

Determine the maximum reaction velocity,  $V_m$  (mg/ml-min · unit of enzyme) and the saturation constant,  $K_M$  (mg/ml).

- b. The same authors studied the effect of temperature on the maximum rate of the hydrolysis of corn starch by glucoamylase. The results are tabulated next. Determine the activation energy ( $\Delta E$  cal/g mole) for the soluble and immobilized enzyme reaction.

T, °C	$V_{\max}$ (m mol/min $10^6$ )	
	Soluble	Azo-immobilized
25	0.62	0.80
35	1.42	1.40
45	3.60	3.00
55	8.0	6.2
65	16.0	11.0

- c. Using these results, determine if immobilized enzyme is diffusion limited.

[Courtesy of A. E. Humphrey from “Collected Coursework Problems in Biochemical Engineering” compiled by H. W. Blanch for 1977 Am. Soc. Eng. Educ. Summer School.]

- 3.11.** Michaelis–Menten kinetics are used to describe intracellular reactions. Yet  $[E_0] \approx [S_0]$ . In in vitro batch reactors, the quasi-steady-state hypothesis does not hold for  $[E_0] \approx [S_0]$ . The rapid equilibrium assumption also will not hold. Explain why Michaelis–Menten kinetics and the quasi-steady-state approximation are still reasonable descriptions of intracellular enzyme reactions.

- 3.12.** You are working for company A and you join a research group working on immobilized enzymes. Harry, the head of the lab, claims that immobilization improves the stability of the enzyme. His proof is that the enzyme has a half-life of 10 days in free solution, but under identical conditions of temperature, pH, and medium composition, the measured half-life of a packed column is 30 days. The enzyme is immobilized in a porous sphere 5 mm in diameter. Is Harry’s reasoning right? Do you agree with him? Why or why not?

- 3.13.** The following data were obtained from enzymatic oxidation of phenol by phenol oxidase at different phenol concentrations.

S (mg/l)	10	20	30	50	60	80	90	110	130	140	150
$v$ (mg/l-h)	5	7.5	10	12.5	13.7	15	15	12.5	9.5	7.5	5.7

- What type of inhibition is this ?
- Determine the constants  $V_m$ ,  $K_m$ , and  $K_{si}$ .
- Determine the oxidation rate at  $[S] = 70$  mg/l.

- 3.14.** Uric acid is degraded by uricase enzyme immobilized in porous Ca-alginate beads. Experiments conducted with different bead sizes result in the following rate data:

Bead Diameter, Dp (cm)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Rate, $v$ (mg/l.h)	200	198	180	140	100	70	50	30

- Determine the effectiveness factor for particle sizes Dp = 0.5 cm and Dp = 0.7 cm.
- The following data were obtained for Dp = 0.5 cm at different bulk uric acid concentrations. Assuming negligible liquid film resistance, calculate  $V_m$  and  $K_s$  for the enzyme. Assume no substrate or product inhibition.

$S_0$ (mg UA/l)	10	25	50	100	200	250
$v$ (mg UA/l.h)	10	20	30	40	45	46