BT209

Bioreaction Engineering

17/04/2023

Tutorial: Heterogeneous reaction

Problem 1

L-Lactate 2-monooxygenase from *Mycobacterium smegmatis* is immobilised in spherical agarose beads. The enzyme catalyses the reaction:

$$\underset{\text{(lactic acid)}}{C_3H_6O_3} + O_2 \rightarrow \underset{\text{(acetic acid)}}{C_2H_4O_2} + CO_2 + H_2O.$$

Beads 4 mm in diameter are immersed in a well-mixed solution containing 0.5 mM oxygen. A high lactic acid concentration is provided so that oxygen is the rate-limiting substrate. The effective diffusivity of oxygen in agarose is 2.1×10^{-9} m^2 s^{-1}. K_m for the immobilised enzyme is 0.015 mM; v_{max} is 0.12 mol s^{-1} per kg enzyme. The beads contain 0.012 kg enzyme m^{-3} gel. External mass-transfer effects are negligible.

(a) Plot the oxygen concentration profile inside the beads.
(b) What fraction of the catalyst volume is active?
(c) Determine the largest bead size that allows the maximum conversion rate?

Solution

(a)

as C_{As} is $0.5\ \text{mM}/0.015\ \text{mM} = 33$ times the value of K_m, as a first approximation we can consider the kinetics to be effectively zero order with $k_0 = v_{max}$. Converting the units of k_0 to a per volume of gel basis:

$$k_0 = v_{max} = 0.12\,\text{mol}\,\text{s}^{-1}\,\text{kg}^{-1}\left(0.012\,\text{kg}\,\text{m}^{-3}\right).\left|\frac{32\,\text{g}}{1\,\text{mol}}\right|.\left|\frac{1\,\text{kg}}{1000\,\text{g}}\right| = 4.61\times10^{-5}\,\text{kg}\,\text{s}^{-1}\,\text{m}^{-3}$$

Converting C_{As} to units of kg m^{-3}:

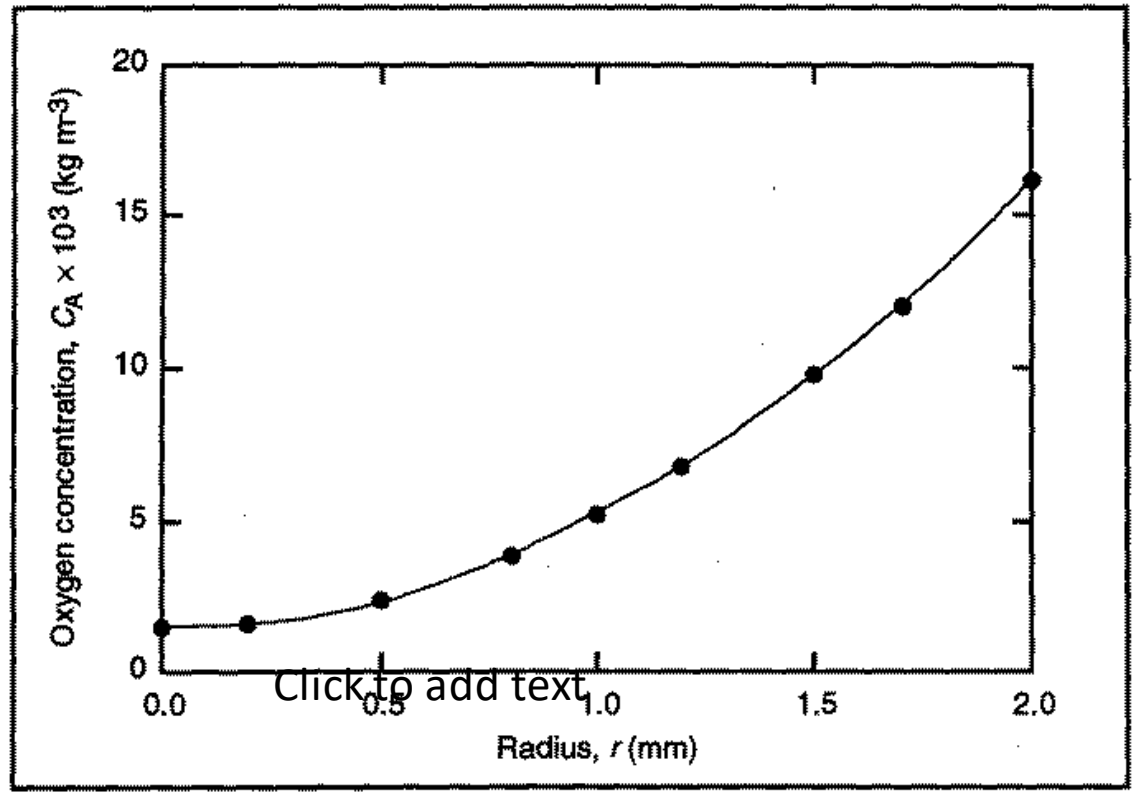
$$C_{As} = 0.5\,\text{mM} = 0.5\times10^{-3}\,\text{gmol}\,\text{l}^{-1}.\left|\frac{1000\,\text{l}}{1\,\text{m}^3}\right|.\left|\frac{32\,\text{g}}{1\,\text{gmol}}\right|.\left|\frac{1\,\text{kg}}{1000\,\text{g}}\right| = 0.016\,\text{kg}\,\text{m}^{-3}$$

For zero-order reaction, the equation used to determine the substrate concentration inside the beads depends on whether C_A remains > 0 throughout the particle. The maximum particle radius for which this occurs can be calculated

$$R_{max} = \sqrt{\frac{6\,\mathcal{D}_{Ae}\,C_{As}}{k_0}} = \sqrt{\frac{6\left(2.1\times10^{-9}\,\text{m}^2\,\text{s}^{-1}\right)0.016\,\text{kg}\,\text{m}^{-3}}{4.61\times10^{-5}\,\text{kg}\,\text{s}^{-1}\,\text{m}^{-3}}} = 0.0021\,\text{m} = 2.1\,\text{mm}$$

Therefore, the maximum particle diameter for $C_A > 0$ everywhere is 4.2 mm. Because the immobilised-enzyme beads are smaller than this, $C_A > 0$ and the oxygen concentration profile can be calculated using the equation for zero-order reaction and spherical geometry Values for C_A as a function of r are listed and plotted below.

Radius, r (m)	Oxygen concentration, C_A (kg m^{-3})
2.0×10^{-3}	1.60×10^{-2}
1.7×10^{-3}	1.19×10^{-2}
1.5×10^{-3}	9.60×10^{-3}
1.2×10^{-3}	6.63×10^{-3}
1.0×10^{-3}	5.02×10^{-3}
0.8×10^{-3}	3.71×10^{-3}
0.5×10^{-3}	2.28×10^{-3}
0.2×10^{-3}	1.51×10^{-3}
0.0	1.37×10^{-3}



As the minimum value of C_A at the centre of the bead is still about 2.9 times K_m, the assumption of zero-order kinetics is reasonable.

(b)
As $C_A > 0$ everywhere within the bead, for zero-order reaction this means that the entire bead volume is active.

Answer: 1.0

(c)
For zero-order reaction, the maximum conversion rate occurs when the oxygen concentration is greater than zero everywhere in the particle. The largest bead size for this to occur was calculated in **(a)** is 4.2 mm.

Answer: 4.2 mm

Problem 2

Enzyme is immobilised in 8 mm diameter agarose beads at a concentration of 0.018 kg protein m^{-3} gel. Ten beads are immersed in a well-mixed solution containing 3.2×10^{-3} kg m^{-3} substrate. The effective diffusivity of substrate in agarose gel is 2.1×10^{-9} m^2 s^{-1}. Kinetics of the enzyme can be approximated as first order with specific rate constant 3.11×10^5 s^{-1} per kg protein. Mass transfer effects outside the particles are negligible. Plot the steady-state substrate concentration profile as a function of particle radius.

$R = 4 \times 10^{-3}$ m; $\mathscr{D}_{Ae} = 2.1 \times 10^{-9}$ m^2 s^{-1}. In the absence of external mass-transfer effects, $C_{As} = 3.2 \times 10^{-3}$ kg m^{-3}.

$$\text{Volume per bead} = \frac{4}{3}\pi R^3 = \frac{4}{3}\pi\,(4 \times 10^{-3}\text{ m})^3 = 2.68 \times 10^{-7}\text{ m}^3.$$

Therefore, 10 beads have volume 2.68×10^{-6} m^3. The amount of enzyme present is:

$$2.68 \times 10^{-6}\text{ m}^3\,(0.018\text{ kg m}^{-3}) = 4.83 \times 10^{-8}\text{ kg}.$$

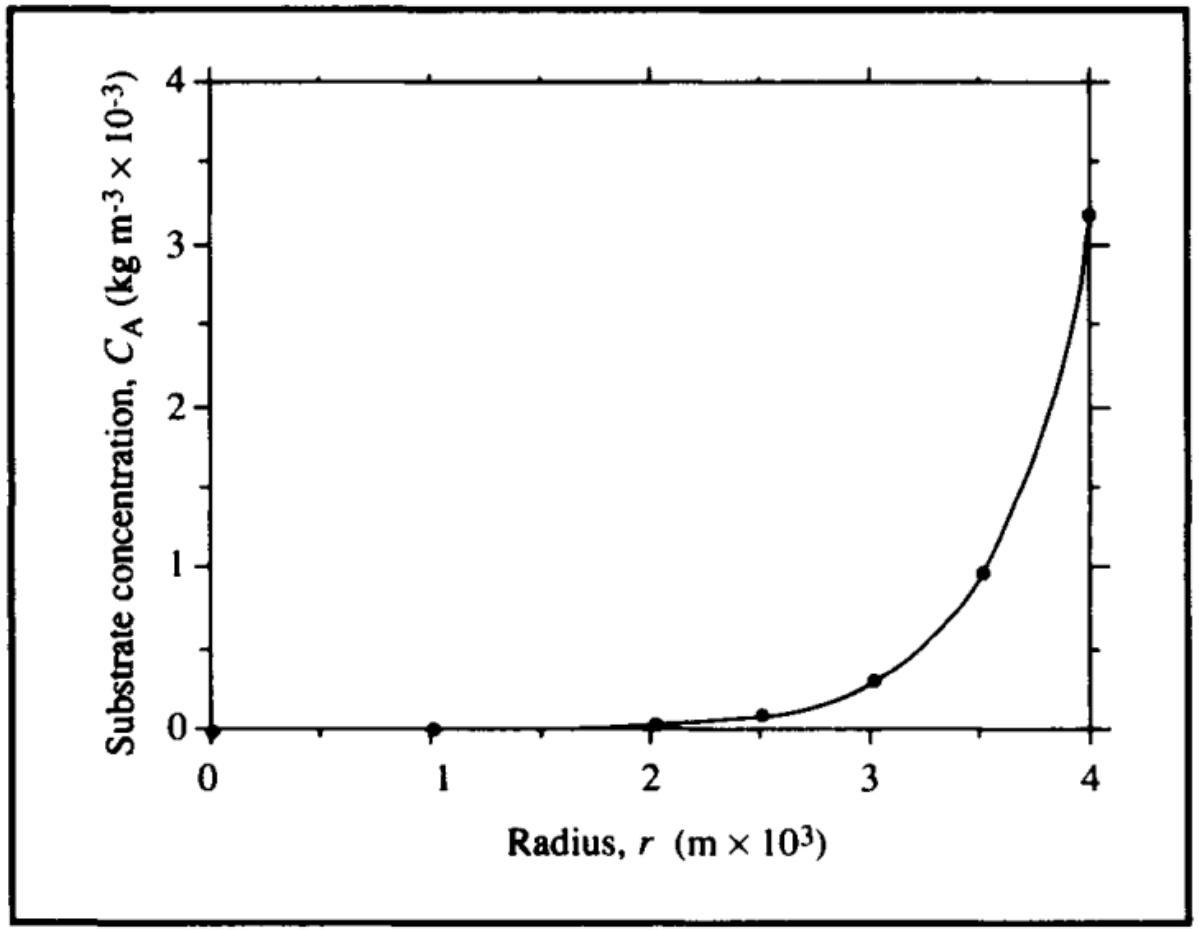
Therefore:

$$k_1 = 3.11 \times 10^5\text{ s}^{-1}\text{ kg}^{-1}\,(4.83 \times 10^{-8}\text{ kg}) = 0.015\text{ s}^{-1}$$

and:

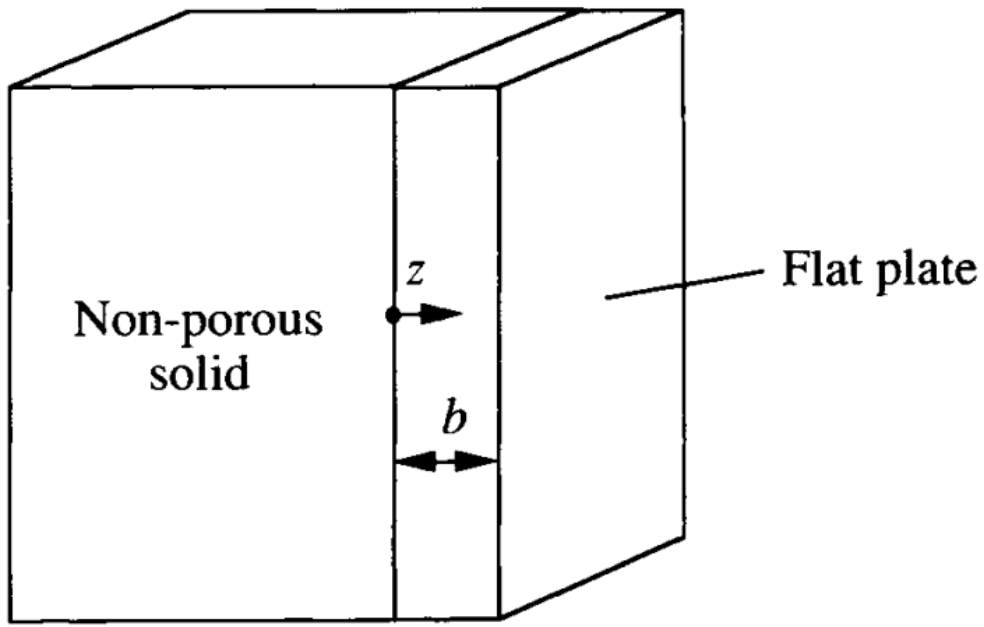
$$R\sqrt{\frac{k_1}{\mathscr{D}_{Ae}}} = 10.693.$$

$$C_A = C_{As}\,\frac{R}{r}\,\frac{\sinh\left(r\sqrt{k_1/\mathscr{D}_{Ae}}\right)}{\sinh\left(R\sqrt{k_1/\mathscr{D}_{Ae}}\right)}. \qquad \sinh\left(R\sqrt{k_1/\mathscr{D}_{Ae}}\right) = \frac{e^{10.693} - e^{-10.693}}{2} = 2.202\times10^4.$$



the particle to reach virtually zero 2 mm from the centre.

Equations for flat-plate geometry are used to analyse reactions in cell films attached to inert solids; the biofilm constitutes the flat plate. Even if the surface supporting the biofilm is curved rather than flat, if the film thickness b is very small compared with the radius of curvature, equations for flat-plate geometry are applicable. To simplify mathematical treatment and keep the problem one-dimensional, the flat plate is assumed to have infinite length. In practice, this assumption is reasonable if its length is much greater than its thickness. If not, it must be assumed that the ends of the plate are sealed to eliminate axial concentration gradients. What is the substrate concentration profile inside this biofilm without external boundary-layer effects assuming zero order reaction. D_{Ae} is the effective diffusivity inside the biofilm and C_{AS} is the concentration of A in surface. Assume



$$C_A = C_{As} + \frac{k_0}{2\mathscr{D}_{Ae}} (z^2 - b^2)$$