BEL403, Enzyme Engineering

Minor II

9th Oct 2014

0 minutes 20 marks

1. The enzyme α-glucosidase (EC 3.2.1.20) catalyzes the hydrolysis of maltose into glucose. α -glucosidase is competitively inhibited by the product glucose and inhibited at high maltose concentrations in a partial uncompetitive mode. Determine a kinetic rate expression in terms of the dissociation constants for: the secondary enzyme–substrate complex (K_M), the secondary enzyme–product complex (K_P), the tertiary enzyme–substrate–substrate complex (K_0), and the maximum reaction rates of product formation from the enzyme–substrate active complex (V_{max}) and the enzyme–substrate–substrate partially active complex (V^0_{max}). The molar concentrations of maltose and glucose are [M] and [G], respectively. (5)

2. 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)

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

3. 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. (7.5)

4. 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.
   a. If one of the reactors is PBR and the other is CSTR, determine the right sequence of reactor (for maximum conversion).
   b. 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.

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. (7.5)