

60 minutes

1. A certain arylesterase (EC 3.1.1.29) catalyzes the hydrolysis of phenyl acetate (S) into phenol (P) and acetic acid (A). Phenol is a (total) competitive inhibitor but also exerts (total) uncompetitive inhibition. Determine the kinetic expression for the initial rate of phenylacetate hydrolysis. (5)
2. Mushroom tyrosinase is immobilised in 2-mm spherical beads for conversion of tyrosine to DOPA in a continuous, well-mixed bubble column. The Michaelis constant for the immobilised enzyme is 2 gmol m^{-3} . A solution containing 15 gmol m^{-3} tyrosine is fed into the reactor; because of the high cost of the substrate, the desired conversion is 99%. The reactor is loaded with beads at a density of $0.25 \text{ m}^3 \text{m}^{-3}$; all enzyme is retained within the reactor. The intrinsic V_{\max} for the immobilized enzyme is $1.5 \times 10^{-2} \text{ gmol s}^{-1} \text{ per m}^3 \text{ beads}$. The effective diffusivity of tyrosine in the beads is $7 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$; external mass-transfer effects are negligible. Immobilisation stabilises the enzyme so that deactivation is minimal over the operating period. Determine the reactor volume needed to treat 18 m^3 tyrosine solution per day. (7.5)
3. Protein A is 500 kDa intracellular protein with negatively charged N-terminus. Explain how this protein can be purified using flow chart diagram. (2.5)
4. The trisaccharide raffinose (α -D-galactosylsucrose) is a contaminant in sugar beet juice that retards sucrose crystallization. Raffinose (R) is hydrolyzed by soluble α -galactosidase (α -D-galactoside galactohydrolase, EC 3.2.1.22) from *Aspergillus niger* into sucrose and galactose. Its kinetic parameters are:
 $V_{\max} = 2500 \text{ } \mu\text{mol}_{\text{hydrolyzed raffinose}} \cdot \text{g}^{-1} \text{ enzyme protein min}^{-1}$
 $K_M = 70 \text{ mM}$
A batch reactor is loaded with 10 g/L of enzyme preparation with 14% protein and 300 g/L of raffinose (Mol. Wt: 504.42 g/mol). Calculate time required for 90% substrate conversion. (5)

BBL433, Enzyme Science Engineering
Minor II

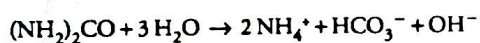
27th March 2018
20 marks

60 minutes

1. A BSTR of 600 L working volume is used for the removal of lactose from spent cheese whey (40 g/L) with fungal β -galactosidase at 50 °C and pH 4.5. The enzyme is strongly competitively inhibited by the product, galactose; its kinetic parameters under such conditions are K_m 90mM and K_i 9 mM. The operation is designed to remove 80% of the lactose after 5 hours of reaction.

- (a) Determine the amount of enzyme required in katal (1 katal is the amount of enzyme that hydrolyzes 1 mole of lactose per second under these conditions).
(b) A mutant β -galactosidase has been developed through directed evolution, in which K_M is reduced to one-third and K_I is increased three times. What amount of this mutant enzyme will be required to perform the same task? (7.5)

2. A system is being developed to remove urea from the blood of patients with renal failure. A prototype fixed-bed reactor is set up with urease immobilised in 2-mm gel beads; buffered urea solution is recycled rapidly through the bed so that the system is well mixed. The urease reaction is:



K_m for the immobilised urease is 0.54 g/L. The volume of beads in the reactor is 250 cm³, the total amount of urease is 10⁻⁴g, and the turnover number is 11 000 g NH₄⁺ per g enzyme per second. The effective diffusivity of urease in the gel is 7 x 10⁻⁶ cm²s⁻¹; external mass-transfer effects are negligible. The reactor is operated continuously with a liquid volume of 1 litre. The feed stream contains 0.42 g/L urea; the desired urea concentration is 0.02 g/L. Ignoring enzyme deactivation, what volume of urea solution can be treated in 30 min? (7.5)

3. Write short notes on

(2.5 x 2)

- a. Enzyme formulation
- b. Any one of carrier less immobilization method