Department of Biochemical Engineer and Biotechnology
Enzyme Science and Engineering

24th March 2023

60 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. 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 the 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 reactors and discuss the result. (7.5)

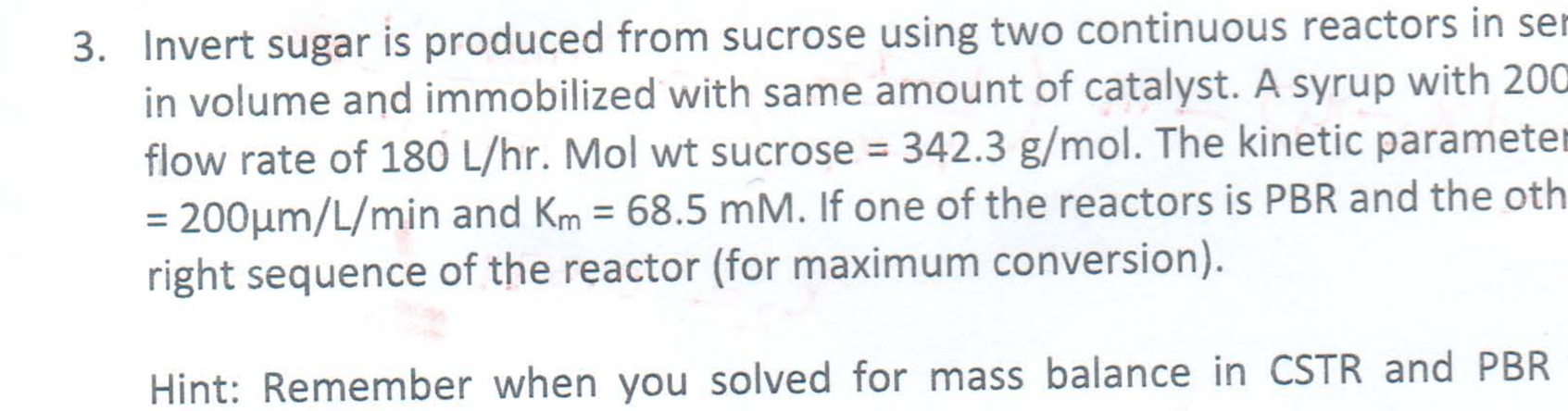
3. Invert sugar is produced from sucrose using two continuous reactors in series, both of them 5000 L in volume and immobilized with same amount of catalyst. A syrup with 200 g/L of sucrose is fed at a flow rate of 180 L/hr. Mol wt sucrose = 342.3 g/mol. The kinetic parameters of the enzyme are V_{max} = 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 the reactor (for maximum conversion).

   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)

4. Write a short note on the following (2.5x2)
   a. Cross-linked enzyme aggregate
   b. High-throughput enzyme screening

Q1.



2

2

$$v = v_{max}[EM] + v^{\circ}_{max}[EMM] = \frac{v_{max}\cdot\frac{m}{K_m} + v^{\circ}_{max}\cdot\frac{m^2}{K_m\cdot K_0}}{1 + \frac{m}{K_m} + \frac{G}{K_p} + \frac{m^2}{K_m K_0}}$$

1

2

Q2. Competitive inhibition by product — 7.5

$$U = \frac{U_{max} \cdot S/K_m}{1 + S/K_m + P/K_i}$$ — 1.5

CSTR :

$$\frac{S_0 - S}{\tau} = \frac{U_{max} \cdot S}{K_m + S + (S_0 - S)\frac{K_m}{K_i}}$$ — 2

$$x = 0.645$$ — 1

PBR : $$\frac{U}{F \cdot S_0} = \frac{1}{U_{max}}\left[\frac{K_m(1 + S_0/K_I)}{S_0}\ln(1-x) + x\left(1 - \frac{K_m}{K_I}\right)\right]$$ — 2

$$x = 0.956$$ — 1

Q.3 Case I (First CSTR → PBR

CSTR → $$\frac{S_i x}{K_m} + \frac{x}{1-x} = \frac{U_{max} \cdot U}{K_m \cdot F}$$ — 1

$x_1 = 0.467$ — 1

PBR → $$\frac{S_i}{K_m}(x_2 - x_1) - \ln\left(\frac{1-x_2}{1-x_1}\right) = \frac{U_{max} \cdot U}{K_m \cdot F}$$ — 1

$x_2 = 0.871$ — 1

Case II (PBR → CSTR)

PBR → $$\frac{S_i x_1}{K_m} - \ln(1-x_1) = \frac{U_{max} \cdot U}{K_m \cdot F}$$ — 1

$x_1 = 0.491$ — 1

CSTR → $$\frac{S_i}{K_m}(x_2 - x_1) + \frac{(x_2 - x_1)}{1-x_2} = \frac{U_{max} \cdot U}{K_m \cdot F}$$ — 1

$\Rightarrow x_2 = 0.825$ — 1

∴ CSTR followed by PBR is preferred.