

EB – Exercícios

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Conteúdo

Questão 1	2	Questão 5	6
Questão 2	3	Questão 7	7
Questão 3	4	Questão 8	8
Questão 4	5		

Questão 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:

$$Y_{X/S} = 85 \text{ g (biomass)/mol (glucose)}$$

$$Y_{X/O_2} = 39 \text{ g (biomass)/mol (O}_2\text{)}$$

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

Q1 a.

Show that the measured values of $Y_{X/S}$ and Y_{X/O_2} are consistent.

Resposta



$$Y_{X/S} = \frac{85 \text{ g}_X}{\text{mol}_S} = \frac{1 \text{ mol}_X}{a \text{ mol}_S} \frac{M_{wB} \text{ g}_X}{1 \text{ mol}_X} \implies \\ \implies a = M_{wB}/85 = 23.6/85 \cong 0.278$$

$$Y_{X/O_2} = \frac{39 \text{ g}_X}{\text{mol}_{O_2}} = \frac{1 * M_{wB} \text{ g}_X}{b \text{ mol}_{O_2}} \implies \\ \implies b = \frac{M_{wB}}{39} = \frac{23.6}{39} \cong 0.605$$

Balanço Mássico

$$\begin{cases} \text{C :} & 6a = 1 + d \\ \text{O :} & 6a + 2b = 0.46 + 2d + e \\ \text{N :} & c = 0.18 \\ \text{H :} & 12a + 3c = 1.7 + 2e \end{cases}$$

$$\therefore d \cong 6 * 0.278 - 1 \cong 0.666;$$

$$2(6a + 2b) - (12a + 3c) = 4b - 3c = \\ = 2(0.46 + 2d + e) - (1.7 + 2e) = 0.92 + 4d - 1.7 \implies \\ \implies b \cong \frac{0.92 + 4 * 0.666 - 1.7 + 3 * 0.18}{4} \cong 0.606 \cong 0.605$$

Balanço Energetico

$$\begin{cases} \gamma_S = 6 * \gamma_C + 12 * \gamma_H + 6 * \gamma_O = 6 * 4 + 12 * 1 + 6 * (-2) = 24 \\ \gamma_{O_2} = 2 * \gamma_O = -4 \\ \gamma_{NH_3} = 1 * \gamma_N + 3 * \gamma_H = 1 * (-3) + 3 * 1 = 0 \\ \gamma_X = 1 * \gamma_C + 1.7 * \gamma_H + 0.46 * \gamma_O + 0.18 * \gamma_N = 4.24 \\ \gamma_{CO_2} = 1 * \gamma_C + 2 * \gamma_O = 1 * 4 + 2 * (-2) = 0 \\ \gamma_{H_2O} = 2 * \gamma_H + \gamma_O = 0 \end{cases}$$

$$a\gamma_S + b\gamma_{O_2} + c\gamma_{NH_3} = a24 + b - 4 = \\ = \gamma_X + d\gamma_{CO_2} + e\gamma_{H_2O} = 4.24 \implies \\ \implies b = (24 * a - 4.24)/4 \cong (24 * 0.278 - 4.24)/4 \cong \\ \cong 0.606 \cong 0.605$$

Q1 b.

A batch culture of this organism initially contains $0.01 \text{ g}_{\text{biomass}}$ and $20 \text{ mmol}_{\text{glucose}}$. 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.

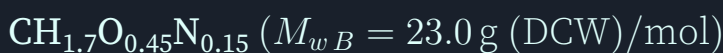
Resposta

$$Y_{X/S} = \frac{85 \text{ g}_X}{\text{mol}_S} = -\frac{\Delta X}{\Delta S} = \frac{X_1 - X_0}{S_0 - S_1} \implies \\ \implies S_1/\text{mmol} = S_0 - \frac{X_1 - X_0}{85 * 10^{-3}} = 20 - \frac{1 - 0.01}{85 * 10^{-3}} \cong 8.353$$

The non nule amount of glucose indicates that the arrest was due to the lack of another nutrient: NH_3, O_2 , micronutrients

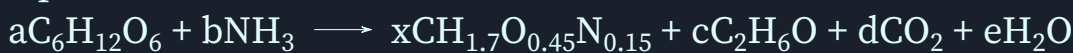
Questão 2

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.

Resposta



Q2 a.

What is the maximum biomass yield coefficient ($Y_{X/S}$ g (DCW)/mol (glucose)), and under what conditions is it obtainable?

Resposta

$$DCW = X$$

$$Y_{X/S \max} = \frac{x \text{ mol}_X}{a \text{ mol}_S} \frac{23.0 \text{ g}_X}{\text{mol}_X};$$

Balanço energético:

$$\begin{cases} \gamma_{\text{NH}_3} = \gamma_{\text{CO}_2} = \gamma_{\text{H}_2\text{O}} = 0 \\ \gamma_S = 6 * 4 + 12 + 6 * (-2) = 24 \\ \gamma_X = 4 + 1.7 + 0.45 * (-2) + 0.15 * (-3) = 4.35 \end{cases}$$

$$Y_{X/S \max} : c = 0$$

$$\therefore a \gamma_S = x \gamma_X \implies a = x 24 / 4.35 \cong x 0.181 \implies$$

$$\implies \frac{Y_{X/S \max}}{\text{g (X) / mol (S)}} = x 23 / x 0.181 \cong 126.897$$

Q2 b.

What is the maximum ethanol yield coefficient ($Y_{E/S}$ mol (EtOH)/mol (glucose)), and under what conditions is it obtainable?

Resposta

$$\text{EtOH} = E$$

$$Y_{E/S \max} = \frac{c \text{ mol}_E}{a \text{ mol}_X}$$

Balanço Energético

$$\begin{cases} \gamma_S = 24 \\ \gamma_X = 4.35 \\ \gamma_E = 2 * 4 + 6 - 2 = 12 \end{cases}$$

$$Y_{E/S \max} \implies x = b = 0;$$

$$a \gamma_X = c \gamma_E \implies a = c \gamma_E / \gamma_X = c 12 / 4.35 = c / 2 \implies$$

$$\implies Y_{E/S \max} = \frac{c \text{ mol}_E}{(c/2) \text{ mol}_X} = 2 \frac{\text{mol (E)}}{\text{mol (X)}}$$

Questão 3

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



The culture medium contains 10 mmol_S glucose (C₆H₁₂O₆) 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_{O₂}.

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

Resposta



Q3 a.

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

Resposta

$$Y_{X/S} : Y_{X/S} = \frac{x \text{ mol}_X}{a \text{ mol}_S} \frac{25.2 \text{ g}_X}{\text{mol}_X} =$$

$$S_1 : Y_{X/S} = -\frac{\Delta X}{\Delta S} = \frac{\Delta X}{S_0 - S_1};$$

Balanço Energético

$$\begin{cases} \gamma_S = 6 * 4 + 12 - 6 * 2 & = 24 \\ \gamma_X = 4 + 1.6 - 0.55 * 2 - 0.20 * 3 & = 3.9 \\ \gamma_{\text{O}_2} & = -4 \\ \gamma_{\text{NH}_3} = \gamma_{\text{CO}_2} = \gamma_{\text{H}_2\text{O}} & = 0 \end{cases}$$

$$a : a \gamma_S + b \gamma_{\text{O}_2} = a 24 - b 4 =$$

$$= x \gamma_X = x 3.9$$

$$a : b : Y_{X/\text{O}_2} = \frac{x \text{ mol}_X}{b \text{ mol}_{\text{O}_2}} \frac{25.2 \text{ g}_X}{\text{mol}_X} = \frac{\Delta X}{\Delta \text{O}_2} = \frac{0.3 \text{ g}_X}{15 \text{ E-}3 \text{ mol}_{\text{O}_2}} \implies$$

$$\implies b = \frac{x * 25.2 * 15 \text{ E-}3}{0.3} = x 1.26 \implies$$

$$\implies a = \frac{x 3.9 + b * 4}{24} = \frac{x 3.9 + x 1.26 * 4}{24} = x 0.3725 \implies$$

$$\implies \frac{Y_{X/S}}{\text{g (X)/mol (S)}} = \frac{25.2 x}{a} = \frac{25.2 x}{x 0.3725} \cong 67.651 \wedge$$

$$\wedge S_1/\text{mmol} = S_0/\text{mmol} - \frac{\Delta X}{Y_{X/S} \text{ E-}3}/\text{mmol} \cong$$

$$\cong 10 - \frac{0.3}{67.651 \text{ E-}3} \cong 5.565$$

Q3 b.

Estimate how much CO₂ was produced (mmol).

Resposta

$$X_{\text{CO}_2 1} : Y_{\text{CO}_2/X} = \frac{\Delta X_{\text{CO}_2}}{\Delta X} = \frac{X_{\text{CO}_2 1} - X_{\text{CO}_2 0}}{\Delta X} = \frac{X_{\text{CO}_2 1}}{0.3} =$$

$$= \frac{d \text{ mol}_{\text{CO}_2}}{x \text{ mol}_X} \frac{\text{mol}_X}{25.2 \text{ g}_X};$$

Balanço Mássico:

$$\left\{ \text{C} : a * 6 = x + d \right.$$

$$d = a * 6 - x = x 0.3725 * 6 - x = x 1.235 \implies$$

$$\implies \frac{X_{\text{CO}_2 1}}{\text{mmol}_{\text{CO}_2}} = 0.3 \frac{d \text{ E}^3}{25.2 x} = 0.3 \frac{x 1.235 \text{ E}^3}{25.2 x} =$$

$$= 14.702$$

Questão 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:

$$\text{CH}_{1.8}\text{O}_{0.5}\text{N}_{0.17} \quad (M_{wB} = 24.2 \text{ g (DCW)/mol}).$$

Resposta



Q4 a.

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

Resposta

$$Y_{X/S} : Y_{X/S \max} = \frac{x \text{ mol}_X}{a \text{ mol}_S} \frac{24.2 \text{ g}_X}{\text{mol}_X};$$

$$Y_{X/S \max} \implies b = d = e = 0;$$

Balanço Energético:

$$\begin{cases} \gamma_S = 3 * 4 + 4 - 3 * 2 = 10 \\ \gamma_X = 4 + 1.8 - 0.5 * 2 - 0.17 * 3 = 4.29 \\ \gamma_{\text{NH}_3} = 0 \end{cases}$$

$$a : a \gamma_S = a 10 =$$

$$= x \gamma_X = x 4.29 \implies a = x 0.429 \implies$$

$$\implies \frac{Y_{X/S \max}}{\text{g (X)/mol (S)}} = \frac{x 24.2}{a} = \frac{x 24.2}{x 0.429} \cong 56.410$$

The culture described above is performed aerobically and excretion of metabolites into the extracellular medium was not detected. It was determined that $45 \text{ mmol}_{\text{CO}_2}$ is released for every g_{DCW} of biomass produced.
Q4 b.

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

Resposta

$$Y_{X/S} : Y_{X/S} = \frac{x 24.2 \text{ g}_X}{a \text{ mol}_S};$$

$$\text{Balanço Mássico: } \left\{ \text{C : } a * 3 = x + d \right.$$

$$a : d : Y_{\text{CO}_2/X} = \frac{d \text{ mol}_{\text{CO}_2}}{x 24.2 \text{ mol}_X} = \frac{45 \text{ mmol}_{\text{CO}_2}}{\text{g}_X} \implies$$

$$\implies d = x 1.089 \implies$$

$$\implies a = (x + d)/3 = (x + x 1.089)/3 \cong x 0.696 \implies$$

$$\frac{Y_{X/S}}{\text{g (X)/mol (S)}} \cong \frac{x 24.2}{x 0.696} \cong 34.753$$

Q4 c.

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

Resposta

The second case uses the substrate only for growing while the second part is used on the cell respiration and maintenance thus the lower growth.

Questão 5

The chemical reaction for glucose respiration is as follows:



Candida utilis yeast converts glucose to CO₂ and H₂O as it grows. Its empirical formula is:



The substrate biomass yield is 0.5 (w/w). The source of nitrogen is ammonia.

Resposta



Q5 a.

Formulate the free electron balance equation.

Resposta

Balanço Energético:

$$\begin{cases} \gamma_S = 6 * 4 + 12 - 6 * 2 = 24 \\ \gamma_X = 4 + 1.85 - 0.55 * 2 - 0.2 * 3 = 4.15 \\ \gamma_{O_2} = -4 \\ \gamma_{NH_3} = \gamma_{CO_2} = \gamma_{H_2O} = 0 \end{cases}$$
$$a \gamma_S + b \gamma_{O_2} = a 24 - b 4 =$$
$$= x \gamma_X = x 4.15$$

Q5 b.

Assess oxygen requirements (oxygen/glucose yield) when growth occurs or when cells only breathe glucose (i.e., cell maintenance).

Resposta

(i)

$$Y_{O_2/S} : Y_{O_2/S} = \frac{b \text{ mol}_{O_2}}{a \text{ mol}_S} = \frac{((a 24 - x 4.15)/4) \text{ mol}_{O_2}}{a \text{ mol}_S} =$$
$$= \frac{(6 - x 1.0375/a) \text{ mol}_{O_2}}{\text{mol}_S};$$

$$a : Y_{X/S} = \frac{x \text{ mol}_X}{a \text{ mol}_S} \frac{25.4663 * 1.05 \text{ g}_X}{\text{mol}_X} \frac{\text{mol}_S}{180.56 \text{ g}_S} = \frac{0.5 \text{ g}_X}{\text{g}_S} \implies$$
$$\implies a = \frac{x 25.4663 * 1.05}{0.5 * 180.56} \cong x 0.296 \implies$$

$$\implies \frac{Y_{O_2/S}}{\text{mol } (O_2)/\text{mol } (S)} = 6 - \frac{x 1.0375}{a} \cong$$
$$\cong 6 - \frac{x 1.0375}{x 0.296} \cong 2.497$$

(ii)

$$Y_{O_2/S} : Y_{O_2/S} = \frac{b \text{ mol}_{O_2}}{a \text{ mol}_S};$$

Balanço Energético:

$$\begin{cases} \gamma_S = 24 \\ \gamma_{O_2} = -4 \\ \gamma_{CO_2} = \gamma_{H_2O} = 0 \end{cases}$$

Maintenance: $c = x = 0$;

$$b : a \gamma_S + b \gamma_{O_2} = a 24 - b 4 = 0 \implies b = a 6 \implies$$

$$\implies Y_{O_2/S} = \frac{6 \text{ mol}_{O_2}}{\text{mol}_S}$$

Q5 c.

C. utilis is also capable of using ethanol as a carbon source. Compare the maximum thermodynamic yields of ethanol growth and glucose growth.

Resposta

- For glucose: $S_0, a_0C_6H_{12}O_6 + c_0NH_3 \longrightarrow x_0CH_{1.84}O_{0.55}N_{0.2}$
- for ethanol: $S_1, a_1C_2H_6O + c_1NH_3 \longrightarrow x_1CH_{1.84}O_{0.55}N_{0.2}$

$$\frac{Y_{X/S_0}}{Y_{X/S_1}} : Y_{X/S_i} = \frac{x_i \text{ mol}_X}{a_i \text{ mol}_{S_i}};$$

$$\text{Balanço Energético: } \begin{cases} \gamma_{S_0} = 24 \\ \gamma_{S_1} = 2 * 4 + 6 - 2 = 12 \\ \gamma_X = 4.15 \\ \gamma_{NH_3} = 0 \end{cases}$$

$$a_i : a_i \gamma_{S_i} = x_i \gamma_X \implies$$

$$\implies \frac{Y_{X/S_0}}{Y_{X/S_1}} = \frac{\frac{x_0 \text{ mol}_X}{(x_0 4.15/24) \text{ mol}_{S_0}}}{\frac{x_1 \text{ mol}_X}{(x_1 4.15/12) \text{ mol}_{S_1}}} = \frac{2 \text{ mol}_{S_1}}{\text{mol}_{S_0}}$$

Glucose can produce the double of biomass compared to ethanol, its more efficient

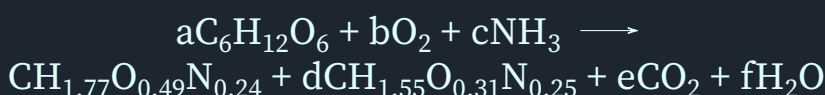
Questão 7

A recombinant protein is produced using genetically modified *Escherichia coli*. It was found that protein formation is proportional to cell growth. Glucose and ammonia are used as carbon and nitrogen sources respectively. The empirical formulas for biomass and protein are $\text{CH}_{1.77}\text{O}_{0.49}\text{N}_{0.24}$ and $\text{CH}_{1.55}\text{O}_{0.31}\text{N}_{0.25}$ respectively. It was experimentally determined that the biomass to glucose yield is 0.48 (w/w) and that the protein to glucose yield is 0.096 (w/w)

Q7 a.

Assess ammonia requirements.

Resposta



$$\begin{cases} M_{wX} = 12 + 1.77 + 16 * 0.49 + 14 * 0.24 = 24.97 \\ M_{wP} = 12 + 1.55 + 16 * 0.31 + 14 * 0.25 = 22.01 \\ M_{wS} = 12 * 6 + 12 + 16 * 6 = 180 \end{cases}$$

$$Y_{\text{NH}_3/\text{X}} = \frac{c \text{ mol}_{\text{NH}_3}}{\text{mol}_X} = \frac{(0.24 + 0.25 d) \text{ mol}_{\text{NH}_3}}{\text{mol}_X};$$

$$\begin{aligned} d \text{ mol}_P &= Y_{P/S} a \text{ mol}_S = Y_{P/S} \frac{1 \text{ mol}_X}{Y_{X/S}} = \\ &\left(\frac{0.096 \text{ g}_P}{\text{g}_S} \right) \frac{1 \text{ mol}_X}{0.48 \left(\frac{\text{g}_X}{\text{g}_S} \right) \frac{1}{M_{wX} \text{ g (X)}/\text{mol (X)}}} \frac{\text{mol}_P}{M_{wP} \text{ g}_P} = \\ &= \left(\frac{0.096 M_{wX}}{0.48 M_{wP}} \right) \text{ mol}_P = \left(\frac{0.096 * 24.97}{0.48 * 22.01} \right) \text{ mol}_P \cong \\ &\cong 0.227 \text{ mol}_P \implies \end{aligned}$$

$$\begin{aligned} \implies \frac{Y_{\text{NH}_3/\text{X}}}{\text{mol (NH}_3)/\text{mol (X)}} &= c = 0.24 + 0.25 d \cong \\ &\cong 0.24 + 0.25 * 0.227 \cong 0.297 \end{aligned}$$

Q7 b.

Assess oxygen requirements.

Resposta

$$Y_{\text{O}_2/\text{X}} = \frac{b \text{ mol}_{\text{O}_2}}{\text{mol}_X};$$

$$\begin{cases} \gamma_S = 4 * 6 + 12 - 2 * 6 = 24 \\ \gamma_X = 4 + 1.77 - 2 * 0.49 - 3 * 0.24 = 4.07 \\ \gamma_P = 4 + 1.55 - 2 * 0.31 - 3 * 0.25 = 4.18 \\ \gamma_{\text{O}_2} = -4 \\ \gamma_{\text{NH}_3} = \gamma_{\text{CO}_2} = \gamma_{\text{H}_2\text{O}} = 0 \end{cases}$$

$$24a - 4b = 4.07 + 4.18d;$$

$$a = \frac{\text{mol (X)}/\text{mol (S)}}{Y_{X/S}} = \frac{\text{mol (X)}/\text{mol (S)}}{0.48 \frac{\text{g (X)}}{\text{g (S)}} \frac{M_{wS} \text{ g (S)}/\text{mol (S)}}{M_{wX} \text{ g (X)}/\text{mol (X)}}} = \frac{24.97}{0.48 * 180} \cong 0.289$$

$$\implies b \cong 6a - \frac{4.07 + 4.18d}{4} = 6 \left(\frac{1 + d}{6} \right) - \frac{4.07 + 4.18d}{4} = 1 - \frac{4.07}{4}$$

$$\implies a * 6 + b * 2 = 0.49 + d * 0.31 + e * 2 + f \implies$$

$$\implies b = (0.49 + d * 0.31 + e * 2 + f - a * 6)/2$$

Q7 c.

If the biomass yield to glucose were the same, what would be the ammonia and oxygen requirements for a wild strain of *E. coli* that fails to synthesize the protein?

Resposta

(i) Ammonia

$$Y_{\text{NH}_3/\text{X}} = \frac{c \text{ mol}_{\text{NH}_3}}{\text{mol}_X} = \frac{0.24 \text{ mol}_{\text{NH}_3}}{\text{mol}_X}$$

(ii) Oxygen

Questão 8

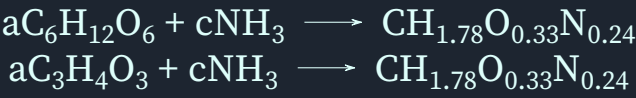
Aerobacter aerogenes is grown aerobically on glucose (C₆H₁₂O₆) or pyruvate (C₃H₄O₃) as a carbon source. The empirical formula for biomass is CH_{1.78}N_{0.24}O_{0.33} (MWB = 22.5 g/mol) and its degree of reduction is = 4.4. The following yields were experimentally determined

	Y _{X/S}			Y _{X/O₂}	
Substrate	g/g	g/mol	g/g (C)	g/g	g/mol
Glucose	0.40	0.72	1.01	1.11	35.5
Pyruvato	0.20	17.9	0.49	0.48	15.4

Q8 a.

Compare the biomass yield per mole of glucose and per mole of pyruvate with their respective thermodynamic maxima. Which of the two substrates is more efficient with respect to biosynthesis? Justique.

Resposta



$$\frac{\max Y_{X/G}}{\exp Y_{X/G}} = \frac{\left(\frac{\text{mol}_X}{a \text{ mol}_G} \frac{22.5 \text{ g}_X}{\text{mol}_X}\right)}{}$$

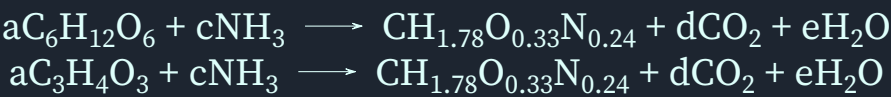
$$Y_{X/P} = \frac{\text{mol}_X}{a \text{ mol}_P} \frac{22.5 \text{ g}_X}{\text{mol}_X};$$

$$\gamma(\text{C}_3\text{H}_4\text{O}_6) = 3 * 2 + 4 * 1 - 1 * 2 = 8$$

Q8 b.

Considering the case of growth on glucose, assess whether excretion of metabolites into the extracellular medium will be expected.

Resposta



$$\begin{aligned} \gamma(\text{C}_6\text{H}_{12}\text{O}_6) a + \gamma(\text{O}_2) b &= (6 * 4 + 12 * 1 - 6 * 2) a - 4 b = 24 a - 4 b = \\ &= \gamma(\text{biomassa}) = 4.4; \end{aligned}$$