BIOPROCESS ENGINEERING

Basic Concepts

Second Edition (2002)

Michael L. Shuler
School of Chemical Engineering
Cornell University

Fikret Kargi
Department of Environmental Engineering
Dokuz Eylül University
Izmir, Turkey

7.3. STOICHIOMETRIC CALCULATIONS

7.3.1. Elemental Balances

A material balance on biological reactions can easily be written when the compositions of substrates, products, and cellular material are known. Usually, electron–proton balances are required in addition to elemental balances to determine the stoichiometric coefficients in bioreactions. Accurate determination of the composition of cellular material is a major problem. Variations in cellular composition with different types of organisms are shown in Table 7.3. A typical cellular composition can be represented as $CH_{1.8}O_{0.5}N_{0.2}$. One mole of biological material is defined as the amount containing 1 gram atom of carbon, such as $CH_{\alpha}O_{\beta}N_{\delta}$.

Consider the following simplified biological conversion, in which no extracellular products other than H_2O and CO_2 are produced.

$$CH_mO_n + a\,O_2 + b\,NH_3 \longrightarrow c\,CH_{\alpha}O_{\beta}N_{\delta} + d\,H_2O + e\,CO_2 \qquad (7.3)$$

TABLE 7.3 Date on Elemental Composition of Several Microorganisms

Microorganism	Limiting Nutrient	μ (h^{-1})	Composition (% by wt) C	H	N	O	P	S	Ash	Empirical Chemical Formula	Formula "Molecular" Weight
Bacteria			53.0	7.3	12.0	19.0			8	$CH_{1.660}N_{0.20}O_{0.27}$	20.7
Bacteria			47.1	7.8	13.7	31.3				$CH_2N_{0.25}O_{0.5}$	25.5
Aerobacter aerogenes			48.7	7.3	13.9	21.1			8.9	$CH_{1.79}N_{0.24}O_{0.33}$	22.5
Klebsiella aerogenes	Glycerol	0.1	50.6	7.3	13.0	29.0				$CH_{1.74}N_{0.22}O_{0.43}$	23.7
K aerogenes	Glycerol	0.85	50.1	7.3	14.0	28.7				$CH_{1.73}N_{0.24}O_{0.43}$	24.0
Yeast			47.0	6.5	7.5	31.0			8	$CH_{1.66}N_{0.13}O_{0.40}$	23.5
Yeast			50.3	7.4	8.8	33.5				$CH_{1.75}N_{0.15}O_{0.5}$	23.9
Yeast			44.7	6.2	8.5	31.2	1.08	0.6		$CH_{1.64}N_{0.16}O_{0.52}P_{0.01}S_{0.005}$	26.9
Candida utilis	Glucose	0.08	50.0	7.6	11.1	31.3				$CH_{1.82}N_{0.19}O_{0.47}$	24.0
C. utilis	Glucose	0.45	46.9	7.2	10.9	35.0				$CH_{1.84}N_{0.2}O_{0.56}$	25.6
C. utilis	Ethanol	0.06	50.3	7.7	11.0	30.8				$CH_{1.82}N_{0.19}O_{0.46}$	23.9
C. utilis	Ethanol	0.43	47.2	7.3	11.0	34.6				$CH_{1.84}N_{0.2}O_{0.55}$	25.5

With permission, from B. Atkinson and F. Mavituna, *Biochemical Engineering and Biotechnology Handbook*, Macmillan, Inc., New York, 1983.

where CH_mO_n represents 1 mole of carbohydrate and $CH_\alpha O_\beta N_\delta$ stands for 1 mole of cellular material. Simple elemental balances on C, H, O, and N yield the following equations:

$$\begin{aligned} &\text{C:} \quad 1 = c + e \\ &\text{H:} \quad m + 3b = c\alpha + 2d \\ &\text{O:} \quad n + 2a = c\beta + d + 2e \\ &\text{N:} \quad b = c\delta \end{aligned} \tag{7.4}$$

The respiratory quotient (RQ) is

$$\text{RQ} = \frac{e}{a} \tag{7.5}$$

Equations 7.4 and 7.5 constitute five equations for five unknowns a, b, c, d, and e. With a measured value of RQ, these equations can be solved to determine the stoichiometric coefficients.

7.3.2. Degree of Reduction

In more complex reactions, as in the formation of extracellular products, an additional stoichiometric coefficient is added, requiring more information. Also, elemental balances provide no insight into the energetics of a reaction. Consequently, the concept of *degree of reduction* has been developed and used for proton–electron balances in bioreactions. The degree of reduction, γ, for organic compounds may be defined as the number of equivalents of available electrons per gram atom C. The available electrons are those that would be transferred to oxygen upon oxidation of a compound to CO_2, H_2O, and NH_3. The degrees of reduction for some key elements are C = 4, H = 1, N = −3, O = −2, P = 5, and S = 6. The degree of reduction of any element in a compound is equal to the valence of this element. For example, 4 is the valence of carbon in CO_2 and −3 is the valence of N in NH_3. Degrees of reduction for various organic compounds are given in Table 7.4. The following are examples of how to calculate the degree of reduction for substrates.

Methane (CH_4): $1(4) + 4(1) = 8, \quad \gamma = 8/1 = 8$

Glucose ($C_6H_{12}O_6$): $6(4) + 12(1) + 6(-2) = 24, \quad \gamma = 24/6 = 4$

Ethanol (C_2H_5OH): $2(4) + 6(1) + 1(-2) = 12, \quad \gamma = 12/2 = 6$

A high degree of reduction indicates a low degree of oxidation. That is, $\gamma_{CH_4} > \gamma_{EtOH} > \gamma_{glucose}$.

Consider the aerobic production of a single extracellular product.

$$\underset{\text{substrate}}{CH_mO_n} + aO_2 + bNH_3 \longrightarrow \underset{\text{biomass}}{cCH_\alpha O_\beta N_\delta} + \underset{\text{product}}{dCH_xO_yN_z} + eH_2O + fCO_2 \tag{7.6}$$

The degrees of reduction of substrate, biomass, and product are

$$\gamma_s = 4 + m - 2n \tag{7.7}$$

TABLE 7.4 Degree of Reduction and Weight of One Carbon Equivalent of One Mole of Some Substrates and Biomass

Compound	Molecular Formula	Degree of Reduction, γ	Weight, m
Biomass	$CH_{1.64}N_{0.16}O_{0.52}$ $P_{0.0054}S_{0.005}$[a]	4.17 (NH_3) 4.65 (N_2) 5.45 (HNO_3)	24.5
Methane	CH_4	8	16.0
n-Alkane	$C_{15}H_{32}$	6.13	14.1
Methanol	CH_4O	6.0	32.0
Ethanol	C_2H_6O	6.0	23.0
Glycerol	$C_3H_8O_3$	4.67	30.7
Mannitol	$C_6H_{14}O_6$	4.33	30.3
Acetic acid	$C_2H_4O_2$	4.0	30.0
Lactic acid	$C_3H_6O_3$	4.0	30.0
Glucose	$C_6H_{12}O_6$	4.0	30.0
Formaldehyde	CH_2O	4.0	30.0
Gluconic acid	$C_6H_{12}O_7$	3.67	32.7
Succinic acid	$C_4H_6O_4$	3.50	29.5
Citric acid	$C_6H_8O_7$	3.0	33.5
Formic acid	CH_2O_2	2.0	46.0
Oxalic acid	$C_2H_2O_4$	1.0	45.0

With permission, from B. Atkinson and F. Mavituna, *Biochemical Engineering and Biotechnology Handbook*, Macmillan, Inc., New York, 1983.

$$\gamma_b = 4 + \alpha - 2\beta - 3\delta \tag{7.8}$$

$$\gamma_p = 4 + x - 2y - 3z \tag{7.9}$$

Note that for CO_2, H_2O, and NH_3 the degree of reduction is zero.

Equation 7.6 can lead to elemental balances on C, H, O, and N, an available electron balance, an energy balance, and a total mass balance. Of the equations, only five will be independent. If all the equations are written, then the extra equations can be used to check the consistency of an experimental data set. Because the amount of water formed or used in such reactions is difficult to determine and water is present in great excess, the hydrogen and oxygen balances are difficult to use. For such a data set, we would typically choose a carbon, a nitrogen, and an available-electron balance. Thus,

$$c + d + f = 1 \tag{7.10}$$

$$c\delta + dz = b \tag{7.11}$$

$$c\gamma_b + d\gamma_p = \gamma_s - 4a \tag{7.12}$$

With partial experimental data, it is possible to solve this set of equations. Measurements of RQ and a yield coefficient would, for example, allow the calculation of the remaining coefficients. It should be noted that the coefficient, c, is $Y_{X/S}$ (on a molar basis) and d is $Y_{P/S}$ (also on a molar basis).

An energy balance for aerobic growth is

$$Q_0 c\gamma_b + Q_0 d\gamma_p = Q_0\gamma_s - Q_0 4a \tag{7.13}$$

If Q_0, the heat evolved per equivalent of available electrons transferred to oxygen, is constant, eq. 7.13 is *not* independent of eq. 7.12. Recall that an observed regularity is 26.95 kcal/g equivalent of available electrons transferred to oxygen, which allows the prediction of heat evolution based on estimates of oxygen consumption.

Equations 7.12 and 7.13 also allow estimates of the fractional allocation of available electrons or energy for an organic substrate. Equation 7.12 can be rewritten as

$$1 = \frac{c\gamma_b}{\gamma_s} + \frac{d\gamma_p}{\gamma_s} + \frac{4a}{\gamma_s} \tag{7.14a}$$

$$1 = \xi_b + \xi_p + \varepsilon \tag{7.14b}$$

where ε is the fraction of available electrons in the organic substrate that is transferred to oxygen, ξ_b is the fraction of available electrons that is incorporated into biomass, and ξ_p is the fraction of available electrons that is incorporated into extracellular products.

Example 7.1

Assume that experimental measurements for a certain organism have shown that cells can convert two-thirds (wt/wt) of the substrate carbon (alkane or glucose) to biomass.

a. Calculate the stoichiometric coefficients for the following biological reactions:

$$\text{Hexadecane: } C_{16}H_{34} + a\,O_2 + b\,NH_3 \longrightarrow c(C_{4.4}H_{7.3}N_{0.86}O_{1.2}) + d\,H_2O + e\,CO_2$$

$$\text{Glucose: } C_6H_{12}O_6 + a\,O_2 + b\,NH_3 \longrightarrow c(C_{4.4}H_{7.3}N_{0.86}O_{1.2}) + d\,H_2O + e\,CO_2$$

b. Calculate the yield coefficients $Y_{X/S}$ (g dw cell/g substrate), Y_{X/O_2} (g dw cell/g O_2) for both reactions. Comment on the differences.

Solution

a. For hexadecane,

amount of carbon in 1 mole of substrate = 16(12) = 192 g

amount of carbon converted to biomass = 192(2/3) = 128 g

Then, $128 = c(4.4)(12)$; $c = 2.42$.

amount of carbon converted to CO_2 = 192 − 128 = 64 g

$$64 = e\,(12), \qquad e = 5.33$$

The nitrogen balance yields

$$14b = c(0.86)(14)$$

$$b = (2.42)(0.86) = 2.085$$

The hydrogen balance is

$$34(1) + 3b = 7.3c + 2d$$

$$d = 12.43$$

The oxygen balance yields

$$2a(16) = 1.2c(16) + 2e(16) + d(16)$$

$$a = 12.427$$

For glucose,

amount of carbon in 1 mole of substrate = 72 g

amount of carbon converted to biomass = 72(2/3) = 48 g

Then, $48 = 4.4c(12)$; $c = 0.909$.

amount of carbon converted to CO_2 = 72 − 48 = 24 g

$$24 = 12e; \qquad e = 2$$

The nitrogen balance yields

$$14b = 0.86c(14)$$

$$b = 0.782$$

The hydrogen balance is

$$12 + 3b = 7.3c + 2d$$

$$d = 3.854$$

The oxygen balance yields

$$6(16) + 2(16)a = 1.2(16)c + 2(16)e + 16d$$

$$a = 1.473$$

b. For hexadecane,

$$Y_{X/S} = \frac{2.42(\text{MW})_{\text{biomass}}}{(\text{MW})_{\text{substrate}}}$$

$$Y_{X/S} = \frac{2.42(91.34)}{226} = 0.98 \text{ gdw cells/g substrate}$$

$$Y_{X/O_2} = \frac{2.42(\text{MW})_{\text{biomass}}}{12.43(\text{MW})_{O_2}}$$

$$Y_{X/O_2} = \frac{2.42(91.34)}{(12.43)(32)} = 0.557 \text{ gdw cells/g } O_2$$

For glucose,

$$Y_{X/S} = \frac{(0.909)(91.34)}{180} = 0.461 \text{ gdw cells/g substrate}$$

$$Y_{X/O_2} = \frac{(0.909)(91.34)}{(1.473)(32)} = 1.76 \text{ gdw cells/g } O_2$$

The growth yield on more reduced substrate (hexadecane) is higher than that on partially oxidized substrate (glucose), assuming that two-thirds of all the entering carbon is incorporated in cellular structures. However, the oxygen yield on glucose is higher than that on the hexadecane, since glucose is partially oxidized.

7.4. THEORETICAL PREDICTIONS OF YIELD COEFFICIENTS

In aerobic fermentations, the growth yield per available electron in oxygen molecules is approximately 3.14 ± 0.11 gdw cells/electron when ammonia is used as the nitrogen source. The number of available electrons per oxygen molecule (O_2) is four. When the number of oxygen molecules per mole of substrate consumed is known, the growth yield coefficient, $Y_{X/S}$, can easily be calculated. Consider the aerobic catabolism of glucose.

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

The total number of available electrons in 1 mole of glucose is 24. The cellular yield per available electron is $Y_{X/S} = 24(3.14) = 76$ gdw cells/mol.

The predicted growth yield coefficient is $Y_{X/S} = 76/180 = 0.4$ gdw cells/g glucose. Most measured values of $Y_{X/S}$ for aerobic growth on glucose are 0.38 to 0.51 g/g (see Table 6.1).

The ATP yield ($Y_{X/ATP}$) in many anaerobic fermentations is approximately 10.5 ± 2 gdw cells/mol ATP. In aerobic fermentations, this yield varies between 6 and 29. When the energy yield of a metabolic pathway is known (N moles of ATP produced per gram of substrate consumed), the growth yield $Y_{X/S}$ can be calculated using the following equation:

$$Y_{X/S} = Y_{X/ATP}\,N$$

Example 7.2

Estimate the theoretical growth and product yield coefficients for ethanol fermentation by *S. cerevisiae* as described by the following overall reaction:

$$C_6H_{12}O_6 \longrightarrow 2\,C_2H_5OH + 2\,CO_2$$

Solution Since $Y_{X/ATP} \approx 10.5$ gdw/mol ATP and since glycolysis yields 2 ATP/mol of glucose in yeast,

$$Y_{X/S} \approx 10.5 \text{ gdw/mol ATP} \cdot 2\frac{\text{moles ATP}}{180 \text{ g glucose}}$$

or

$$Y_{X/S} \approx 0.117 \text{ gdw/g glucose}$$

For complete conversion of glucose to ethanol by the yeast pathway, the maximal yield would be

$$Y_{P/S} = \frac{2(46)}{180} = 0.51 \text{ g ethanol/g glucose}$$

while for CO_2 the maximum yield is

$$Y_{CO_2/S} = \frac{2(44)}{180} = 0.49 \text{ g ethanol/g glucose}$$

In practice, these maximal yields are not obtained. The product yields are about 90% to 95% of the maximal values, because the glucose is converted into biomass and other metabolic by-products (e.g., glycerol or acetate).

7.5. SUMMARY

Simple methods to determine the reaction stoichiometry for bioreactors are reviewed. These methods lead to the possibility of predicting yield coefficients for various fermentations using a variety of substrates. By coupling these equations to experimentally measurable parameters, such as the respiratory quotient, we can infer a great deal about the progress of a fermentation. Such calculations can also assist in initial process design equations by allowing the prediction of the amount of oxygen required (and consequently heat generated) for a certain conversion of a particular substrate. The prediction of yield coefficients is not exact, because unknown or unaccounted for metabolic pathways and products are present. Nonetheless, such calculations provide useful first estimates of such parameters.

SUGGESTIONS FOR FURTHER READING

ATKINSON, B., AND F. MAVITUNA, *Biochemical Engineering and Biotechnology Handbook*, Macmillan, Inc., New York, 1983.

BAILEY, J. E., AND D. F. OLLIS, *Biochemical Engineering Fundamentals*, 2d ed., McGraw-Hill Book Co., New York, 1986.

ERICKSON, L. E., AND D. Y.-C. FUNG, *Handbook on Anerobic Fermentations*, Marcel Dekker, Inc., New York, 1988. (Five chapters deal with bioenergetics, stoichiometry, and yields.)

———, I. G. MINKEVICH, AND V. K. EROSHIN, Application of Mass and Energy Balance Regularities in Fermentation, *Biotechnol. Bioeng. 20*:1595, 1978.

MINKEVICH, I. G., Mass and Energy Balance for Microbial Product Synthesis: Biochemical and Cultural Aspects, *Biotechnol. Bioeng. 25*:1267, 1983.

ROELS, J. A., *Energetics and Kinetics in Biotechnology*, Elsevier Science Publishing, New York, 1983.