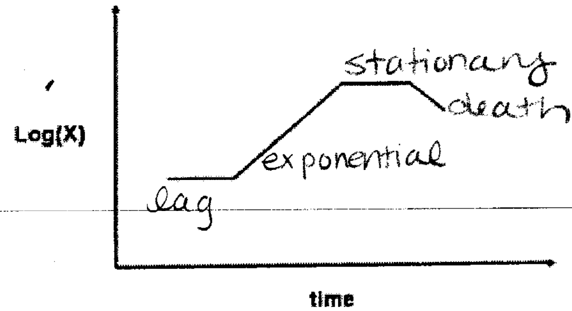
Name: Key

Chemical Engineering 170A

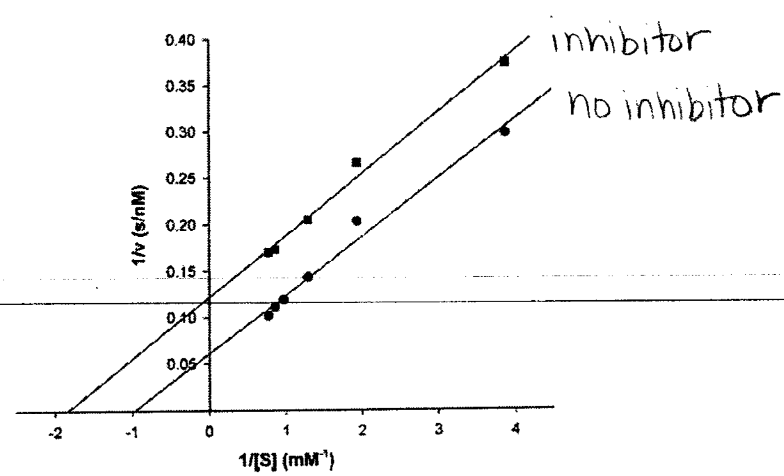
October 8, 2007

Midterm Exam I (100 pts)
Closed Book and Closed Notes
One 8.5 x 11 in. page of notes (front and back) allowed

1. Label the four phases of cell growth in the graph below (4 pts).



2. In a recent paper in Bioorg. Med. Chem. Lett. 15 (2005), Támas Sperka and colleagues investigated beta-lactam compounds as potential inhibitors of the enzyme HIV-1-protease. One compound, I/11a, appeared to inhibit the enzyme, giving the following Lineweaver-Burke plots for the enzymatic reaction with and without the inhibitor.



a) What kind of inhibition does compound I/11a exert on the HIV-1-protease (2 pts)?

uncompetitive Vmax + KM change

b) Estimate V_{max} for the enzymatic reaction with the inhibitor (3 pts).

$1/V_{max}$ inhibitor = 0.12 s/nM ; V_{max} = 8.33 nM/s

3. A peptide has the following sequence

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Please answer the following questions with single letter codes only

a) Are there any residues negatively charged (-1 charge) at pH 8 (2 pts)?

D, E

b) Are there any hydrophobic residues (2 pts)?

A, L, I, G

c) Are there any residues that absorb light at 280 nm (1 pt)?

No

4. A population of cells is growing in a batch reactor with a mass specific growth rate $\mu = 0.023\ \text{min}^{-1}$ and a number specific growth rate of $\nu = 0.023\ \text{min}^{-1}$.

a) What is the doubling time of the cells during exponential growth (3 pts)?

$$t_d = \frac{\ln 2}{\mu} = 30.1 \text{ mins}$$

b) Is the growth balanced or unbalanced (1 pt)?

Balanced

c) What is the definition of an unstructured and segregated cell growth model (1 pt)?

Unstructured - model views the cell as one entity and does not take the RNA, DNA, protein into account.

Segregated - model views the cells as several different types of populations.

5. The growth of filamentous organisms (such as molds) in a rich medium is described by:

$$\frac{dM}{dt} = \gamma M^{2/3}$$

where M is the dry weight (dw) concentration of the biomass (in mg/l). I observe that an overnight (12 hour) culture seeded with 1 mg/l of cells grows to 8 mg/l. By what dilution factor should I dilute this 8 mg/l culture if I want a concentration of 4 mg/l tomorrow (after 24 hours) (6 pts)?

12 hour culture seeded w/ 1mg/l → 8mg/l

$$\frac{dM}{dt} = \gamma M^{2/3}$$

$$\int \frac{dM}{M^{2/3}} = \gamma dt$$

$$3M^{1/3} \int_{M_0}^{M} = \gamma t$$

(+2 integration)

$$3(M^{1/3} - M_0^{1/3}) = \gamma t$$

$$3(8^{1/3} - 1^{1/3}) = \gamma (12 \text{ hrs})$$

$$3\left(2\left(\frac{mg}{l}\right)^{1/3} - 1\left(\frac{mg}{l}\right)^{1/3}\right) = 12\gamma \text{ hr}$$

$$1\left(\frac{mg}{l}\right)^{1/3} = 4\gamma \text{ hr}$$

(2) for value of γ

$$\gamma = 1/4 (mg/l)^{1/3} hr^{-1}$$

Have 8 mg/mL culture
want 4 mg/mL in 24 hrs

$$M = \left(\frac{\gamma t}{3} + M_0^{1/3}\right)^3$$

(1) for setting up for M

$$M = \left[\frac{0.25 (mg/l)^{1/3} hr^{-1} 24 hr}{3} + (8 mg/l)^{1/3}\right]^3$$

(1) final answer

$$M = (4 (mg/l)^{1/3})^3; \quad M = 64 \text{ mg/l}$$

→ dilute culture by 1/16th to get 4mg/L

6. β-lactamase is an enzyme that confers resistance to β-lactam antibiotics, such as penicillin. It is known that β-lactamase exhibits the following kinetic parameters when the substrate is benzylpenicillin

$k_{cat} = 2 \times 10^3\ s^{-1}$
$K_M = 2 \times 10^{-5}$ M

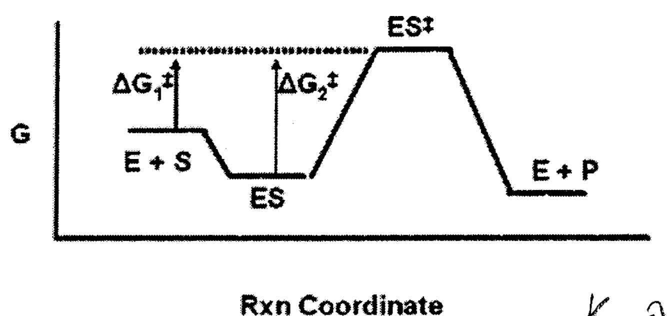
a) Calculate the rate of substrate consumption if the β-lactamase concentration is 10 mM and the benzylpenicillin concentration is 200 μM (10 pts).

$$\frac{dS}{dt} = V = \frac{V_{max}[S]}{K_M + [S]} = \frac{K_{cat}[E_o][S]}{K_M + [S]}$$ 5 pts. equation

$$\frac{dS}{dt} = \frac{2\times10^3 s^{-1}\,[10\,mM][200\,\mu M] \times \frac{1\,mmole}{10^3\,\mu mole}}{2\times10^{-5} M \times \frac{10^3\,mmoles}{1\,mole} + 200\,\mu M \times \frac{1\,mmole}{10^3\,\mu moles}}$$ 5 pts. calculation

$$\frac{dS}{dt} = \frac{4000\,mM^2/s}{0.22\,mM} = 18.18\,\frac{M}{s}$$

b) Using transition state theory, calculate $\Delta G_1^\ddagger$ and $\Delta G_2^\ddagger$ in the free energy scheme shown below for room temperature (25°C) (15 pts).



$K_T \approx 1$

$$\Delta G^\ddagger = -RT\ln(K^\ddagger) = -RT\ln\left(\frac{Kh}{K_T K_B T}\right)$$ 5 pts. equation

$\Delta G^\ddagger_1 \propto \frac{kcat}{K_M} = K$

$$\Delta G^\ddagger_1 = -\frac{8.314 J}{K\cdot mole} \times 298K \times \ln\left(\frac{\frac{2\times10^3}{s} \times 6.626\times10^{-34} J\cdot s\ 1M}{1.381\times10^{-23}\frac{J}{K} \cdot 298\,K \cdot 2\times10^{-5} M}\right)$$

5 pts. $\Delta G^\ddagger_1$ calculation

$\Delta G^\ddagger_1 = 27.34$ kJ/mole

$\Delta G^\ddagger_2 \propto kcat$

$$\Delta G^\ddagger_2 = -\frac{8.314 J}{k\cdot mole} \times 298K \times \ln\left(\frac{\frac{2\times10^3}{s} \times 6.626\times10^{-34} J\cdot s}{1.381\times10^{-23} J/K \cdot 298}\right)$$

5 pts. $\Delta G^\ddagger_2$ calculation

$\Delta G^\ddagger_2 = 54.15$ kJ/mole

7. The experimental observations for the growth of the organism *Californicus* show that the cells convert 2/3 (wt fraction) of the substrate glucose to biomass. The growth of the cells can be described by the following biological reaction:

$$C_6H_{12}O_6 + aO_2 + bNH_3 \rightarrow cC_{4.4}H_{7.3}O_{1.2}N_{0.86} + dH_2O + eCO_2$$

a) Calculate the stoichiometric coefficients a, b, c, d, e (10 pts).

$$\frac{2/3 \text{ gm cells}}{1 \text{ gm glucose}} \times \frac{180 \text{ gm glucose}}{1 \text{ mole}} \times \frac{1 \text{ mole}}{91.34 \text{ g cells}} = \frac{1.31 \text{ mole cell}}{\text{mole glucose}}$$

2 points $c = 1.31$

Equations 1.5 point each 4 × 1.5 = 6

(C): $6 = 4.4(1.31) + e$; $e = 0.236$ 0.5

(H): $12 + 3b = 7.3(1.31) + 2d$

$12 + 3b = 9.563 + 2d$

$2.437 + 3b = 2d$

(O): $6 + 2a = 1.2(1.31) + d + 2(0.236)$

O: $6 + 2a = 1.572 + d + 0.472$

$3.956 + 2a = d$

(N): $b = 0.86(1.31)$

$b = 1.13$ 0.5

$d = \frac{2.437 + 3(1.13)}{2}$

$d = 3.17$ 0.5

$a = \frac{d - 3.956}{2}$

$a = \frac{3.17 - 3.956}{2} = -0.393$ 0.5

b) Calculate the yield coefficients $Y_{X/S}$ (gdw cells/g substrate), $Y_{X/O}$ (gdw cells/g O_2), RQ, and the heat of combustion ΔH_c (15 pts).

(2) $Y_{x/s} = \frac{\text{mass cells}}{\text{mass glucose}} = \frac{2/3 \text{ gm cells}}{1 \text{ gm glucose}} = 2/3$

(2) $Y_{x/o} = \frac{\text{mass cells}}{\text{mass } O_2} = \frac{1.31 \text{ mole cells}}{-0.393 \text{ moles } O_2} \times \frac{91.34 \text{ g cells}}{1 \text{ mole}} \times \frac{1 \text{ mole } O_2}{32 \text{ gm}}$

$= -9.515$

(2) $RQ = \frac{e}{a} = \frac{0.236}{-0.393} = -0.60$

ΔH_c; $Q_o = \frac{12(-\Delta H_c)}{\sigma_b \gamma_b} - 3.328$ — equation

$Q_o = 27 \frac{\text{kcal}}{e^-}$ 2 points

$\frac{(Q_o + 3.328)\sigma_b \gamma_b}{12} = -\Delta H_c$

$\sigma_b = 0.58$ 2 points (wt. fract)

$\Delta H_c = -\frac{(27 + 3.328)\left(4.53 \frac{e^-}{\text{mol C}} \times \frac{0.58 \text{ g C}}{\text{g biomass}}\right)}{12 \text{ gC/mol C}}$

$\gamma_b = \frac{4.4(4) + 7.3 - 2(1.2) - 3(0.86)}{4.4}$

$\gamma_b = 4.53$ 3 points

$\Delta H_c = -6.64$ kcal/gm cells 2 points

8. Urea dissolved in aqueous solution is degraded to ammonia and CO_2 by the enzyme urease immobilized on porous spherical poly(HEMA) supports. The diameter of the support is 50 μm and the bulk urea concentration S_o is 200 mM. The effective diffusivity of urea through the poly(HEMA) support is 3.8×10^{-6} cm²/s and the enzyme has the following kinetic parameters:

$K_M = 20$ mM urea
$V_{max} = 10$ mmoles urea/cm³·s

a) Calculate the Thiele modulus, ϕ, and the effectiveness factor, η_I for this reaction (10 pts).

½

$$\phi = \frac{R}{3}\left(\frac{V_{max}}{K_m D_{eff}}\right)^{1/2} = \frac{25\,\mu m}{3}\left(\frac{\frac{10\text{ mmoles}}{cm^3 \cdot s}}{\frac{20\text{ mmoles}}{L}\cdot\frac{1\,L}{1000\,cm^3}\cdot 3.8\times10^{-6}\frac{cm^2}{s}}\right)$$

5 pts. ϕ calc (2.5 equation + 2.5 calculating)

$$\phi = \frac{25\,\mu m}{3} \times \frac{1\,cm}{10^4\,\mu m} \times \frac{11470.8}{cm}$$

$$\phi = 9.56$$

$$\beta = S_o/K_M = \frac{200\text{ mM}}{20\text{ mM}} = 10$$

using chart, $\eta_I = 0.70$ 5 pts. η_I

b) What is the overall rate of urea consumption, V_{obs} (15 pts)?

$$\eta_I = \frac{V_{obs}}{V_{S=S_o}}$$

5 pts. equation for V_{obs} $S=S_o$

$$V_{S=S_o} = \frac{V_{max} S_o}{K_M + S_o} = \frac{\frac{10\text{ mmoles}}{cm^3 \cdot s} \times 200\text{ mM}}{20\text{ mM} + 200\text{ mM}}$$

$$= 9.09\ \frac{\text{mmoles}}{cm^3 \cdot s}$$ 5 pts. calculation

$$V_{obs} = V_{S=S_o}(\eta_I)$$

$$V_{obs} = 9.09\ \frac{\text{mmoles}}{cm^3\, s}(0.7)$$

5 pts. solving for V_{obs} using η_I

$$V_{obs} = 6.36\ \frac{\text{mmoles}}{cm^3 \cdot s}$$

#7 on a carbon basis

a) Glucose carbon mass = $6(12) = 72\,g$ $\qquad \frac{2}{3}(72g) = (4.4)c(12)$

Biomass " " $= 4.4(c)(12)$ $\qquad c = 0.91$

Atom Balances:

C: $6 = 4.4(0.91) + e \Rightarrow e = 2$

H: $12 + 3b = 7.3(0.91) + 2d \Rightarrow d = 3.85$

N: $b = 0.91(0.86) \quad b = 0.78$

O: $6 + 2a = 1.2(0.91) + d + 2(2) \Rightarrow a = 1.47$

b) $Y_{X/S} = \frac{(0.91) \times 91.34\,g/mole}{180\,g/mole} = 0.461\,\frac{gdw\ cells}{g\ substrate}$

$Y_{X/O} = \frac{0.91 \times 91.34\,g/mole}{1.47 \times 32\,g/mole\ O_2} = 1.76\,\frac{gdw\ cells}{g\ O_2}$

$RQ = \frac{e}{a} = \frac{2}{1.47} = 1.36$

ΔH_C: same answer

however, can use

$$-\Delta H_C = 8.076C + 34.462\left(H - \frac{O}{8}\right)$$

$C = 0.58 \quad H = 0.08$

$O = 0.21 \quad -\Delta H_C = -6.56\,\frac{kcal}{g\ cells}$