Problem 1.1

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

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

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:

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

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

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.

Problem 1.2

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

$$CH_{1.7}O_{0.45}N_{0.15} \ (MW_B = 23.0 \ g \ DCW/mole)$$
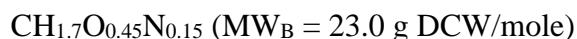
Carbon and nitrogen sources are glucose ($C_6H_{12}O_6$) and ammonium salts, respectively. Possible products of the growth reaction are biomass, ethanol (C_2H_6O), carbon dioxide and water. Ethanol growth and formation depend on growing conditions.

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?

Problem 1.3

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

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

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).

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).

Problem 1.5

The chemical reaction for glucose respiration is as follows:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$
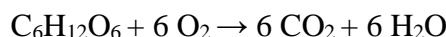
Candida utilis yeast converts glucose to CO_2 and H_2O as it grows. Its empirical formula is $CH_{1.84}O_{0.55}N_{0.2}$ plus 5% (w/w) ash. The substrate biomass yield is 0.5 (w/w). The source of nitrogen is ammonia.

a) Formulate the free electron balance equation.

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

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

Problem 1.6

The bacteria *Klebsiella aerogenes* grows in glycerol aerobically in a culture medium containing ammonia. The biomass contains 8% (w/w) of ash and the organic fraction has the empirical formula $CH_{1.75}O_{0.43}N_{0.22}$. It has been experimentally determined that 0.4 g of biomass is formed per gram of glycerol consumed. Evaluate oxygen yield on a mass basis.

Problem 1.7

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)

a) Assess ammonia requirements.

b) Assess oxygen requirements.

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?

Problem 1.8

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 $CH_{1.78}N_{0.24}O_{0.33}$ ($MW_B = 22.5$ g/mol) and its degree of reduction is = 4.4. The following yields were experimentally determined

Substrate	$Y_{x/s}$			$Y_{x/o2}$	
	g/g	g/mol	g/g-C	g/g	g/mol
Glucose	0.40	72.7	1.01	1.11	35.5
Pyruvate	0.20	17.9	0.49	0.48	15.4

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.

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