Aulas Teórico-Práticas / Problem Solving Classes

Professor: Filomena Freitas

1 aula semanal (3 horas) / 1 weekly class (3 hours)

Turnos:	3ª feira (Tuesday)	08:00-11:00	(Ed. VII, 3.4)
	3ª feira (Tuesday)	13:30-16:30	(Ed. IV, 110)
	4ª feira (Wednesday)	08:00-11:00	(ED. 2.2)

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Resolução de Problemas / Problem solving

- Os enunciados são disponibilizados no CLIP / Problem sheets available at CLIP (*Documentação de apoio - Problemas*)
- Os problemas são resolvidos na aula, pelos alunos em conjunto com a docente / Problems are solved in class by the students and the Professor
- As soluções dos problemas são disponibilizadas no CLIP / Problems' solutions are available at CLIP

Coeficientes de rendimento / Yield coefficients

$C_6H_{12}O_6$	+	$6\ O_2$	$\rightarrow$	$6\ CO_2$	+	$6\ H_2O$
1 mol		*6 mol*		*6 mol*		*6 mol*

Coeficiente de rendimento da glucose em função do oxigénio

Yield coeficient for glucose on an oxygen basis

$$Y_{glc/O_2} = \frac{1\ mol\ glucose}{6\ mol\ O_2} = 0{,}167\ mol\_glc/mol\_O_2$$

Coeficientes de rendimento / Yield coefficients

$C_6H_{12}O_6$	+	$6\ O_2$	$\rightarrow$	$6\ CO_2$	+	$6\ H_2O$
1 mol		*6 mol*		*6 mol*		*6 mol*

Coeficiente de rendimento da água em função do oxigénio

Yield coeficient for water on an oxygen basis

$$Y_{H_2O/O_2} = \frac{6\ mol\ H_2O}{6\ mol\ O_2} = 1{,}0\ mol\_H_2O/mol\_O_2$$

Coeficientes de rendimento / Yield coefficients

$$C_6H_{12}O_6 \quad + \quad 6\ O_2 \quad \rightarrow \quad 6\ CO_2 \quad + \quad 6\ H_2O$$

Conversão de unidades

Units conversion

M (H_2O) = 18 g/mol

$$Y_{H_2O/O_2} = \frac{6\ mol\ H_2O}{6\ mol\ O_2} = \frac{6 \times 18\ g\ H_2O}{6\ mol\ O_2} = \frac{108\ g\ H_2O}{6\ mol\ O_2} = 18\ g\_H_2O/mol\_O_2$$

Coeficientes de rendimento / Yield coefficients

$$C_6H_{12}O_6 \quad + \quad 6\ O_2 \quad \rightarrow \quad 6\ CO_2 \quad + \quad 6\ H_2O$$

Conversão de unidades

Units conversion

M (H_2O) = 18 g/mol

$$Y_{H_2O/O_2} = \frac{6\ mol\ H_2O}{6\ mol\ O_2} = \frac{6\times 18\ g\ H_2O}{6\times 32\ g\ O_2} = \frac{108\ g\ H_2O}{192\ g\ O_2} = 0{,}56\ g\_H_2O/g\_O_2$$

M (O_2) = 32 g/mol

Coeficientes de rendimento / Yield coefficients

Units:

mol / mol

g / mol

mol / g

g / g

Problem 1.1

Consider the culture of a bacterium with the following empirical formula:

$$CH_{1.7}O_{0.46}N_{0.18} \ (MW_B = 23.6 \text{ g/mole})$$

This bacterium grows aerobically in a culture medium using glucose as a carbon source ($C_6H_{12}O_6$).

Glucose and oxygen yield coefficients were experimentally determined:

$$Y_{X/S} = 85 \text{ g biomass/mole glucose}$$

$$Y_{X/O2} = 39 \text{ g biomass/mole } O_2$$

This organism does not excrete appreciable amounts of metabolites under growing conditions.

a) Show that the measured values of $Y_{X/S}$ and $Y_{X/O2}$ are consistent.

b) A batch culture of this organism initially contains 0.01 g of biomass and 20 mmol of glucose. After a few hours of cultivation, the cells stopped growing. The total biomass in the culture is 1.0 g. Estimate the final amount of glucose in the culture medium (in mmol) and speculate on the likely cause of cell growth arrest.

Problema 1.1-a)

⇒ escrever a equação da reação: Write the equation of the reaction

$$C_6H_{12}O_6 + O_2 + NH_3 \rightarrow CH_{1.7}O_{0.46}N_{0.18} + \underbrace{CO_2 + H_2O}_{\text{Respiration products}}$$

$C_6H_{12}O_6$: Carbon source (glucose)

O_2: Aerobic

NH_3: Nitrogen source (ammonia)

$CH_{1.7}O_{0.46}N_{0.18}$: Biomass/ cells

Problema 1.1-a)

⇒ equação da reação: equation of the reaction

$$a\,C_6H_{12}O_6 + b\,O_2 + c\,NH_3 \rightarrow CH_{1.7}O_{0.46}N_{0.18} + d\,CO_2 + e\,H_2O$$

⇒ Mostrar que os valores medidos de $Y_{X/S}$ e $Y_{X/O2}$ são consistentes:

Show that the measured values of $Y_{X/S}$ and $Y_{X/O2}$ are consistent

A partir do valor de Yx/s experimental (enunciado) vamos calcular Yx/o2 → verificar se dá o valor indicado no enunciado para Yx/o2

From the experimental Yx/s value (problem sheet) we will calculate Yx/o2 → check if it gives the value indicated for Yx/o2

Problema 1.1-a)

⇒ equação da reação: equation of the reaction

$$a\,C_6H_{12}O_6 \quad + \quad b\,O_2 \quad + \quad c\,NH_3 \rightarrow CH_{1.7}O_{0.46}N_{0.18} \quad + \quad d\,CO_2 \quad + \quad e\,H_2O$$

⇒ Mostrar que os valores medidos de $Y_{X/S}$ e $Y_{X/O2}$ são consistentes:

Show that the measured values of $Y_{X/S}$ and $Y_{X/O2}$ are consistent

X – biomassa (células, microrganismo, bactéria, levedura,...)

X – biomass (cells, microorganism, bactéria, yeast,…)

S – substrato, fonte de carbono (ex. glucose, glicerol,...)

S – substrate, carbon source (e.g., glucose, glycerol)

Problema 1.1-a)

$\Rightarrow$ equação da reação: equation of the reaction

$$a\,C_6H_{12}O_6 + b\,O_2 + c\,NH_3 \rightarrow CH_{1.7}O_{0.46}N_{0.18} + d\,CO_2 + e\,H_2O$$

$\Rightarrow$ coeficientes de rendimento: yield coefficients

$$Y_{x/s} = \underbrace{85\ g\_X/mol\_S}_{\text{(experimental)}}$$

Pela estequiometria da reação, temos:

By reaction stoichiometry, we have:

$$Y_{x/s} = \frac{1\ mol\ X}{a\ mol\ S} = \underbrace{\frac{23{,}6\ g\_X}{a\ mol\_S}}_{\text{(teórico)}}$$

Problema 1.1-a)

$\Rightarrow$ equação da reação: equation of the reaction

$$a\,C_6H_{12}O_6 + b\,O_2 + c\,NH_3 \rightarrow CH_{1.7}O_{0.46}N_{0.18} + d\,CO_2 + e\,H_2O$$

$\Rightarrow$ coeficientes de rendimento: yield coefficients

$$Y_{x/s} = 85\ g\_X/mol\_S$$

Pela estequiometria da reação, temos:

By reaction stoichiometry, we have:

$$Y_{x/s} = \frac{1\ mol\ X}{a\ mol\ S} = \frac{23{,}6\ g\_X}{a\ mol\_S}$$

$$85\ g\_X/mol\_S = \frac{23{,}6\ g\_X}{a\ mol\_S} \Leftrightarrow a = 0{,}278$$

Problema 1.1-a)

⇒ equação da reação: equation of the reaction

$$a\,C_6H_{12}O_6 + b\,O_2 + c\,NH_3 \rightarrow CH_{1.7}O_{0.46}N_{0.18} + d\,CO_2 + e\,H_2O$$

⇒ coeficientes de rendimento:

$$Y_{x/o2} = ?$$

Pela estequiometria da reação, temos:

$$Y_{x/o2} = \frac{1\ mol\ X}{b\ mol\ O2} = \frac{23{,}6\ g\_X}{b\ mol\_O2}$$

Calcular b

Problema 1.1-a)

$\Rightarrow$ podemos usar um de dois métodos: we can use one of two methods

- ❖ Balanço aos elementos Balance to the elements
- ❖ Balanço energético Energy balance

❖ Balanço aos elementos Balance to the elements

$$a\ C_6H_{12}O_6 + b\ O_2 + c\ NH_3 \rightarrow CH_{1.7}O_{0.46}N_{0.18} + d\ CO_2 + e\ H_2O$$

C	$6a = 1 + d$
H	$12a + 3c = 1.7 + 2e$
O	$6a + 2b = 0.46 + 2d + e$
N	$c = 0.18$

$a = 0{,}278$

❖ Balanço aos elementos Balance to the elements

voltando aos balanços

$6a = 1 + d \leftrightarrow 6 \times 0.28 = 1 + d \leftrightarrow$ **$d = 0.666$**

$12a + 3c = 1.7 + 2e \leftrightarrow 12 \times 0.28 + 3 \times 0.18 = 1.7 + 2e \leftrightarrow$ **$e = 1.088$**

$6a + 2b = 0.46 + 2d + e \leftrightarrow 6 \times 0.28 + 2b = 0.46 + 2 \times 0.68 + 1.1 \leftrightarrow$ **$b = 0.601$**

$$Y_{x/o2} = \frac{23{,}6\ g\_X}{b\ mol\_O2} = \frac{23{,}6}{0{,}601} = 39{,}27\ g\_X/mol\_O2$$

(teórico)

$$Y_{x/o2} = 39\ g\_X/mol\_O2$$

(experimental)

Os valores são semelhantes → os valores de Yx/s e Yx/o2 são consistentes

The values are similar → Yx/s and Yx/o2 are consistent

❖ Balanço energético Energy balance

crescimento ~ reação redox
growth ~ redox reaction

Para cada elemento / for each element

$\gamma_C = +4$

$\gamma_H = +1$

$\gamma_O = -2$

$\gamma_N = -3$

Para cada composto / for each molecule

$\gamma_{glucose} = 6\times(+4) + 12\times(+1) + 6\times(-2) = +24$

$\gamma_{O2} = 2\times(-2) = -4$

$\gamma_{NH3} = 1\times(-3) + 3\times(+1) = 0$

$\gamma_{biomassa} = 1\times(+4) + 1.7\times(+1) + 0.46\times(-2) + 0.18\times(-3) = +4.24$

$\gamma_{CO2} = 1\times(+4) + 2\times(-2) = 0$

$\gamma_{H2O} = 2\times(+1) + 1\times(-2) = 0$

$$a \times \gamma_{glucose} + b \times \gamma_{O2} + c \times \gamma_{NH3} = \gamma_{biomassa} + d \times \gamma_{CO2} + e \times \gamma_{H20}$$

$$24a - 4b + 0 = 4.24 + 0 + 0 \leftrightarrow \mathbf{24a - 4b = 4.24}$$

$$a = 0{,}278$$

$$24a - 4b = 4.24 \leftrightarrow 24 \times 0.278 - 4b = 4.24 \leftrightarrow \mathbf{b = 0.601}$$

❖ Balanço energético Energy balance

$$Y_{x/o2} = \frac{23{,}6\ g\_X}{b\ mol\_O2} = \frac{23{,}6}{0{,}601} = 39{,}27\ g_X/mol\_O2$$

(teórico)

$$Y_{x/o2} = 39\ g_X/mol\_O2$$

(experimental)

Os valores são semelhantes → os valores de Yx/s e Yx/o2 são consistentes

The values are similar → Yx/s and Yx/o2 are consistent

Problema 1.1-b)

initial:

0.01 g biomass

20 mmol glucose

→

final:

1 g biomass

?? glucose

$$Y_{X/S} = \frac{\Delta X}{\Delta S} = \frac{X_{final} - X_{inicial}}{S_{inicial} - S_{final}} = \frac{1 - 0.01}{0.02 - S_{final}}$$

$$Y_{X/S} = 85g\_biomassa/mol\_O2$$

coeficiente rendimento experimental

$$= \frac{1 - 0.01}{0.02 - S_{final}} = 85 \quad \Leftrightarrow S_{final} = 0.0084 mol = 8.4 mmol\_glucose$$

=> ainda havia glucose no final => crescimento parou por falta de outro nutriente:

NH_3, O_2, micronutrientes

=> there was still glucose left at the end => growth stopped due to lack of another nutrient: NH3, O2, micronutrients

Problem 1.2

Consider an anaerobic fermentation by a yeast whose empirical biomass formula is as follows:

$$CH_{1.7}O_{0.45}N_{0.15} \text{ } (MW_B = 23.0 \text{ g DCW/mole})$$

Carbon and nitrogen sources are glucose ($C_6H_{12}O_6$) and ammonium salts, respectively. Possible products of the growth reaction are biomass, ethanol (C_2H_6O), carbon dioxide and water. Ethanol growth and formation depend on growing conditions.

a) What is the maximum biomass yield coefficient ($Y_{x/s}$, g DCW/mole glucose), and under what conditions is it obtainable?

b) What is the maximum ethanol yield coefficient ($Y_{e/s}$, moles EtOH/mole glucose), and under what conditions is it obtainable?

Problema 1.2-a)

⇒ escrever a equação da reação:
write the reaction equation:

$$C_6H_{12}O_6 \quad + \quad NH_3 \rightarrow CH_{1.7}O_{0.45}N_{0.15} \quad + \quad \cancel{C_2H_6O} \quad + \quad CO_2 \quad + \quad H_2O$$

rendimento máximo em biomassa => não há produção de etanol
maximum biomass yield => no ethanol production

$$a\,C_6H_{12}O_6 \quad + \quad b\,NH_3 \rightarrow CH_{1.7}O_{0.45}N_{0.15} \quad + \quad c\,CO_2 \quad + \quad d\,H_2O$$

Problema 1.2-a)

pelo método do balanço energético

Energy balance

$$\left.\begin{aligned} \gamma_{\text{glucose}} &= +24 \\ \gamma_{\text{NH3}} &= 0 \\ \gamma_{\text{biomassa}} &= +4.35 \\ \gamma_{\text{CO2}} &= 0 \\ \gamma_{\text{H2O}} &= 0 \end{aligned}\right\}$$

$$a \times \gamma_{\text{glucose}} + \underbrace{\cancel{b \times \gamma_{\text{NH3}}}}_{=0} = \gamma_{\text{biomassa}} \underbrace{\cancel{+ c \times \gamma_{\text{CO2}}}}_{=0} \underbrace{\cancel{+ d \times \gamma_{\text{H20}}}}_{=0}$$

$$a \times \gamma_{\text{glucose}} = \gamma_{\text{biomassa}} \Leftrightarrow 24a = 4.35 \Leftrightarrow a = 0.18$$

$$(Y_{X/S})_{\max} = \frac{1mol\_biomassa}{a} = \frac{23}{a} = 127.8 gbiomassa/ mol\_glucose$$

Problema 1.2-b)

$$C_6H_{12}O_6 \quad + \quad \cancel{NH_3} \quad \rightarrow \quad CH_{1.7}\cancel{O_{0.45}}N_{0.15} \quad + \quad C_2H_6O \quad + \quad CO_2 \quad + \quad H_2O$$

rendimento máximo em etanol=> não há produção de biomassa

maximum ethanol yield=> no biomass production

$$a\,C_6H_{12}O_6 \quad \rightarrow \quad C_2H_6O \quad + \quad b\,CO_2 \quad + \quad c\,H_2O$$

pelo método do balanço energético

Energy balance

$$\left.\begin{aligned} \gamma_{\text{glucose}} &= +24 \\ \gamma_{\text{etanol}} &= +12 \end{aligned}\right\}$$

$$a \times \gamma_{\text{glucose}} = \gamma_{\text{etanol}} + \underbrace{\cancel{b \times \gamma_{\text{CO2}}}}_{=\,0} + \underbrace{\cancel{c \times \gamma_{\text{H20}}}}_{=\,0}$$

$$a \times \gamma_{\text{glucose}} = \gamma_{\text{etanol}} \Leftrightarrow 24a = 12 \Leftrightarrow a = 0.5$$

$$(Y_{E/S})_{\max} = \frac{1mol\_e\tan ol}{a} = \frac{1}{a} = \frac{1}{0.5} = 2\ mol\ etanol/mol\ glucose$$

Problem 1.3

Considere uma cultura em descontínuo de determinado microrganismo, cuja fórmula empírica da biomassa é a seguinte

$$CH_{1.6}O_{0.55}N_{0.20} \text{ } (MW_B = 25.2 \text{ g/mole}).$$

The culture medium contains 10 mmol glucose ($C_6H_{12}O_6$) and ammonium sulfate in large excess. During the cultivation time there was an effective growth of 0.3 g of dry weight and a total consumption of 15 mmol of O_2.

This organism does not excrete appreciable amounts of metabolites under growing conditions.

a) Estimate the substrate yield coefficient, $Y_{X/S}$ (g DCW/mole glucose), and the final amount of glucose in the medium (mmol).

b) Estimate how much CO_2 was produced (mmol).

Problema 1.3-a)

⇒ escrever a equação da reação:
write the reaction equation

$$a\ C_6H_{12}O_6 + b\ O_2 + c\ NH_3 \rightarrow CH_{1.6}O_{0.55}N_{0.20} + d\ CO_2 + e\ H_2O$$

⇒ calcular Yx/s:

$$Y_{x/s} = \frac{1\ mol\ X}{a\ mol\ S} = \frac{25{,}2\ g\ X}{a\ mol\ S}$$

calcular a

Problema 1.3-a)

pelo método do balanço energético

Energy balance

$$\left.\begin{array}{l} \gamma_{glucose} = +24 \\ \gamma_{O2} = -4 \\ \gamma_{NH3} = 0 \\ \gamma_{biomassa} = +3.9 \\ \gamma_{CO2} = 0 \\ \gamma_{H2O} = 0 \end{array}\right\}$$

$$a \times \gamma_{glucose} + b \times \gamma_{O2} + c \times \underbrace{\cancel{\gamma_{NH3}}}_{=0} = \gamma_{biomassa} + d \times \underbrace{\cancel{\gamma_{CO2}}}_{=0} + e \times \underbrace{\cancel{\gamma_{H20}}}_{=0}$$

$$a \times \gamma_{glucose} + b \times \gamma_{O2} = \gamma_{biomassa} \Leftrightarrow 24a - 4b = 3.9$$

$$\Leftrightarrow a = 0,373$$

$$Y_{x/o2} = \frac{1\ mol\ X}{b\ mol\ O2} = \frac{25,2\ g\ X}{b\ mol\ O2}$$

$$\frac{25,2\ g\ X}{b\ mol\ O2} = 20\ gX/molO2$$

$$b = 1,26$$

$$Y_{x/o2} = \frac{\Delta X}{\Delta O2} = \frac{0,3\ g\ X}{0,015\ mol\ o2} = 20gX/molO2$$

(experimental)

$$Y_{x/s} = \frac{1\ mol\ X}{a\ mol\ S} = \frac{25{,}2\ g\ X}{a\ mol\ S} = \frac{25{,}2\ gX}{0{,}373\ molS} = 67{,}56\ gX/molS$$

$$Y_{x/s} = \frac{\Delta X}{\Delta S} = \frac{0{,}3\ g\ X}{\Delta S}$$

$$\frac{0{,}3\ g\ X}{\Delta S} = 67{,}56\ gX/molS$$

$$\Delta S = 0{,}00444\ molS$$

$$\Delta S = S\_inicial\ - S\_final$$

$$0{,}00444 = 0{,}01\ mol\ - S\_final$$

$$S_{final} = 0{,}00556mol = 5{,}56\ mmol$$

Problema 1.3-b)

balanço ao carbono:
carbon balance

$$a\ C_6H_{12}O_6 \quad + \quad b\ O_2 \quad + \quad c\ NH_3 \rightarrow CH_{1.6}O_{0.55}N_{0.20} \quad + \quad d\ CO_2 \quad + \quad e\ H_2O$$

$$6a = 1 + d \Leftrightarrow \underset{\uparrow}{6 \times 0.373} = 1 + d \Leftrightarrow d = 1.24$$

Probl. 1.3-a)

1 mol biomass ↔ d mol CO_2

25.2 g biomass ↔ 1.24 mol CO_2

0.3 g biomass ↔ ?

? = 0.01476 mol CO_2 = 14.76 mmol CO_2

Problem 1.4

In a bacterial culture, pyruvate ($C_3H_4O_3$) is used as a carbon source for growth. The source of nitrogen is ammonia salts. The empirical formula for biomass is $CH_{1.8}O_{0.5}N_{0.17}$ (MW_B = 24.2 g DCW/mol).

a) Based on the above information, estimate the maximum theoretical biomass yield per mole of pyruvate (g DCW/mol).

The culture described above is performed aerobically and excretion of metabolites into the extracellular medium was not detected. It was determined that 45 mmol CO_2 is released for every g DCW of biomass produced.

b) Estimate current biomass yield per mole of pyruvate (g DCW/mol).

c) Explain the difference in results obtained in the items a) and b).

Problema 1.4

Escrever a equação da reação:

Write the reaction equation

$$a\ C_3H_4O_3 \quad + \quad bO_2 \quad + \quad c\ NH_3 \quad \rightarrow \quad CH_{1.8}O_{0.5}N_{0.17} \quad + \quad d\ CO_2 \quad + \quad e\ H_2O$$

a) Rendimento máximo: consideramos apenas a conversão de substrato em biomassa

Maximum yield: we only consider the conversion of substrate to biomass

$$Y_{x/s} = \frac{1\ mol\_X}{a\ mol\_S} = \frac{24{,}2\ g\_X}{a\ mol\_S} = \frac{24{,}2\ g\_X}{0{,}429\ mol\_S} = 56{,}41\ g\_X/mol\_S$$

Calcular a: balanço energético

Determine a: energy balance

$\gamma(CH_{1,8}O_{0,5}N_{0,17}) = 4{,}29$

$\gamma(C_3H_4O_3) = 10$

$a \times \gamma(C_3H_4O_3) = \gamma(CH_{1,8}O_{0,5}N_{0,17})$

$10a = 4{,}29$

$a = 0{,}429$

Problema 1.4

b) Rendimento atual: consideramos a equação da reação completa

Current yield: we consider the complete reaction equation

$$a\ C_3H_4O_3 \ + \ bO_2 \ + \ c\ NH_3 \rightarrow CH_{1.8}O_{0.5}N_{0.17} \ + \ d\ CO_2 \ + \ e\ H_2O$$

$$Y_{x/s} = \frac{1\ mol\_X}{a\ mol\_S} = \frac{24{,}2\ g\_X}{a\ mol\_S}$$

Calcular a / Determine a

Vamos usar dados de produção de CO_2

Let's use CO2 production data

$$45\ mmol_{CO2}/g\_X = Y_{CO2/X}$$

$$0{,}045\ mol_{CO2}/g\_X = \frac{d\ mol_{CO2}}{24{,}2\ g\_X}$$

$$d = 1{,}089$$

$$Y_{CO2/X} = \frac{d\ mol_{CO2}}{1\ mol\_X} = \frac{d\ mol_{CO2}}{24{,}2\ g\_X}$$

Problema 1.4

b) Rendimento atual: consideramos a equação da reação completa

Current yield: we consider the complete reaction equation

$$a\, C_3H_4O_3 + bO_2 + c\, NH_3 \rightarrow CH_{1.8}O_{0.5}N_{0.17} + d\, CO_2 + e\, H_2O$$

$$Y_{x/s} = \frac{1\, mol\_X}{a\, mol\_S} = \frac{24{,}2\ g\_X}{a\, mol\_S} = \frac{24{,}2\ g\_X}{0{,}696\, mol\_S} = 34{,}77\ g\_X/mol\_S$$

Calcular a / Determine a

Carbon balance

$$3a = 1 + d \Leftrightarrow a = 0{,}696$$

Problema 1.4

c) Comparação / comparison

Rendimento máximo
Maximum yield

$$Y_{x/s} = 56,41\ g\_X/mol\_S$$

Rendimento atual
Real yield

$$Y_{x/s} = 34,77\ g\_X/mol\_S$$

Considera que parte do susbtrato é utilizado para respiração e manutenção celular; menos substrato fica disponível para crescimento → menor Yx/s

Considers that part of the substrate is used for respiration and cell maintenance; less substrate is available for growth → lower Yx/s