

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)

## Aulas Teórico-Práticas / Problem Solving Classes

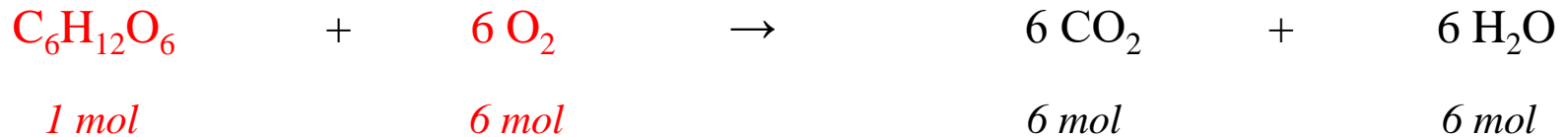
Professor: Filomena Freitas

1 aula semanal (3 horas)	Turnos:	3ª feira (Tuesday)	08:00-11:00
/ 1 weekly class (3 hours)		3ª feira (Tuesday)	13:30-16:30
		4ª feira (Wednesday)	08:00-11:00

### 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

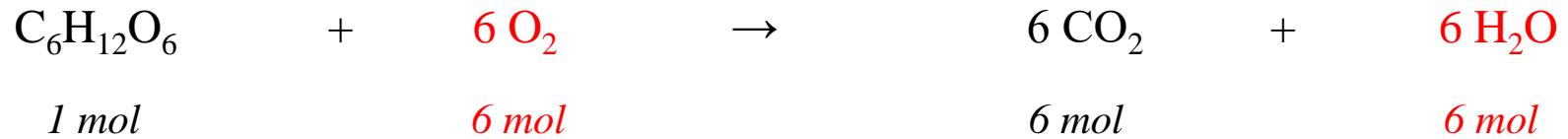


Coeficiente de rendimento da glucose em função do oxigênio

Yield coefficient for glucose on an oxygen basis

$$Y_{glc/O_2} = \frac{1 \text{ mol glucose}}{6 \text{ mol O}_2} = 0,167 \text{ mol\_glc/mol\_O}_2$$

## Coeficientes de rendimento / Yield coefficients

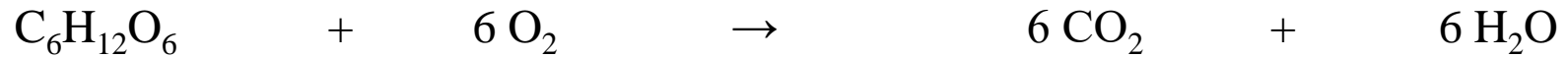


Coeficiente de rendimento da água em função do oxigênio

Yield coefficient for water on an oxygen basis

$$Y_{\text{H}_2\text{O}/\text{O}_2} = \frac{6 \text{ mol H}_2\text{O}}{6 \text{ mol O}_2} = 1,0 \text{ mol\_H}_2\text{O/mol\_O}_2$$

## Coeficientes de rendimento / Yield coefficients

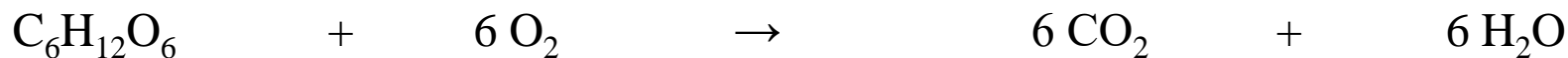


Conversão de unidades

Units conversion

$$M(\text{H}_2\text{O}) = 18 \text{ g/mol}$$

$$Y_{\text{H}_2\text{O}/\text{O}_2} = \frac{6 \text{ mol H}_2\text{O}}{6 \text{ mol O}_2} = \frac{6 \times 18 \text{ g H}_2\text{O}}{6 \text{ mol O}_2} = \frac{108 \text{ g H}_2\text{O}}{6 \text{ mol O}_2} = 18 \text{ g}_{\text{H}_2\text{O}}/\text{mol}_{\text{O}_2}$$

Coeficientes de rendimento / Yield coefficients

Conversão de unidades

Units conversion

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

$M(H_2O) = 18 \text{ g/mol}$   
 $M(O_2) = 32 \text{ g/mol}$

## 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:



This bacterium grows aerobically in a culture medium using glucose as a carbon source ( $\text{C}_6\text{H}_{12}\text{O}_6$ ).

Glucose and oxygen yield coefficients were experimentally determined:

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

$$Y_{X/O_2} = 39 \text{ g biomass/mole O}_2$$

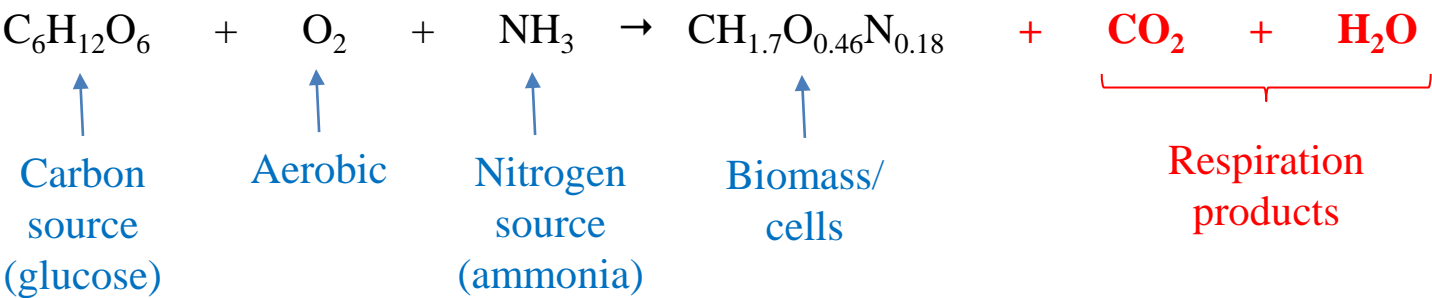
*This organism does not excrete appreciable amounts of metabolites under growing conditions.*

- a) Show that the measured values of  $Y_{X/S}$  and  $Y_{X/O_2}$  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



**Problema 1.1-a)**

⇒ equação da reação: equation of the reaction



⇒ Mostrar que os valores medidos de  $Y_{x/s}$  e  $Y_{x/o_2}$  são consistentes:

Show that the measured values of  $Y_{x/s}$  and  $Y_{x/o_2}$  are consistent

A partir do valor de  $Y_{x/s}$  experimental (enunciado) vamos calcular  $Y_{x/o_2}$  → verificar se dá o valor indicado no enunciado para  $Y_{x/o_2}$

From the experimental  $Y_{x/s}$  value (problem sheet) we will calculate  $Y_{x/o_2}$  → check if it gives the value indicated for  $Y_{x/o_2}$

**Problema 1.1-a)**

⇒ equação da reação: equation of the reaction



⇒ Mostrar que os valores medidos de  $Y_{X/S}$  e  $Y_{X/O_2}$  são consistentes:

Show that the measured values of  $Y_{X/S}$  and  $Y_{X/O_2}$  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)

⇒ equação da reação: equation of the reaction



⇒ coeficientes de rendimento: yield coefficients

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

Pela estequiometria da reação, temos:

By reaction stoichiometry, we have:

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

**Problema 1.1-a)**

⇒ equação da reação: equation of the reaction



⇒ coeficientes de rendimento: yield coefficients

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

Pela estequiometria da reação, temos:

By reaction stoichiometry, we have:

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

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

**Problema 1.1-a)**

⇒ equação da reação: equation of the reaction



⇒ coeficientes de rendimento:

$$Y_{x/o_2} = ?$$

Pela estequiometria da reação, temos:

$$Y_{x/o_2} = \frac{1 \text{ mol } X}{b \text{ mol } O_2} = \frac{23,6 \text{ g}_X}{\textcolor{red}{b} \text{ mol}_{O_2}}$$



Calcular b

## Problema 1.1-a)

⇒ 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**



C	$6a = 1 + d$	
H	$12a + 3c = 1.7 + 2e$	
O	$6a + 2b = 0.46 + 2d + e$	
N	$c = 0.18$	$a = 0,278$



❖ **Balço aos elementos Balance to the elements***voltando aos balanços*

$$6a = 1 + d \leftrightarrow 6 \times 0.28 = 1 + d \leftrightarrow \mathbf{d = 0.666}$$

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

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

$$Y_{x/o2} = \frac{23,6 \text{ g}_X}{b \text{ mol}_{O2}} = \frac{23,6}{0,601} = 39,27 \text{ g}_X/\text{mol}_{O2}$$

(teórico)

$$Y_{x/o2} = 39 \text{ g}_X/\text{mol}_{O2}$$

(experimental)

Os valores são semelhantes  $\rightarrow$  os valores de  $Y_{x/s}$  e  $Y_{x/o2}$  são consistentes

The values are similar  $\rightarrow$   $Y_{x/s}$  and  $Y_{x/o2}$  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*

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

$$\gamma_{\text{O}_2} = 2 \times (-2) = -4$$

$$\gamma_{\text{NH}_3} = 1 \times (-3) + 3 \times (+1) = 0$$

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

$$\gamma_{\text{CO}_2} = 1 \times (+4) + 2 \times (-2) = 0$$

$$\gamma_{\text{H}_2\text{O}} = 2 \times (+1) + 1 \times (-2) = 0$$

$$a \times \gamma_{\text{glucose}} + b \times \gamma_{\text{O}_2} + c \times \gamma_{\text{NH}_3} = \gamma_{\text{biomassa}} + d \times \gamma_{\text{CO}_2} + e \times \gamma_{\text{H}_2\text{O}}$$

$$24a - 4b + 0 = 4.24 + 0 + 0 \quad \leftrightarrow \quad 24a - 4b = 4.24$$

$$a = 0,278$$

$$24a - 4b = 4.24 \quad \leftrightarrow \quad 24 \times 0.278 - 4b = 4.24 \quad \leftrightarrow \quad b = 0.601$$

**❖ Balanço energético Energy balance**

$$Y_{x/o_2} = \frac{23,6 \text{ g}_X}{b \text{ mol}_{O_2}} = \frac{23,6}{0,601} = 39,27 \text{ g}_X/\text{mol}_{O_2}$$

(teórico)

$$Y_{x/o_2} = 39 \text{ g}_X/\text{mol}_{O_2}$$

(experimental)

Os valores são semelhantes → os valores de  $Y_{x/s}$  e  $Y_{x/o_2}$  são consistentes

The values are similar →  $Y_{x/s}$  and  $Y_{x/o_2}$  are consistent

## Problema 1.1-b)



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

$$Y_{X/S} = 85 \text{ g}_{\text{biomassa}} / \text{mol}_{\text{O}_2} \quad \text{coeficiente rendimento experimental}$$

$$= \frac{1 - 0.01}{0.02 - S_{final}} = 85 \Leftrightarrow S_{final} = 0.0084 \text{ mol} = 8.4 \text{ mmol}_{\text{glucose}}$$

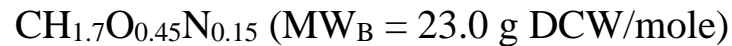
=> *ainda havia glucose no final => crescimento parou por falta de outro nutriente:*

*NH<sub>3</sub>, O<sub>2</sub>, micronutrientes*

=> there was still glucose left at the end => growth stopped due to lack of another nutrient:  
NH<sub>3</sub>, O<sub>2</sub>, micronutrients

## Problem 1.2

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



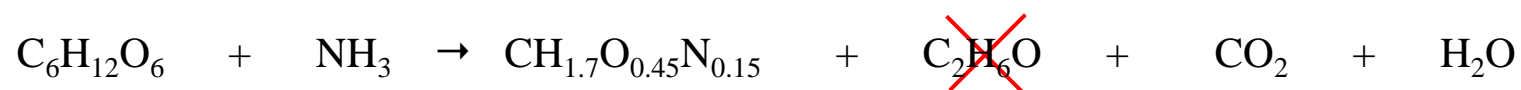
Carbon and nitrogen sources are glucose ( $\text{C}_6\text{H}_{12}\text{O}_6$ ) and ammonium salts, respectively. Possible products of the growth reaction are biomass, ethanol ( $\text{C}_2\text{H}_6\text{O}$ ), carbon dioxide and water. Ethanol growth and formation depend on growing conditions.

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

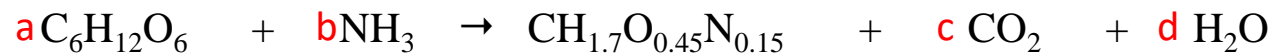
⇒ escrever a equação da reação:

write the reaction equation:



*rendimento máximo em biomassa => não há produção de etanol*

*maximum biomass yield => no ethanol production*



## Problema 1.2-a)

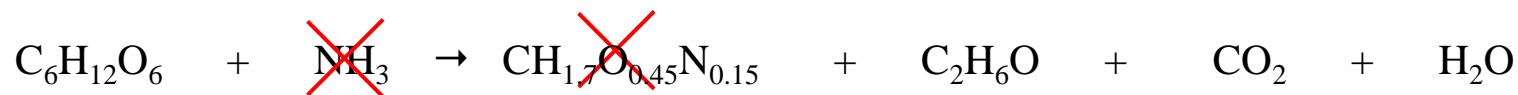
*pelo método do balanço energético*

*Energy balance*

$$\left. \begin{array}{l} \gamma_{\text{glucose}} = +24 \\ \gamma_{\text{NH}_3} = 0 \\ \gamma_{\text{biomassa}} = +4.35 \\ \gamma_{\text{CO}_2} = 0 \\ \gamma_{\text{H}_2\text{O}} = 0 \end{array} \right\} \begin{array}{l} a \times \gamma_{\text{glucose}} + \cancel{b \times \gamma_{\text{NH}_3}} = \gamma_{\text{biomassa}} + \cancel{c \times \gamma_{\text{CO}_2}} + \cancel{d \times \gamma_{\text{H}_2\text{O}}} \\ \phantom{a \times \gamma_{\text{glucose}}} = 0 \phantom{= 0} \phantom{= 0} \\ a \times \gamma_{\text{glucose}} = \gamma_{\text{biomassa}} \Leftrightarrow 24a = 4.35 \Leftrightarrow a = 0.18 \end{array}$$

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

## Problema 1.2-b)



*rendimento máximo em etanol=> não há produção de biomassa*

*maximum ethanol yield=> no biomass production*



*pelo método do balanço energético*

*Energy balance*

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

$$a \times \gamma_{\text{glucose}} = \gamma_{\text{etanol}} + \cancel{b \times \gamma_{\text{CO}_2}} + \cancel{c \times \gamma_{\text{H}_2\text{O}}}$$

$$\phantom{a \times \gamma_{\text{glucose}}} = 0 \phantom{+} = 0$$

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

$$(Y_{e/s})_{\max} = \frac{1 \text{ mol etanol}}{a} = \frac{1}{0.5} = 2 \text{ mol etanol/mol glucose}$$



**Problem 1.3**

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



The culture medium contains 10 mmol glucose ( $\text{C}_6\text{H}_{12}\text{O}_6$ ) 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  $\text{O}_2$ .

*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  $\text{CO}_2$  was produced (mmol).

## Problema 1.3-a)

⇒ escrever a equação da reação:  
write the reaction equation



⇒ calcular  $Y_{x/s}$ :

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

calcular a

## Problema 1.3-a)

*pelo método do balanço energético**Energy balance*

$$\left. \begin{array}{l} \gamma_{\text{glucose}} = +24 \\ \gamma_{\text{O}_2} = -4 \\ \gamma_{\text{NH}_3} = 0 \\ \gamma_{\text{biomassa}} = +3.9 \\ \gamma_{\text{CO}_2} = 0 \\ \gamma_{\text{H}_2\text{O}} = 0 \end{array} \right\} \begin{array}{l} a \times \gamma_{\text{glucose}} + b \times \gamma_{\text{O}_2} + \cancel{c \times \gamma_{\text{NH}_3}} = \gamma_{\text{biomassa}} + \cancel{d \times \gamma_{\text{CO}_2}} + \cancel{e \times \gamma_{\text{H}_2\text{O}}} \\ \phantom{a \times \gamma_{\text{glucose}} + b \times \gamma_{\text{O}_2}} = 0 \phantom{+ \gamma_{\text{biomassa}}} \phantom{+ \gamma_{\text{CO}_2}} = 0 \phantom{+ \gamma_{\text{H}_2\text{O}}} \\ \\ a \times \gamma_{\text{glucose}} + b \times \gamma_{\text{O}_2} = \gamma_{\text{biomassa}} \Leftrightarrow 24a - 4b = 3.9 \\ \phantom{a \times \gamma_{\text{glucose}} + b \times \gamma_{\text{O}_2}} \Leftrightarrow a = 0,373 \end{array}$$

$$Y_{x/o_2} = \frac{1 \text{ mol } X}{b \text{ mol } \text{O}_2} = \frac{25,2 \text{ g } X}{b \text{ mol } \text{O}_2} \qquad \frac{25,2 \text{ g } X}{b \text{ mol } \text{O}_2} = 20 \text{ gX/molO}_2$$

$$b = 1,26$$

$$Y_{x/o_2} = \frac{\Delta X}{\Delta \text{O}_2} = \frac{0,3 \text{ g } X}{0,015 \text{ mol } \text{O}_2} = 20 \text{ gX/molO}_2$$

(experimental)

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

$$Y_{x/s} = \frac{\Delta X}{\Delta S} = \frac{0,3 \text{ g } X}{\Delta S} \qquad \frac{0,3 \text{ g } X}{\Delta S} = 67,56 \text{ g } X / \text{mol } S$$

$$\Delta S = 0,00444 \text{ mol } S$$

$$\Delta S = S_{\text{inicial}} - S_{\text{final}}$$

$$0,00444 = 0,01 \text{ mol} - S_{\text{final}}$$

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

## Problema 1.3-b)

*balanço ao carbono:*  
*carbon balance*



$$6a = 1 + d \Leftrightarrow 6 \times 0.373 = 1 + d \Leftrightarrow d = 1.24$$

↑  
 Probl. 1.3-a)

$$1 \text{ mol biomass} \leftrightarrow d \text{ mol CO}_2$$

$$25.2 \text{ g biomass} \leftrightarrow 1.24 \text{ mol CO}_2$$

$$0.3 \text{ g biomass} \leftrightarrow ?$$

$$? = 0.01476 \text{ mol CO}_2 = 14.76 \text{ mmol CO}_2$$

**Problem 1.4**

In a bacterial culture, pyruvate ( $\text{C}_3\text{H}_4\text{O}_3$ ) is used as a carbon source for growth. The source of nitrogen is ammonia salts. The empirical formula for biomass is  $\text{CH}_{1.8}\text{O}_{0.5}\text{N}_{0.17}$  ( $MW_B = 24.2$  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  $\text{CO}_2$  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) **Rendimento máximo: consideramos apenas a conversão de substrato em biomassa**

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

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

Calcular a: **balanço energético**

Determine a: energy balance

$$\gamma(\text{CH}_{1.8}\text{O}_{0.5}\text{N}_{0.17}) = 4,29$$

$$a \times \gamma(\text{C}_3\text{H}_4\text{O}_3) = \gamma(\text{CH}_{1.8}\text{O}_{0.5}\text{N}_{0.17})$$

$$\gamma(\text{C}_3\text{H}_4\text{O}_3) = 10$$

$$10a = 4,29$$

$$a = 0,429$$

## Problema 1.4

- b) Rendimento atual: consideramos a equação da reação completa  
 Current yield: we consider the complete reaction equation



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

Calcular  $a$  / Determine  $a$

Vamos usar dados de produção de  $\text{CO}_2$

Let's use  $\text{CO}_2$  production data

$$45 \text{ mmol}_{\text{CO}_2}/\text{g}_X = Y_{\text{CO}_2/X}$$

$$0,045 \text{ mol}_{\text{CO}_2}/\text{g}_X = \frac{d \text{ mol}_{\text{CO}_2}}{24,2 \text{ g}_X}$$

$$Y_{\text{CO}_2/X} = \frac{d \text{ mol}_{\text{CO}_2}}{1 \text{ mol}_X} = \frac{d \text{ mol}_{\text{CO}_2}}{24,2 \text{ g}_X}$$

$$d = 1,089$$



## Problema 1.4

- b) Rendimento atual: consideramos a equação da reação completa  
Current yield: we consider the complete reaction equation



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

Calcular  $a$  / Determine  $a$

Carbon balance

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

## Problema 1.4

c) Comparação / comparison

Rendimento máximo  
Maximum yield

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

Rendimento atual  
Real yield

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



Considera que parte do substrato é utilizado para respiração e manutenção celular; menos substrato fica disponível para crescimento → menor  $Y_{x/s}$

Considers that part of the substrate is used for respiration and cell maintenance; less substrate is available for growth → lower  $Y_{x/s}$