BEL403, Enzyme Engineering
Minor II

25/4/2018
20 marks

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 m^3m^{-3}; all enzyme is retained within the reactor. The intrinsic V_{max} for the immobilized enzyme is 1.5×10^{-2} gmol s^{-1} per m^3 beads. The effective diffusivity of tyrosine in the beads is 7×10^{-10} m^2 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 (a-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 $\mu mol_{hydrolyzed\ raffinose} \cdot g^{-1}_{enzyme\ protein}\ min^{-1}$
   K_M= 70 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

60 minutes 20 marks

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 katals (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 KM is reduced to one-third and KI 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:

$$(NH_2)_2CO + 3\,H_2O \rightarrow 2\,NH_4^+ + HCO_3^- + OH^-$$

   K_m for the immobilised urease is 0.54 g/L. The volume of beads in the reactor is 250 cm^3, the total amount of urease is 10^{-4}g, 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} cm $^2s^{-1}$; 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