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: \( S + E_1 \rightarrow E_1 + P_1 \)
  Reaction #2: \( S + E_2 \rightarrow E_2 + P_2 \)
Intermediate products \( P_1 \) and \( P_2 \) combine spontaneously to form the final product \( P_3 \):
  \( P_1 + P_2 \rightarrow P_3 \)

Enzyme kinetic constants from the given graph are:

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

**Reaction #2**
- \( v_{m2} = 2 \times 10^{-5} \text{ mg/cm}^2 \cdot \text{sec} \)
- \( K_{m2} = 0.11 \text{ mg/cm}^3 \)
- \( v_2(s) = \frac{v_{m2}s}{K_{m2} + s} \)

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

- Mass transfer coefficient: \( k_L = 6 \times 10^{-5} \text{ cm/sec} \)
- Substrate concentration in the bulk liquid: \( s_b = 0.5 \text{ mg/cm}^3 \)
- Mass transfer: \( J(s) = k_L \left( s_b - s \right) \)
- \( s = 0.01, 0.02, \ldots, s_b \)

When there is only one enzyme present at one time, the intersection of the two curves \( v_1 \) & \( J \) gives solution to Reaction #1, and that of \( v_2 \) and \( J \) gives solution to Reaction #2.

When there are two enzymes present simultaneously, the intersection of \( v_1 + v_2 \) & \( J \) gives solution to combined Reaction #1 and Reaction #2.
Determine surface concentration of substrate at steady-state:

\[ s := s_b \quad \text{... Initial guess} \quad \text{Given} \quad J(s) = v_1(s) + v_2(s) \quad s := \text{Find}(s) \quad s = 0.15 \quad \text{mg/cm}^3 \]

Rate of consumption of substrate due to Reaction #1
\[ v_1(s) = 9.431 \times 10^{-6} \quad \text{mg/cm}^2\text{-sec} \]

Rate of consumption of substrate due to Reaction #2
\[ v_2(s) = 1.155 \times 10^{-5} \quad \text{mg/cm}^2\text{-sec} \]

Total rate of consumption of substrate due to both reactions
\[ v_1(s) + v_2(s) = 2.098 \times 10^{-5} \quad \text{mg/cm}^2\text{-sec} \]

Mass transfer of substrate to surface (check)
\[ J(s) = 2.098 \times 10^{-5} \quad \text{mg/cm}^2\text{-sec} \]

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

\[ \eta := \frac{v_1(s) + v_2(s)}{v_1(s) + v_2(s)} \quad \eta = 0.781 \]

**Part c)** Ratio of \( P_2 \) to \( P_1 \)
\[ \text{ratio} := \frac{v_2(s)}{v_1(s)} \quad \text{ratio} = 1.225 \]

**Part d)** Find \( s_b \) that leads to equimolar amount of \( P_1 \) and \( P_2 \) (i.e., \( v_1 = v_2 \)), while \( k_L \) remains unchanged. We first find the value of substrate concentration on the surface such that \( v_1 = v_2 \).

\[ s := s_b \quad \text{... initial guess} \quad \text{Given} \quad v_1(s) = v_2(s) \quad s := \text{Find}(s) \quad s = 0.079 \quad \text{mg/cm}^3 \]

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., \( v_1 + v_2 = J \)).

\[ J(s) := k_L \cdot (s - s) \quad s := s \quad \text{... initial guess} \quad \text{Given} \quad v_1(s) + v_2(s) = J(s) \quad s := \text{Find}(s) \quad s = 0.357 \quad \text{mg/cm}^3 \]

Plot \( s := 0, 0.01 \ldots s_b \quad J(s) := k_L \cdot (s - s) \)

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