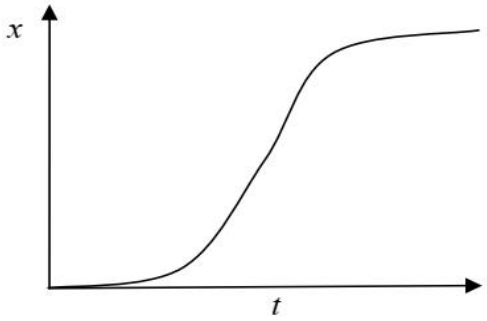

Figure 5. Graph of logistic growth.

If product is something cells are making:

Product synthesis kinetics

1) $\frac{1}{x}\frac{dP}{dt} = \alpha\mu$ growth associated (e.g. ethanol)

$P \equiv \frac{\text{product}}{\text{volume}}$

2) $\frac{1}{x}\frac{dP}{dt} = \beta$ not growth associated (e.g. antibiotics, proteins, antibodies)

$P = \beta\int_0^t x dt$ integrate for amount of product.

2. Kinetics of Non-Covalent Biomolecular Interactions

Noncovalent Interactions

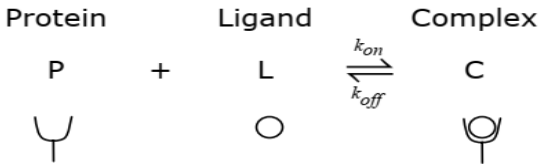

Figure 1. Protein-ligand binding.

Association rate = $k_{on}C_pC_L$

Dissociation rate = $k_{off}C_c$

@ equilibrium, $k_{on}C_pC_L = k_{off}C_c$

$$\frac{C_pC_L}{C_c} = \frac{k_{off}}{k_{on}} = K_d$$

$\frac{1}{\text{s}}$

$\frac{L}{\text{mol s}}$

In general, for protein-protein interactions, $k_{on} \approx 10^5 mol^{-1}s^{-1}$

half-time for complex dissociation $\tau_{1/2} = \dfrac{\ln 2}{k_{off}}$

K_d		$\tau_{1/2}$	types
mM	stronger interactions	milliseconds	non-specific stickiness
μM (micromolar)		milliseconds-seconds	cell surface, multi valent
nM		minutes-hours	antibodies, enzymes
pM		hours-weeks	growth factors
fM (femtomolar)		weeks-months	hycholase inhibitors

Fractional saturation $Y = \dfrac{C_c}{C_{p,o}} = \dfrac{C_c}{C_c + C_p}$

$$K_d = \frac{C_p C_L}{C_c} \rightarrow Y = \frac{C_L}{C_L + K_d}$$

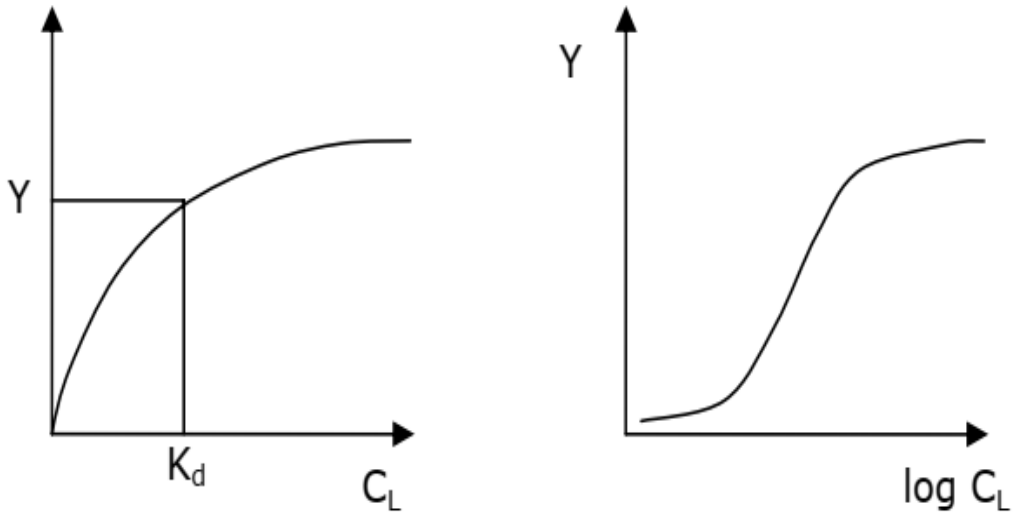

Figure 2. Left: Graph of fractional saturation versus ligand concentration. Right: Graph of fractional saturation versus the logarithm of ligand concentration.

If $C_{p,o} \approx C_{L,o}$, then at equilibrium, $C_L \neq C_{L,o}$

$$Y = \frac{C_{L,o} - yC_{p,o}}{C_{L,o} - yC_{p,o} + K_d}$$

$$Y = \frac{K_d + C_{L,o} + C_{p,o} - \sqrt{(K_d + C_{L,o} + C_{p,o})^2 - 4C_{p,o}C_{L,o}}}{2C_{p,o}}$$

If instead $C_{L,o} \gg C_{p,o}$, $C_L \approx C_{L,o}$

$$Y = \frac{C_{L,o}}{C_{L,o} + K_d}$$

How quickly is equilibrium reached?

$$\frac{dC_c}{dt} = k_{on}C_L C_p - k_{off}C_c$$

If $C_{L,o} \gg C_{p,o}$ "pseudo-1st order"

$$k_{on}C_L = k_{on}C_{L,o}$$

$$C_{p,o} = C_p + C_c$$

(complexed)

$$C_p = C_{p,o} - C_c$$

$$\frac{dC_c}{dt} = k_{on}C_{L,o}C_p - k_{off}C_c = k_{on}C_{L,o}(C_{p,o} - C_c) - k_{off}C_c$$

$$= k_{on}C_{p,o}C_{L,o} - (k_{on}C_{L,o} + k_{off})C_c$$

$$\frac{dC_c}{dt} = k_{on}C_{L,o}C_p - k_{off}C_c = k_{on}C_{L,o}(C_{p,o} - C_c) - k_{off}C_c$$
$$= k_{on}C_{p,o}C_{L,o} - (k_{on}C_{L,o} + k_{off})C_c$$

$$\Rightarrow C_c(t) = C_{p,o}\frac{C_{L,o}}{C_{L,o} + K_d}(1 - e^{-k_{obs}t})$$

$$k_{obs} = k_{on}C_{L,o} + k_{off}$$

$$\frac{\ln 2}{k_{obs}} = \text{half-time for reaching equilibrium}$$

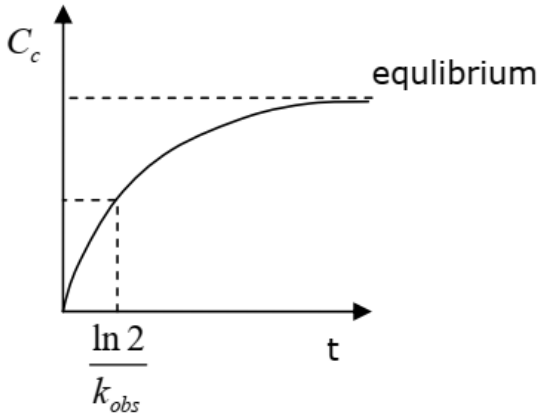

Figure 3. Concentration of complex versus time. Equilibrium is approached at long times.

Biosensor

Surface plasmon resonance (label-free)

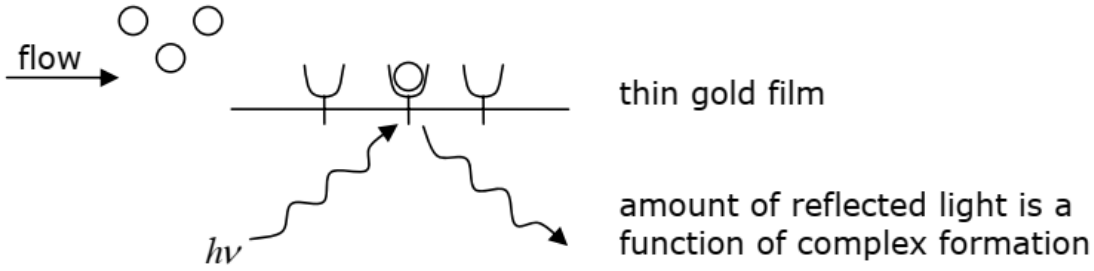

Figure 4. Schematic of how surface plasmon resonance works.

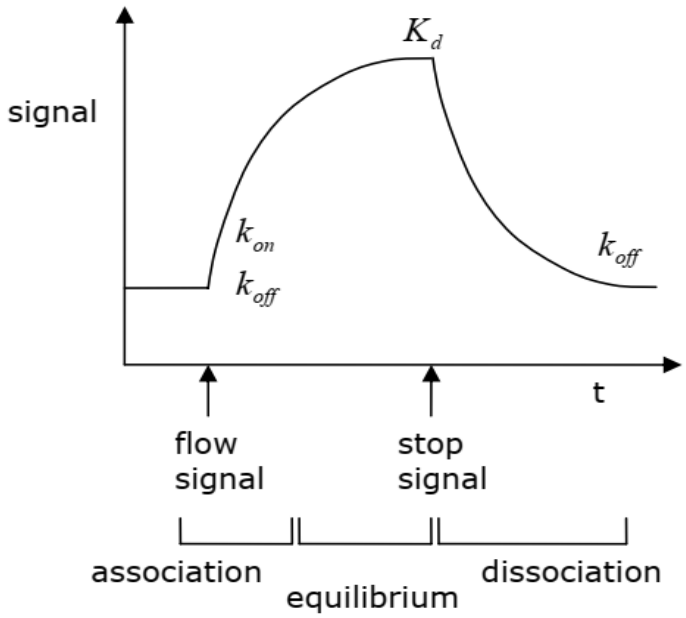

Figure 5. Signal of detector versus time.

redundant estimates: k_{off} in both association & dissociation, $K_d = \frac{k_{off}}{k_{on}}$ in equilibrium phase

best approach: fit one set of parameters to three phases of experiment. (global least squares)

Multivalency (Avidity)

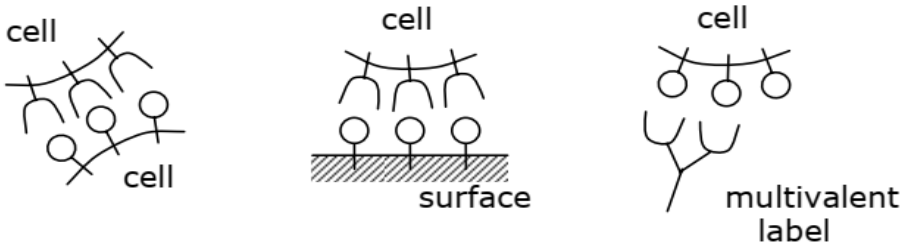

Figure 6. Three examples of multiple protein-ligand binding.

How does multivalency effect apparent interaction strength?

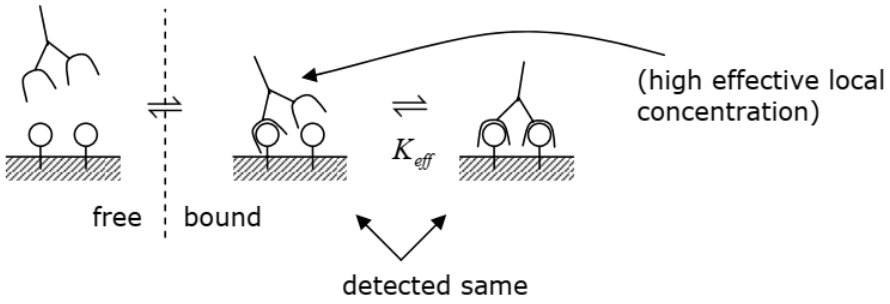

Figure 7. Multivalent binding equilibrium.