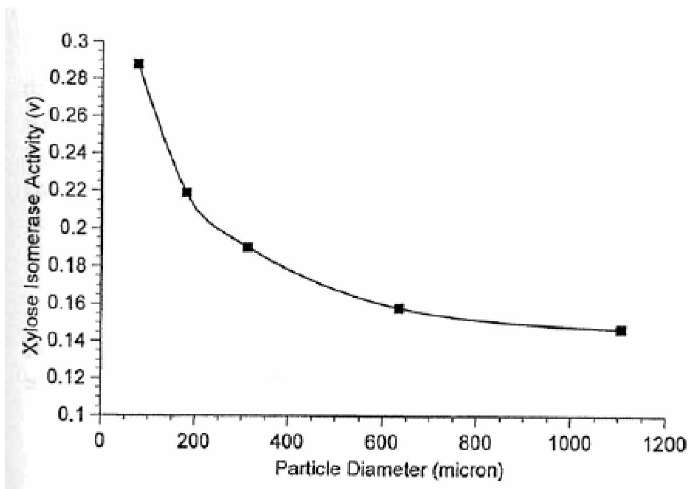
Practice Problem sheet (BBL433)

1. Sweetzyme Q is a commercial preparation of glucose isomerase immobilized in a porous spherical particle (Novo Industries, Denmark). Plotted below are initial rates of xylose isomerization catalyzed by Sweetzyme Q (in units of μmols xylose isomerized/mg enzyme·min). The external concentration of xylose is 1.5 M the density of the catalyst is 1.4 g/cm3, and the measured KM is 0.209M. Assume that the maximum activity plotted in the figure would not increase if the particle diameter were further reduced. (ref. Blanch and Clark, Biochemical Engineering, p.155).
    i) Estimate the effective diffusivity of xylose in the Sweetzyme Q catalyst.
    ii) What is the largest potential source of error in the calculation in part i.



2. A batch reactor containing the enzyme invertase is used to hydrolyse a 2 % w/v solution of sucrose (molecular weight 342) into a mixture of glucose and fructose. If the V_{max} and K_m of the enzyme used are 2 mmol min^{-1} l^{-1} and 5 mM respectively, determine the % conversion after 40 minutes.

3. Determine the kinetic parameters and comment on the following data which was obtained for the inhibition of an enzyme.

Substrate concentration (mM)	Uninhibited rate (μmol/min)	+ 1 mM Inhibitor (μmol/min)
10	0.86	0.35
20	1.33	0.63
30	1.64	0.86
40	1.85	1.04
50	2.00	1.20

4. Given the expression

5. $(V_{max}/Vol_S) .t = [S]_0\mathbf{X} - K_m Ln(1 - \mathbf{X})$

determine the percentage hydrolysis of 5 litres of 2 M lactose in 15 hours. (K_m = 2 mM; V_{max} = 10 mmol/min).

6. Enzymatic isomerisation of glucose to fructose can be kinetically expressed by the rate expression

$$V = \frac{0.128\,(G) - \,0.098\,(F)}{0.096 + 0.383\,(G) + \,0.25\,(F)}$$

Where (G) and (F) represent the concentrations of glucose and fructose respectively. If the feed substrate concentration is 1.0 M and the desired conversion is 40% compare the productivity of the above reaction if carried out in CSTR/PFR of the same volume and enzyme loading.

7. You are comparing different processes and reactor types for degradation of pollutant A in a waste stream. Pollutant A concentration in the stream to be treated is $[A]_0$=10 mM and the flow rate of the stream is 1000 L/min. In presence of a dissolved enzyme, E, pollutant A decomposes by Michaelis-Menten kinetics, with μ_{max} = 50 mM/min and K_M = 5 mM when the total enzyme concentration is 10^{-3} mM. What reactor volume do you need to achieve 99% conversion of A in i) CSTR, ii) PFR?
What reactor volume do you need for CSTR and PFR if you are willing to pay for more enzyme and raise the enzyme concentration to 10^{-2} mM? Considering that capital cost is proportional to reactor size and operating cost to enzyme concentration used, what information would you need to determine reactor type and enzyme concentration to be used. Explain clearly.

8. A yeast invertase inactivates thermally following a second order kinetics. Derive a relation between fraction of conversion, time and enzyme in a batch reactor. You can assume the enzyme follows a Michaelis Menten behavior.
(5)

$$-\frac{dE}{dt} = k_D \cdot E^2$$

9. Penicillin G is hydrolyzed with immobilized penicillin G acylase (PGA) from Bacillus megaterium in order to produce the β lactam nucleus 6-aminopenicillanic acid (6APA). CPBR and CSTR

configurations have been proposed for the treatment of 100 g/L of penicillin G potassium salt ($C_{16}H_{17}KN_2O_4S$) solution. The product 6APA is a competitive inhibitor for PGA. The michaelis constant for penicillin G is 0.06 M and the 6APA inhibition constant is 0.25 M. Both the reactors are loaded with equal amount of enzyme and volume of the reactors are 2000 L and substrate is fed at 100 L/hr. V_{max} = 30 mmol/L/hr. Calculate the state state conversion achieved in both the reactors and discuss the result.

10. Invert sugar is produced from sucrose using two continuous reactor in series, both of them 5000 L in volume and immobilized with same amount of catalyst. A syrup with 200 g/L of sucrose is fed at a flow rate of 180 L/hr. Mol wt sucrose = 342.3 g/mol. The kinetic parameters of the enzyme are V_{max} = 200µm/L/min and K_m = 68.5 mM.
    If one of the reactors is PBR and the other is CSTR, determine the right sequence of reactor (for maximum conversion).
    In what fraction the flow rate has to be adjusted in the second reactor to increase the performance of unfavorable sequence equal to the favorable sequence determined in part a.

    i) Hint: Remember when you solved for mass balance in CSTR and PBR the integration limit for conversion is zero to X. It is not the case here.

11. Invertase catalyses the reaction:

    $C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$

    Sucrose glucose fructose

    Invertase from *Aspergillus oryzae* is immobilized in porous resin particles of diameter 1.6 mm at a density of 0.1 µmol enzyme/g. The effective diffusivity of sucrose in the resin is 1.3×10^{-11} m^2s^{-1}. The resin is placed in a spinning basket reactor operated so that external mass-transfer effects are eliminated. At a sucrose concentration of 0.85 kg/m^3, the observed rate of conversion is 1.25×10^{-3} kgs^{-1} m^{-3} resin. Km for the immobilized enzyme is 3.5 kg/m^3.Calculate the effectiveness factor.