p3-19.mcd

Two enzymes immobilized on nonporous polymeric beads. (Problem 3.19 of Shuler & Kargi) Instructor: Nam Sun Wang

Two enzymes with the same substrate are co-immobilized on the same surface.

Reaction #1:  $S + E_1 \longrightarrow E_1 + P_1$ 

Reaction #2:  $S + E_2 \longrightarrow E_2 + P_2$ 

Intermediate products P<sub>1</sub> and P<sub>2</sub> combine spontaneously to form the final product P<sub>3</sub>.

$$P_1 + P_2 \longrightarrow P_3$$

Enzyme kinetic constants from the given graph are:

Reaction #1 
$$v_{m1} := 1.1 \cdot 10^{-5} \text{ mg/cm}^2 \cdot \text{sec}$$
  $K_{m1} := 0.025 \text{ mg/cm}^3$   $v_1(s) := \frac{v_{m1} \cdot s}{K_{m1} + s}$   
Reaction #2  $v_{m2} := 2 \cdot 10^{-5} \text{ mg/cm}^2 \cdot \text{sec}$   $K_{m2} := 0.11 \text{ mg/cm}^3$   $v_2(s) := \frac{v_{m2} \cdot s}{K_{m2} + s}$ 

Part a) Find total rate of substrate disappearance, based on the following operating parameters.

 $k_{\rm L} := 6 \cdot 10^{-5} \, {\rm cm/sec}$ Mass transfer coefficient:

Substrate concentration in the bulk liquid:  $s_h = 0.5 \text{ mg/cm}^3$ 

 $J(s) := k L \cdot (s_b - s)$ Mass transfer

 $s = 0.01, 0.02 \dots s_{h}$ 



When there is only one enzyme present at one time, the intersection of the two curves v1 & J gives solution to Reaction #1, and that of v<sub>2</sub> and J gives solution to Reaction #2.

v

When there are two enzymes present simultaneously, the intersection of v1+v2 & J give solution to combined Reaction #1 and Reaction #2.

Determine surface concentration of substrate at steady-state:

 $s := s_b$ ... Initial guessGiven $J(s) = v_1(s) + v_2(s)$ s := Find(s)s = 0.15 $mg/cm^3$ Rate of consumption of substrate due to Reaction #1 $v_1(s) = 9.431 \cdot 10^{-6}$  $mg/cm^2 \cdot sec$ Rate of consumption of substrate due to Reaction #2 $v_2(s) = 1.155 \cdot 10^{-5}$  $mg/cm^2 \cdot sec$ Total rate of consumption of substrate due to both reactions $v_1(s) + v_2(s) = 2.098 \cdot 10^{-5}$  $mg/cm^2 \cdot sec$ Mass transfer of substrate to surface (check) $J(s) = 2.098 \cdot 10^{-5}$  $mg/cm^2 \cdot sec$ 

**Part b)** Overall effectiveness factor is the ratio of observed rate with mass transfer to the intrinsic rate without mass transfer limitation.

effectiveness factor 
$$\eta := \frac{v_1(s) + v_2(s)}{v_1(s_b) + v_2(s_b)}$$
  $\eta = 0.781$   
**Part c)** Ratio of P<sub>2</sub> to P<sub>1</sub> ratio  $:= \frac{v_2(s)}{v_1(s)}$  ratio = 1.225

**Part d)** Find s<sub>b</sub> that leads to equimolar amount of P<sub>1</sub> and P<sub>2</sub> (i.e.,  $v_1 = v_2$ ), while k<sub>L</sub> remains unchanged. We first find the value of substrate concentration on the surface such that  $v_1 = v_2$ .

 $s := s_b$  ... initial guess Given  $v_1(s) = v_2(s)$  s := Find(s) s = 0.079 mg/cm<sup>3</sup> We then find the value of  $s_b$  that satisfies the condition where total rate of substrate consumption equals to rate of substrate mass transfer (i.e.,  $v_1 + v_2 = J$ ).

$$\begin{split} &J(s_b) \coloneqq k_L \cdot \left(s_b - s\right) \\ &s_b \coloneqq s \quad ... \text{ initial guess } \quad \text{Given } v_1(s) + v_2(s) = J(s_b) \quad s_b \coloneqq \text{Find}\left(s_b\right) \quad s_b = 0.357 \text{ mg/cm}^3 \\ &\text{Plot} \quad s \coloneqq 0, 0.01 \dots s_b \quad J(s) \coloneqq k_L \cdot \left(s_b - s\right) \end{split}$$



The two curves  $v_1 \& v_2$  intersect at the same value of s as the two curves  $v_1+v_2 \& J$  does.