EB – Exercicios

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Conteúdo

Questão 1

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

$$CH_{1.7}O_{0.46}N_{0.18} \ (M_{wB} = 23.6\,\text{g/mol})$$

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:

$$Y_{X/S} = 85\,\text{g (biomass)/mol (glucose)}$$
$$Y_{X/O2} = 39\,\text{g (biomass)/mol (O2)}$$

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

Q1 a.

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

Resposta

$$aC_6H_{12}O_6 + bO_2 + cNH_3 \longrightarrow CH_{1.7}O_{0.46}N_{0.18} + dCO_2 + eH_2O$$

$$Y_{X/S} = \frac{85\,g_X}{mol_S} = \frac{1\,mol_X}{a\,mol_S}\frac{M_{wB}\,g_X}{1\,mol_X} \Longrightarrow$$
$$\Longrightarrow a = M_{wB}/85 = 23.6/85 \cong 0.278$$

$$Y_{X/O_2} = \frac{39\,g_X}{mol_{O_2}} = \frac{1*M_{wB}\,g_X}{b\,mol_{O_2}} \Longrightarrow$$
$$\Longrightarrow b = \frac{M_{wB}}{39} = \frac{23.6}{39} \cong 0.605$$

Balanço Mássico

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

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

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

Balanço Energetico

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

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

Q1 b.

A batch culture of this organism initially contains $0.01\,g_{biomass}$ and $20\,mmol_{glucose}$. 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.
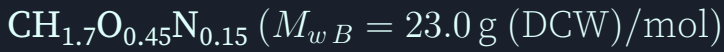
Resposta

$$Y_{X/S} = \frac{85\,g_X}{mol_S} = -\frac{\Delta X}{\Delta S} = \frac{X_1 - X_0}{S_0 - S_1} \Longrightarrow$$
$$\Longrightarrow S_1/mmol = S_0 - \frac{X_1 - X_0}{85*10^{-3}} = 20 - \frac{1 - 0.01}{85*10^{-3}} \cong 8.353$$

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:

$$CH_{1.7}O_{0.45}N_{0.15} \ (M_{wB} = 23.0 \, \text{g (DCW)/mol})$$

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.

Resposta

$$aC_6H_{12}O_6 + bNH_3 \longrightarrow xCH_{1.7}O_{0.45}N_{0.15} + cC_2H_6O + dCO_2 + eH_2O$$

Q2 a.

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

Resposta

$DCW = X$

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

Balanço energético:

$$\begin{cases} \gamma_{NH_3} = \gamma_{CO_2} = \gamma_{H_2O} = 0 \\ \gamma_S = 6*4 + 12 + 6*(-2) = 24 \\ \gamma_X = 4 + 1.7 + 0.45*(-2) + 0.15*(-3) = 4.35 \end{cases}$$

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

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

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

Q2 b.

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

Resposta

EtOH=E

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

Balanço Energético

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

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

$$a\,\gamma_X = c\,\gamma_E \implies a = c\,\gamma_E/\gamma_X = c\,12/24 = c/2 \implies$$

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

Questão 3

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

$$CH_{1.6}O_{0.55}N_{0.20} \ (M_{wB} = 25.2 \, \text{g/mol})$$

The culture medium contains $10 \, \text{mmol}_S$ glucose ($C_6H_{12}O_6$) and ammonium sulfate in large excess. During the cultivation time there was an effective growth of $0.3 \, \text{g}$ of dry weight and a total consumption of $15 \, \text{mmol}_{O_2}$.
This organism does not excrete appreciable amounts of metabolites under growing conditions.

Resposta

$$aC_6H_{12}O_6 + bO_2 + cNH_3 \longrightarrow xCH_{1.6}O_{0.55}N_{0.20} + dCO_2 + eH_2O$$

Q3 a.

Estimate the substrate yield coefficient, $Y_{X/S}$ $(\text{g}(X)/\text{mol}(S))$, 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

$$\begin{cases} \gamma_S = 6*4 + 12 - 6*2 & = 24 \\ \gamma_X = 4 + 1.6 - 0.55*2 - 0.20*3 & = 3.9 \\ \gamma_{O_2} & = -4 \\ \gamma_{NH_3} = \gamma_{CO_2} = \gamma_{H_2O} & = 0 \end{cases}$$

$$a : a\,\gamma_S + b\,\gamma_{O_2} = a\,24 - b\,4 =$$
$$= x\,\gamma_X = x\,3.9$$

$$a : b : Y_{X/O_2} = \frac{x \, \text{mol}_X}{b \, \text{mol}_{O_2}} \frac{25.2 \, \text{g}_X}{\text{mol}_X} = \frac{\Delta X}{\Delta O_2} = \frac{0.3 \, \text{g}_X}{15 \, \text{E} -3 \, \text{mol}_{O_2}} \Longrightarrow$$

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

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

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

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

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

Q3 b.

Estimate how much CO_2 was produced (mmol).

Resposta

$$X_{CO_2 1} : Y_{CO_2/X} = \frac{\Delta X_{CO_2}}{\Delta X} = \frac{X_{CO_2 1} - X_{CO_2 0}}{\Delta X} = \frac{X_{CO_2 1}}{0.3} =$$
$$= \frac{d \, \text{mol}_{CO_2}}{x \, \text{mol}_X} \frac{\text{mol}_X}{25.2 \, \text{g}_X};$$

Balanço Mássico:

$$\begin{cases} C : a*6 = x + d \end{cases}$$

$$d = a*6 - x = x\,0.3725*6 - x = x\,1.235 \Longrightarrow$$

$$\Longrightarrow \frac{X_{CO_2 1}}{\text{mmol}_{CO_2}} = 0.3\frac{d \, \text{E} \, 3}{25.2 \, x} = 0.3\frac{x\,1.235 \, \text{E} \, 3}{25.2 \, x} =$$
$$= 14.702$$

Questão 4

In a bacterial culture, pyruvate ($C_3H_4O_3$) is used as a carbon source for growth. The source of nitrogen is ammonia salts. The empirical formula for biomass is:

$$CH_{1.8}O_{0.5}N_{0.17} \ (M_{wB} = 24.2 \ g \ (DCW)/mol).$$

Resposta

$$aC_3H_4O_3 + bNH_3 + cO_2 \longrightarrow xCH_{1.8}O_{0.5}N_{0.17} + dCO_2 + eH_2O$$

Q4 a.

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

Resposta

$$Y_{X/S \ max} : Y_{X/S \ max} = \frac{x \ mol_X}{a \ mol_S} \frac{24.2 \ g_X}{mol_X};$$

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

Balanço Energético:

$$\begin{cases} \gamma_S = 3*4 + 4 - 3*2 = 10 \\ \gamma_X = 4 + 1.8 - 0.5*2 - 0.17*3 = 4.29 \\ \gamma_{NH_3} = 0 \end{cases}$$

$$a : a\,\gamma_S = a\,10 =$$
$$= x\,\gamma_X = x\,4.29 \implies a = x\,0.429 \implies$$

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

The culture described above is performed aerobically and excretion of metabolites into the extracellular medium was not detected. It was determined that $45 \ mmol_{CO_2}$ is released for every g_{DCW} of biomass produced.

Q4 b.

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

Resposta

$$Y_{X/S} : Y_{X/S} = \frac{x\,24.2 \ g_X}{a \ mol_S};$$

Balanço Mássico: $\begin{cases} C : & a*3 = x + d \end{cases}$

$$a : d : Y_{CO_2/X} = \frac{d \ mol_{CO_2}}{x\,24.2 \ mol_X} = \frac{45 \ mmol_{CO_2}}{g_X} \implies$$
$$\implies d = x\,1.089 \implies$$

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

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

Q4 c.

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

Resposta

The second case uses the substrate only for growing whilas 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:

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O$$

Candida utilis yeast converts glucose to CO_2 and H_2O as it grows. Its empirical formula is:

$$CH_{1.84}O_{0.55}N_{0.2} \text{ plus } 5\% \ (w/w) \text{ ash.}$$

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

Resposta

$$aC_6H_{12}O_6 + bO_2 + cNH_3 \longrightarrow xCH_{1.84}O_{0.55}N_{0.2} + dCO_2 + eH_2O$$

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)

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

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

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

(ii)

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

Balanço Energético:

$$\begin{cases} \gamma_S = 24 \\ \gamma_{O_2} = -4 \\ \gamma_{CO_2} = \gamma_{H_2O} = 0 \end{cases}$$
Manteinance: $c = x = 0$;

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

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

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, $a_1C_2H_6O + c_1NH_3 \longrightarrow x_1CH_{1.84}O_{0.55}N_{0.2}$

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

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

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

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 $CH_{1.77}O_{0.49}N_{0.24}$ and $CH_{1.55}O_{0.31}N_{0.25}$ 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

$$aC_6H_{12}O_6 + bO_2 + cNH_3 \longrightarrow$$
$$CH_{1.77}O_{0.49}N_{0.24} + dCH_{1.55}O_{0.31}N_{0.25} + eCO_2 + fH_2O$$
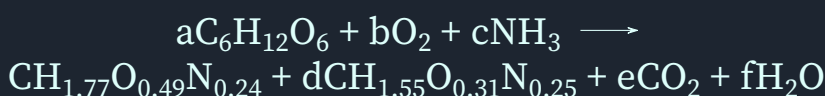
$$Y_{NH_3/X} = \frac{c\,mol_{NH_3}}{mol_X} \implies$$

$$\implies c = 0.24 + 0.25\,d;$$

$$d\,mol_P = Y_{P/S}\,a\,mol_S = Y_{P/S}\frac{1\,mol_X}{Y_{X/S}} =$$

$$\left(\frac{0.096\,g_P}{g_S}\right)\frac{1\,mol_X}{\left(\frac{g_X}{g_S}\right)};$$

$$Y_{P/S} = \frac{d\,mol_P}{a\,mol_S} = \frac{0.096\,g_P}{g_S}\frac{mol_P}{\begin{pmatrix}1*12\\+1.55*1\\+0.31*16\\+0.25*14\end{pmatrix}g_P}\frac{\begin{pmatrix}6*12\\+12*1\\+6*16\end{pmatrix}g_S}{mol_S}$$

Q7 b.

Assess oxygen requirements.

Resposta

$$Y_{O_2/X} = \frac{b\,mol_{O_2}}{mol_X} \implies$$

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

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

$$Y_{NH_3/X} = \frac{c\,mol_{NH_3}}{mol_X} = \frac{0.24\,mol_{NH_3}}{mol_X}$$
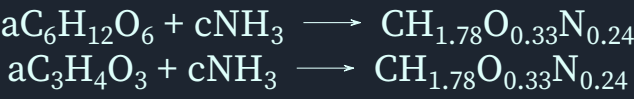
(ii) Oxygen

Questão 8

Aerobacter aerogenes is grown aerobically on glucose ($C_6H_{12}O_6$) or pyruvate ($C_3H_4O_3$) 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/O_2}	
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

$$aC_6H_{12}O_6 + cNH_3 \longrightarrow CH_{1.78}O_{0.33}N_{0.24}$$
$$aC_3H_4O_3 + cNH_3 \longrightarrow CH_{1.78}O_{0.33}N_{0.24}$$

$$\frac{\max Y_{X/G}}{\exp Y_{X/G}} = \frac{\left(\frac{mol_X}{a\,mol_G} \frac{22.5\,g_X}{mol_X} \right)}{};$$

$$Y_{X/P} = \frac{mol_X}{a\,mol_P} \frac{22.5\,g_X}{mol_X};$$

$$\gamma(C_3H_4O_6) = 3*2 + 4*1 - 1*2 = 8$$

Q8 b.

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

Resposta

$$aC_6H_{12}O_6 + cNH_3 \longrightarrow CH_{1.78}O_{0.33}N_{0.24} + dCO_2 + eH_2O$$
$$aC_3H_4O_3 + cNH_3 \longrightarrow CH_{1.78}O_{0.33}N_{0.24} + dCO_2 + eH_2O$$
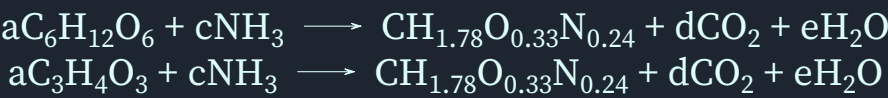
$$\gamma(C_6H_{12}O_6)\,a + \gamma(O_2)\,b = (6*4 + 12*1 - 6*2)\,a - 4\,b = 24\,a - 4\,b =$$
$$= \gamma(\text{biomassa}) = 4.4;$$