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: $\mathrm{S}+\mathrm{E}_{1} \longrightarrow \mathrm{E}_{1}+\mathrm{P}_{1}$
Reaction \#2: $\mathrm{S}+\mathrm{E}_{2} \longrightarrow \mathrm{E}_{2}+\mathrm{P}_{2}$
Intermediate products $P_{1}$ and $P_{2}$ combine spontaneously to form the final product $P_{3}$.

$$
P_{1}+P_{2} \longrightarrow P_{3}
$$

Enzyme kinetic constants from the given graph are:
Reaction \#1 $\quad \mathrm{v}_{\mathrm{m} 1}:=1.1 \cdot 10^{-5} \mathrm{mg} / \mathrm{cm}^{2} \cdot \mathrm{sec} \quad \mathrm{K}_{\mathrm{m} 1}:=0.025 \mathrm{mg} / \mathrm{cm}^{3} \quad \mathrm{v}_{1}(\mathrm{~s}):=\frac{\mathrm{v}_{\mathrm{m} 1} \cdot \mathrm{~s}}{\mathrm{~K}_{\mathrm{m} 1}+\mathrm{s}}$
Reaction \#2 $\quad \mathrm{v}_{\mathrm{m} 2}:=2 \cdot 10^{-5} \mathrm{mg} / \mathrm{cm}^{2}$. sec $\quad \mathrm{K}_{\mathrm{m} 2}:=0.11 \mathrm{mg} / \mathrm{cm}^{3} \quad \mathrm{v}_{2}(\mathrm{~s}):=\frac{\mathrm{v}_{\mathrm{m} 2} \cdot \mathrm{~s}}{\mathrm{~K}_{\mathrm{m} 2}+\mathrm{s}}$

Part a) Find total rate of substrate disappearance, based on the following operating parameters.
Mass transfer coefficient: $\quad \mathrm{k}_{\mathrm{L}}:=6 \cdot 10^{-5} \mathrm{~cm} / \mathrm{sec}$
Substrate concentration in the bulk liquid: $\mathrm{s}_{\mathrm{b}}:=0.5 \mathrm{mg} / \mathrm{cm}^{3}$
Mass transfer $\quad \mathrm{J}(\mathrm{s}):=\mathrm{k}_{\mathrm{L}} \cdot \mathrm{s}_{\mathrm{b}}-\mathrm{s}$
$\mathrm{s}:=0.01,0.02 . . \mathrm{s}_{\mathrm{b}}$


Determine surface concentration of substrate at steady-state:

$$
\mathrm{s}:=\mathrm{s}_{\mathrm{b}} \quad \cdots \text { Initial guess } \quad \text { Given } \quad \mathrm{J}(\mathrm{~s})=\mathrm{v}_{1}(\mathrm{~s})+\mathrm{v}_{2}(\mathrm{~s})
$$

Rate of consumption of substrate due to Reaction \#1
Rate of consumption of substrate due to Reaction \#2

$$
\mathrm{v}_{1}(\mathrm{~s})=9.431 \cdot 10^{-6} \quad \mathrm{mg} / \mathrm{cm}^{2} \cdot \mathrm{sec}
$$

Total rate of consumption of substrate due to both reactions
$v_{2}(s)=1.155 \cdot 10^{-5} \quad \mathrm{mg} / \mathrm{cm}^{2}$. sec

Mass transfer of substrate to surface (check) $\quad J(s)=2.098 \cdot 10^{-5} \mathrm{mg} / \mathrm{cm}^{2} \cdot \mathrm{sec}$
Part b) Overall effectiveness factor is the ratio of observed rate with mass transfer to the intrinsic rate without mass transfer limitation.

$$
\begin{aligned}
& \text { effectiveness factor } \eta:=\frac{v_{1}(s)+v_{2}(s)}{v_{1}\left(s_{b}\right)+v_{2}(s b)} \quad \eta=0.781 \\
& \text { Part c) Ratio of } P_{2} \text { to } P_{1} \quad \text { ratio }:=\frac{v_{2}(s)}{v_{1}(s)} \quad \text { ratio }=1.225
\end{aligned}
$$

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 $\mathrm{v}_{1}=\mathrm{v}_{2}$.

$$
\mathrm{s}:=\mathrm{s}_{\mathrm{b}} \quad \ldots \text { initial guess } \quad \text { Given } \quad \mathrm{v}_{1}(\mathrm{~s})=\mathrm{v}_{2}(\mathrm{~s}) \quad \mathrm{s}:=\operatorname{Find}(\mathrm{s}) \quad \mathrm{s}=0.079 \mathrm{mg} / \mathrm{cm}^{3}
$$

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., $\mathrm{v}_{1}+\mathrm{v}_{2}=\mathrm{J}$ ).

$$
\mathrm{J}\left(\mathrm{~s}_{\mathrm{b}}\right):=\mathrm{k}_{\mathrm{L}} \cdot\left(\mathrm{~s}_{\mathrm{b}}-\mathrm{s}\right)
$$

$$
\mathrm{s}_{\mathrm{b}}:=\mathrm{s} \quad \ldots \text { initial guess } \quad \text { Given } \quad \mathrm{v}_{1}(\mathrm{~s})+\mathrm{v}_{2}(\mathrm{~s})=\mathrm{J}\left(\mathrm{~s}_{\mathrm{b}}\right) \quad \mathrm{s}_{\mathrm{b}}:=\operatorname{Find}\left(\mathrm{s}_{\mathrm{b}}\right) \quad \mathrm{s}_{\mathrm{b}}=0.357 \mathrm{mg} / \mathrm{cm}^{3}
$$

$$
\text { Plot } \mathrm{s}:=0,0.01 . . \mathrm{s}_{\mathrm{b}} \quad \mathrm{~J}(\mathrm{~s}):=\mathrm{k}_{\mathrm{L}} \cdot\left(\mathrm{~s}_{\mathrm{b}}-\mathrm{s}\right)
$$

The two curves $\mathrm{v}_{1} \& \mathrm{v}_{2}$ intersect at the same value of $s$ as the two curves $\mathrm{v}_{1}+\mathrm{v}_{2}$ \& J does.

