Midterm 1

CHE 170A: Biochemical Engineering

October 8, 2010

Name: _____ SID: _____

Problem 1: _____ pts/20 pts

Problem 2: _____ pts/20 pts

Problem 3: _____ pts/20 pts

Problem 4: _____ pts/20 pts

Problem 5: _____ pts/20 pts

Total: _____ pts/100 pts

Problem 1. Enzymatic Conversion of Two Substrates (20 pts)

Suppose that two substrates are required for an enzymatic conversion according to the following mechanism:

$$E + S_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES_1$$

$$E\,S_1 + S_2 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E\,S_1S_2$$

$$E\,S_1S_2 \xrightarrow{k_5} E + P$$

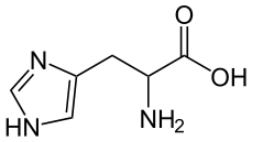
a) Write a mass balance for the total enzyme concentration E_o. (3 pts)

b) Write a rate equation for the formation of product P. (3 pts)

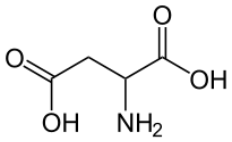
c) Assuming rapid equilibrium, write expressions for the reversible steps. (4 pts)

d) Draw the free energy diagram of this enzymatic reaction and compare it to the free energy diagram for the uncatalyzed reaction. (5 pts)

e) Histadine and aspartic acid are important amino acids in this reaction. What is the total charge of the two amino acids at pH 5.8 where the reaction takes place? (5 pts)



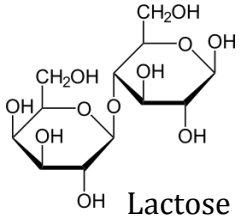
Histidine

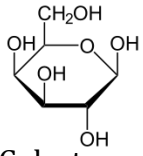


Aspartic acid

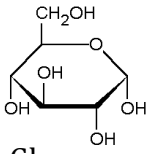
Problem 2. Lactose Hydrolysis

The kinetic model of lactose hydrolysis by *Aspergillus niger* lactase can be described as follows (Scott *et al.,* 1985):


Lactose


Galactose

+


Glucose

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P + Q$$

$$E + P \underset{k_5}{\overset{k_4}{\rightleftharpoons}} EP$$

where

S = lactose P = galactose Q = glucose E = free enzyme

In this mechanism, glucose is released from the enzyme-substrate complex first, leaving the enzyme-galactose complex, which subsequently releases galactose.

a) Derive the rate equation for the production of galactose using the Briggs-Haldane approach. (12 pts)

b) What kind of inhibition occurs with galactose (i.e., what type of inhibitor is galactose)? (4 pts)

c) Calculate K_M^{app} if [P] = 0.4 mM, K_M =83 mM and K_I = 20 mM. (4 pts)

Problem 3. Immobilized Enzymes

Two enzymes are immobilized on the same nonporous beads. For enzyme A, the substrate is S_1. For enzyme B, the substrate is S_2. The product of the first reaction is S_2. That is,

$$S_1 \longrightarrow S_2 \longrightarrow P$$

The relevant parameters of the reaction are given below:

$$k_{s1} = 4\text{x}10^{-5}\ \text{cm/s} \qquad v_{max} = 3\text{x}10^{-6}\ \text{mg cm}^{-2}\ \text{s}^{-1} \qquad K_M = 12\ \text{mg L}^{-1}$$

a) What is the surface concentration of S_1 for a bulk S_1 concentration of 60 mg/L? (8 pts)

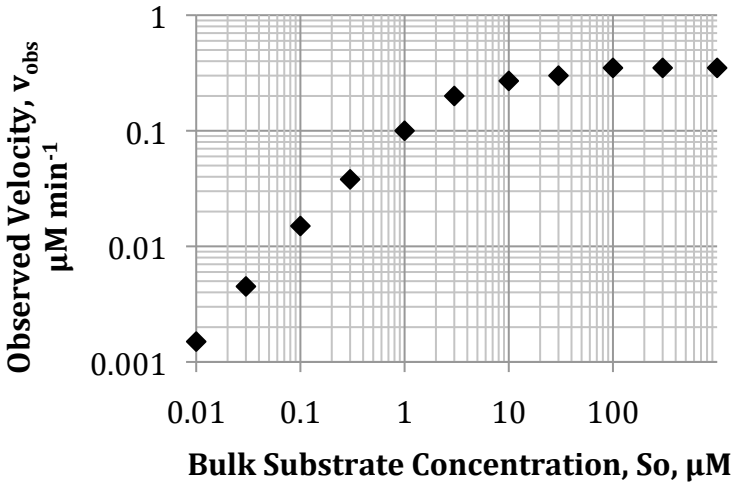
b) What is the rate of S_2 formation for the above value of S_1? (3 pts)

c) What is the value of Da when S_1 = 8 mg/L? What is limiting in this case?

d) What is the value of the η_E for $\overline{Da}$ = 0.4 with a bulk concentration of S_1 = 8 mg/L? (5 pts)

Problem 4. Immobilized Enzymes Embedded Within Spherical Particles

An enzyme is immobilized in porous, spherical particles of 3.0 mm diameter. The enzyme is characterized through a series of initial rate experiments. The substrate, S_o, penetrates the particles with an effective diffusivity of 1.0×10^{-6} cm^2/s. The enzyme loading (moles of enzyme per liter of particle) is known to be 1.5 μM.



a) At low substrate concentrations (S_o <0.1 μM), does intraparticle diffusion affect the rate significantly? Justify your answer. (6 pts)

b) At high substrate concentrations (S_o >100 μM), does intraparticle diffusion affect the rate significantly? Justify your answer. (6 pts)

c) Estimate the values of k_{cat} (s^{-1}) and K_M (μM) for the immobilized enzyme. (8 pts)

Problem 5. Glutamate production by *Corynebacterium glutamicum*

A research and development group is interested in producing glutamate. They measured the glutamate production by *Corynebacterium glutamicum* from glucose.

$$0.49C_6H_{12}O_6 + 0.758O_2 + \beta NH_3 \rightarrow CH_{1.3}N_{0.879}O_{0.322} + 0.429C_5H_9NO_4 + 1.497H_2O + \kappa CO_2$$

(glucose) (biomass) (glutamate)

Answer the following questions based on the balance above:

a) During the fermentation, the technician measured the respiratory quotient, RQ, as 0.25. How many g-atoms of C in CO_2 are produced from 10 grams of substrate? (5 pts)

b) Calculate β and κ. (5 pts)

c) What fraction of chemical energy in the substrate is transferred to the extracellular product, glutamate? 5 pts)

d) If 500 kg of glutamate are needed every day to make a profit, what concentration of glucose do the operators need to start with in a 10^4 L tank? (5 pts)

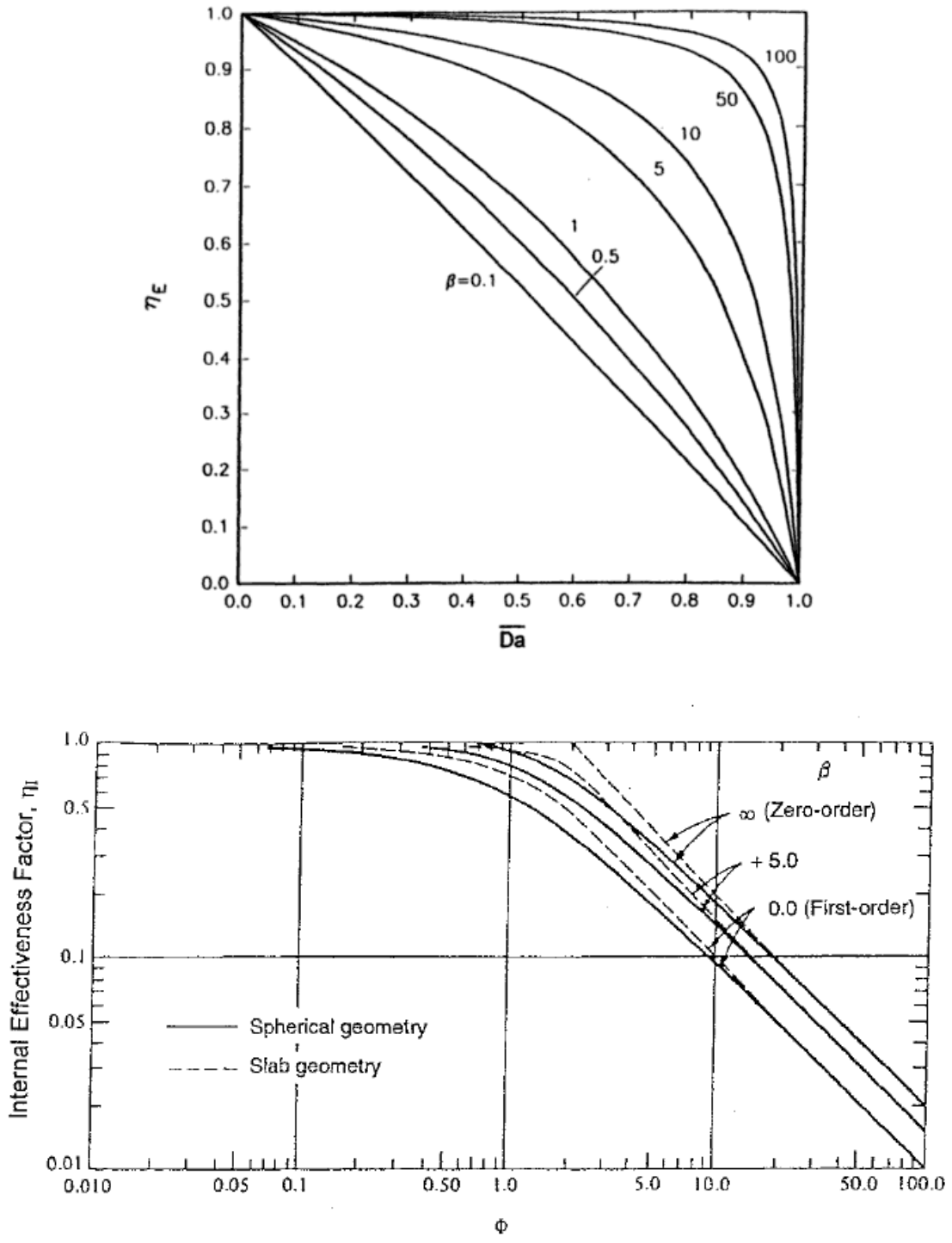


Figure 2.13. *Effectiveness factor, η_I, as a function of the observable modulus, Φ, and the dimensionless substrate concentration, β.*