

### Problem 3.1

The bacterium A is used to produce biomass from methanol in a 1000 m<sup>3</sup> reactor that can be operated in batch or continuously. The biomass/substrate yield coefficient is 0.41 g/g, K<sub>s</sub>=0.7 mg/l and the maximum specific growth rate is 0.44 h<sup>-1</sup>. The medium contains 4% (w/v) methanol and is intended to achieve a substrate conversion of 98%. If operated in batch, an inoculum of 0.01% (w/v) is used and the waiting time between batches is 20 h. If operated continuously, there are 25 days a year that the reactor is not in operation. Disregarding cell maintenance and death requirements, compare the annual biomass production of the two reactors.

$$t_b = \frac{1}{\mu_{max}} \ln \left[ 1 + \frac{S_0 - S_f}{\left( \frac{1}{Y_{x/s}} + \frac{m_s}{\mu_{max}} \right) X_0} \right] \quad \text{onde } t_b \text{ é o tempo de batch}$$

### Problem 3.2

Consider a microorganism that follows the Monod equation, in which  $\mu_{max} = 0.7 \text{ h}^{-1}$  e  $K_s = 3.5 \text{ g.Litro}^{-1}$ .

- In a continuous stirred reactor under steady state, without cell death, fed at a flow rate of 20 Liter h<sup>-1</sup>, if  $S_0 = 40 \text{ g.Litro}^{-1}$  and  $Y_{x/s} = 0.9 \text{ g.cell/g-substrate}$ , which reactor volume will correspond to the maximum cell production rate?
- Determine the substrate concentration at the outlet of the 1st reactor.
- If  $Y_{p/x} = 0.6 \text{ g P/g cel}$ , what concentration of product would be obtained in the 1st reactor (consider that it is a type I process).
- If you double and quadruple the substrate concentration, what would be the dilution rate corresponding to maximum productivity? And if it changes to a quarter?
- For the same flow rate, what volume is needed for another reactor, placed in series with the first, so that the substrate concentration at the outlet of this second reactor is 0.4 g.Litro<sup>-1</sup>?
- Calculate the overall productivity (in cells) obtained in this system.

### Problema 3.3

Consider a 100 L CSTR where a crop that obeys the  $S \rightarrow X$  equation grows. It is known that Monod's law is valid, that the consumption of glucose (substrate) due to maintenance is not negligible and that:  $\mu_{\max} = 0.37 \text{ h}^{-1}$ ;  $K_S = 1.3 \text{ g/L}$ ;  $m_S = 0.0135 \text{ g S/(gX.h)}$ ;  $Y'_{X/S} = 0.36 \text{ g/g}$ . The reactor is operated at 5 l/h and  $S_0 = 20 \text{ g/l}$ .

- Calculate the substrate and biomass concentration in the outlet stream of the CSTR.
- If operating at the following dilution rates: 25%, 50% or 99% less than the critical washout rate, what would be the substrate and biomass concentrations in the outlet streams? Explain the results obtained.

### Problem 3.4

Consider two bacterial cultures (1 and 2) producing a product P in a steady state CSTR. The CSTR is fed with sterile medium that contains 50 mM glucose (the growth-limiting substrate, S). In a set of experiments, the dilution rate D is gradually increased, having obtained the experimental results summarized in the table.

- Explain the shape of the X vs. curves. D for both cultures. Why does the cell density increase with increasing inflow to  $D < 0.35 \text{ h}^{-1}$ ?
- At which dilution rate would a CSTR operate when product formation is type I (associated with growth)? justify your answer (approximate values)
- At which dilution rate would a CSTR operate when the product is type III (product formation dissociated from growth)? justify your answer (approximate values)

D (h <sup>-1</sup> )	x1 (g/l)	x2 (g/l)
0.05	1.58	2.82
0.1	2.17	2.83
0.15	2.47	2.84
0.2	2.65	2.83
0.25	2.76	2.84
0.3	2.83	2.83
0.35	2.86	2.85
0.4	2.84	2.84
0.45	2.74	2.74
0.5	2.25	2.25
0.52	1.32	1.32
0.53	0	0

### Problem 3.5

Ethanol ( $C_2H_5OH$ ) is produced by a microorganism cultured anaerobically in CSTR at steady state. The empirical formula for biomass is  $CH_2O_{0.45}N_{0.19}$  under growing conditions. The CSTR is fed with sterile medium that contains a high concentration of glucose (20 mM), an excess of ammonium salts, and does not contain ethanol. When the dilution rate is  $D=0.1\text{ h}^{-1}$ , the output current contains 2 mM glucose and 0.45 g/L biomass. Ethanol is known to be the only metabolite excreted by cells.

- Determine the ethanol concentration (mM) in the outlet stream.
- The chosen dilution rate probably does not optimize biomass productivity. Briefly explain the above statement and speculate on why you chose  $D=0.1\text{ h}^{-1}$ .

### Problem 3.6

The specific growth rate for a CSTR process with inhibition is given by the following equation:

$$\mu = \frac{\mu_m S}{K_s + S + I \frac{K_s}{K_i}}$$

Where:  $S_0 = 10\text{ g/l}$ ;  $K_s = 1\text{ g/l}$ ;  $I = 0.05\text{ g/l}$   
 $Y_{x/s} = 0.1\text{ g}_{\text{cel}}/\text{g}_{\text{sub}}$ ;  $X_0 = 0$ ;  $K_i = 0.01\text{ g/l}$   
 $\mu_{\text{max}} = 0.5\text{ h}^{-1}$ ;  $K_d = 0$

- Determine the cell and substrate concentration in the CSTR reactor as a function of  $D$  when the inhibitor concentration is equal to zero.
- If inhibitor is added to CSTR, determine substrate and cell concentration as a function of  $D$ .
- Determine the cell productivity,  $DX$ , as a function of the dilution rate for situation a) and b).
- If you double the concentration of inhibitor added to the reactor how is the cell and substrate concentration affected?

### Problema 3.7

A system consisting of two reactors in series is used to produce a secondary metabolite. The volume of each reactor is  $0.5 \text{ m}^3$  and the feed rate of the medium is  $50 \text{ l/h}$ . Micellar growth takes place in the first reactor while the second is used to produce product. The substrate concentration in the feed is  $10 \text{ g/l}$ . The kinetic and stoichiometric parameters for the microorganism are as follows:

$$Y'_{x/s} = 0.5 \text{ gcell/ gsubstrate}$$

$$K_s = 1.0 \text{ gsub /l}$$

$$\mu_{\max} = 0.12 \text{ h}^{-1}$$

$$m_s = 0.025 \text{ gsub/gcel.h}$$

$$V_p = 0.16 \text{ gProd/gcel.h}$$

$$Y_{p/s} = 0.85 \text{ gProd/gsub}$$

Assuming that product synthesis is negligible in the first reactor and growth is negligible in the second reactor, calculate:

- The concentration of cells and substrate entering the second reactor.
- Global Substrate Conversion.
- The final concentration of the product.