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Review

Thermodynamic considerations in constructing energy balances for cellular growth

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Energy balances play an increasingly important role in quantitative descriptions of growth processes, both for biological research and for process design in biotechnology. This contribution contains a detailed description of how such balances can be set up and develops guidelines concerning the selection of accurate standard and reference states. Thermodynamic energy balances require a rigorous definition of the thermodynamic state of all components involved in the process, since the enthalpies of formation of any substance in different states may differ considerably. Biological processes generally take place in the aqueous state. This is particularly true for organisms living in an aqueous environment which is not in direct contact with the atmosphere. Thus, the aqueous state often defines the correct standard state for energy balances if the energy exchange is to be calculated for individual cells or for a specified unit of live biomass. If, however, a whole fermentor or growth vessel defines the system boundary, gaseous compounds such as O_2 and CO_2 are often exchanged predominantly in the gaseous state, which then defines the standard state to be used for these compounds. It is not possible to assign a well-defined state for living biomass, which may be described as a solid in an aqueous environment. For biomass, the corrections for the aqueous environment have been ignored in the past. Experimental work reported in this paper shows that this is basically justified, the magnitude of the corrections being too small to make a noticeable difference. Sample calculations concerning real, measured growth stoichiometries show that the differences between results based on correct standard states and approximate balances simply based on pure compound data are quite negligible for at least moderately aerobic growth. In anaerobic and fermentative growth, however, neglect in selecting the proper standard states results in errors up to 70%. Equally important are corrections for side-effects and side-reactions, such as evaporation and neutralization, which can give rise to 50% errors in anaerobic growth. Either constituent elements or combustion products of the involved chemical species may be used as reference states for constructing energy balances. Choosing the latter often simplifies calculations. Tables containing standard enthalpies of combustion for most important chemicals not only in their pure, but also in the biologically important aqueous standard states are presented.

I. Introduction

Energy balances play a considerable role in quantitative descriptions of biological growth processes. In research, they form the basis for biological calorimetry [1–6]. Enabling scientists to calculate the enthalpy changes during growth processes, such balances form the basis for understanding the energy exchange between living matter and its environment and for formulating theories about bioenergetics and biological growth efficiency [2,7–9]. In biotechnology, enthalpy balances are indispensable for the design of cooling facilities and can be utilized for the development, monitoring and control of bioprocesses [10–13].

Although biological growth processes are extremely complex in their nature and may involve open non-steady-state systems which exchange energy and matter with their surroundings, it is often still possible to consider the total process as a set of rather simple physico-chemical reactions. In doing so, it is common practice to approximate the enthalpic content of the reacting species in a very simple way by the molar enthalpies of the pure substrates and products of the growth reaction. In principle, however, one has to take into account that the reactions of biological origin almost always take place in an aqueous environment. As a result, the conventional standard states of solids, liquids and gases may not be appropriate approximations of the actual thermodynamic state (aqueous, gaseous, etc.) of participating substrates and products in describing the biological reactions [2,14–16].

In his work, Battley [14–16] paid considerable attention to correction factors and pointed out that no satisfactory standard state exists for the cellular material formed during a growth reaction. Attention has to be paid to the fact that the actual thermodynamic state, the 'wet' state of the cellular material, cannot easily be accounted for [2,17,18].

By not using the actual thermodynamic state of all reactants and products of a biological process, considerable errors may be introduced in the energy balance equations. Gnaiger [19] showed these errors to be as high as 70% when incorrect states are used, and Battley [15] estimated the discrepancies to be as high as 48% for an anaerobic growth reaction, compared to between 1 to 8% for an aerobic growth reaction with yeast. The aim of this paper is to investigate and demonstrate the importance of using correct thermodynamic states in establishing an energy balance of defined open systems. First, a general introduction is given into constructing energy balance equations for various experimental and biological systems. Basic data are presented for the calculation of enthalpy changes during growth.

A particular goal of this work was to investigate the importance of thermodynamic data for the actual 'wet' state of live biomass. Corresponding measurements are reported.

The importance of following these guidelines is then demonstrated in a discussion of numerical examples concerning aerobic and anaerobic growth processes.

II. Mass and energy balances for open and non-steady-state systems

II-A. General remarks on balances

Most thermodynamic expressions found in standard textbooks are based on mass and energy balances for closed systems which proceed from a defined initial

state to a time-invariant final state. On the other hand, open systems are routinely described in nonequilibrium thermodynamics. There, however, the emphasis shifts from energy balances to the time rates of entropy production and Gibbs energy changes of irreversible processes [20–21]. In the following analysis of the energetics of biological systems, general energy balances derived from classical thermodynamics are combined with the formalism of nonequilibrium thermodynamics. Since living systems do not reach a final thermodynamic equilibrium state, the changes of the thermodynamic parameters and the exchanged quantities are measured over defined periods of time.

Growth processes often involve open biological systems. This is very obvious when formulating balances around a certain quantity of biomass acting as a catalytic unit that transforms a number of substrates into more biomass and products. Similar situations are, however, also encountered when constructing balances around whole experimental devices such as fermentors, perfusion chambers of calorimeters or respirometers. Even culture vessels used in 'batch' experiments, which usually are regarded as being closed systems operating at non-steady state, often involve discontinuous or continuous feed streams, for instance in the form of injections or aeration of the liquid. Therefore, the most important principles involved in formulating mass and energy balances for open non-steady-state systems will be reviewed in this section.

Fig. 1 depicts an open system exchanging energy and matter with its surroundings. Before the formulation of any balance can even be attempted, the system boundaries must be clearly defined. In two definitions that are often used, the system boundaries are drawn around an experimental device such as a culture vessel, calorimeter or fermentor, or the system is chosen to be a unit of living biomass with its natural boundaries. The systems thus defined may be at steady state or in transients.

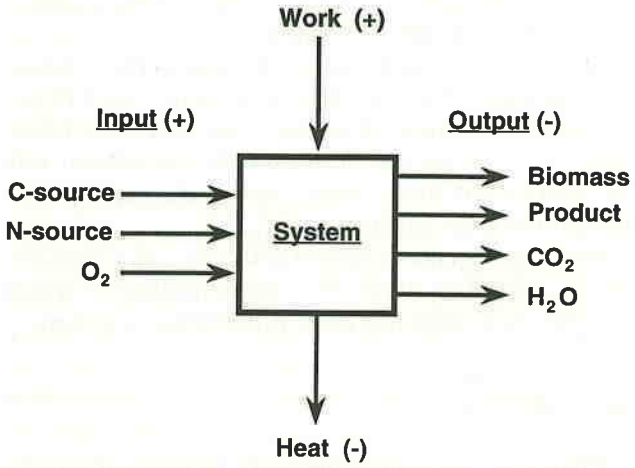


Fig. 1. Open system exchanging matter and energy with its environment. The system may either represent a unit live biomass such as a living cell or an experimental device such as a culture vessel and it may be at steady state or in a transient.

General balances for any considered quantity have the following structure:

$$\text{change of system} = \text{external} + \text{internal} \tag{2.1}$$

The external fluxes shown in Fig. 1 cross the system boundaries and correspond to the transfer term in Eqn. 2.1. In the system analysis any output is defined by a negative sign. The internal term on the right-hand side of Eqn. 2.1 describes the difference between all internal sources and sinks for the considered quantity and thus corresponds to the net rate of its production within the system, due to chemical reactions, and transition processes from one form into another. Any imbalance between input fluxes, output fluxes, sources and sinks must result in an accumulation or depletion of the stock of the considered entity in the system (left-hand term of Eqn. 2.1). At steady state all internal source terms are completely compensated by the external (transfer) terms, hence the change of the system is zero. One of the great potentials of thermodynamic balances is the fact that this 'change of the system' can be related to observable changes in state variables of the system, such as T, P, and composition.

II-B. Mass and molar balances

For open non-steady-state systems, it is advantageous to set up the mass balance first as a basis for complete energy balances. For the general mass balances, Eqn. 2.1 takes the following form:

$$\frac{dm}{dt} = \frac{d_{ext}m}{dt} + \frac{d_{int}m}{dt} \tag{2.2}$$

Total mass is conserved in all biochemical energy transformations. Therefore, internally mass is neither generated nor destroyed, $d_{int}m/dt = 0$.

This is different when mass or molar balances are written for each chemical substance involved in the system because they may be produced or consumed by chemical reactions. In order to account for the presence of chemicals in different phases, separate molar balances will be written for each chemical species instead of formulating a single balance for a chemical compound. A species is a chemical compound in a defined thermodynamic state such as solid, liquid, gaseous, or aqueous. Any chemical compound may therefore give rise to several different species according to the states of aggregation in which it can exist and according to whether it is pure (e.g., liquid) or dissolved in a solvent (e.g., aqueous). In this case, any

species i may be generated or consumed within the system as a result of chemical and biochemical reactions, phase transitions, and dilution in a solvent, thus giving rise to an internal term $d_r n_i/dt$. The subscript r denotes a general reaction, encompassing all processes mentioned above.

$$\frac{dn_i}{dt} = \frac{d_{ext}n_i}{dt} + \frac{d_r n_i}{dt} \tag{2.3}$$

The net generation rate of i (positive for products, negative for reactants) is obtained by summing up the effects of all these simultaneous processes j:

$$\frac{d_r n_i}{dt} = \sum_j \nu_{i,j} \frac{d_r \xi_j}{dt} \tag{2.4}$$

where $\nu_{i,j}$ is the stoichiometric number of species i in the j-th process (positive for products, negative for reactants; $+1$ or -1 if the process is just a phase transition). ξ_j is the extent of the j-th process with defined stoichiometry (see List of symbols). Any chemical reaction j can be written as a mass balance, $0 = \Sigma \nu_i M_i$, where M_i is the molar mass of i [g mol^{-1}].

Due to the lack of homogeneity in living systems, a rigorous calculation of $d_r \xi_j/dt$ requires defining local reaction rates which vary from one point to another. The rate, $d_r n_i/dt$, for the whole system must then be computed by integrating over the whole reaction volume. In practice, however, biological systems can often be treated as 'pseudohomogeneous' [7] by choosing the system size in a way to permit averaging over a large number of cells or organisms. Although the interior of each cell is highly inhomogeneous and a cell may be in a different phase of its cell cycle than its neighbors, the states and rates anywhere in the culture may be characterized by average values for a large population. If the cell culture is well mixed and the system size is chosen large enough, these average values will be the same for the whole system as for several subdomains at various locations. Such a system is said to be pseudohomogeneous.

Substituting Eqn 2.4 into Eqn 2.3, the complete molar balance for species i in a pseudohomogeneous system reads (Fig. 2a),

$$\frac{dn_i}{dt} = \frac{d_{ext}n_i}{dt} + \sum_j \nu_{i,j} \frac{d_r \xi_j}{dt} \tag{2.5}$$

The time-course of the amount of i in the system can be described by integrating Eqn. 2.5 with respect to time. However, it must be emphasized that the terms $d_{ext}n_i/dt$ and $d_r \xi_j/dt$ do not represent real-time derivatives, but flows or rates. They are thus differentiated from real-time derivatives by an appropriate subscript after the d. When integrating Eqn. 2.5, the flows have to be treated as constants or as explicit functions

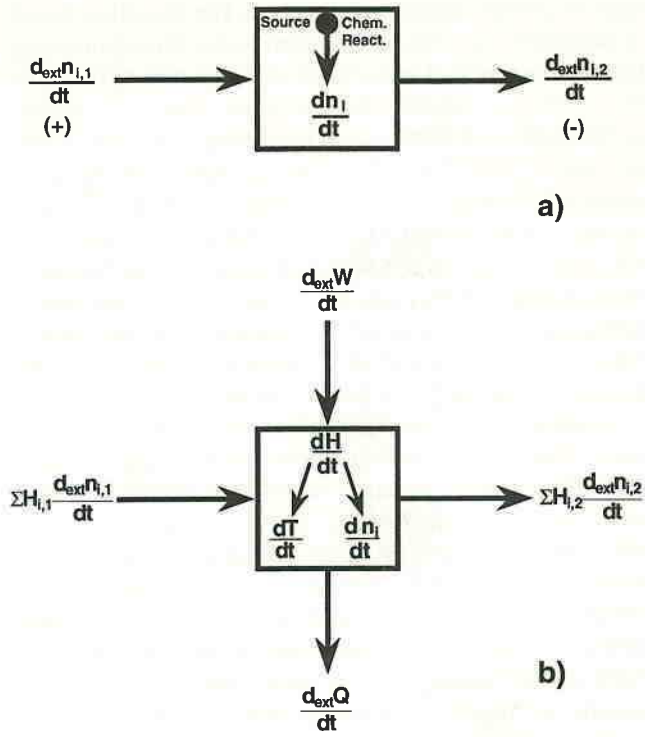


Fig. 2. Structures of molar and energy balances. (a) According to the molar balance, Eqn. 2.5, the change of the number of moles of species i stored within the system, n_i, is determined by the flows of substance i in and out of the system and by the rates of production or consumption by chemical reactions. (b) According to the enthalpy balance, Eqn. 2.10, the change of the enthalpy of the system is determined by the flows of work and heat into or out of the system as well as by the enthalpy imported or exported by flows of matter exchanged with the surroundings. Also shown is the fact that the enthalpy of the system is related to the measurable properties temperature and composition (n_1, $n_2 \ldots n_i$) of the system by Eqn. 2.11.

of time, but not as differentials. Only the left-hand side of Eqn. 2.5 reflects the change of the system and only it represents a real-time derivative.

In non-equilibrium thermodynamics, the internal terms in Eqn. 2.5 are considered as 'generalized flows'. $d_r \xi_j/dt$ is the extent of reaction per unit time, representing an internal chemical flow. In conjunction with the generalized force, these flows yield entropy production or power dissipation [21].

For a homogeneous system with only one phase, dn_i may be related to observable time derivatives of system variables by writing this exact differential as follows,

$$\frac{dn_i}{dt} = V\frac{dc_i}{dt} + c_i\frac{dV}{dt} \tag{2.6}$$

Therefore, the various flows of i by external transfer and internal generation (Eqn. 2.5) are directly linked to time derivatives of the observable system properties concentration, c_i, and volume, V.

II-C. Enthalpy balances

Under certain conditions, such as at constant pressure, the principles set forth in the previous sections can also be used to construct an enthalpy balance. This approach will be followed hereafter. The rigorous proof of the resulting equation (2.10) is provided in Appendix 1.

Consider a pseudohomogeneous system at constant pressure, in which reactions occur and which exchanges both mass and energy with its surroundings, such as depicted in Fig. 2b. For this system, Eqn. 2.1 translates into the following expression for enthalpy,

$$\frac{dH}{dt} = \frac{d_{ext}Q}{dt} + \frac{d_{ext}W}{dt} + \frac{d_{con}H}{dt} + \frac{d_{int}H}{dt} \tag{2.7}$$

It can be shown from the first law of thermodynamics that the rate of enthalpy production within the system is zero at constant pressure (see Appendix 1),

$$\frac{d_{int}H}{dt} = 0 \tag{2.8}$$

For open systems the net rate of enthalpy input, the so-called 'external enthalpy transfer', comprises three terms. Enthalpy may be exchanged with the environment in the form of (1) heat flow, $d_{ext}Q/dt$ and (2) work per unit time, which is power, $d_{ext}W/dt$ (unit = W). It should be born in mind that this work term does not include pressure/volume work. Rather, it refers to work such as stirring power in a fermentor, electrical work in bioelectrochemical systems or mechanical work done by muscle cells. Enthalpy is also imported by convection (3), i.e. by mass streams entering or leaving the system. The convective enthalpy transfer $d_{con}H/dt$ is often separated into an isothermal and a non-isothermal part denoted by $d_{mat}H/dt$ and by d_QH/dt, respectively:

$$\frac{d_{con}H}{dt} = \frac{d_{mat}H}{dt} + \frac{d_QH}{dt} \tag{2.9a}$$

The non-isothermal enthalpy import is the rate at which enthalpy is imported because of the fact that inflowing matter may be warmer or cooler than the system, whereas $d_{mat}H/dt$ groups all other effects of convective energy transport. The former plays a particular role in certain forms of biological calorimetry and in physiology. It is sometimes called 'convective heat exchange' and combined with $d_{ext}Q/dt$, although it represents internal energy rather than heat in the thermodynamic sense.

The enthalpy flows represented in Eqn. 2.9a are related to $d_{ext}n_i/dt$ appearing in the molar balance (Eqn. 2.5) as follows:

$$\frac{d_{mat}H}{dt} = \sum_e \sum_i H_{i,e}(T) \frac{d_{ext}n_{i,e}}{dt} \tag{2.9b}$$

$$\frac{d_QH}{dt} = \sum_e \sum_i (H_{i,e} - H_{i,e}(T)) \frac{d_{ext}n_i}{dt} \tag{2.9c}$$

In this equation, $d_{ext}n_{i,e}/dt$ represents the flow of i into the systems at exchange site e, whereas $H_{i,e}$ is the partial molar enthalpy of species i entering through exchange site e. $H_{i,e}(T)$ is the partial molar enthalpy of species i at the condition of exchange site e, except for the temperature, which is taken at the system temperature T.

Exchange sites may be feed streams or overflow tubes if the culture vessel defines the system boundary, or different sites on the surface of an organism through which it exchanges mass with its surroundings if the organism defines the system boundary. Either system is expected to take up mass in varying magnitudes and compositions through the different exchange sites. Therefore, both $H_{i,e}$ and $d_{ext}n_{i,e}/dt$ are expected to vary from one exchange site to another (for an explanation for H_i, see list of symbols).

In a biological system, $d_{ext}n_i/dt$ might even be a continuously varying function from one point to another over its surface. Here, again, the surface and surrounding medium will be described as pseudohomogeneous by accounting only for an average term of $H_{i,e}d_{ext}n_{i,e}/dt$. In the experimental vessel, however, different exchange sites may still have to be considered for a given substance.

Substituting Eqns. 2.8 and 2.9 into Eqn. 2.7 yields the following differential form of the enthalpy balance,

$$\frac{dH}{dt} = \frac{d_{ext}Q}{dt} + \frac{d_{ext}W}{dt} + \sum_e \sum_i H_{i,e}(T) \frac{d_{ext}n_{i,e}}{dt} + \sum_e \sum_i (H_{i,e} - H_{i,e}(T)) \frac{d_{ext}n_{i,e}}{dt} \tag{2.10}$$

It must be stressed again that the only exact differential in Eqn. 2.10 is usually dH/dt, whereas $d_{ext}Q/dt$, $d_{ext}W/dt$ and $d_{ext}n_{i,e}/dt$ really represent exchange rates or external flows.

The left-hand-side term of Eqn. 2.10 represents the accumulation or depletion of the enthalpy stored in the system due to imbalances between input and output flows. The great potential of enthalpy balances stems from the fact that the enthalpy of the system, albeit an abstract quantity, can often be related in an unequivocal way to the measurable properties of the system, as shown in Fig. 2.

At constant pressure, the enthalpy of the system is related to temperature, T, of the system and to n_i of the various compounds in their specified thermodynamic state within the system. The enthalpy change

must therefore be given by a total differential as follows:

$$\frac{dH}{dt} = C_p \frac{dT}{dt} + \sum_i H_{i,s} \frac{dn_i}{dt} \tag{2.11}$$

where $H_{i,s}$ is the partial molar enthalpy of species i at the conditions within the system.

In practical calculations, Eqn. 2.10 is combined with Eqn. 2.11 to eliminate dH/dt:

$$C_p \frac{dT}{dt} + \sum_i \frac{dn_i}{dt} H_{i,s} = \frac{d_{ext}Q}{dt} + \frac{d_{ext}W}{dt} + \sum_e \sum_i H_{i,e}(T) \frac{d_{ext}n_i}{dt} + \sum_e \sum_i (H_{i,e} - H_{i,e}(T)) \frac{d_{ext}n_i}{dt} \tag{2.12}$$

Eqn. 2.12 represents a very general energy balance for non-steady-state open reacting systems. It can in theory be applied to check the energy budget of a system or to predict the heat dissipation rate, but its use requires measuring not only all rates at which the system exchanges each species with its environment, but also the rates at which each species is accumulated or depleted within the system (dn_i/dt) as well as the rate of temperature change.

In order to cut down on the necessary measurements, the change of the number of moles of i in the system can be calculated from a molar balance such as Eqn. 2.5. It accounts for the fact that in open multiphase systems the change of the number of moles of i in the system, dn_i/dt, can be brought about by chemical or biochemical reactions, by phase transitions and physical transformations, and by an exchange of entity i with the surroundings. The combination of Eqns 2.5 and 2.12 will be demonstrated for a somewhat simplified case in subsection II-D.

II-D. Application to simple real cases

II-D.1. Development of a realistic simplified enthalpy balance

In the cases considered below, we will make the following simplifying assumptions in addition to keeping the pressure constant.

(i) Any given biochemical species may enter the system by at most one exchange site and leave it by a maximum of one other exchange site. It is obvious that the state of a species in the exit stream may differ from the one in the feed stream with respect to concentration and temperature. If a unit of cellular biomass defines the system boundary, only one exchange site will be accounted for, which is the surface of the cells. All metabolites either enter or leave through this one site.

(ii) The system is assumed to be pseudohomogeneous in the sense defined above. This postulate implies that the conditions in the exit streams must reflect the interior of the system, such that temperature and concentrations in each of the emerging phases are equal to the temperature and concentrations in the different phases within the system:

$$T_{out} = T \tag{2.13a}$$

$$H_{i,out} = H_{i,s} \tag{2.13b}$$

(iii) A small number of 'processes' are assumed to describe all transformation phenomena occurring in the system. They include the physical transitions of the same compound from one thermodynamic state to another, spontaneous chemical reactions such as acid-base reactions, and the transformations of matter catalyzed by cellular growth. Despite the enormous complexity of the network of biochemical transformation resulting from cellular metabolism, it is assumed that the overall growth process can be approximated by one or several 'reactions' with fixed stoichiometry.

(iv) The concentration dependence of the partial molar enthalpy H_i is neglected. This is justified for the gaseous species by the fact that gas mixtures behave ideally at the pressure range encountered in biological experiments. Aqueous mixtures are far from ideal, but it will be assumed that solute concentrations are so low that the partial molar enthalpy of i is not much different from the molar enthalpy of i at infinite dilution (the slight difference existing between liquid substances in a highly concentrated, pure and an infinitely diluted state is allowed for by treating these two states as different species). As a result, the partial molar enthalpies in gaseous and liquid mixture may be approximated by the molar enthalpies of the respective chemical in the pure gaseous and in the aqueous form at infinite dilution, respectively. Such states, which are defined unequivocally with respect to state of aggregation and concentration, are known as *standard states*. They include the pure gaseous (g), the pure liquid (l), the pure solid (s) and the aqueous (aq) state at infinite dilution. In conclusion, all partial molar enthalpies, H_i, will be replaced by concentration independent standard molar enthalpies H_i°.

(v) Standard molar heat capacities are assumed to be independent of temperature. The temperature dependence of H_i° may thus be expressed as

$$H_i^\circ(T) = C_{pi}(T - T_{ref}) \tag{2.14}$$

where T_{ref} is an arbitrary reference temperature, frequently chosen at 25°C.

Under the assumptions (i) and (iv), the species balance (Eqn. 2.5) and the enthalpy balance (Eqn. 2.12) reduce to:

$$\frac{dn_i}{dt} = \frac{d_{in}n_i}{dt} - \frac{d_{out}n_i}{dt} + \sum_j \nu_{i,j} \frac{d_r \xi_j}{dt} \tag{2.15}$$

$$C_p\frac{dT}{dt}+\sum_i H^\circ_{i,s}\frac{dn_i}{dt}=\frac{d_{ext}Q}{dt}+\frac{d_{ext}W}{dt}+\sum_i H^\circ_{i,s}\left(\frac{d_{in}n_i}{dt}-\frac{d_{out}n_i}{dt}\right)$$
$$\times\sum_i(H^\circ_{i,in}-H^\circ_{i,s})\frac{d_{in}n_i}{dt}$$
$$-\sum_i(H^\circ_{i,out}-H^\circ_{i,s})\frac{d_{out}n_i}{dt} \qquad (2.16)$$

$H^\circ_{i,s}$, $H^\circ_{i,in}$ and $H^\circ_{i,out}$ stand for the standard molar enthalpy of substance i at, respectively, the temperature of the system and of the mixtures by which it enters and leaves the system.

Allowing for Eqns. (2.13) and (2.14), Eqn (2.16) simplifies to:

$$C_p\frac{dT}{dt}+\sum_i H^\circ_{i,s}\frac{dn_i}{dt}=\frac{d_{ext}Q}{dt}+\frac{d_{ext}W}{dt}+\sum_i H^\circ_{i,s}\left(\frac{d_{in}n_i}{dt}-\frac{d_{out}n_i}{dt}\right)$$
$$+\sum_i C_{p,i}(T_{in}-T)\frac{d_{in}n_i}{dt} \qquad (2.17)$$

By substitution of the molar balance (2.15) into Eqn (2.17) one finally obtains:

$$C_p\frac{dT}{dt}+\sum_j \Delta_r H^\circ_{B,j}\left(\frac{d_r\xi_j}{dt}\right)+\sum_i(C_{pi}[T-T_{in}])\frac{d_{in}n_i}{dt}$$
$$=\frac{d_{ext}Q}{dt}+\frac{d_{ext}W}{dt} \qquad (2.18)$$

where

$$\Delta_r H^\circ_{B,j}=\sum_i \nu_{i,j}H^\circ_{i,s} \qquad (2.19)$$

Eqn. 2.18 represents the enthalpy balance for a non-steady-state, open, pseudohomogeneous multiphase system in which several biochemical, chemical and physical transformation processes occur simultaneously.

The first left-hand term of Eqn. 2.18 represents the change of enthalpy of the system with time due to temperature variations (see Fig. 2b). It contains the only real-time derivative. The other derivatives actually represent rates or flows and must be treated as constants or functions of time when integrating Eqn. 2.18.

The second left-hand term quantifies the enthalpy change resulting from all the species transformations. $\Delta_r H^\circ_{B,j}$ as defined by Eqn. 2.19 is the standard enthalpy change associated with the reaction. $\Delta_r H_{B,j}$ really expresses the enthalpy change of a closed system per one formula unit of reaction advancement, provided the other system variables, P and T, are kept constant and no other processes occur.

$$\Delta_r H_B=\left(\frac{\partial H}{\partial \xi_j}\right)_{T,P,_{ex}n_i} \qquad (2.20)$$

The subscript B indicates 'per mole of B', which also means that the reaction stoichiometry is defined in a form where the stoichiometry number ($\nu_{B,j}$) of species B is either $+1$ or -1.

If process j is a biological or a chemical reaction, $\Delta_r H^\circ$ is known as the standard molar reaction enthalpy and $d_r\xi_j/dt$ as the corresponding reaction rate. If process j is a phase transition or dilution from the liquid to the aqueous state, $\Delta_r H^\circ_B$ represents the standard enthalpy of the phase change or of dilution. As may be seen from Eqn. 2.19, $\Delta_r H^\circ_B$ must be evaluated at system temperature.

The third term of Eqn. 2.18 represents the exchange of enthalpy between the system and its environment due to the fact that some of the substances are heated up (or cooled down) to the system temperature as they flow through the system. The two right-hand terms express the net rate of enthalpy import due to the exchange of heat and work (excluding P-V work) between the system and its environment.

It should be noted that Eqn. 2.18 does not reflect the entire system change, because the $d_{mat}H$ term cancelled out. Whereas Eqn. 2.12 represents a system analysis, Eqn. 2.18 focuses on the analysis of transformation. In order to use it, the transformations occurring in the system have to be described in terms of one or several 'processes' with determined stoichiometry. For each of them, the enthalpy change must be computed and the rate determined. This analysis is illustrated in the next sections using a specific hypothetical example.

II-D.2. Illustration of the simplified enthalpy balance around a culture vessel

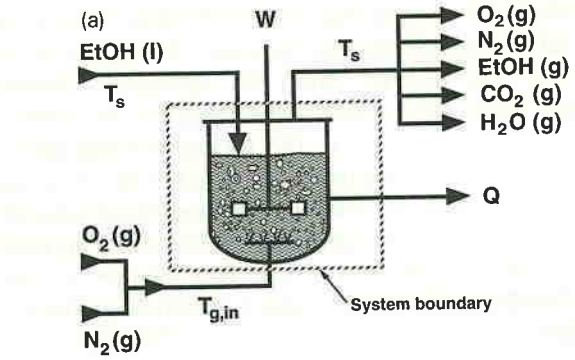
For the illustration of how to use the simplified enthalpy balance (Eqn. 2.18), a simple realistic case has been chosen. Fig. 3A shows a stirred and thermostated fed-batch culture, which is fed with pure ethanol (1), resulting in a negligible volume change. The culture is also continuously fed by air (in Fig. 3A represented as a flow of O_2 (g) and N_2 (g)) at a temperature ($T_{g,in}$), which differs from the culture temperature (T). The central metabolic reaction taking place is a result of the catabolic activity of the microorganism *Theorymyces hypotheticum*. Since no growth is occurring, the biocatalyst, *T. hypotheticum*, simply catabolizes the reaction of ethanol to carbon dioxide and water by respiration according to the following stoichiometry:

$$CH_3CH_2OH(aq)+3O_2(aq)\rightarrow 2CO_2(aq)+3H_2O(l) \qquad (2.21)$$

Ethanol is assumed to be fed at a higher rate than it is consumed in the microbial process, hence it will accumulate in the culture broth. The system is thus not at steady state. A fraction of the introduced ethanol will leave the culture by evaporation (ethanol (g)),

owing to aeration of the culture. The same situation, including both accumulation and evaporation, exists for the water which is produced in the metabolic process. Oxygen and nitrogen enter and leave the system in the same state, except for the temperature, which will be T in the exit stream.

The enthalpy and species balances will be set-up in this example by drawing the system boundaries around the whole experimental growth vessel as shown in Fig. 3A. The definition of the processes in this system forms the next step. It requires an identification of all major species occurring in the system, such as shown in Table IA. A list of all possible processes may then be formulated (Table IB). The analysis is obviously simplified by the fact that the only activity of *T. hypotheticum* consists of catalyzing a very simple metabolic transformation with known stoichiometry (reaction 21). A more complex situation will be treated in Section IV.



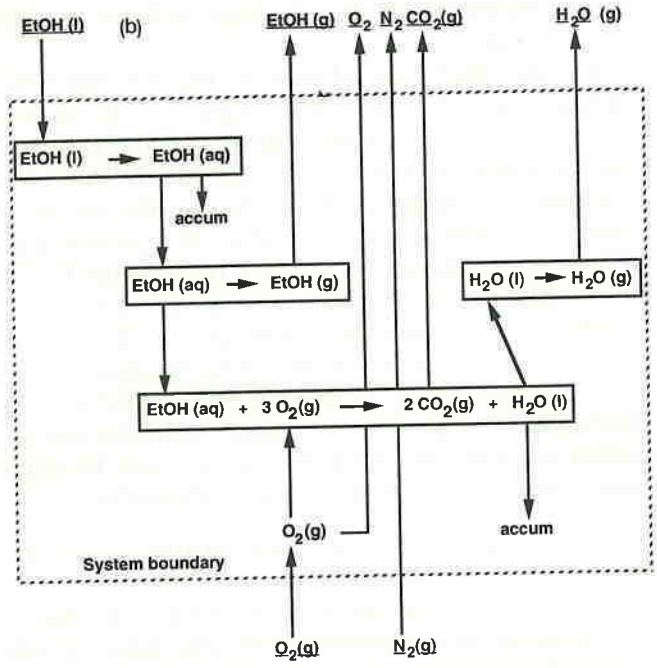


Fig. 3. Energy balance for illustrative example around whole bioreactor. (a) System boundary. (b) Simplified scheme of reactions and transformations occurring within the system.

TABLE IA

List of species in hypothetical example

Chemical compound	State	Species
CH_3CH_2OH	liquid	EtOH(l)
CH_3CH_2OH	aqueous	EtOH(aq)
CH_3CH_2OH	vapor	EtOH(g)
O_2	gas	O_2(g)
O_2	aqueous	O_2(aq)
CO_2	aqueous	CO_2(aq)
CO_2	gas	CO_2(g)
H_2O	liquid	H_2O(l)
H_2O	vapor	H_2O(g)
N_2	gas	N_2(g)

TABLE IB

List of possible processes in hypothetical example

Process	Stoichiometry	Process
Dilution of EtOH	EtOH(l) → EtOH(aq)	dil
Evaporation of EtOH	EtOH(aq) → EtOH(g)	V
Absorption of O_2	O_2(g) → O_2(aq)	abs
Stripping of CO_2	CO_2(aq) → CO_2(g)	str
Evaporation of H_2O	H_2O(l) → H_2O(g)	V
Metabolic reaction	EtOH(aq) + 3 O_2(aq) → 2 CO_2(aq) + 3 H_2O(l)	r21

Even so, the description of dynamics of this simple system consists of six processes. Evaluation of Eqn. 2.18 would thus require measurements of six reaction and transition rates in addition to the two net import rates for the two species O_2(g) and N_2(g) that undergo a temperature change. In order to keep the measurements to a minimum, it is paramount to reduce the number of processes as much as possible by lumping those that proceed at proportional rates.

An application of a molar balance such as Eqn. 2.15 to each species will immediately reveal that not all the processes listed in Table IB are independent of each other. The balance on aqueous O_2, for example, states:

$$\frac{dn_{O(aq)}}{dt} = 0 - 0 + 1\frac{d_r\xi_{abs}}{dt} - 3\frac{d_r\xi_{r21}}{dt} \qquad (2.22)$$

In this balance, the dn_i/dt term must be negligible in comparison with the consumption and the absorption rate due to the low solubility of oxygen in the liquid phase. Typical O_2 consumption rates by *T. hypotheticum* cultures are on the order of 0.1 mol l^{-1} h^{-1}, whereas the solubility of O_2 is only about $2 \cdot 10^{-4}$ mol l^{-1}. The stock of O_2 dissolved in the liquid phase would thus be exhausted in only about 7 s without aeration.

It is therefore obvious that pratically all of the oxygen consumed by the culture over several hours must have been supplied by the gas phase, so that the

rate of O_2 absorption is on average equal to its consumption rate. Thus, according to Eqn. 2.22:

$$\frac{d_r\xi_{abs}}{dt} = +3\frac{d_r\xi_{r21}}{dt} \tag{2.23}$$

A similar consideration reveals that the amount of CO_2 accumulating in the liquid phase is usually negligible compared with the amount stripped into the gas phase. The stripping rate is thus coupled to the CO_2 production rate. Hence, the reaction scheme can be simplified (see Fig. 3B) by lumping the absorption of O_2 and the stripping of CO_2 with the metabolic reaction, yielding a process represented by Eqn. 2.24:

$$CH_3CH_2OH(aq) + 3O_2(g) \rightarrow 2CO_2(g) + 3H_2O(l) \tag{2.24}$$

A similar lumping is possible neither for the evaporation of ethanol nor for water. Since both EtOH(aq) and $H_2O(l)$ may accumulate in the broth or evaporate, a balance on these species will reveal that their evaporation rate is not coupled to their production rate (see Fig. 3B). Since, on the other hand, neither EtOH(g) nor $H_2O(g)$ can accumulate to a significant extent in the system, their evaporation rates must be equal to the rate at which these species leave the reactor in the gas phase. These rates can be measured.

The above considerations show that species balances such as Eqn. 2.15 are also routinely applied to link the $d_r\xi_j/dt$ terms to measurable flow rates or accumulation rates. Applying it to, for instance, gaseous O_2 and assuming that there is no change of the number of moles of O_2 (g) in the system yields:

$$\frac{d_r\xi_{r24}}{dt} = \frac{1}{3}\left(\frac{d_{in}n_{O_2(g)}}{dt} - \frac{d_{out}n_{O_2(g)}}{dt}\right) \tag{2.25}$$

Eqn. 2.25 enables determining the reaction rate $d_r\xi_{r24}/dt$ from the measurable rate of oxygen uptake.

Likewise, the balance on liquid ethanol states:

$$0 = +1\cdot\frac{d_{in}n_{EtOH(l)}}{dt} - 1\cdot\frac{d_r\xi_{dil}}{dt} \tag{2.26}$$

Since it is unthinkable that undiluted, liquid ethanol accumulates in the broth, the dn_i/dt term must be zero and the dilution proceeds at a rate equal to the feed rate.

In summary, all the transformations may be represented by the reaction scheme shown in Fig. 3B. Of the six processes listed in Table Ib, only four independent reactions remain because at steady state of $O_{2(aq)}$ and $CO_{2(aq)}$, the O_2 absorption and the CO_2 stripping can be lumped with the biochemical reaction. The rates of these four processes must be measured. These processes are listed together with their standard molar enthalpy change in Table II.

After this analysis, the energy balance, Eqn. 2.18, can now be assembled. The first term is zero, because the temperature is assumed to be constant. The second term consists of a sum over all reactions and transitions as follows:

$$\sum_j \Delta_r H^\circ_{B,j}\frac{d_r\xi_j}{dt} = \Delta_r H^\circ_{24}\cdot\frac{d_r\xi_{r24}}{dt} + \Delta_{dil}H^\circ_{EtOH}\frac{d_{in}n_{EtOH(l)}}{dt} + \Delta_v H^\circ_{EtOH}\frac{d_{out}n_{EtOH(g)}}{dt} + \Delta_v H^\circ_{H_2O}\frac{d_{out}n_{H_2O(g)}}{dt} \tag{2.27}$$

The third term has one expression for O_2 and one for N_2:

$$\sum_i (C_{pi}[T - T_{in}])\frac{d_{in}n_i}{dt} = \left[C_{pO_2(g)}\frac{d_{in}n_{O_2(g)}}{dt} + C_{pN_2(g)}\frac{d_{in}n_{N_2(g)}}{dt}\right](T - T_{in}) \tag{2.28}$$

Thus, although N_2 does not react and is absent from Table II, it still influences the balance because of the temperature change it undergoes.

According to Eqn. 2.18, the sum of Eqns. 2.27 and 2.28 yields the sum of the heat flux and the mechanical power exchanged with the environment, which, in our example, corresponds to the power uptake by the stir-

TABLE II

Independent processes in hypothetical example

Process	Stoichiometry	Process	Rate	$\Delta_r H^\circ$
Metabolic reaction	$EtOH(aq) + 3O_2(g) \rightarrow 2CO_2(g) + 3H_2O(l)$	r24	$\frac{d_r\xi_{r24}}{dt}$	$-1H^\circ_{EtOH(aq)} - 3H^\circ_{O_2(g)} + 2H^\circ_{CO_2(g)} + 3H^\circ_{H_2O(l)} = \Delta_r H^\circ_{24}$
Dilution of EtOH	$EtOH(l) \rightarrow EtOH(aq)$	dil	$\frac{d_{in}n_{EtOH(l)}}{dt}$	$H^\circ_{EtOH(aq)} - H^\circ_{EtOH(l)} = \Delta_{dil}H^\circ_{EtOH}$
Evaporation of EtOH	$EtOH(aq) \rightarrow EtOH(g)$	V	$\frac{d_{out}n_{EtOH(g)}}{dt}$	$H^\circ_{EtOH(g)} - H^\circ_{EtOH(aq)} = \Delta_v H^\circ_{EtOH}$
Evaporation of H_2O	$H_2O(l) \rightarrow H_2O(g)$	V	$\frac{d_{out}n_{H_2O(g)}}{dt}$	$H^\circ_{W(g)} - H^\circ_{W(l)} = \Delta_v H^\circ_W$

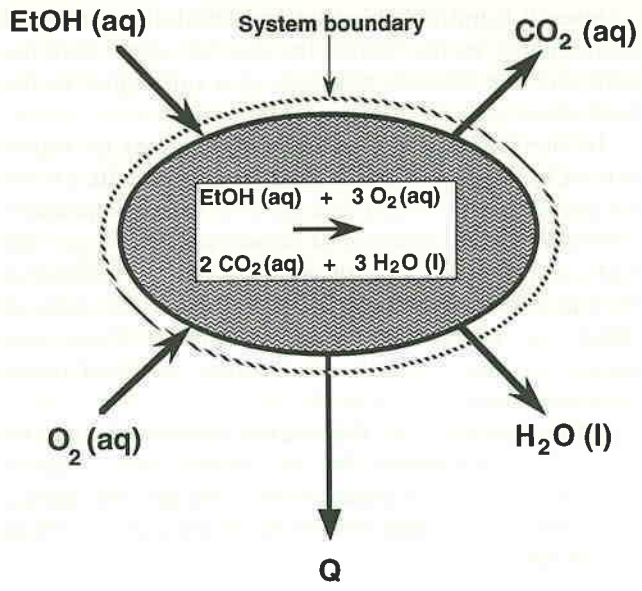


Fig. 4. Energy balance for illustrative example around a single unit of catalytic biomass.

rer. Therefore, the explicit expression of the energy balance for our example reads:

$$\Delta_r H^\circ_{24} \cdot \frac{d_r \xi_{r24}}{dt} + \Delta_{dil} H^\circ_{EtOH} \cdot \frac{d_{in} n_{EtOH(l)}}{dt} + \Delta_v H^\circ_{EtOH} \frac{d_{out} n_{EtOH(g)}}{dt} + \Delta_v H^\circ_{H_2O} \frac{d_{out} n_{H_2O(g)}}{dt} + \left[C_{pO_2(g)} \frac{d_{in} n_{O_2(g)}}{dt} + C_{pN_2(g)} \frac{d_{in} n_{N_2(g)}}{dt} \right] [T - T_{in}] + \frac{d_{ext} Q}{dt} + \frac{d_{ext} W}{dt} \quad (2.29)$$

II-D.3. Illustration of the simplified enthalpy balance around a unit of living biomass

The same hypothetical experiment as in the previous section will be used for this illustration, but let us assume that this time one is interested in the exchange of energy at the cellular level. A unit of live *T. hypotheticum* will now form the basis for drawing the system boundary as shown in Fig. 4. This unit could be a single cell, but in order to ensure pseudohomogeneous conditions one could include a large quantity, e.g., 1 kg of live biomass within the boundary, or else one ought to select an 'average' single cell.

The biomass comprising this system operates at steady state. It exchanges matter with its environment and transforms it exactly as represented by Eqn. 2.21. No temperature gradients exist between microbial cells and its environment and the culture neither absorbs nor performs any work apart from volume changes. The enthalpy balance thus simply reads:

$$\Delta_r H^\circ_{21} \frac{d_r \xi_{r21}}{dt} = \frac{d_{ext} Q}{dt} \quad (2.30)$$

where

$$\Delta_r H^\circ_{21} = - H^\circ_{EtOH(aq)} - 3H^\circ_{O_2(aq)} + 2H^\circ_{CO_2(aq)} + 3H^\circ_{H_2O(l)} \quad (2.31)$$

Eqn. 2.30 enables computing the power released by the cells in form of heat, but the heat measured in a calorimeter will often be different because the system boundaries of calorimeters rather resemble those drawn in Fig. 3A than in Fig. 4.

III. Calculation of reaction enthalpies

III-A. Use of enthalpies of formation

The recommended equation for constructing correct enthalpy balances for growth reactions is Egn. 2.18. In order to evaluate the standard reaction enthalpy $\Delta_r H^\circ_B$ based on Eqn. 2.19, values have to be assigned to the molar enthalpies. This requires defining a zero point, or reference state, common to all involved species. It is customary to use the constituent elements of the respective compounds in their standard state at 25°C as a reference state and thus to set the standard molar enthalpies equal to the standard enthalpy of formation $\Delta_f H_i^\circ$ of the respective compound (see Fig. 5). Hence

$$\Delta_r H^\circ = \sum_i \nu_i \Delta_f H_i^\circ \quad (3.1)$$

The standard enthalpy of formation, $\Delta_f H_i^\circ$, expresses the reaction enthalpy when converting the constituent elements of compound i in their standard states into this compound. Calculating $\Delta_r H^\circ_B$ by Eqn. 3.1 can be thought of as an application of Hess' Law to a sequence of reactions, which consist of decomposing all reactants into their constituent elements in their standard states, followed by reforming the products from there as shown in Fig. 5.

Standard enthalpies of formation have been tabulated for a number of compounds (see Appendix 2, Table A-I). The exact definitions of the standard and reference states are explained in Appendix 2.

III-B. Use of modified enthalpies of combustion

Biological experiments often involve respiratory metabolism corresponding to oxidations of organic compounds into CO_2 and H_2O. Therefore, the energy balances are simplified with the standard molar enthalpies of the chemicals computed using their combustion products as a reference. Both $H_2O(l)$ and $CO_2(g)$ formed in the combustion process have a zero enthalpy and drop out of the balance. This computation of $\Delta_r H^\circ$ could also be thought of as an application of Hess' Law to a sequence of reactions beginning with a complete combustion of all reactants followed

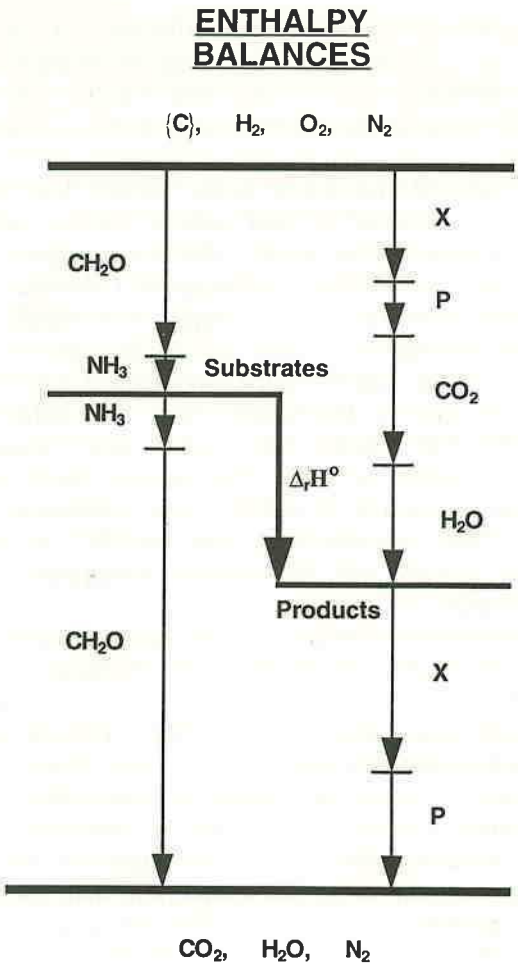


Fig. 5. Calculation of the standard enthalpy of the growth reaction, $\Delta_r H^\circ$, using to two different reference states. $\Delta_r H^\circ$ appears as the vertical difference between the energy levels for the sum of all 'products' and for the sum of all 'substrates'. The arrows above these levels refer to the enthalpies of formation needed in Eqn. 3.1, which uses the constituent elements as a reference, whereas the arrows below these levels refer to the enthalpies of combustion, needed in Eqn. 3.2, which uses the completely combusted state as a reference. The arrows indicate the individual products of $\nu_i \Delta_f H_i^\circ$ and $\nu_i \Delta_c H_i^\circ$ appearing in these equations. The length of the arrows indicate schematically the quantities $\Delta_f H_i^\circ$ and $\Delta_c H_i^\circ$ multiplied with $|\nu_i|$. The arrows in the upper part of the diagram thus indicate the enthalpy change, i.e., the enthalpy 'needed' to form the involved reactants (left-hand) and products (right-hand). The arrows in the lower part show the amount of enthalpy recovered (as heat) when combusting the substrates and products. As opposed to arrows associated with $\Delta_f H_i^\circ$, those associated with $\Delta_c H_i^\circ$ must face away from the substrates and products because the combustion reaction is defined in this direction. CH_2O stands for 1 C-mol of carbon and energy substrate, {C} for elemental carbon in its standard state. X for the biomass formed, P for product. Using either reference state yields the same value for $\Delta_r H$.

by formation of the products from the combustion products as shown in Fig. 5. Hence

$$\Delta_r H^0 = -\sum_i \nu_i \Delta_c H_i^\circ \qquad (3.2)$$

where $\Delta_c H_i^\circ$ are the standard enthalpies of combustion. The minus sign in Eqn. 3.2 reflects the fact that enthalpies of combustion, in contrast to $\Delta_f H_i^\circ$, do not express the enthalpy needed to form the respective compound from the reference state, but the reverse.

Standard enthalpies of combustion are tabulated for many pure compounds at 25°C. The exact definitions of the reference and standard states used for tabulating enthalpies of combustion are discussed in Appendix 2. It is, however, more difficult to find values referring to the aqueous standard state needed for biological calculations. Thus, the $\Delta_c H_i^\circ$ is known for pure crystaline glucose but not directly tabulated for glucose in the aqueous, infinitely dilute state. Values of $\Delta_c H_i^\circ$ for the aqueous states (caq) of the respective compounds were therefore calculated from $\Delta_f H_i^\circ$ and included in Table A-I, Appendix 2, as $\Delta_{caq} H_i^\circ$ (combustions aqueous). While these values generally represent modified standard enthalpies of combustion, they necessarily also include transitions which do not involve a combustion at all, such as the transition of O_2 and CO_2 from the aqueous standard to the pure gaseous reference state.

As shown in Fig. 5, the results obtained for $\Delta_r H_B^\circ$ must be independent of choosing either the constituent elements or the combustion products as reference states. However, consistent standard states of reactants and products of the reaction r must be chosen in any case.

In order to illustrate the compatible use of either appropriate enthalpy of formation or modified combustion values, the following reactions will be used to calculate $\Delta_r H^\circ_{\text{glucose},3.3}$ (Eqn. 3.3) and $\Delta_r H^\circ_{\text{glucose},3.4}$ (Eqn. 3.4), respectively:

$$\alpha,\beta\text{-D}-C_6H_{12}O_6(\text{aq}) \rightarrow 2C_2H_6O\,(\text{aq}) + 2CO_2(\text{g}) \qquad (3.3)$$

$$\alpha,\beta\text{-D}-C_6H_{12}O_6\,(\text{aq}) + 6\,O_2(\text{g}) \rightarrow 6CO_2\,(\text{g}) + 6H_2O\,(\text{l}) \qquad (3.4)$$

When calculating $\Delta_r H^\circ$ (25°C) of Eqns. 3.3 and 3.4 by using modified enthalpy of combustion values, Eqn. 3.2 is applied and values from Table A-I, Appendix 2 are used:

$$\Delta_r H^\circ_{\text{glucose},3.3} = (-1)(2813.6) + 2(1356.8) + 2(0)$$

$$= -100\ \text{kJ/mol of glucose} \qquad (3.5)$$

$$\Delta_r H^\circ_{\text{glucose},3.4} = (-1)(2813.6) + (-6)(0.0) + 6(0.0) + 6(0.0)$$

$$= -2813.6\ \text{kJ/mol of glucose} \qquad (3.6)$$

When calculating $\Delta_r H^\circ$ of Eqns. 3.3 ad 3.4 by using enthalpy of formation values, Eqn. 3.1 is applied and values from Table A-I, Appendix 2 are used:

$$\Delta_r H^\circ_{\text{glucose},3.3} = (-1)(1262.4) + (2)(-287.7) + 2(-393.51)$$

$$= -100\ \text{kJ/mol of glucose} \qquad (3.7)$$

$$\Delta_r H^\circ_{\text{glucose},3.4} = (-1)(1262.4) + (-6)(0.0) + 6(-393.51) + 6(-285.83) = -2813.6 \text{ kJ/mol of glucose} \quad (3.8)$$

III-C. Enthalpy of combustion of biomass

While data on enthalpies of formation and combustion are readily available for all chemically defined compounds (Table A-I, Appendix 2), it appears more difficult to estimate the enthalpic content of biomass from the literature because of a considerable scatter of sometimes as much as 50% (for reviews, see Refs. 22,23). Unfortunately, determining the heat of combustion of dried biomass experimentally using combustion calorimetry is difficult and tedious. The preparation of representative samples requires great care [23,24], which may explain some of the scatter found in the literature. Table III summarizes values for the enthalpy of combustion of dried ash-free biomass samples prepared according to optimized and standardized procedures.

It is also possible to estimate the enthalpy of combustion of dried biomass from its elemental composition [25]. It is widely known that $\Delta_c H_i^\circ$ correlates well with its degree of reduction γ_x° [23], which is defined for a C-mol of dry ash-free biomass having the 'chemical' formula of $CH_{x1}O_{x2}N_{x3}$ as follows:

$$\gamma_x^\circ = 4 + x_1 - 2x_2 \quad (3.9)$$

According to Cordier et al. [23], the best correlation is the following:

$$\Delta_c H_x^\circ = -Q_o \gamma_x^\circ \quad (3.10)$$

where Q_o has the same value of 115 kJ C-mol^{-1}, irrespective of the type of biomass.

According to Section II, dried biomass is, however, never the standard state required to construct correct energy balances for growth experiments. No values o $\Delta_f H_i^\circ$ or $\Delta_c H_i^\circ$ values for hydrated, live biomass, hav been published, and it is unknown whether these val ues are significantly different from those in Table II or not.

A series of experiments were recently done at th University of Lund (S) and Geneva (CH) to addres this question. In the former, dry yeast samples wer rehydrated in an LKB 8700 Reaction Calorimeter. I the latter, lyophilized yeast samples were equilibrate with an atmosphere of 54% relative humidity at 25°C and the water content of the biomass was determine by thermogravity. The samples were then dehydrate in a BT 250 Setaram Calorimeter using vacuum o $4 \cdot 10^{-2}$ mmHg at 25°C. The samples were subse quently rehydrated in a 2107 LKB Calorimeter wit water. Other samples were dried to 100°C at atmos pheric pressure and subsequently rehydrated in th calorimeter.

The measured enthalpy change during rehydratatio range from about 65 to 90 J/g dry biomass, whereas the dehydration values vary between 25 and 90 J/g The low reproducibility of the values obtained durin the dehydratation is due to the difficulty of controllin the rate at which the vacuum is established in th calorimeter. Nevertheless, it can be concluded fro these measurements that $\Delta_c H^\circ$ values for hydrate biomass appear to be less exothermic than those fo dried samples by an amount on the order of 1–2 kJ/C-mol.

IV. Enthalpy balance in aerobic and anaerobic growth

IV-A. The importance of side-reactions

The growth reaction (for instance, Eqn. 4.1) an side-reactions occurring in aqueous solution must b

TABLE III

Elemental composition and standard enthalpy of combustion of dry ash-free microbial biomass

Recalculated from Cordier et al. [23] Gürakan et al. [24], Larsson et al. [5] and Oelz, R. and Gustafsson, L. unpublished data. γ_x° is the Karas degree of reduction and M_x is the C-molar mass (see list of symbols).

Strain	Ref.	Substrate	x_1	x_2	x_3	γ_x°	M_x (g C-mol^{-1})	$\Delta_c H_x^\circ$ (kJ C-mol^{-1})
Escherichia coli	23	glucose	1.70	0.42	0.25	4.86	23.99	552.7
Methylotrophus methylophilus	23	methanol	1.72	0.40	0.25	4.92	23.58	561.6
Lactobacillus helveticus	24	glucose	1.62	0.38	0.23	4.86	22.92	536.1
Saccharomyces cerevisiae	24	glucose	1.65	0.49	0.18	4.67	24.17	512.9
Saccharomyces cerevisiae	5	glucose	1.71	0.52	0.17	4.67	24.44	522.8
Saccharomyces cerevisae	–	glucose	1.69	0.55	0.19	4.59	25.18	559.4
Zygosaccharomyces bailii	24	glucose	1.64	0.54	0.13	4.56	24.14	495.1
Debaromyces nepaliensis	24	glucose	1.80	0.63	0.09	4.54	25.07	523.5
Kluyveromyces fragilis	23	glucose	1.75	0.52	0.15	4.71	24.16	523.3
Kluyveromyces fragilis	23	galactose	1.75	0.53	0.17	4.69	24.56	534.9
Kluyveromyces fragilis	23	lactose	1.78	0.57	0.16	4.64	25.19	542.6

considered in the enthalpy balance of growth. The most important side-reactions include acid-base equilibria, ion- or ligand-binding equilibria and phase equilibria [26,27]. In fermentation, the enthalpy change per unit glucose catabolized can be as low as 3.5% of catabolic enthalpies in aerobic respiration [5,28]. Therefore, the relative importance of side-reactions increases up to 30-times with the proportion of the fermentative contribution to the growth reaction.

Enthalpies of neutralization in proton buffering are exothermic for most cellular and environmental buffers and for buffers used in growth media (Table A-II, Appendix 2). Net proton formation in growth (Eqn. 4.1) is important owing to the production of organic acids, and carbonic acid at pH > 6 if the excreted CO_2 remains in the aqueous phase. Protons are also produced in the dissociation of ammonium ion following the uptake of ammonia or in proton exchange during import of ammonium ion as a nitrogen source. On the contrary, ammonia excretion due to catabolism of protein or amino acids decreases the net proton production [18].

The relative importance of the enthalpy of neutralization depends on the stoichiometric number of protons in the growth reaction (typically 0.02 to 0.05 H^+/C-mol glucose with CO_2 in the gas phase), the enthalpy of neutralization of the buffer system (−5 to −50 kJ/mol H^+), and the enthalpy change of the reaction (−10 to −470 kJ/C-mol glucose). Taking catabolism in the absence of growth as the simplest example, then the effect of side-reactions on aerobic respiration is < 5%, in most cases < 3% [19,28]. Transfer of all CO_2 into the gas phase changes the catabolic enthalpy from −476 to −469 kJ/C-mol glucose (or kJ/mol O_2), the more exothermic value referring to a buffer system at pH 7 and an enthalpy of neutralization of −9 kJ/mol H^+ [3].

In lactic acid fermentation, however, the enthalpy of neutralization may be as high as 55% of the catabolic reaction enthalpy of −18.2 kJ/C-mol (−54.7 kJ/mol lactate) in aqueous solution at pH 7 (Table A-III, Appendix 2). For biological systems (microbes and mammalian cells), the calorimetrically observed heat change ranges from −18 to −23 kJ/C-mol lactate as a function of different buffer systems (−55 to −70 kJ/mol; Ref. 3).

IV-B. The importance of selecting correct standard states

To illustrate the importance of using the correct thermodynamic states of the reactants and products for the formulation of energy balances of biological reactions, three growth reactions representing a respiratory, a mixed respiratory-fermentative, and a purely fermentative growth process of the yeast *Saccharomyces cerevisiae* are used as examples. For all three processes the batch mode of cultivation was used in defined medium with glucose as the only carbon and energy source. For the respiratory and mixed respiratory-fermentative processes the growth conditions are as described by Larsson et al. [5]. The medium used was yeast nitrogen base without amino acids (YNB, Difco), supplemented with glucose to a concentration of 0.5% (w/v) and ammonium sulfate as the nitrogen source. The liquid culture (250 ml) was incubated aerobically at 30°C. Samples for determination of biomass, glucose, ethanol, glycerol, acetate, succinate and malate were withdrawn at selected intervals and analyzed as described by these authors. The elemental and heat of combustion analyses of the biomass were performed in separate experiments on the same strain, by a method published recently [24]. The respective elemental compositions, as given in Eqns. 4.1 and 4.2, have the corresponding heat of combustion values of −522.8 kJ/C-mol and −559.4 kJ/C-mol of biomass (Table III).

When *S. cerevisiae* was grown under the conditions described above, the well-known mixed respiratory-fermentative metabolism was obtained [29]. This metabolism was represented by a first phase of growth during which the glucose was totally consumed, partly being fermented to ethanol, glycerol and acetate, partly being respired to CO_2 and H_2O, and partly being converted into biomass. This phase of respiro-fermentative metabolism was followed by a transition to a purely respiratory metabolism, during which the ethanol was partly oxidized to CO_2 and H_2O and partly converted into biomass [5,29]. The overall growth reaction is therefore represented by a respiratory catabolism [4], according to Eqn. 4.1:

$$\alpha,\beta\text{-D-}C_6H_{12}O_6(aq) + 0.298NH_4^+(aq) + 4.002O_2(g) \rightarrow 1.751CH_{1.71}O_{0.52}N_{0.17}(?) + 0.298H^+(aq) + 0.010C_3H_8O_3(aq) + 0.071C_2H_4O_2(aq) + 4.077CO_2(g) + 4.768H_2O(l) \quad (4.1)$$

During the first glucose consuming phase, the so-called respiro-fermentative metabolism was obtained, during which phase there was a continuous change in proportion of respiration to fermentation. In the middle of this phase fermentative metabolism dominated. Only 2.5% of the total carbon was respired, while 80% of the total carbon was fermented to ethanol, according to Eqn. 4.2:

$$\alpha,\beta\text{-D-}C_6H_{12}O_6(aq) + 0.128NH_4^+(aq) + 0.149O_2(g) \rightarrow 0.676CH_{1.69}O_{0.55}N_{0.19}(?) + 0.128H^+(aq) + 1.601C_2H_6O(aq) + 0.095C_3H_8O_3(aq) + 0.018C_2H_4O_2(aq) + 1.801CO_2(g) + 0.402H_2O(l) \quad (4.2)$$

In order to have a purely anaerobic growth reaction, the stoichiometry given by Battley [14] is used in the example below (Eqn. 4.3). However, the biomass composition was assumed to be the same as for the respiro-fermentative metabolism (Eqn. 4.2), thereby introducing some minor changes in the original stoichiometry. The same states of the reactants and products as stated in Eqns. 4.1 and 4.2 were assumed. This equation is used only as a real example of a purely anaerobic growth process. The stoichiometry should not be compared with the previous equations (4.1 and 4.2), since these were obtained with a different strain and with a different medium and growth conditions.

$$\alpha,\beta\text{-D-}C_6H_{12}O_6(aq) + 0.111NH_4^+(aq)$$
$$\rightarrow 0.586CH_{1.69}O_{0.55}N_{0.19}(?) + 0.111H^+(aq)$$
$$+ 1.292C_2H_6O(aq) + 0.429C_3H_8O_3(aq) + 1.543CO_2(g)$$
$$+ 0.079H_2O(l) \qquad (4.3)$$

The enthalpy change of the growth reactions can, for example, be calculated by the enthalpies of modified combustion reactions ($\Delta_{caq}H°$, Table A-I, Appendix 2), which will give the same result as if the enthalpies of formation were used ($\Delta_fH°$, Table A-I, Appendix 2), as discussed in subsection III-B. The effect of choosing different thermodynamic states in the growth reactions given by Eqns. 4.2 and 4.3, is demonstrated in Tables IV and V, respectively.

By comparing the data obtained in these tables, it is seen that the enthalpy change of fermentative metabolism is most affected by the choice of thermodynamic states of the reactants and products, while already a small involvement of aerobic metabolism markedly reduces the effect of incorrectly chosen states. Thus, during purely or mainly fermentative metabolism, the neglect of the prevailing experimental conditions may give rise to serious errors in the calculation of the enthalpy change of a metabolic process. For more or less aerobic processes these changes are negligible, which can be illustrated by calculating the enthalpy change of Eqn. 4.1, with the given set of states and comparing that with the enthalpy change values that will be obtained by choosing the set of states given in columns A and B in Tables IV and V. The values obtained are -1923.3, -1955.8 and -1907.8 kJ/mol glucose, respectively. In other words, an error of less than 3% was introduced in the enthalpy calculations by choosing incorrect states of the reactants and products for a respiratory metabolism.

In the interpreted correct choice of states given in Eqns. 4.1, 4.2 and 4.3, protons were produced because of dissociation of the ammonium anion being used as the nitrogen source. This proton production caused a pH reduction from 4.5 to 2.6 [5]. The protons being produced can be calculated to cause a pH reduction to about 2.4 if not buffered. The fraction of the protons being buffered will cause an enthalpy change which has to be corrected for, as discussed in subsection IV-A. The correction in these cases (Table A-II, Appendix 2) is of minor importance, being at most -1 kJ per mol of glucose consumed. However, a medium including a buffer with stronger capacity would have provoked a greater importance of a buffering effect correction.

TABLE IV

Calculated enthalpy change values ($\Delta_rH_i^o$, kJ mol^{-1} of glucose consumed) of a mixed respiratory-fermentative metabolism, with the stoichiometry as given in Eqn. 4.2

To illustrate the importance of choosing correct thermodynamic states of the reactants and products three different choices have been made in columns A–C. (A) The correct states for a combustion reaction in a bomb calorimeter (c, l and g), (B) everything in the aqueous state except for the biomass where the dry state was used, (C) the actual states of the growth reaction as given in Eqn. 4.2, again except for the biomass where the dry state was used. The deviation (%) in enthalpy change is calculated with the actual states in column C as the reference. The stoichiometric coefficients (ν) are given and the experimentally obtained enthalpy of combustion of the biomass was -559.4 kJ/C-mol of dry biomass.

Substance	State	ν	Mixed respiratory-fermentative $\Delta_rH°$ (kJ·mol^{-1})		
			A	B	C
Glucose	c	−1.000	−2803.0		
	aq	−1.000		−2813.6	−2813.6
Ammonia	g	−0.128	−49.1		
	aq	−0.128		−44.6	
Ammonium ion	aq	−0.128			−37.8
Oxygen	g	−0.149	0		0
	aq	−0.149		+1.8	
Biomass	dry	0.676	+378.2	+378.2	+378.2
Ethanol	l	1.601	+2188.3		
	aq	1.601		+2172.2	+2172.2
Glycerol	l	0.095	+157.2		
	aq	0.095		+156.6	+156.6
Acetic acid	l	0.018	+15.7		
	aq			+15.7	+15.7
Carbon dioxide	g	1.801	0		
	aq	1.801		−35.7	0
Water	l	0.402	0	0	0
Hydrogen ion	aq	0.128			0
Σ			−112.7	−169.4	−128.7
Deviation (%)			−12.4	+31.6	0

IV-C. The importance of temperature corrections

Both $\Delta_fH_i^o$ and $\Delta_{caq}H_i^o$ values in tables such as Table A-I, Appendix 2 are usually given for a tempera-

TABLE V

Calculated enthalpy change values ($\Delta_r H_i^\circ$, kJ mol^{-1} of glucose consumed) of a fermentative metabolism, with the stoichiometry as given in Eqn. 4.3

To illustrate the importance of choosing correct thermodynamic states of the reactant and products, three different choices have been made in columns A–C. (A) The correct states for a combustion reaction in a bomb calorimeter (c, l and g); (B) everything in the aqueous state except for the biomass where the dry state was used; (C) the actual states of the growth reaction as given in Eqn. 4.3, again except for the biomass where the dry state was used. The deviation (%) in enthalpy change is calculated with the actual states in column C as the reference. The stoichiometric coefficients (ν) are given and the experimentally obtained enthalpy of combustion of the biomass was −559.4 kJ/C-mol of dry biomass.

Substance	State	ν	Fermentative $\Delta_r H^\circ$ (kJ mol^{-1})		
			A	B	C
Glucose	c	−1.000	−2803.0		
	aq	−1.000		−2813.6	−2813.6
Ammonia	g	−0.111	−42.6		
	aq	−0.111		−38.7	
Ammonium ion	aq	−0.111			−32.8
Biomass	dry	0.586	+327.8	+327.8	+327.8
Ethanol	l	1.292	+1765.9		
	aq	1.292		+1753.0	+1753.0
Glycerol	l	0.429	+709.7		
	aq	0.429		+707.2	+707.2
Carbon dioxide	g	1.543	0		0
	aq	1.543		−30.6	
Water	l	0.079	0	0	0
Hydrogen ion	aq	0.111			0
Σ			−42.2	−94.9	−58.4
Deviation (%)			−27.7	+62.5	0

ture at 25°C. Use of Eqns. 3.1 and 3.2 will thus yield the standard enthalpy of reaction at 25°C ($\Delta_f H^\circ$ (25°)). If the growth reaction for which $\Delta_r H^\circ$ has to be calculated occurs at another temperature, the standard enthalpy of reaction must in principle, be corrected for this difference by using Kirchhoff's Law:

$$\Delta_r H^\circ(T) = \Delta_r H^\circ(25°C) + \sum_i \nu_i C_{pi}(T - 25°) \quad (4.4)$$

The term $\Sigma_i \nu_i C_{pi}$ is called the heat capacity of reaction, $\Delta_r C_p$.

The growth reactions described in the previous sections were carried out at 30°C. Using the heat capacity values given in Table A-I, Appendix 2, both $\Delta_r C_p$ and the correction of $\Delta_r H^\circ$ for the 5°C difference were calculated for growth reactions 4.1–4.3 and reported in Table VI. Since the C_p of biomass is not known, the calculations were performed once neglecting biomass altogether and once assuming an average C_p of 70 J C-mol^{-1} K^{-1}. As may be seen, the correction amounts to between a fraction and about 1 kJ/mol of consumed glucose.

Even in thermophilic growth reactions, this correction remains unimportant. Recently, Gerhard [30] reported energy balances on batch growth of *Methanobacterium thermoautotrophicum* at 60°C on CO_2 and H_2. After an initial growth phase, Gerhard [30] observed completely decoupled catabolism for prolonged periods of time during which CO_2 and H_2 were completely transformed into CH_4 and H_2O. The $\Delta_r H^\circ$ of this reaction was reported as 253.09 kJ/mol of CO_2 at 25°C. Even for this reaction, the temperature correction calculated according to the above methodology is only 1.34 kJ/mol of CO_2 consumed.

It may be concluded that temperature corrections may be safely neglected for most energy balance calculations for cellular growth.

TABLE VI

Temperature correction of $\Delta_r H^\circ$ for the difference of the reference (25°C) and the actual growth temperature (30°C)

	Growth stoichiometry		
	Eqn. 4.1	Eqn. 4.2	Eqn. 4.3
Neglecting biomass			
$\Delta_r C_P$, J mol^{-1} K^{-1}	77	−18.5	−4.5
$\Delta_r H^\circ(30°) - \Delta_r H^\circ(25°)$, kJ mol^{-1}	0.4	−0.09	0.02
Assuming C_p = 70 J C-mol^{-1} K^{-1} for biomass			
$\Delta_r C_P$, J mol^{-1} K^{-1}	199	28.9	36
$\Delta_r H^\circ(30°) - \Delta_r H^\circ(25°)$, kJ mol^{-1}	1.0	0.14	0.18

Conclusions

Enthalpy balances can be generalized for open systems at constant pressure. A relatively simple treatment is possible even for multiphase systems if they can be described as pseudohomogeneous, so that it suffices to account for only average values of the reaction rates, the physical transition rates and the flow rates describing the exchange of matter with the environment. The thermodynamic state of such a multiphase system and its rate of change is computed by performing separate molar balances for each chemical species. A species is a chemical compound in a given thermodynamic state as defined by the state of aggregation and according to whether the compound is pure or dissolved in a solvent. In this treatment it is also assumed that the physical transitions from one species to another and the transformations between the different species that are catalyzed by living cells can be described by one or several overall 'processes' with

fixed stoichiometry. The resulting enthalpy balance (Eqn. 2.18) is generally applicable to closed adiabatic and closed isothermal systems, open systems at steady state, and also to open systems in transient.

The reaction and transition enthalpies appearing in this balance may be approximated by standard 'process' enthalpies provided that appropriate standard states are chosen. This choice must reflect the state in which the various substrates and growth products participate in any particular process for which the standard enthalpy change must be evaluated. It thus depends on the definition of the individual processes by which the system dynamics is described. The correct definition of these processes themselves depends on how the system boundaries are drawn.

If, the system boundaries are, for instance, drawn around a whole vessel used for growing an aerobic microbial culture, it is often possible to lump the transition from gaseous to aqueous O_2 and the stripping of the CO_2 produced with the growth reaction. Hence, the gaseous standard states would be used for O_2 and CO_2. If the carbon and energy substrate has been dissolved in the medium before inoculation, it exists in the mixture in the aqueous state and thus the aqueous species determines its standard state.

If, in the same experiment only the cells are defined as 'system', all processes consume and produce aqueous infinitely dilute species and hence the aqueous standard states should be chosen throughout.

For the biomass forming during a growth experiment, the 'standard state' one usually applies corresponds to homogenized dried biomass samples, despite the fact that the real state of biomass during growth is different. Experiments on the enthalpic content of 'wet' suspended and live biomass are reported in this paper for the first time. They show that the heat of transition between the two states is of the order of only 1–2 kJ/C-mol.

In constructing enthalpy balances describing growth experiments, either constituent elements or the combustion products of the involved chemical and biochemical species may be used as reference state for calculating tabulated molar enthalpies. Choosing the latter often simplifies calculations as CO_2 and H_2O may drop out at the balance, depending on the exact standard states used. Standard enthalpies of combustion and enthalpies of combustion modified for other biologically imported standard states were compiled allowing for the various standard states (Table A-I, Appendix 2).

Standard enthalpies of reactions ought, in principle, to be corrected for differences between the actual growth and reference temperature (25°C). Calculations show this correction to be negligible in all practical cases even for thermophilic growth.

The sensitivity of enthalpy change calculations on the selection of standard states was investigated by performing numerical calculations on various yeast growth experiments. As expected, the results are virtually invariant to the choice of standard states if the metabolism is to at least a modest extent respiratory. The enthalpy changes then become so large that differences due to standard states can safely be neglected. Working with enthalpies for the pure compounds in their most stable state of aggregation then yields virtually correct results. Enthalpy changes associated with anaerobic and fermentative metabolisms are, on the other hand, so small that the proper selection of standard states becomes a major prerequisite for meaningful results. According to preliminary experimental results, the correction that one must make in principle for the hydrated, live biomass as compared to dried homogenized samples, appears to remain quite modest even for fully fermentative growth.

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Appendix 1

Thermodynamic derivation of Eqn. 2.10

The first law of thermodynamics states that internal energy can neither be created nor destroyed:

$$d_{int}U = 0 \tag{A1}$$

Accounting for this in an internal energy balance according to Eqn. 1 for an open system yields the following form of the 1st law:

$$\frac{d_{sys}U}{dt} = \frac{d_{ext}Q}{dt} + \frac{d_{ext}W}{dt} + \sum_e \sum_i U_{i,e} \frac{d_{ext}n_{i,e}}{dt}$$

$$+ \sum_e \sum_i PV_{i,e} \frac{d_{ext}n_{i,e}}{dt} - P\frac{dV}{dt} \tag{A2}$$

In this equation, U_{ie}, and $V_{i,e}$ denote, respectively, the partial molar internal energy and the partial molar

volume of substance i in the mixture entering through exchange site e. The term $\Sigma_i PV_{i,e}\ \mathrm{d}_{ext}n_{i,e}/\mathrm{d}t$ represents the work required to push the mixture entering through exchange site e into the system. The term $-P\mathrm{d}V/\mathrm{d}t$ reflects the work done by the environment due to variations in system volume. (Note that both P and V may remain constant in open systems.)

Together with the definition for enthalpy and its time derivative, one obtains:

$$\frac{\mathrm{d}_{sys}H}{\mathrm{d}t} = \frac{\mathrm{d}_{ext}Q}{\mathrm{d}t} + \frac{\mathrm{d}_{ext}W}{\mathrm{d}t} + \sum_e\sum_i H_{i,e}\frac{\mathrm{d}_{ext}n_{i,e}}{\mathrm{d}t} + V\frac{\mathrm{d}P}{\mathrm{d}t} \qquad \text{(A3)}$$

which reduces to Eqn. 2.10 for $P = \text{const.}$

Appendix 2

Standard states

The standard state for gases (g) is 'the (hypothetical) state of the pure substance B in the gaseous phase at the standard pressure', $P = 100$ kPa, and exhibiting ideal gas behavior [31]. For the conversion of values referred to the old standard pressure of 101.325 kPa (1 standard atmosphere), to the standard pressure defined as 1 bar (10^5 Pa), see Freemann [32]. In the standard state, the enthalpy of formation is the same as that of the real gas at the same temperature and at zero pressure. The standard state for liquid (1), solid (s) or crystalline solid (cr) substances is 'the state of the pure substance B in the liquid or solid phase at standard pressure'. The standard state for a solute in aqueous solution (aq) is the state of the (hypothetical) ideal solution of unit activity which, for enthalpies of formation, is equivalent to infinite dilution.

Reference states

The reference state for a substance at a specified temperature is its most stable state or, for enthalpies of combustion [33] and for biological energy balance calculations, the state selected to be close to that obtained experimentally, such that large corrections are avoided. Care must be taken to ensure that consistent

TABLE A-I

*Standard molar enthalpies of formation ($\Delta_f H°$), standard molar enthalpy of combustion * or of non-standard combustion reactions, ($\Delta_{caq}H°$, $\Delta_{caq}H_c°$), for calculating metabolic enthalpy changes, and heat capacity (C_p) for selected compounds at 25°C*

The heat capacity values were obtained from Willhoit [35]. Units $\Delta_f H°$ and $\Delta_{caq}H°$(kJ/mol); $\Delta_{caq}H_c°$ (kJ/C-mol); C_p (J/K mol)

Substance	Formula	State	$\Delta_f H°$	$\Delta_{caq}H°$	$\Delta_{caq}H_c°$	C_p	Ref
Oxygen	O_2	g	0.0	0.0 *		29.4	def
		aq	−12.1	+12.1			36
Hydrogen ion	H^+	aq	0.0	0.0			def
Water	H_2O	l	−285.83	0.0 *		75.3	34
Carbon dioxide	CO_2	g	−393.51	0.0 *		37.1	37
		aq	−413.3	+19.8			37
		eq,b	−405.8				37
Carbonic acid	H_2CO_3	aq	−699.1	+19.8			37
		eq,b	−691.6				37
Bicarbonate ion	HCO_3^-	aq	−689.9	+10.6			37
Ammonia	NH_3	g	−45.8	−383.6 *			38
		aq	−81.1	−348.2			38
Ammonia ion	NH_4^+	aq	−133.1	−295.6		79.9	38
α-D-Glucose	$C_6H_{12}O_6$	c	−1273.0	−2803.0 *	−467.2		34
α,β-D-Glucose		aq	−1262.4	−2813.6	−468.9	305.4	34,35
Ethanol	C_2H_6O	l	−277.7	−1366.8 *	−683.4	112.0	34
		aq	−287.7	−1356.8	−678.4		34,35
Glycerol	$C_3H_8O_3$	l	−669.6	−1654.3 *	−551.4		39
		aq	−675.4	−1648.5	−549.5	238.5	39
Acetic acid	$C_2H_4O_2$	l	−484.1	−874.5 *	−437.3		34
		aq	−485.2	−873.4	−436.7	154.8	34,35
Acetate ion	$C_2H_3O_2^-$	aq	−485.5	−873.1	−436.6		34,35
L-Lactic acid	$C_3H_6O_3$	c	−694.1	−1344.0 *	−448.0		40
L-Lactic acid		aq	−686.3	−1351.8	−450.6		40
L-Lactate ion	$C_3H_5O_3^-$	aq	−686.5	−1351.6	−450.5		40
Succinate acid	$C_4H_6O_4$	c	−940.5	−1491.0 *	−372.8		34
		aq	−912.2	−1519.3	−379.8		35
Hydrogen-Succinate ion	$C_4H_5O_4^-$	aq	−908.9	−1522.6	−380.7		35
Succinate ion	$C_4H_4O_4^{-2}$	aq	−908.7	−1522.8	−380.7		35

TABLE A-II

Enthalpies of neutralization, $\Delta_b H_{H^+}$ of physiological buffer systems

If available, the values refer to ionic strength near 0.2 mol/l. For further experimental details consult original references. $\Delta_b H_{H^+}$ corresponds to the reaction $A^- + H^+ \rightarrow AH$.

Buffer system	T (°C)	$\Delta_b H_{H^+}$ (kJ/mol H^+)	pk_a	Ref.
bicarbonate				
HCO_3^-/CO_2(aq)	0	−19	6.6	41
	25	−8.7 – −7.7	6.4	41,35
	37	−5	6.3	41
$CaCO_3$ (c)/HCO_3^-	25	−28		35,42
carboxyl groups				
succinate	25	−0.3	5.6	35,43
citrate	25	+3.4	6.4	35,43
phosphate groups				
$HPO_4^{2-}/H_2PO_4^-$	0	−10	6.9	44
	25	−4.2	6.9	44
	37	−3.6	pH 6.6	45
pyrophosphate	25	−0.4	6.8	43
glucose-1-*P*	25	+1.8	6.5	43
glycerol-1-*P*	25	+3.1	6.7	43
nucleotide-*P*	25	+3.4 – +7.3	6.7–7.7	43,44
ribose-5-*P*	25	+11.3	6.7	43
amine groups				
glycylglycine	20	−48	pH 8.0	43,46
Tris	25	−48 – −49	8.0–8.3	46
Imidazole groups				
histidine	25	−30	6.0	43
H. in protein	0	−32	6.5	44
H. in myoglobin	25	−30	6.6	43
H. in ribonuclease	10–32	−34 – −17	5.8–6.7	43
carnosine	0	−32	7.3	44
cysteine (-SH)	25	−36	8.4	43
ferrihemoproteins	20–25	−43 – −7	8.0–9.0	43
intracellular				
mitochondria				
- in phosphate media	25	−27 – +4		47
- in chloride and				
acetate media	25	−27 – −18		47
erythrocytes	37	−17		48
muscle tissue	0	−31 – −25	pH 7.0	44
	25	−28 – −22	pH 7.0	44
	37	−26 – −20	pH 7.0	44

TABLE A-III

Enthalpies of catabolic reaction in lactic acid fermentation as a function of the buffer system at pH 7 and 25°C

α,β-D-glucose (aq) = 2L-(+)-lactate ion (aq) + $2H^+$ (aq)

Enthalpy of neutralization $\Delta_b H_{H^+}$ (kJ/mol H^+)	Catabolic enthalpy change		Dev. (%)	Ref.
	kJ/mol glucose	kJ/C-mol		
0	−109.4	−18.2	0	
−9	−127.4	−21.2	16	
−25	−159.4	−26.6	46	
S. faecalis	−128	−21.3	17	45
Mammalian				
cells	−126	−21.0	15	1

reference states are adopted for all quantities in an energy balance equation.

The reaction of formation refers to the formation of the substance from elements in their reference state [31]. Therefore, the enthalpy of formation of the elements in their reference state is zero by definition. For hydrogen, nitrogen and oxygen the reference state is the ideal diatomic gas (H_2, N_2 and O_2) at standard fugacity of 100 kPa, which is equal to the standard state of the gas. For C, S and P, the reference state is crystalline graphite, the rhombic modification of sulfur and the crystalline white, α-modification of phosphorus at 100 kPa pressure.

For enthalpies of formation of aqueous ions, the proton, H^+(aq), is by convention in its reference state, i.e., $\Delta_f H^\circ_{H^+} = 0$ [31,34].

The generalized combustion reaction of CHNOS compounds is [34],

$$C_cH_hN_nO_oS_s + ((4c + h - 2o + 6s)/4)O_2(g) + (116s - h/2)H_2O(l) \rightarrow cCO_2(g) + (n/2)N_2(g) + s[H_2SO_4 \cdot 115H_2O]$$

List of symbols

c	concentration, mol m^{-3}
C_p	heat capacity, kJ mol^{-1} K^{-1}
$\Delta_r C_p$	heat capacity of the reaction, kJ mol^{-1} K^{-1}
H	enthalpy, kJ
$H_{i,e}$	partial molar enthalpy of speices at conditions in the exchange stream, kJ mol^{-1}, see footnote 1
$H_{i,s}$	partial molar enthalpy of species i at conditions within the system, kJ mol^{-1}
H_i°	standard molar enthalpy of i, kJ mol^{-1}
$H^\circ_{i,out}$	standard molar enthalpy of species i in the exit stream, kJ mol^{-1}
$H^\circ_{i,in}$	standard molar enthalpy of species i in the entering stream, kJ mol^{-1}
$H^\circ_{i,s}$	standard molar enthalpy of species i in the system, kJ mol^{-1}
$\Delta_c H_i^\circ$	standard enthalpy of combustion of i, kJ mol^{-1}
$\Delta_{caq} H_i^\circ$	standard enthalpy of combustion of i modified for the aqueous state, kJ mol^{-1}
$\Delta_f H_i^\circ$	standard enthalpy of formation of i, kJ mol^{-1}
$\Delta_r H^\circ$	standard enthalpy of reaction r, kJ mol^{-1}, [a]
$\Delta_r H^\circ_{B,j}$	standard enthalpy of the j-th specified reaction or process, kJ mol^{-1}, [a]

[a] The term 'reaction' is used in a general way and includes chemical and biochemical reactions, entire growth processes, phase transitions, and other transitions between various standard states.

m	mass, kg
M_i	molar mass of i, g mol^{-1}
M_x	C-molar mass of dry ash-free biomass, g C-mol^{-1}
n	number of moles
P	pressure, kPa
Q	heat, kJ [b]
Q_o	proportionality coefficient between $\Delta_c H_x$ and γ_x^o, kJ C-mol^{-1}
T	temperature, K
t	time, s
U	internal energy, kJ
V	volume, m^3
W	work, kJ
γ_x^o	degree of reduction of biomass (Eqn. 3.9)
ξ_j	extent of the j-th process or reaction, see footnote 2
$\nu_{i,j}$	stoichiometric coefficient of species i in process j

Subscripts

B	per mol of B
e	e-th exchange site
ext	external [c]
i	referring to i-th chemical species; chemical compound in a defined thermodynamic state
j	referring to j-th process
in	entering the system [c]
int	internal [c]
mat	matter exchanged with surroundings
out	leaving the system [c]
r	reaction [d]
ref	referring to a reference state
s	system
x	referring to biomass

(1) Note concerning partial molar enthalpy

The partial molar H_i describes the change of the total enthalpy of a given mixture provoked by the addition of an infinitessimal amount of i, all other conditions staying constant. H_i is

$$H_i = \left(\frac{\partial H}{\partial n_i}\right)_{n_{j,\mathrm{ext}} n_i, T, P}$$

Very loosely speaking, H_i may be regarded as the enthalpy 1 mol of i contains when inside the mixture. H_i is different from the molar enthalpy of pure i even if the pure compound has the same state of aggregation as the mixture because the molecular interactions acting on i in the mixture are different. If all H_i in a mixture are known, the enthalpy of the mixture may be computed following a mathematical theorem as follows:

$$H = \sum_i H_i \cdot n_i$$

(2) Note concerning the advancement of reaction

The advancement of a reaction ξ_j is particularly useful in its derivative form, which represents a normalized rate of reaction. In a closed system, the rates of change of n_i due to reaction j is different from one species i to another. These rates have, however, the same values if each of them is divided by its stoichiometric coefficient:

$$\frac{1}{\nu_1}\frac{\mathrm{d}n_1}{\mathrm{d}t} = \frac{1}{\nu_2}\frac{\mathrm{d}n_2}{\mathrm{d}t} = \ldots\ldots = \frac{1}{\nu_i}\frac{\mathrm{d}n_i}{\mathrm{d}t} = \frac{\mathrm{d}_r\xi}{\mathrm{d}t}$$

This 'normalized' reaction rate defines $\mathrm{d}_r\xi/\mathrm{d}t$. Provided this normalized reaction rate is known, the rate of change of n_i due to reaction j may be computed for any i by:

$$\frac{\mathrm{d}n_i}{\mathrm{d}t} = \nu_i \frac{\mathrm{d}_r\xi}{\mathrm{d}t}$$

[b] The term 'heat' only comprises sensible heat exchanged with the environment by conduction, radiation, or convective heat transfer at a solid surface. Any sensible or latent heat imported by matter flowing into the system is accounted for in the $\mathrm{d}_{\mathrm{mat}}H/\mathrm{d}t$ term (Eqn. 2.9).

[c] Quantities and operators lacking any of these indices refer to system conditions and to exact differentials, respectively.

[d] See footnote a on p. 238. In connection with enthalpy changes, this r may be replaced by v for evaporation, by abs for absorption, by str for stripping, or by dil for dilution.

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