Appendix C Calculation of Energetic and Biochemical Equivalents of Respiratory Oxygen Consumption

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1 Introduction

The evaluation of metabolic energy loss is the major aim of respiratory studies in ecological energetics and for the construction of energy budgets. The polarographic oxygen sensor is becoming the prominent tool for indirect calorimetry in laboratory as well as in field investigations (Parts II and III). In this context a comprehensive outline of the calculation procedures for conversion of oxygen consumption data to energy equivalents is warranted. A more rigorous treatment of the subject will be presented elsewhere [6].

2 Stoichiometry

2.1 Respiratory Quotient and Nitrogen Quotient

The respiratory exchange ratio or respiratory quotient, RQ or ν_{CO_2/O_2} (moles of CO_2 liberated per mole of O_2 consumed), represents a well-known concept for the estimation of the oxycaloric equivalent in relation to the relative proportions of carbohydrate and lipid respired. The oxidation of carbohydrate involves equal amounts of carbon dioxide and oxygen ($RQ = 1$), but, due to the more reduced state of lipids, less CO_2 is liberated per mole of oxygen in the oxidation of fat ($RQ = 0.72$). The RQ for protein varies as a function of the excretory product. Taking alanine as an example,

$$NH_2CHCH_3COOH + 3\ O_2 \rightarrow 2.5\ CO_2 + 1.5\ H_2O + 0.5\ CO(NH_2)_2 \qquad (1a)$$

$$NH_2CHCH_3COOH + 3\ O_2 \rightarrow 3\ CO_2 + 2\ H_2O + NH_3, \qquad (1b)$$

the respiratory quotient for urea, RQ_{urea}, equals 0.83, whereas for ammonia RQ_{NH_3} is 1.0.

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The ammonia RQ ranged from 0.94 to 0.99 for bacterial, plant and animal proteins as calculated from their amino acid compositions [6].

In addition to the respiratory quotient, the nitrogen quotient, NQ [ν_{N/O_2}, moles of N excreted per mole of O_2 consumed, i.e., moles of NH_3 per O_2 or 2 x moles of $CO(NH_2)_2$ per O_2], must be known in order to attain accurate energetic and biochemical interpretations of oxygen consumption data. For proteins from various sources, NQ averages 0.27 ± 0.01 (Table 1).

RQ and NQ are often expressed in terms of volume ratios. These values are unclear if the use of ideal gas volumes (V_o = 22.414 dm^3 mol^{-1}), or of real gas volumes, V_m [dm^3 mol^{-1}], at STP [7] for oxygen (22.392), CO_2 (22.262), and NH_3 (22.117) is not specified. Real volume-based values of RQ should be multiplied by 1.006 and those of NQ by 1.012 to convert them to molar ratios.

2.2 Oxygen Consumption and Respiratory Substrates

The proportion of different respiratory substrates can be calculated on the basis of oxygen consumption measurements and accompanying RQ and NQ values. The principal approach for distinguishing between two substrate categories in maintenance metabolism has been outlined in classical textbooks [9]. Equation (2) summarises the stoichiometric analysis extended to separate the mass fractions of carbohydrate, lipid and protein, w_K, w_L and w_P respectively. With reference atomic compositions shown in Table 2 we obtain for ammonioteles (see Fig. 1),

$$w_K = \frac{-0.72 + RQ - 0.9259 \times NQ}{-0.3070 + 0.5870 \times RQ - 0.1298 \times NQ} \quad (2a)$$

$$w_L = \frac{1 - RQ - 0.1111 \times NQ}{-0.7432 + 1.4212 \times RQ - 0.3142 \times NQ} \quad (2b)$$

$$w_P = \frac{NQ}{-0.3646 + 0.6971 \times RQ - 0.1541 \times NQ} \; . \quad (2c)$$

Corrected RQ values have to be inserted into Eq. (2) if a mole fraction x_{urea} of nitrogen is excreted as urea.

$$RQ \text{ (corrected)} = RQ \text{ (measured)} + 0.5 \times y_{urea} \times NQ. \quad (2d)$$

Similarly, the loss of ash-free organic biomass catabolised per mole of oxygen respired, $\Delta_k W_{O_2}$ [g (mol O_2)$^{-1}$], can be assessed as a function of RQ and NQ (Fig. 2),

$$\Delta_k W_{O_2} = 29.63 - 56.66 \times RQ + 12.52 \times NQ. \quad (3)$$

If the fractions of the catabolised substrates, w_i, are known without information on CO_2 production and nitrogen excretion, then

$$\Delta_k W_{O_2} = 1/\sum_i \frac{w_i}{\Delta_k W_{O_2}(i)}, \quad (4)$$

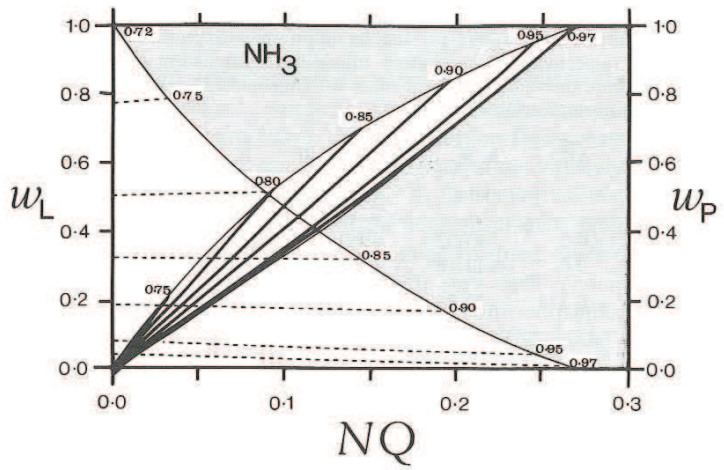

Fig. 1. Mass fractions of respiratory substrates in aerobic dissipative metabolism of ammoniotelic organisms as a function of the nitrogen quotient, NQ, and the respiratory quotient, RQ (*numbers*). *Full lines* mass fraction of protein, w_P, for different values of RQ; *broken lines* mass fraction of lipid, w_L, for different values of RQ. The mass fraction of carbohydrate, w_K, is obtained as

$$w_K = 1 - w_L - w_P$$

RQ/NQ combinations extending into the *hatched area* indicate the presence of anabolic or partial anoxic metabolism [see Eq. (2)]

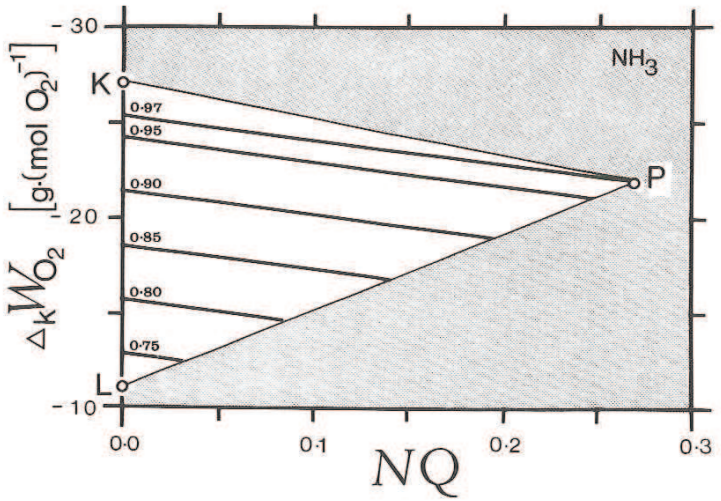

Fig. 2. Organic mass (ash-free dry weight) catabolised per mole of oxygen respired, $\Delta_k W_{O_2}$, as a function of the nitrogen quotient, NQ, and the respiratory quotient, RQ, with ammonia as the excretory product. The *full lines* display $\Delta_k W_{O_2}$ for different RQ values indicated by *numbers*. *Circles* (K, L, P) designate the pure reference substrates [see Eq. (3)]

Table 1. Substrate specific respiratory quotients, RQ_i, nitrogen quotients, NQ_i, catabolic weight equivalents of oxygen consumption, $\Delta_k W_{O_2}(i)$, and oxycaloric and oxyenthalpic equivalents, $\Delta_k H_{O_2}(i)$ and $\Delta_c H_{O_2}(i)$ respectively, in respiratory (dissipative) metabolism of aquatic organisms. For a protein substrate the values are given either for ammoniotelic organisms (protein → NH_3) or for ureotelic organisms (protein → urea) [6]

Substrate, i	RQ_i	NQ_i	$\Delta_k W_{O_2}(i)$ g mol^{-1}	$\Delta_k H^\circ_{O_2}(i)$ kJ mol^{-1}	$\Delta_k H'_{O_2}(i)$ kJ mol^{-1}	$\Delta_c H_{O_2}(i)$ kJ mol^{-1}
Carbohydrate	1.0	0.0	−27.02	−471	−478	−473
Lipid	0.72	0.0	−11.16	−440	−445	−441
Protein → NH_3	0.97	0.27	−21.95	−447	−451	−527
Protein → urea	0.84			−438	−443	

Table 2. Chemical and thermodynamic specification of standard carbohydrate (glycogen, starch), lipid (triacylglycerol) and protein (average amino acid composition). $\Delta_c h_i$ is the specific enthalpy of combustion obtained by bomb calorimetry [2]. $\Delta_c H_C(i)$ is the substrate specific molar enthalpy of combustion based on carbon

Substrate, i	Atomic composition	$\Delta_c h_i$ kJ g^{-1}	$\Delta_c H_C(i)$ kJ (mol C)$^{-1}$
Carbohydrate	$(C_6 H_{10} O_5)_n$	−17.5	−473
Lipid	$(C_{18.8} H_{33.0} O_2)_3$	−39.5	−611
Protein	$(C_{4.83} H_{7.58} O_{1.50} N_{1.35} S_{0.025})_n$	−24.0	−543

where i indicates the three substrates (K, L and P), and $\Delta_k W_{O_2}(i)$ are the substrate-specific weight equivalents of oxygen consumption (Table 1).

3 Indirect Calorimetry: Two Objectives

Insufficient attention has been paid to the conceptual disparity of two closely related functions of indirect calorimetry. The classical aim encountered a recent rennaissance with aquatic animals: Calorimetrically determined rates of heat dissipation are compared with heat changes of metabolic reactions (Chaps. II.3, II.4) [1, 5, 9]. Oxygen uptake measurements, $\dot{N}_{O_2}$, are converted to rates of *heat* dissipation, $\dot{Q}$, with appropriately derived oxy*caloric* equivalents, $\Delta_k H_{O_2}$,

$$_k\dot{Q} = \Delta_k H_{O_2} \times \dot{N}_{O_2}. \quad (5)$$

In the context of physiological and ecological energy balance studies, however, the oxy*enthalpic* equivalent, $\Delta_c H_{O_2}$, should be used to convert oxygen consumption to values of *enthalpies of combustion* of the aerobically catabolised biomass.

3.1 The Oxycaloric Equivalent, $\Delta_k H_{O_2}$

Chemical stoichiometries and the corresponding enthalpies of reaction form the basis of calculating the caloric equivalent of oxygen consumption in dissipative metabolism, $\Delta_k H_{O_2}$, in units [kJ (mol O_2)$^{-1}$] (for conversion factors between different units see Tables 3 and 4)[2]. Earlier calculations were based on enthalpies of combustion as determined in bomb calorimeters, e.g. [1, 4, 8, 9]. For calculating heat dissipation of aquatic organisms, consideration of the aqueous phase is more appropriate than reliance upon thermochemical values pertaining to dried solids or undissolved gases [5, 11]. Enthalpies of solution and dilution are very small in comparison to the large enthalpies of oxidation, but they were, together with enthalpies of dissociation, incorporated in the present calculations of the standard oxycaloric equivalent, $\Delta_k H^\circ_{O_2}$. An important side reaction is the conversion of ammonia to ammonium ion with an enthalpy of protonation of −52 kJ mol^{-1} [10] and neglect thereof [3] entails a significant error. While other neutralization reactions are very important in anoxic metabolism [5], they increase $\Delta_k H^\circ_{O_2}$ by < 3% under ecological conditions. Their effect is indicated by listing the oxycaloric equivalents, $\Delta_k H'_{O_2}$ for pH 7 and an effective enthalpy of neutralization, $\Delta_b H'_{H+}$, of −8 kJ (mol H^+)$^{-1}$ (Table 1),

$$\Delta_k H'_{O_2} = \Delta_k H^\circ_{O_2} + \nu'_{H^+/O_2} \times \Delta_b H'_{H+}. \quad (6)$$

An approximate calculation of the stoichiometric coefficient of protons released per mole of oxygen, ν'_{H+/O_2}, suffices for practical purposes,

$$\nu'_{H+/O_2} \approx RQ \times \frac{1}{1 + \exp(pK' - pH)} - x_{NH_3} \times NQ, \quad (7)$$

where pK' is the apparent dissociation constant of carbonic acid (6.37 in pure water [7]), and x_{NH_3} is the mole fraction of nitrogen excreted as ammonia (= $1 - x_{urea}$).

For aquatic ammonio-ureotelic organisms the oxycaloric equivalent, $\Delta_k H_{O_2}$ [kJ (mol O_2)$^{-1}$] can be accurately calculated on the basis of RQ, NQ and the nature of the excretory product (Fig. 3),

$$\Delta_k H^\circ_{O_2} = -360 - 111 \times RQ + (77 - 20 \times x_{urea}) \times NQ \quad (8a)$$

$$\Delta_k H'_{O_2} = -360 - 118 \times RQ + (87 - 27 \times x_{urea}) \times NQ. \quad (8b)$$

Equation (8b) is calculated for the conditions pH = 7 and $\Delta_b H'_{H+} = -8$ kJ (mol H^+)$^{-1}$. The oxycaloric equivalent can also be calculated from the mass fractions, w_i (carbohydrate, lipid and protein),

2 In $\Delta_k H_{O_2}$ the suffix "k" stands for "catabolic half cycle" [5] to make it clear that this expression does not apply to coupled catabolic reactions with a net gain of ATP from phosphorylation of ADP, but pertains to the dissipative conversion of substrates to carbon dioxide, water, and nitrogenous endproducts without storage of high energy intermediates (see App. E)

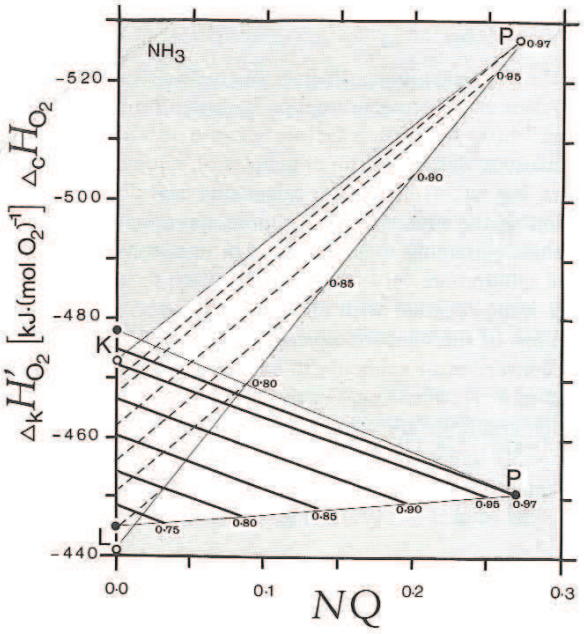

Fig. 3. The oxycaloric and oxyenthalpic equivalent, $\Delta_k H'_{O_2}$ and $\Delta_c H_{O_2}$ as a function of NQ and RQ with ammonia as the excretory product. *Full lines* $\Delta_k H'_{O_2}$ for different values of RQ indicated by numbers; *broken lines* $\Delta_c H_{O_2}$ for different values of RQ [see Eqs. (8, 14)]

$$\Delta_k H_{O_2} = \sum_i w_i \times \frac{\Delta_k W_{O_2}}{\Delta_k W_{O_2}(i)} \times \Delta_k H_{O_2}(i). \tag{9}$$

$\Delta_k W_{O_2} / \Delta_k W_{O_2}(i)$ is a correction factor for converting mass fractions, w_i, into molar fractions of oxygen consumption, $x_{O_2}(i)$ (= oxygen consumed for a particular substrate, i, per total oxygen consumption). $\Delta_k W_{O_2}$ is calculated from Eq. (4) and the substrate specific constants are listed in Table 1.

3.2 The Oxyenthalpic Equivalent, $\Delta_c H_{O_2}$

The fundamental importance of correct energy conversion factors for oxygen consumption data is reflected in the bioenergetic balance equation,

$$P = A + R + U. \tag{10}$$

Production, P, is the result of the net flow of energy into the open system, A (assimilation), and the flow of energy out of the system due to respiration, R, and excretion,

U. The flows of assimilated and excreted energy are bound to the exchange of organic matter. Dry biomass is converted into energy equivalents by bomb calorimetry, yielding specific enthalpies of combustion, $\Delta_c h$ [kJ g^{-1}]. Respiratory energy flow, R, relates to the flow of heat from an isothermal biological system. Actual heat effects occurring in the aqueous phase, however, are not at issue in this context. To satisfy the energy conservation law of thermodynamics in Eq. (10) the same reference state has to be chosen for metabolic loss and assimilatory gain. For this sake oxygen uptake, $\dot{N}_{O_2}$, is converted to the *enthalpy of combustion* equivalent of the catabolised organic mass, ${}_H R$, with consistently derived oxy*enthalpic* equivalents, $\Delta_c H_{O_2}$ [kJ (mol O_2)$^{-1}$],

$${}_H R = R + U = \Delta_c H_{O_2} \times \dot{N}_{O_2}. \tag{11}$$

The oxyenthalpic equivalent is directly related to specific enthalpies of combustion, $\Delta_c h$,

$$\Delta_c H_{O_2} = \Delta_c h \times \Delta_k W_{O_2}. \tag{12}$$

The organic mass that is catabolised per mole of oxygen consumed, $\Delta_k W_{O_2}$ [g (mol O_2)$^{-1}$], is calculated from Eqs. (3) or (4), and $\Delta_c h$ [kJ g^{-1}] is obtained (Table 2) as

$$\Delta_c h = w_K \times \Delta_c h_K + w_L \times \Delta_c h_L + w_P \times \Delta_c h_P. \tag{13}$$

The nitrogen correction which is essential in the oxycaloric equivalent is omitted in deriving the oxyenthalpic equivalent [Eq. (12)]. This renders calculations of the total metabolic energy (enthalpy) flow, ${}_H R$, straightforward and correct. The fate of protein-nitrogen determines merely the partitioning of ${}_H R$ between R and U, without any influence on the conservative total value of ${}_H R$. Appreciation of the different concepts of indirect calorimetry as represented by the oxyenthalpic and oxycaloric coefficient obliterates a frequently encountered bias in energy budget calculations. Metabolic losses were usually calcualted with oxycaloric equivalents. If energy losses in excretion were then considered insignificant, the correction for ammonia or urea excretion, implicit in $\Delta_k H_{O_2}$, should have been avoided. In fact, due to the uncorroborated nitrogen correction, metabolic losses may have been underestimated by up to 15%, since protein is predominantly catabolised by many fish and aquatic invertebrates.

Table 3. Conversion factors for units of the oxycaloric and oxyenthalpic equivalent (energy per amount of oxygen). These factors are based on the ideal molar gas volume (see App. A, Table 6) and on the thermodynamic calorie (= 4.1868 J). Often the thermochemical calorie (= 4.184 J) is used

		kJ (mol O_2)$^{-1}$	kJ (g O_2)$^{-1}$	kJ (dm O_2)$^{-3}$
1 mJ μmol^{-1}	=	1	0.031251	0.044615
1 J mg^{-1}	=	31.9988	1	1.4276
1 J cm^{-3}	=	22.414	0.70046	1
1 kcal mol^{-1}	=	4.1868	0.13084	0.18679
1 cal mg^{-1}	=	133.97	4.1868	5.9772
1 cal cm^{-3}	=	93.843	2.9327	4.1868

Table 4. Conversion factors for units of oxygen consumption (amount of oxygen utilized per time) and heat dissipation (power, energy per time) on the basis of a generalized oxycaloric equivalent, $\Delta_k H_{O_2} = -450$ kJ (mol O_2)$^{-1}$. $_k\dot{W}$ is the rate of weight loss (dry organic matter) due to aerobic catabolism of substrates with a catabolic weight equivalent of oxygen, $\Delta_k W_{O_2} = -19$ g (mol O_2)$^{-1}$. No attention is paid to the sign of the conversion factors. Heat and weight loss, however, should always have a negative sign

		$\dot{N}_{O_2}$		$_k\dot{Q}$		$_k\dot{W}$
		μmol O_2 h^{-1}	nmol O_2 s^{-1}	mW	J h^{-1}	mg h^{-1}
1 μmol O_2 h^{-1}	=	1	0.27778	0.1250	0.450	0.019
1 nmol O_2 s^{-1}	=	3.600	1	0.450	1.620	0.068
1 mg O_2 h^{-1}	=	31.251	8.6809	3.906	14.06	0.594
1 cm^3 O_2 h^{-1}	=	44.615	12.393	5.577	20.08	0.848
1 mW = 1 mJ s^{-1}	=	8.000	2.222	1	3.600	0.152
1 J h^{-1}	=	2.222	0.6173	0.27778	1	0.042
1 cal h^{-1}	=	9.304	2.584	1.1630	4.1868	0.177

Analogous to Eq. (8) the oxyenthalpic equivalent can be derived from *RQ*, *NQ* and constants in Table 1 (Fig. 3),

$$\Delta_c H_{O_2} = -359 - 114 \times RQ - (213 + 56 \times x_{urea}) \times NQ. \quad (14)$$

The highest accuracy in respiratory energetics is accomplished only if the appropriate conversion coefficients are applied (Table 4). These complement the increased fidelity of continuous oxygen uptake measurements achieved by POS.

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