

Problem 3.1

The bacterium A is used to produce biomass from methanol in a 1000 m³ reactor that can be operated in batch or continuously. The biomass/substrate yield coefficient is 0.41 g/g, K_s=0.7 mg/l and the maximum specific growth rate is 0.44 h⁻¹. 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.

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

3.1

methanol → biomass

$$V_{\text{reactor}} = 1000 \text{ m}^3 = 1 \times 10^6 \text{ L}$$

$$Y_{X/S} = 0.41 \text{ g cel / g methanol}$$

$$K_S = 0.7 \times 10^{-3} \text{ g/L}$$

$$\mu_{\max} = 0.44 \text{ h}^{-1}$$

$$S_0 = 4 \% (\text{w/v}) = 40 \text{ g/L}$$

$$\text{98\% conversion: } S_f = S_0 - 0.98 \times S_0 = 0.8 \text{ g/L}$$

EM BATCH

tempo espera = 20 h
waiting time

$$x_0 = 0.01 \% \text{ (w/v)} = 0.1 \text{ g/L}$$

$$Y_{x/S} = \frac{\Delta x}{\Delta S} \quad x_f = 16.17 \text{ g/L}$$

em $1 \times 10^6 \text{ L}$ de reactor $\rightarrow 1.6 \times 10^7 \text{ g cel / batch}$

utilizando a fórmula: $t_b = 11.6 \text{ h}$

$$t_{\text{total}} = t_b + t_{\text{waiting}} = 31.56 \text{ h}$$

por ano: 277 batches \rightarrow 4432 ton biomass / year
in one year

EM CSTR

a biomassa é o produto
despreza-se a manutenção

the biomass is the product
no maintenance

$$D (S_0 - S) = \frac{1}{Y_{x/s}} \mu x$$

em estado estacionário: $D = \mu$ $Y_{x/s} (S_0 - S) = x$
steady-state

$$X = 16 \text{ g/L}$$

$$D = \frac{\mu_{\max} S}{K_s + S} = \mu = 0.4396 \text{ h}^{-1}$$

produtividade = $D x = 7.03 \text{ g/L.h}$
productivity

em $1 \times 10^6 \text{ L}$: $7.03 \times 10^6 \text{ g/h}$

em 340 days: 57365 ton biomass / year

COMPARAÇÃO

biomassa produzida em batch: 4504 ton/year

biomass production in batch

biomassa produzida em CSTR: 57365 ton/year

biomass production in CSTR



CSTR permite maior produção anual

CSTR attains higher production

Problem 3.2

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

- a) In a continuous stirred reactor under steady state, without cell death, fed at a flow rate of 20 Liter h^{-1} , if $S_0 = 40 \text{ g.Liter}^{-1}$ and $Y_{x/s} = 0.9 \text{ g.cell/g-substrate}$, which reactor volume will correspond to the maximum cell production rate?
- b) Determine the substrate concentration at the outlet of the 1st reactor.
- c) 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).
- d) 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?
- e) 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^{-1} ?
- f) Calculate the overall productivity (in cells) obtained in this system.

3.2

$$K_s = 3.5 \text{ g/L}$$

$$\mu_{\max} = 0.7 \text{ h}^{-1}$$

3.2-a)

$$F_0 = 20 \text{ L/h}$$

Assumimos que: Assuming:

$$S_0 = 40 \text{ g/L}$$

estado estacionário
steady-state

$$Y_{X/S} = 0.9 \text{ g cel/g substrate}$$

não há morte celular
no cell death

Máxima produtividade (próxima do washout) obtém-se para:

Maximum productivity is attained for:

$$D_{\max} = \mu_{\max} \left[1 - \left(\frac{K_s}{K_s + S_0} \right)^{1/2} \right]$$

$$D_{\max} = 0.5 \text{ h}^{-1}$$

$$D = F/V \rightarrow V = 40 \text{ L}$$

3.2-b)
$$S = \frac{K_s D}{\mu_{max} - D}$$

$S = 8.75 \text{ g/L}$

3.2-c)

Balanço ao produto:

Balance to the product:

$$\frac{dP}{dt} = \frac{F}{V} (P_0 - P) + \mu X Y_{p/x}$$

\downarrow \downarrow

$$= 0 \qquad \qquad = 0$$

Estado estacionário

steady-state

3.2-c) Balanço ao produto: Balance to the product:

$$\frac{dP}{dt} = \frac{F}{V} (P_0 - P) + \mu X Y_{p/x}$$

$$0 = -\frac{F}{V} P + \mu X Y_{p/x}$$

3.2-c)

Balanço ao produto:

Balance to the product:

$$\frac{dP}{dt} = \frac{F}{V} (P_0 - P) + \mu X Y_{p/x}$$

$$0 = -\frac{F}{V} P + \mu X Y_{p/x}$$

\downarrow \downarrow

$= D$ $= D$

3.2-c) Balanço ao produto: Balance to the product:

$$\frac{dP}{dt} = \frac{F}{V} (P_0 - P) + \mu X Y_{p/x}$$

$$0 = -\frac{F}{V} P + \mu X Y_{p/x}$$

$$0 = -D P + D X Y_{p/x}$$

3.2-c) Balanço ao produto: Balance to the product:

$$\frac{dP}{dt} = \frac{F}{V} (P_0 - P) + \mu X Y_{p/x}$$

$$0 = -\frac{F}{V} P + \mu X Y_{p/x}$$

$$0 = -\cancel{D} P + \cancel{D} X Y_{p/x}$$

$$0 = -P + X Y_{p/x}$$

3.2-c)

Balanço ao produto:

Balance to the product:

$$\frac{dP}{dt} = \frac{F}{V} (P_0 - P) + \mu X Y_{p/x}$$

$$0 = -\frac{F}{V} P + \mu X Y_{p/x}$$

$$0 = -D P + D X Y_{p/x}$$

$$0 = -P + X Y_{p/x}$$

$$P = \textcolor{red}{X} Y_{p/x}$$

**Calcular X**

3.2-c)

$$X = Y_{x/s} (S_0 - S) = 28,125 \text{ g/L}$$

$$P = X Y_{p/x} = 16,875 \text{ g/L}$$

3.2-d) Máxima produtividade (próxima do washout) obtém-se para:
 Maximum productivity (near to washout) is attained for:

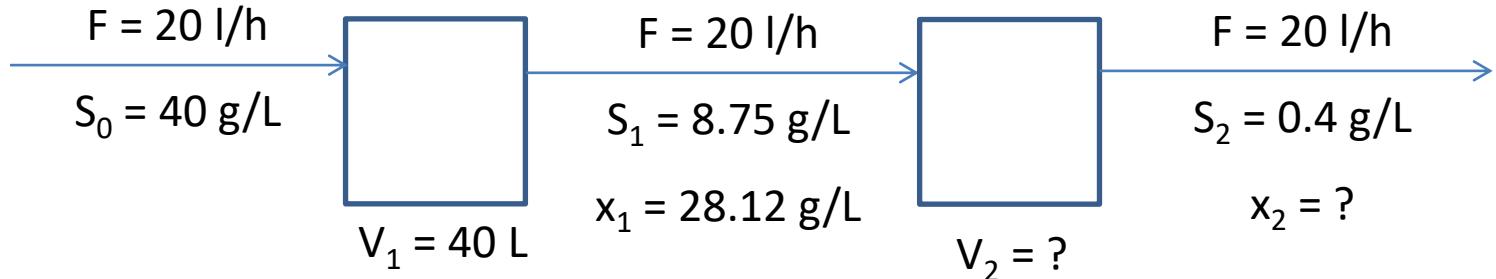
$$D_{\max} = \mu_{\max} \left[1 - \left(\frac{K_s}{K_s + S_0} \right)^{1/2} \right]$$

$$X = Y_{x/S} (S_0 - S)$$

$$S = \frac{K_s D}{\mu_{\max} - D}$$

S_0 (g/L)	D_{\max} (h^{-1})	S (g/L)	X (g/L)	$D \times (g/\text{l.h})$
40	0.5	8,75	28,12	14
80	0.56	14	59,4	33
160	0.6	21	125	75
10	0.34	3,3	6	2

3.2-e)



Balanço ao substrato para 2º reactor:

Balance to the substrate in the 2nd reactor:

$D_2 \neq \mu_2$
Because there are cells
entering the reactor (in
the inlet stream)

$$D_2 (S_1 - S_2) = (\mu_2 x_2) / Y_{x/S}$$

$$\mu_2 = (\mu_{\max} S_2) / (K_s + S_2) = 0.072 \text{ h}^{-1}$$

$$Y_{x/S} = (x_2 - x_1) / (S_1 - S_2) \Leftrightarrow x_2 = 35.64 \text{ g/L}$$

$$D_2 = 0.34 \text{ h}^{-1} \Rightarrow V_2 = 58.8 \text{ L}$$

3.2-f) $D_x \text{ (global)} = x_2 \cdot F / (V_1 + V_2) = 7.21 \text{ g/L.h}$

Problema 3.3

Consider a 100 L CSTR where a culture that obeys the S→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.

3.3

$$V_{\text{reactor}} = 100 \text{ L}$$

$$Y'_{X/S} = 0.36 \text{ g X / g S}$$

$$F = 5 \text{ L/h}$$

$$K_S = 1.3 \text{ g/L}$$

$$S_0 = 20 \text{ g/L}$$

$$\mu_{\text{max}} = 0.37 \text{ h}^{-1}$$

$$m_S = 0.0135 \text{ g S/(gX.h)}$$

3.3-a)

$$D = F / V = 0,05 \text{ h}^{-1}$$

$$S = K_s D / (\mu_{\text{max}} - D) = 0,21 \text{ g/L}$$

$$X = D (S_0 - S) / (D/Y'_{X/S} + m_S) = 6,5 \text{ g/L}$$

3.3-b)

$$D_c = \frac{\mu_{\max} S_0}{K_s + S_0}$$

$$D_c = 0.35 \text{ h}^{-1}$$

Redução de 50%

$$D = 0.175 \text{ h}^{-1}$$

$$S = K_s D / (\mu_{\max} - D) = 1.14 \text{ g/L}$$

$$X = D (S_0 - S) / (D/Y'_{X/S} + m_S) = 6.6 \text{ g/L}$$

Redução de 99%

$$D = 0.0035 \text{ h}^{-1}$$

$$S = K_s D / (\mu_{\max} - D) = 0.012 \text{ g/L}$$

$$X = D (S_0 - S) / (D/Y'_{X/S} + m_S) = 3.04 \text{ g/L}$$

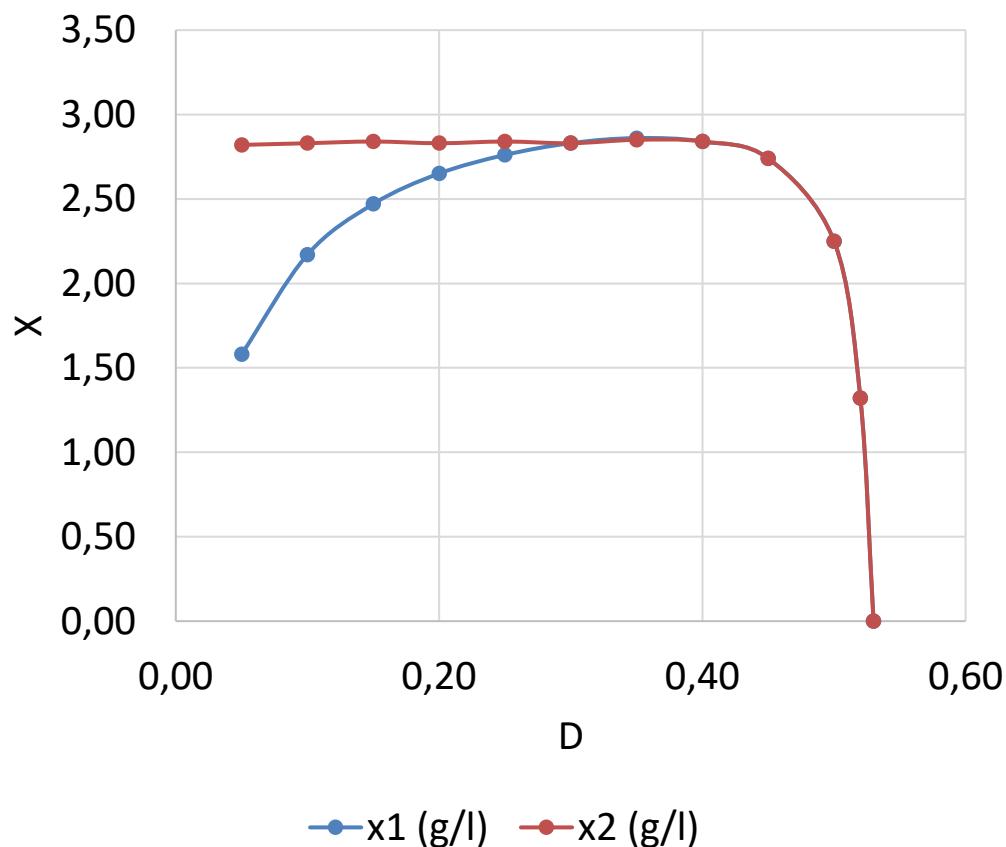
Problem 3.4

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.

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

3.4-a)

$D (h^{-1})$	$x_1 (g/l)$	$x_2 (g/l)$
0,05	1,58	2,82
0,10	2,17	2,83
0,15	2,47	2,84
0,20	2,65	2,83
0,25	2,76	2,84
0,30	2,83	2,83
0,35	2,86	2,85
0,40	2,84	2,84
0,45	2,74	2,74
0,50	2,25	2,25
0,52	1,32	1,32
0,53	0,00	0,00



3.4-a)

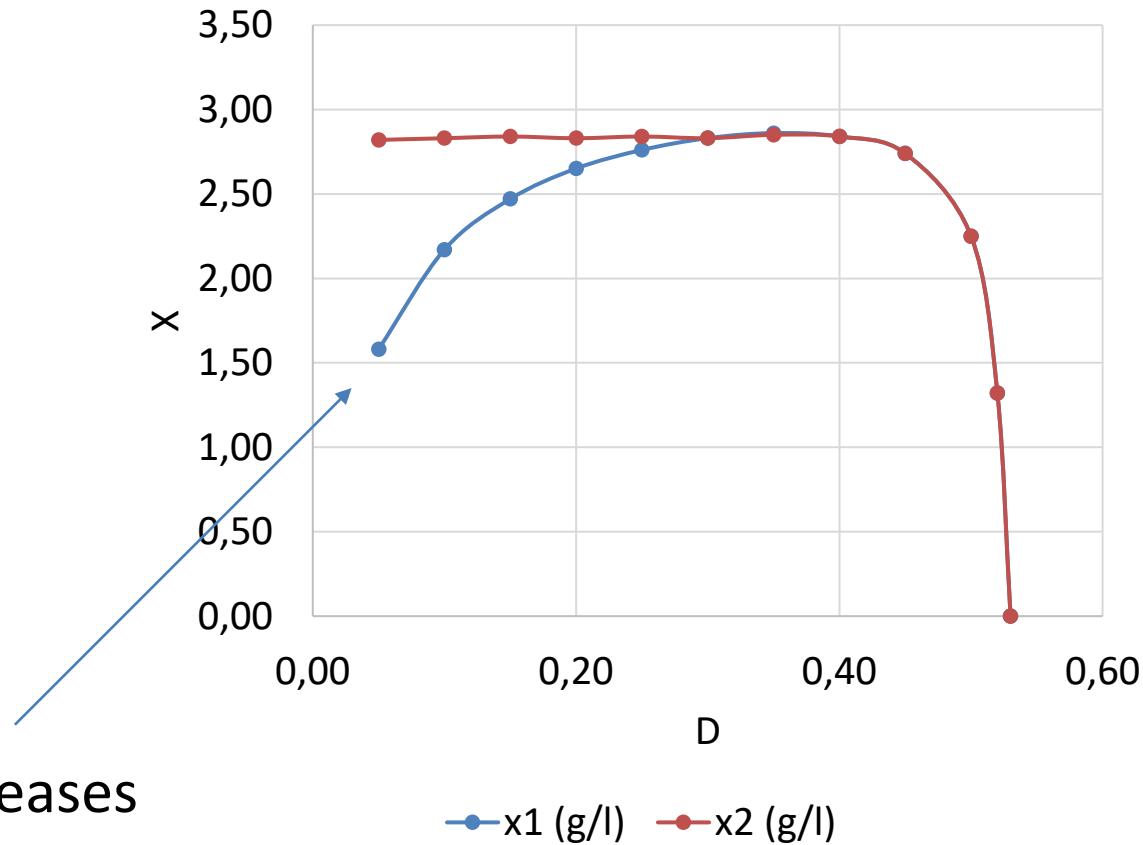
$$X = \frac{D(S_0 - S)}{\frac{D}{Y'_{x/s}} + m_s}$$

$$X = \frac{S_0 - S}{\frac{1}{Y'_{x/s}} + \frac{m_s}{D}}$$

$$D \ll$$

$$\frac{m_s}{D} \gg$$

X decreases



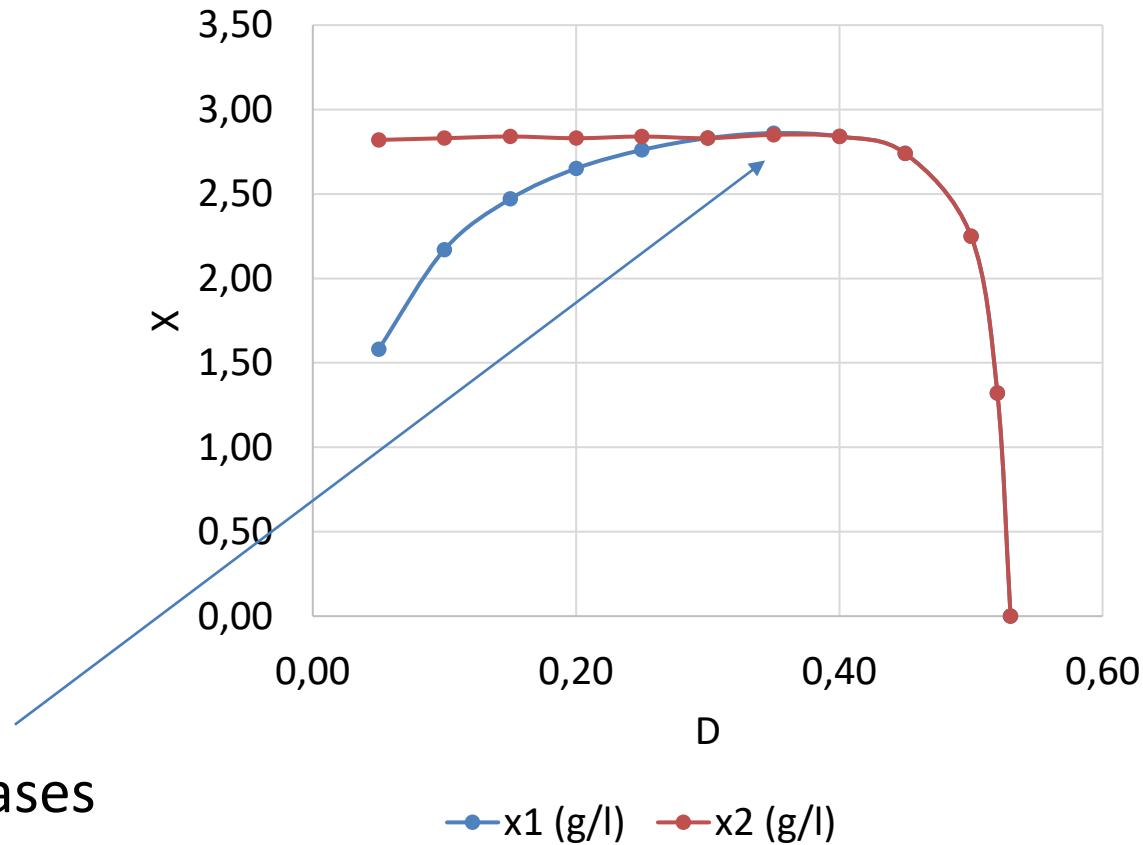
3.4-a)

$$X = \frac{D(S_0 - S)}{\frac{D}{Y'_{x/s}} + m_s}$$

$$X = \frac{S_0 - S}{\frac{1}{Y'_{x/s}} + \frac{m_s}{D}}$$

 $D \gg$ $\frac{m_s}{D} \ll$

X increases

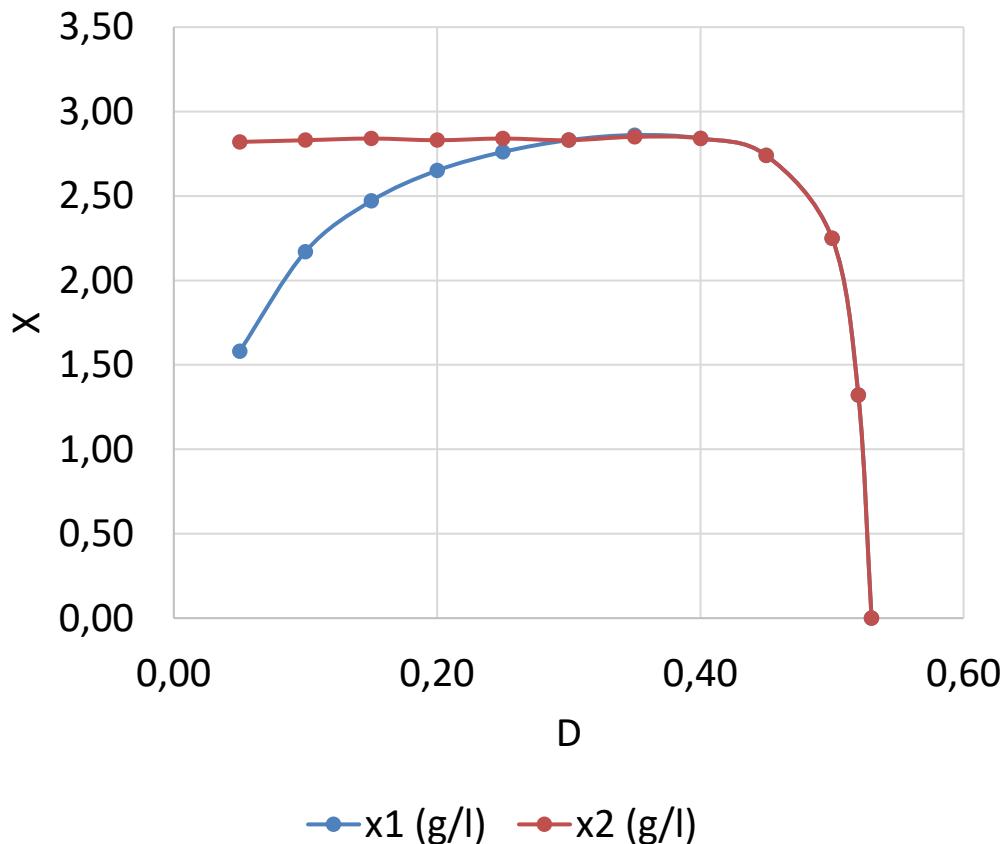


b) At which dilution rate would a CSTR operate when product formation is type I (associated with growth)? justify your answer (approximate values)

10% below D_c

$D (h^{-1})$	$x_1 (g/l)$	$x_2 (g/l)$
0,05	1,58	2,82
0,10	2,17	2,83
0,15	2,47	2,84
0,20	2,65	2,83
0,25	2,76	2,84
0,30	2,83	2,83
0,35	2,86	2,85
0,40	2,84	2,84
0,45	2,74	2,74
0,50	2,25	2,25
0,52	1,32	1,32
0,53	0,00	0,00

D_c



b) At which dilution rate would a CSTR operate when product formation is type I (associated with growth)? justify your answer (approximate values)

10% below D_c

$$D = 0,477 \text{ h}^{-1}$$

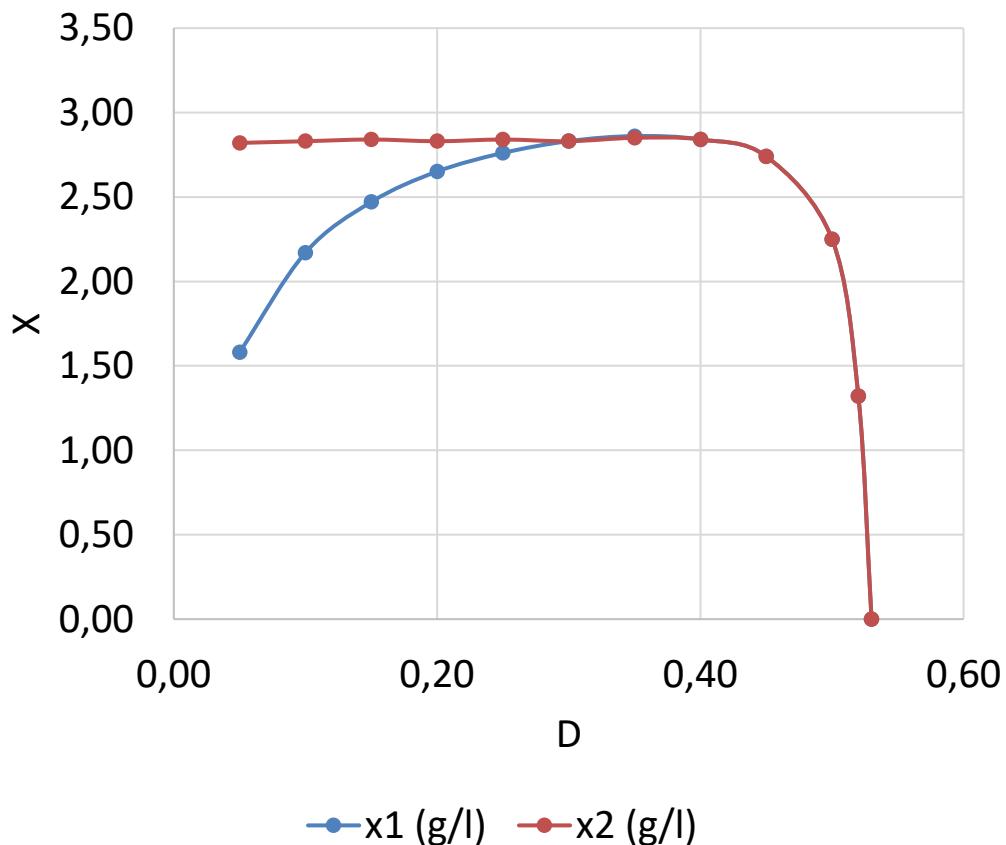
c) At which dilution rate would a CSTR operate when the product is type III (product formation dissociated from growth)? justify your answer (approximate values)

Production is independent from the cell growth rate

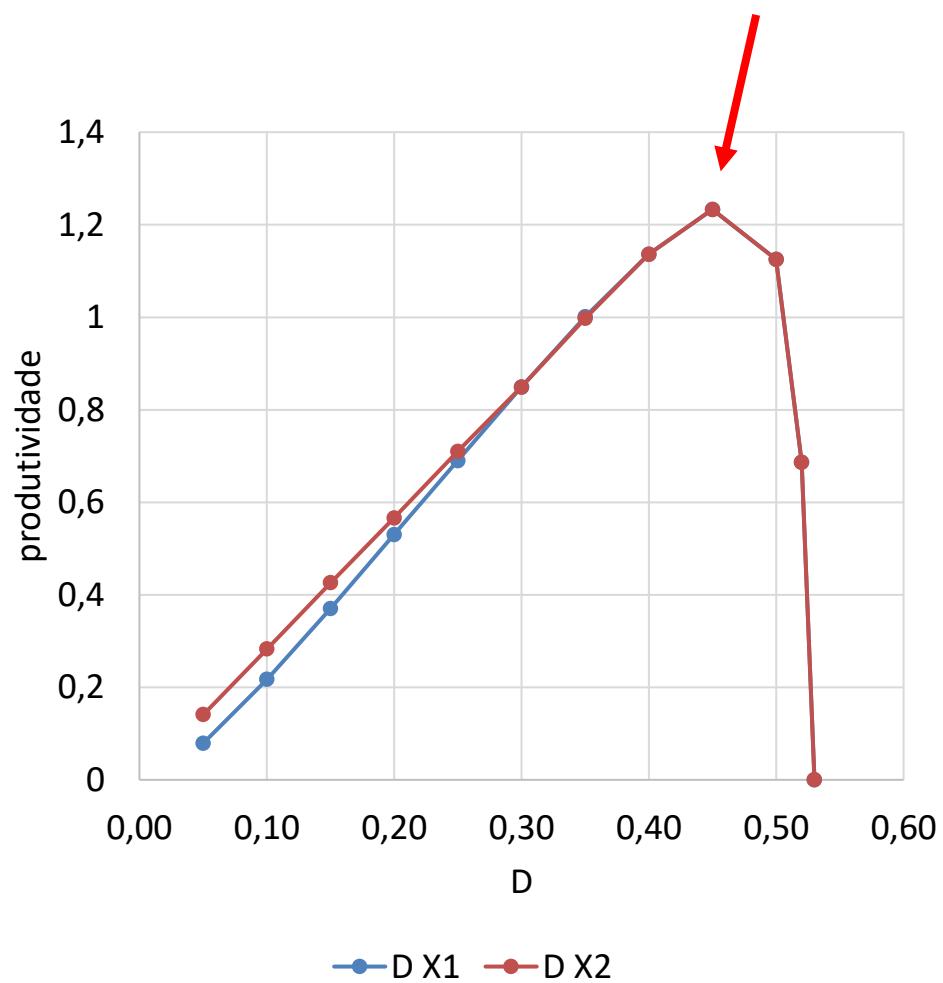
It depends on X

Garantir X máximo

D (h^{-1})	x1 (g/l)	x2 (g/l)
0,05	1,58	2,82
0,10	2,17	2,83
0,15	2,47	2,84
0,20	2,65	2,83
0,25	2,76	2,84
0,30	2,83	2,83
0,35	2,86	2,85
0,40	2,84	2,84
0,45	2,74	2,74
0,50	2,25	2,25
0,52	1,32	1,32
0,53	0,00	0,00



D (h^{-1})	x1 (g/l)	x2 (g/l)
0,05	1,58	2,82
0,10	2,17	2,83
0,15	2,47	2,84
0,20	2,65	2,83
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0,35	2,86	2,85
0,40	2,84	2,84
0,45	2,74	2,74
0,50	2,25	2,25
0,52	1,32	1,32
0,53	0,00	0,00



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 $D=0.1\text{ h}^{-1}$.

$$X_0 = 0 \quad (\text{meio estéril})$$

$$X_f = 0,45 \text{ g/l}$$

$$D = 0,1 \text{ h}^{-1}$$

$$S_0 = 20 \text{ mM}$$

$$S_f = 2 \text{ mM}$$

$$P_0 = 0$$

3.5-a) $P = ?$

Balanço ao produto $P = X Y_{p/x}$

Escrever a equação da reação



$$Y_{p/x} = \frac{c \text{ mol } P}{1 \text{ mol } X} \quad \text{Calcular } c$$

Balanço energético

$$\gamma_S = +24$$

$$a \times \gamma_S = \gamma_X + c \times \gamma_P$$

$$\gamma_P = +12$$

$$24 \textcolor{red}{a} = 4,53 + 12 c$$

$$\gamma_X = +4,53$$

Calcular a

$$\text{(teórico)} \quad Y_{x/s} = \frac{1 \text{ mol } X}{a \text{ mol } S} = \frac{23,86 \text{ g}_X}{a \times 10^3 \text{ mmol}_S} \quad M(\text{biomassa}) = 23,86 \text{ g/mol}$$

$$\text{(exper.)} \quad Y_{x/s} = \frac{\Delta X}{\Delta S} = \frac{X - X_0}{S_0 - S} = 0,025 \text{ g/mmol}_S$$

Balanço energético

$$\gamma_S = +24$$

$$a \times \gamma_S = \gamma_X + c \times \gamma_P$$

$$\gamma_P = +12$$

$$24 \textcolor{red}{a} = 4,53 + 12 c$$

$$\gamma_X = +4,53$$

Calcular a

$$\text{(teórico)} \quad Y_{x/s} = \frac{1 \text{ mol } X}{a \text{ mol } S} = \frac{23,86 \text{ g}_X}{a \times 10^3 \text{ mmol}_S}$$

$$\frac{23,86}{a \times 10^3} = 0,025$$

$$\textcolor{red}{a} = 0,954$$

$$\text{(exper.)} \quad Y_{x/s} = \frac{\Delta X}{\Delta S} = \frac{X - X_0}{S_0 - S} = 0,025 \text{ g/mmol}_S$$

Balanço energético

$$\gamma_S = +24$$

$$a \times \gamma_S = \gamma_X + c \times \gamma_P$$

$$\gamma_P = +12$$

$$24 a = 4,53 + 12 c$$

$$\gamma_X = +4,53$$

$$24 \times 0,954 = 4,53 + 12 c$$

$$c = 1,530$$

$$Y_{p/x} = \frac{c \text{ mol } P}{1 \text{ mol } X} = 1,53 \text{ mol}_P/\text{mol}_X = 0,064 \text{ mol}_P/\text{g}_X$$

$$P = X Y_{p/x} = 0,02886 \text{ mol}_P/l = 28,9 \text{ mM}$$

b) A taxa de diluição escolhida provavelmente não optimiza a produtividade de biomassa. Explique brevemente a afirmação anterior e especule sobre a razão de se ter escolhido $D=0.1\text{ h}^{-1}$.

Etanol: inibidor

D_{\max} maximizes biomass production, but leads to high ethanol production, which inhibits cell growth

Keep D below D_{\max} to avoid inhibiting ethanol concentrations

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?

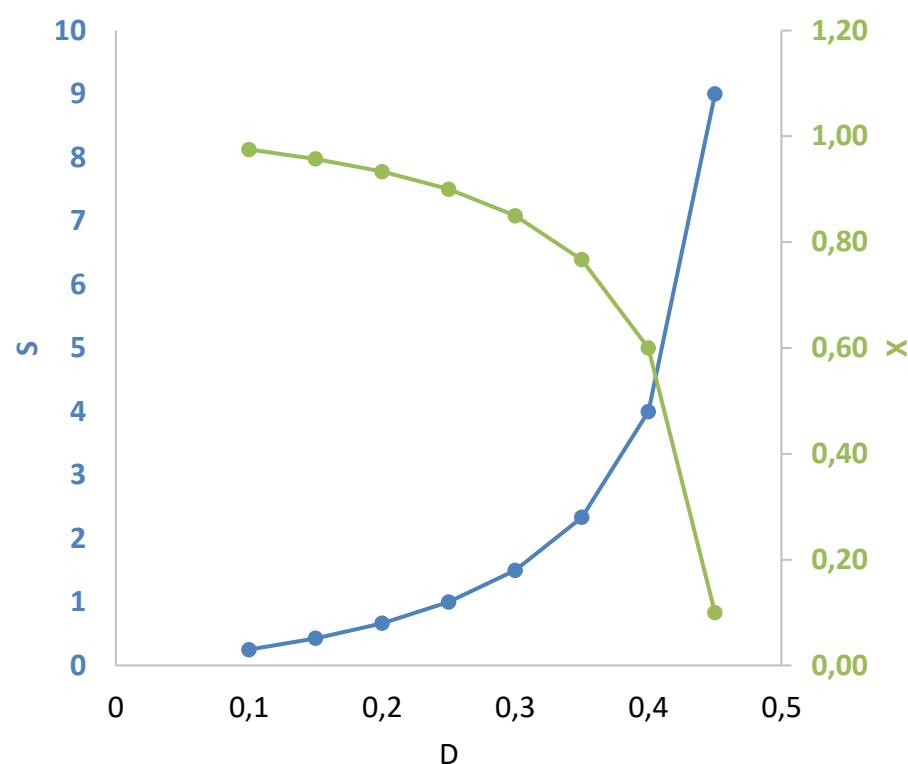
a) Determine the cell and substrate concentration in the CSTR reactor as a function of D when the inhibitor concentration is equal to zero.

$$D = \frac{\mu_m S}{K_s + S + I \frac{K_s}{K_I}}$$

$$S = \frac{K_s D}{\mu_m - D} = \frac{D}{0,5 - D}$$

$$X = Y_{x/s} (S_0 - S)$$

$$= 0,1 \left(10 - \frac{D}{0,5 - D} \right)$$



b) If inhibitor is added to CSTR, determine substrate and cell concentration as a function of D

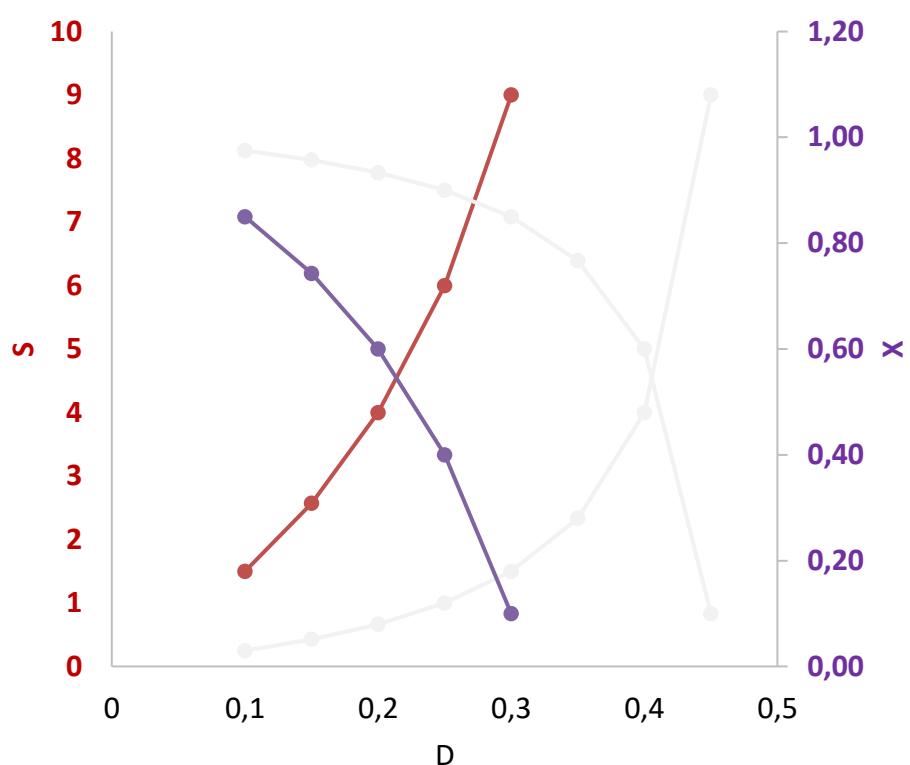
$$D = \frac{\mu_m S}{K_s + S + I \frac{K_s}{K_I}}$$

$$S = \frac{K_s D}{\mu_m - D} \left(1 + \frac{I}{K_I} \right)$$

$$= \frac{6 D}{0,5 - D}$$

$$X = Y_{x/s} (S_0 - S)$$

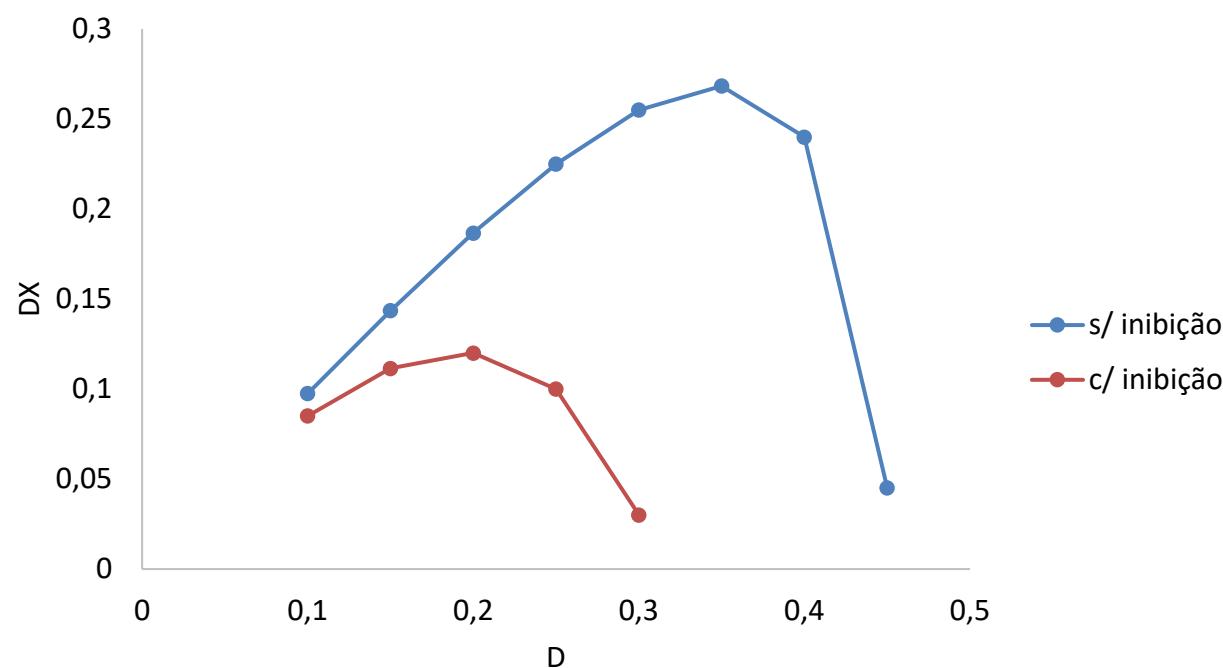
$$= 0,1 \left(10 - \frac{6 D}{0,5 - D} \right)$$



c) Determine the cell productivity, DX , as a function of the dilution rate for situation a) and b).

$$DX(a) = D \times 0,1 \times \left(10 - \frac{D}{0,5 - D} \right) = D - \frac{0,1 D^2}{0,5 - D}$$

$$DX(b) = D \times 0,1 \times \left(10 - \frac{6 D}{0,5 - D} \right) = D - \frac{0,6 D^2}{0,5 - D}$$



d) If you double the concentration of inhibitor added to the reactor how is the cell and substrate concentration affected?

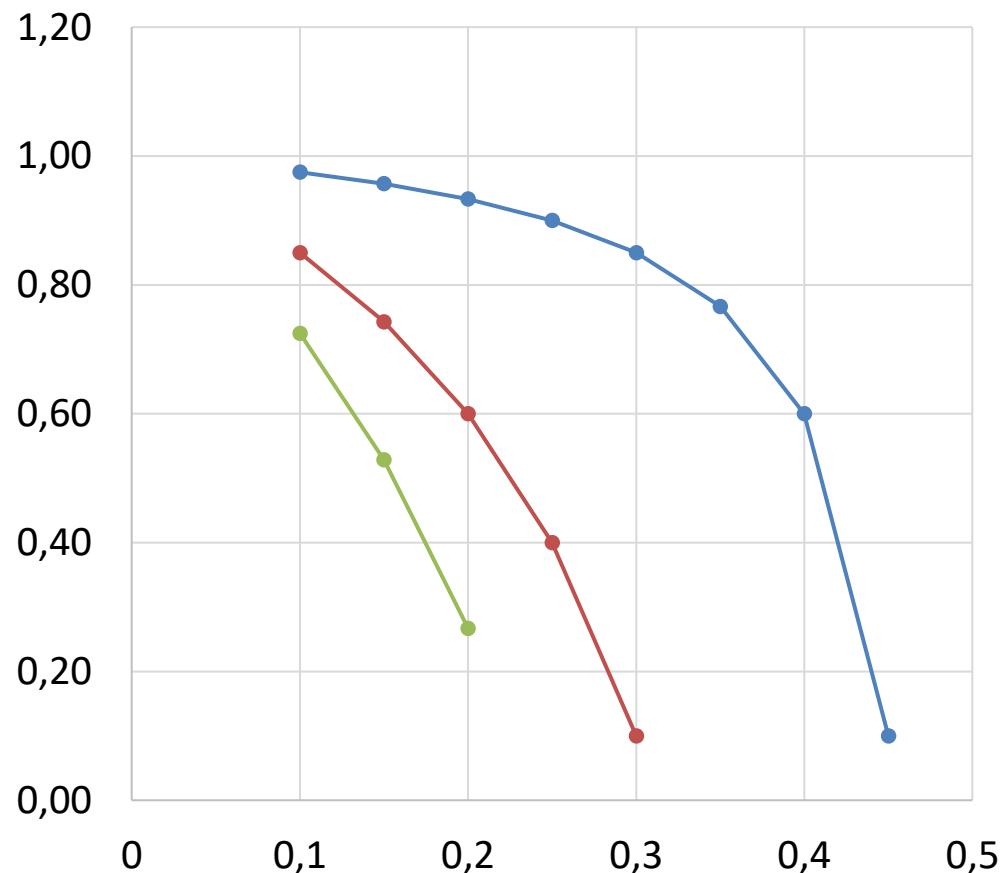
$$D = \frac{\mu_m S}{K_s + S + 2I \frac{K_s}{K_I}}$$

$$S = \frac{K_s D}{\mu_m - D} \left(1 + \frac{2 I}{K_I} \right)$$

$$= \frac{11 D}{0,5 - D}$$

$$X = Y_{x/s} (S_0 - S)$$

$$= 0,1 \left(10 - \frac{11 D}{0,5 - D} \right)$$



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 m^3 and the feed rate of the medium is 50 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 g/l . The kinetic and stoichiometric parameters for the microorganism are as follows:

$$Y'_{x/s} = 0.5 \text{ gcelulas/ gsubstrato}$$

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

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

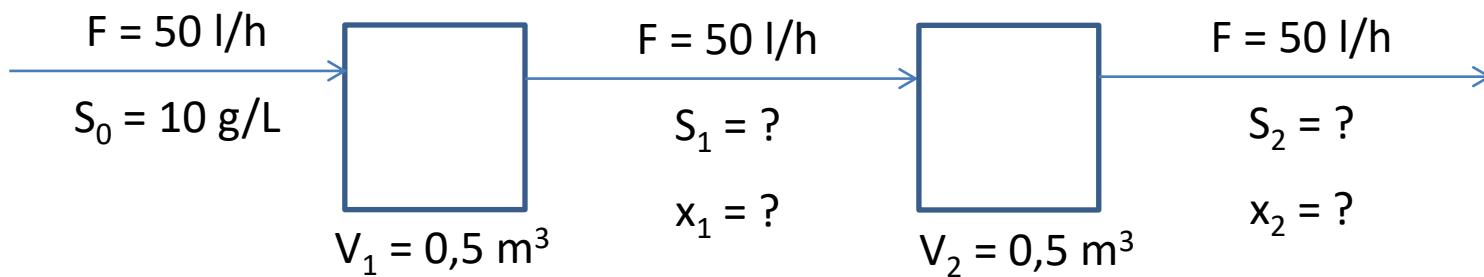
$$m_s = 0.025 \text{ gsub/gcel.h}$$

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

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

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.



a) The concentration of cells and substrate entering the second reactor.

$$S_1 = \frac{K_s D}{\mu_{max} - D} = 5 \text{ g/l}$$

$$D = 0,1 \text{ h}^{-1}$$

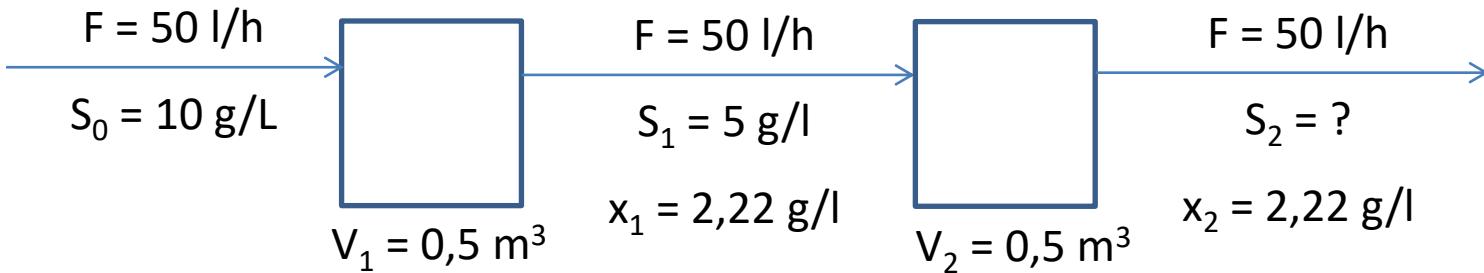
$$X_1 = \frac{D (S_0 - S)}{\frac{D}{Y'_{x/s}} + m_s} = 2,22 \text{ g/l}$$

b) Global Substrate Conversion.

$$S_2 = ?$$

$$X_1 \approx X_2$$

Balanço ao substrato no 2º reator



$$\frac{dS}{dt} = \frac{F}{V} S_1 - \frac{F}{V} S_2 - \frac{1}{Y'_{x/s}} \mu X_2 - \frac{1}{Y_{p/s}} V_p X_2 - m_s X_2 = 0$$

$$= D \quad = D \quad = 0$$

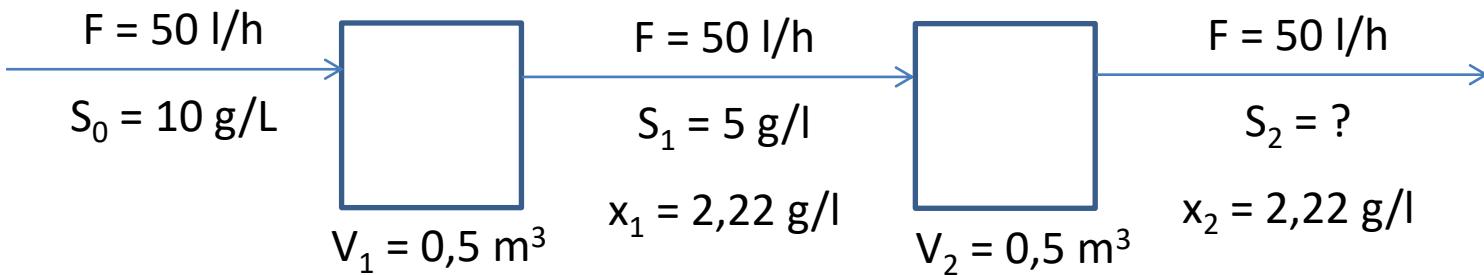
Steady-state

b) Global Substrate Conversion.

$$S_2 = ?$$

$$X_1 \approx X_2$$

Balanço ao substrato no 2º reator



$$\frac{dS}{dt} = \frac{F}{V} S_1 - \frac{F}{V} S_2 - \frac{1}{Y'_{x/s}} \mu X_2 - \frac{1}{Y_{p/s}} V_p X_2 - m_s X_2 = 0$$

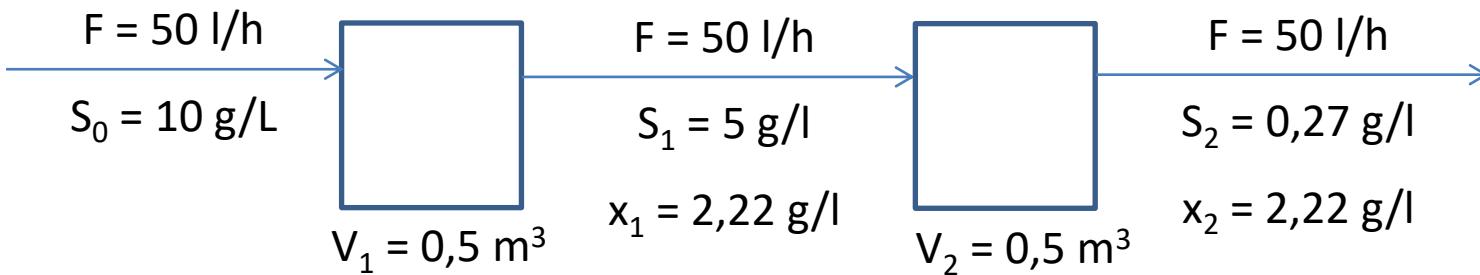
$$S_2 = 0,27 \text{ g/l}$$

$$\text{conversão} = 97,3\%$$

c) The final concentration of the product.

$$P_2 = ?$$

Balanço ao produto no 2º reator



$$\frac{dP}{dt} = - \frac{F}{V} P + V_p X = 0$$

$$P = 3,55 \text{ g/l}$$