BIOCHEMICAL ENGINEERING (10.442/10.542)

ENZYME KINETICS AND BIOREACTORS

PROBLEM SET NUMBER 4

1. The hydration of CO_2 is catalyzed by carbonic anhydrase as follows:

$$H_2O + CO_2 \overset{E}{\Leftrightarrow} HCO_3^- + H^+$$

The following data were obtained for the forward and reverse reaction rates at pH 7.1 and an enzyme concentration of 2.8×10^{-9} *M*.

Hydration		Dehydration	
$1/v$, M^{-1} ($s \times 10^{-3}$)	$(CO_2)(M \times 10^3)$	$1/v$, M^{-1} ($s \times 10^{-3}$)	$(HCO_3)(M \times 10^3)$
36	1.25	95	2
20	2.5	45	5
12	5	29	10
6	20	25	15

v is the *initial* reaction rate at the given substrate concentration. Calculate the forward and reverse catalytic and Michaelis constants.

2. The following data were obtained for an enzyme-catalyzed reaction. Determine V_{max} and K_m by inspection. Plot the data using the Eadie-Hofstee method and determine these constraints graphically. Explain the discrepancy in your two determinations. The initial rate data for the enzyme catalyzed reaction are as follows:

[S], mol/L	v (μmol/min)
5.0×10^{-4}	125
2.0×10^{-4}	125
6.0×10^{-5}	121
4.0×10^{-5}	111
3.0×10^{-5}	96.5
2.0×10^{-5}	62.5
1.6×10^{-5}	42.7
1.0×10^{-5}	13.9
8.0×10^{-6}	7.5

Do these data fit into Michaelis-Menten kinetics? If not, what kind of rate expression would you suggest? Use graphical methods.

3. An enzyme ATPase has a molecular weight of 5×10^4 daltons, a K_M value of 10^{-4} *M* and a $k_2 = 10^4$ molecules ATP/min molecule enzyme at 37ºC. The reaction catalyzed is the following:

$$ATP \xrightarrow{ATPase} ADP + P_i$$

which can also be represented as

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

where S is ATP. The enzyme at this temperature is unstable. The enzyme inactivation kinetics is first order:

$$E = E_0 \exp(-k_d t)$$

where E_0 is the initial enzyme concentration and $k_d = 0.1$ min^{-1}. In an experiment with a partially pure enzyme preparation, 10 μg of total crude protein (containing enzyme) is added to a 1 ml reaction mixture containing 0.02 *M* ATP and incubated at 37ºC. After 12

hours, the reaction ends (that is, $t \rightarrow \infty$) and the inorganic phosphate (P_i) concentration is found to be 0.002 *M*, which was initially zero. What fraction of the crude protein preparation was the enzyme?

4. Upon reaching the stationary phase of growth, a yeast culture was used for the enzymatic conversion of low cost substrate into a high-value pharmaceutical. Using whole cells, the yeast will catalyze the reaction

$$A + O_2 \rightarrow B$$

Because the cost for substrate A is low, it is provided in excess. Accordingly, the rate of the enzymatic reaction is controlled by the dissolved oxygen concentration. In the absence of inhibitors, the kinetics can be described by the Michaelis-Menton equation

$$V = V_{max} \frac{C_s}{K_a + C_s}$$

where C_s = concentration of dissolved oxygen in the liquid adjacent to the cell wall at the yeast, mmole 0_2/liter

$$V = \text{specific reaction velocity, } \frac{\text{moles } 0_2}{\text{hr-g cells (dry)}}$$

Because the yeast are not growing and have low maintenance requirements, virtually 100% of the oxygen consumption is due to the enzymatic reaction with substrate A.

As shown by the data below, the specific oxygen consumption rate, V, was measured at various concentrations of dissolved oxygen, both with and without the presence of inhibitor sulfanilamide.

Dissolved Oxygen, C_s ($\frac{\text{mmoles } 0_2}{\text{liter}}$)	Reaction Velocity with no inhibitor V ($\frac{\text{mmoles } 0_2}{\text{hr - g cell}}$)	Reaction Velocity with 20 μg/ml sulfanilamide V ($\frac{\text{mmoles } 0_2}{\text{hr - g cell}}$)
0.0	0.0	0.0
8.2×10^{-4}	1.05	0.78
1.6×10^{-3}	1.47	0.14
2.5×10^{-3}	1.67	1.37
4.1×10^{-3}	1.87	1.62
5.8×10^{-3}	1.92	1.77
8.2×10^{-3}	1.92	1.78

a) Using this data, determine the constants K_o and V_{max} for the enzymatic reaction without inhibition.

b) Also, determine the nature of the sulfanilamide inhibition and develop an equation which includes its effect on the reaction rate. Calculate the value of any constants used in this equation.

5. a. H.H. Weetall and N.B. Havewola report the following data for the production of dextrose from corn starch using both soluble and immobilized (azo-glass beads) glucoamylase in a fully agitated CSTR system.

   1. Soluble data: $T = 60^{\circ}C$, $S_o = 336$ mg starch/ml, $E_o =$ 11,000 units, volume = 1000 ml.

   2. Immobilized data: $T = 60^{\circ}C$, $S_o = 336$ mg starch/ml, $E_o =$ 46,400 units initially, immobilized, volume = 1000 ml.

Time (min)	Product concentration (mg dextrose/ml)	
	Soluble	Immobilized
0	12.0	18.4
15	40.0	135
30	76.5	200
45	94.3	236
60	120.0	260
75	135.5	258
90	151.2	262
105	150.4	266
120	155.7	278
135	160.1	300
150	164.9	310
165	170.0	306
225	---	316
415	---	320

Determine the maximum reaction velocity, V_m (mg/ml-min • unit of enzyme) and the saturation constant, K_M (mg/ml).

b. The same authors studied the effect of temperature on the maximum rate of the hydrolysis of corn starch by glucoamylase. The results are tabulated next. Determine the activation energy (ΔE cal/g mole) for the soluble and immobilized enzyme reaction.

	V_{max}, m mol/min 10^6	
T, ºC	Soluble	Azo-Immobilized
25	0.62	0.80
35	1.42	1.40
45	3.60	3.00
55	8.0	6.2
65	16.0	11.0

c. Using these results, determine if immobilized enzyme is diffusion limited.