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ABSORBED HEAT AND HEAT OF FORMATION OF DRIED MICROBIAL BIOMASS
Studies on the thermodynamics of microbial growth

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Abstract

As represented by equations in which there is a term representing the biomass, the thermodynamics of biological growth processes is difficult to study without knowing the thermodynamic properties of cellular structural fabric. Measurement of the heat capacity data required to determine the standard entropy, $S^{\circ}_{298.15}$, or the standard absorbed heat, $(H^{\circ}_{298.15}-\Delta H^{\circ}_{0})=Q^{\circ}_{298.15}$, of biomass requires a low-temperature calorimter, and these are not present in most laboratories. Based on a previously described method for entropy, two equations are developed that enable values of the absorbed heat ($Q^{\circ}_{298.15}$) and the absorbed heat of formation, ($\Delta_{f}Q^{\circ}_{298.15}$) for biomass to be calculated empirically which are accurate to within 1% with respect to the biomass substances tested. These equations depend on a previous knowledge of the atomic composition or the unit-carbon formulas of macromolecules or structural cellular fabric.

Keywords: absorbed heat, enthalpy, entropy, free energy, heat content, microbial growth

Introduction

Experiments and determinations in both biological and non-biological calorimetry have experienced many advancements and polemics [1,2]. However in principal, microbial growth processes are no different from conventional chemical processes in that they can be represented by an equation incorporating an initial and a final state that includes cellular biomass [3, 4]. These are called 'growth process' equations, one example of which represents the aerobic growth of *Saccharomyces cerevisiae* on acetic acid [5].

$$C_2H_4O_2(aq)+0.098NH_3(aq)+1.358O_2(aq)+0.007H_2PO_4^-(aq)+0.002SO_4^{2-}(aq)+$$
$$+0.015K^+(aq)+0.004OH^-(aq)\rightarrow 1.380CO_2(aq)+1.656H_2O(l)+ \quad (1)$$
$$+0.620CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.024}(\text{cells})$$

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In Eq. (1) cellular biomass is represented by the last term in the form of a 'unit carbon formula' (UCF), from which a unit carbon formula mass (UCFW) can be calculated, a UCFW also being called a 'carbon-mol' (C-mol) in the European literature. In constructing a UCF, it is essential that the cells which are analyzed have been grown exponentially and do not contain any storage substances. As emphasized by Duclaux [6], storage substances are internal substrates and therefore do not comprise a part of the structural fabric of the cells. Methods of constructing growth process equations can be found in [3–5].

A microbial growth process is spontaneous and therefore accompanied by a negative change in free energy within the growth process system. In a closed system at a constant physiological temperature and pressure, heat is generated by the system as it proceeds from its initial to its final state and becomes transmitted to the environment. In the text to follow the function $(H_T^\circ - H_0^\circ)$, which is usually called the 'enthalpy', will be referred to as Q_T°, representing 'absorbed heat'. The reason for this is so that changes in the magnitude of the absorbed heat of a system as it passes from an initial to a final state can be represented as $\Delta_r Q_T^o$. This is a different quantity from $\Delta_r H_T^o$, representing the change in enthalpy for the system as a whole, which includes both $\Delta_r Q_T^o$, and the change occurring when chemical energy becomes converted into heat as the system passes from its initial to its final state.

As defined, at a constant pressure of 0.1 MPa the standard absorbed heat, Q_T^o, and the standard entropy, S_T^o, of a substance are related to the line integral of its heat capacity as a function of T/K plus the heat exchanges accompanying transitions and phase changes, as follows.

Calculation of the Third Law heat absorbed by a substance at 298.15 K:

$$Q^o_{298.15} = (H^o_{298.15} - H^o_0) = \int_{0\,K}^{298.15\,K} C_p \mathrm{d}T + \sum \Delta_{trs} H^o, \text{ Wagman } \textit{et al.} \text{ [7] p. 2–10} \qquad (2)$$

where $Q^o_{298.15}$ is the quantity of absorbed heat resulting in a temperature rise from 0 K to 298.15 K.

Calculation of the Third Law entropy of a substance at 298.15 K:

$$S^o_{298.15} = \int_{0\,K}^{298.15\,K} \frac{C_p}{T} \mathrm{d}T + \sum \frac{\Delta_{trs} H^o}{T_{trs}} = \int_{0\,K}^{298.15\,K} C_p \mathrm{d}\ln T + \sum \frac{\Delta_{trs} H^o}{T_{trs}},$$

Wagman *et al.* [7] p. 2–9. (3)

In both Eqs (2) and (3) the rightmost term of both equations represents heat changes accompanying transitions and phase changes. The temperature of 298.15 K has been chosen because (1) thermodynamic tabulations are available at that temperature, and (2) it represents roughly the middle of the physiological temperature range when this is taken to be within ca. 278.15 K (5°C) and 328.15 K (55°C). A small number of microbial species will grow at temperatures lower or higher than this. In Eq. (2) the quantity $(H_T^o - H_0^o)$ is here equated with Q_T^o (=total absorbed heat required for the temperature of a substance to be raised from 0 K to *T/K*). For a short discussion about

this please see section 4.3). It is thus different from the traditional q_{rev}, which would apply to a point on a graph of C_p *vs*. T/K, or C_p *vs*. ln$T$/K. For solid substances not undergoing transitions, the right term in either equation does not apply. Values of $Q^{o}_{298.15}$ ($=H^{o}_{298.15}-H^{o}_{0}$) for substances of biological importance are found in the original literature and are given in Table 1. It is apparent that both Eqs (2) and (3) are related to changes in C_p as a function of T/K between the limits 0 and 298.15 K. According to the 3rd Law, at least in the case of perfect crystalline substances there is no absorbed thermal energy at 0 K, although for non-crystalline substances there may be a residual entropy which is usually small compared to the total entropy at 298.15 K. The same would apply to the absorbed heat. However, for the temperature of any substance to be raised above 0 K, thermal energy must be absorbed. The term $Q^{o}_{298.15}$ ($=H^{o}_{298.15}-H^{o}_{0}$) in Eq. (2) represents the quantity of absorbed thermal energy required to raise the temperature of a given substance from 0 to 298.15 K. This quantity of thermal energy can be calculated directly by measuring the quantity of electricity passed in small increments through a wire of known resistance in low-temperature calorimeter so that the incremental temperature rises are small (theoretically, these temperature rises should be infinitely small, but since this is impractical the practice has been to use increments of 5–10 K). Provided that the heat capacities of both the calorimeter and the sample are known for any given temperature, the heat capacity of the sample at that temperature can be determined by difference. As represented by Eq. (2) the symbol $Q^{o}_{298.15}$ represents a function having the dimensions J (unit mass)$^{-1}$ (J mol^{-1} or J g^{-1}), which are those of energy. Because C_p represents thermal energy (heat) per unit mass, these dimensions also represent thermal energy. Equation (3) is a different mathematical treatment of the same C_p data represented in Eq. (2) for the given substance. The symbol S represents a function having the dimensions J (unit mass)$^{-1}$ K^{-1} (J mol^{-1} K^{-1} or J g^{-1} K^{-1}), which are not those of energy, although they are related to it. As emphasized by Klotz and Rosenberg, 'Ultimately, we must realize that entropy is essentially a mathematical function'[8]. So is Q, if not directly measured. However, to convert S into energy, it must be multiplied by T to become TS, the dimensions of TS then becoming J (unit mass)$^{-1}$ (J mol^{-1} or J g^{-1}). Using $S^{o}_{298.15}$ as represented by Eq. (3), $T_{298.15}S^{o}_{298.15}$ also represents the quantity of absorbed thermal energy required to raise the temperature of a given substance from 0 to 298.15 K. The difference in the mathematical treatment of the C_p data in Eqs (2) and (3) is that in Eq. (2) C_p is integrated as a function of T, and in Eq. (3) C_p/T is integrated as a function of T (or C_p as a function of ln$T$). It is probable that the reason for Eq. (3) is that classically S was defined by Clausius as Q_{rev}/T, where Q represents absorbed heat.

There is a caveat with respect to both Eqs (2) and (3), which applies strictly only to substances that are crystalline at 0 K. Disordered substances such as those comprising cellular fabric may have a certain amount of 'residual entropy' constituting an extra entropy contribution which may not be resolved by calorimetric measurements. Because entropy is calculated from C_p data, the same substances that have a residual entropy would be expected to have a residual absorbed heat. At present, to what extent residual entropy of dried cells and biopolymers is significant compared to the total entropy of such substances (or the residual absorbed heat compared to the total absorbed heat) can

only be conjectured because of the lack of sufficient evidence, and in this paper is assumed to be less than 1–2%. Because of this, the values $Q^{\circ}_{298.15}$, and $S^{\circ}_{298.15}$ as calculated using Eqs (2) and (3) must be regarded as upper bounds to their true values.

On the basis of Eqs (2) and (3) both $Q^{\circ}_{298.15}$ and $T_{298.15}S^{\circ}_{298.15}$ represent the quantity of heat required to be absorbed by a given substance to raise it from 0 K to the temperature of 298.15 K, as calculated using different equations, and it might initially appear that their values would be equal [9]. However, using the relationship C_p=0.10 T/K as a model, it has been shown that Q°/TS°=0.5 [10]. On the other hand, no substance has a C_p *vs. T* or a C_p *vs*. ln$T$ curve that is totally straight, although this is approximated for some of them. Using experimental C_p data for a number of these substances of biological importance the values of Q°/TS° at 298.15 K still gave a ratio close to 0.5, with a small standard error [11]. It is apparent that only one quantity of thermal energy can be absorbed by a given mass of a given substance resulting in a temperature rise from 0 to 298.15 K, and that either $Q^{\circ}_{298.15}$ or $T_{298.15}S^{\circ}_{298.15}$ can represent the correct quantity, but not both. The authors proposed that it should be $Q^{\circ}_{298.15}$. A subsequent study examined whether there was any difference in the use of $\Delta Q^{\circ}_{298.15}$ rather than $T_{298.15}\Delta S^{\circ}_{298.15}$ in the calculation of ΔG° accompanying the oxidation or fermentation of catabolic substrates of biological importance in their standard states [12]. It was reported that a difference was clearly evident, especially for those processes for which there was a large entropy change.

For the purpose of testing this idea with growth process equations, it is necessary to have close estimates of both the entropy and the absorbed heat of cellular substance, since these are not generally available from the literature. The determination of values for $S^{\circ}_{298.15}$ and $Q^{\circ}_{298.15}$ is dependent upon acquiring C_p data using a low-temperature calorimeter capable of operating at temperatures down to 5–15 K and applying the Debye or other extrapolation to 0 K. These instruments are costly, and not found in most laboratories. Because of this an empirical, indirect calorimetric method of calculating $\Delta_f S^{\circ}_{298.15}$ and $S^{\circ}_{298.15}$ has been devised that requires only an empirical formula for an organic substance and values for the standard entropies at 298.15 K of the constituent atoms. This method involves the following equations.

$$\Delta_f S^{\circ}_{298.15} = -0.813 \sum S^{\circ}_{298.15}\ \text{(atoms)} \tag{4}$$

and

$$S^{\circ}_{298.15} = 0.187 \sum S^{\circ}_{298.15}\ \text{(atoms)} \tag{5}$$

where $\sum S^{\circ}_{298.15}$ (atoms) represents the sum of the standard entropies of the individual component atoms of an organic substance multiplied by their respective subscripts [13].

This method is accurate to within about 1% for large molecular mass biological substances such as cells, proteins, etc. The purpose of this present investigation is to determine if an analogous method can be devised to calculate values of the absorbed heat (heat content) for similar substances.

Methods

The idea of an organic substance being formed from its elements can be represented by the following equation, representing the formation of cellular substance.

$$c\mathrm{C}+h\mathrm{H}+o\mathrm{O}+n\mathrm{N}+p\mathrm{P}+s\mathrm{S}+k\mathrm{K}\rightarrow \mathrm{C_cH_hO_oN_nP_pS_sK_k} \quad (6)$$

where *c, h, o, n, p, s* and *k* represent the subscripts in, for example, the cellular formula of Eq. (1). This formation process is accompanied by an 'entropy of formation' from the elements $\Delta_f S^o_T$, which varies with the temperature, and for T=298.15 K can be calculated by means of the following equation:

$$\Delta_f S^o_{298.15} = S^o_{298.15} - \sum S^o_{298.15}\ \text{(atoms)} \quad (7)$$

where $S^o_{298.15}$ represents the standard entropy of a substance and $\sum S^o_{298.15}$ (atoms) the sum of the standard entropies of the individual component atoms multiplied by their respective subscripts.

However, values for $\Delta_f S^o_{298.15}$ can also be calculated empirically by means of Eq. (4), from which values for $S^o_{298.15}$ can also be calculated using Eq. (5). The 'absorbed heat of formation' from the elements, $\Delta_f Q^o_{298.15}$, is a concept analogous to that of the 'entropy of formation' from the elements. Thus, in analogy to Eq. (7)

$$\Delta_f Q^\circ_{298.15} = Q^\circ_{298.15} - \sum Q^\circ_{298.15}\ \text{(atoms)} \quad (8)$$

where $Q^o_{298.15}$ represents the standard heat absorbed by a substance at 298.15 K and $\sum Q^o_{298.15}$ (atoms) the sum of the standard heat absorbed by the individual component atoms multiplied by their respective subscripts. Equations analogous to (4) and (5) with respect to entropy could also be expected to apply to calculating the absorbed heat of formation. Thus,

$$\Delta_f Q^o_{298.15} = -\mathrm{c}\sum Q^o_{298.15}\ \text{(atoms)} \quad (9)$$

and

$$Q^o_{298.15} = \mathrm{c}\sum Q^o_{298.15}\ \text{(atoms)} \quad (10)$$

where 'c' in both equations is a constant.

Table 1 lists experimentally determined values of $Q^o_{298.15}$ for atoms and solid organic substances. Values of $\Delta_f Q^o_{298.15}$ are calculated using Eq. (9) and then used to calculate the ratio $\Delta_f Q^o_{298.15} / \sum Q^o_{298.15}$ (atoms). As an example from Table 1, *L*-alanine has the formula $C_3H_7O_2N$ and an experimentally determined value for $Q^o_{298.15}$ (heat content) of 20.034 kJ mol^{-1}. Also from Table 1, the standard heat contents at 298.15 K for the individual atoms comprising *L*-alanine are C(*c*)=1.050, $H_2(g)$=(8.988), $O_2(g)$=(17.390), and $N_2(g)$=(15.512) kJ mol^{-1}. From these

Table 1 Absorbed heat (heat content) data for organic substances of biological interest in the solid state.[a] All values are for 298.15 K

Substance	Formula	1 MW/ Da	2 Q^o/ kJ mol^{-1}	3 $\Delta_f Q^o$/ kJ mol^{-1}	4 ΣQ^o atoms/ kJ mol^{-1}	5 $\frac{\Delta_f Q^o}{\Sigma Q^o \text{ (atoms)}}$
Elements						
Hydrogen [12]	$H_2(g)$	2.016	8.988	0	8.988	
Carbon [15]	$C(c)$	12.011	1.050	0	1.050	
Nitrogen [12]	$N_2(g)$	28.013	15.512	0	15.512	
Phosphorous [15]	$P(c)$	30.974	5.360	0	5.360	
Oxygen [12]	$O_2(g)$	31.998	17.390	0	17.390	
Sulfur [15]	$S(c)$	32.066	4.412	0	4.412	
Potassium [15]	$K(c)$	39.098	7.082	0	7.082	
Zinc [15]	$Zn(c)$	65.39	5.669	0	5.669	
Solid organic substances[a]						
Glycine [16]	$C_2H_5O_2N$	75.07	16.179	–33.537	49.716	–0.6746
L-alanine [16]	$C_3H_7O_2N$	89.09	20.034	–39.720	59.754	–0.6647
L-serine [17]	$C_3H_7O_3N$	105.09	22.653	–45.796	68.449	–0.6691
L-proline [18]	$C_5H_9O_2N$	115.13	24.437	–46.405	70.842	–0.6550
L-valine [19]	$C_5H_{11}O_2N$	117.15	27.581	–52.249	79.830	–0.6545
L-leucine [19]	$C_6H_{13}O_2N$	131.17	31.623	–58.245	89.868	–0.6481
Glycyl glycine [20]	$C_4H_8O_3N_2$	132.12	27.394	–54.355	81.749	–0.6649
L-aspartic acid [21]	$C_4H_7O_4N$	133.10	25.810	–52.384	78.194	–0.6699
L-glutamine [21]	$C_5H_{10}O_3N_2$	146.15	30.051	–61.736	91.787	–0.6726
L-glutamic acid [21]	$C_5H_9O_4N$	147.13	28.766	–59.466	88.232	–0.6740

Table 1 Continued

Substance	Formula	1 MW/ Da	2 Q^o/ kJ mol^{-1}	3 $\Delta_f Q^o$/ kJ mol^{-1}	4 ΣQ^o atoms/ kJ mol^{-1}	5 $\frac{\Delta_f Q^o}{\Sigma Q^o \text{ atoms}}$
Solid organic substances[a]						
L-methionine [22]	$C_5H_{11}O_2NS$	149.21	35.103	–49.139	84.242	–0.5833
L-phenylalanine [18]	$C_9H_{11}O_2N$	165.19	31.947	–52.083	84.030	–0.6198
L-tyrosine [18]	$C_9H_{11}O_3N$	181.19	33.369	–59.356	92.725	–0.6401
L-tryptophane [18]	$C_{11}H_{12}O_2N_2$	204.23	37.211	–61.169	98.380	–0.6218
L-cystine [23]	$C_6H_{12}O_4N_2S$	240.30	42.937	–76.407	114.932	–0.6402
Hexadecanoic acid [23]	$C_{16}H_{32}O_2$	256.43	68.621	–109.377	177.998	–0.6144
Anhydrous biomass						
Bovine zinc insulin [24][b]	$CH_{1.480}O_{0.295}N_{0.256}S_{0.024}Zn_{0.002}$	22.71	4.490	–7.878	12.368	–0.6370
Chymotrypsinogen A [24][c]	$CH_{1.612}O_{0.318}N_{0.282}S_{0.011}$	23.08	4.687	–8.607	13.294	–0.6474
Escherichia coli [25][d]	$CH_{1.595}O_{0.374}N_{0.263}P_{0.023}S_{0.006}K_{0.013}$	24.70	5.003	–8.748	13.751	–0.6362
Saccharomyces cerevisiae [27][e]	$CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.024}$	26.17	5.266	–9.348	14.614	–0.6396

[a]All organic substances are in the crystalline state except for the cells, which have been lyophilized.
[b]This unit carbon formula was calculated from the empirical formula, $C_{508}H_{752}O_{150}N_{130}S_{12}Zn$ taken from [24].
[c]This unit carbon formula was calculated from the empirical formula, $C_{1077}H_{1736}O_{343}N_{304}S_{12}$ taken from [24].
[d]This unit carbon formula was taken from [25]. The heat capacity data were taken from [26].

$$\sum Q^{\circ}_{298.15}\ (\text{Atoms}) = [(3\cdot 1.050)+(3.5\cdot 8.988)+(17.390)+(0.5\cdot 15.512)]$$

$$= (3.150+31.458+17.390+7.756)=59.754\ \text{kJ mol}^{-1}$$

Using Eq. (7),

$$\Delta_f Q^{\circ}_{298.15} = 20.034–59.754 = –39.720\ \text{kJ mol}^{-1}$$

and

$$\Delta_f Q^{\circ}_{298.15}/\sum Q^{\circ}_{298.15}\ (\text{Atoms}) = –39.720/59.754 = –0.6647$$

The same ratios can be calculated with respect to biomass, except that usually these substances can not be represented by a molecular formula. The practice here is then to determine an empirical formula represented in terms of C, H, O, N, P, S and K, and to divide all the subscripts by the subscript for carbon. This results in a formula having a unit quantity of carbon, called a 'unit carbon formula' (UCF) which has a 'unit carbon formula mass' (UCFW) [5]. In the European literature in general this has been more recently called a 'carbon-mol' (C-mol).

Results

In Fig. 1a, values for $\Delta_f Q^{\circ}_{298.15}$ are plotted *vs.* corresponding values for $\sum Q^{\circ}_{298.15}$ (atoms). It is apparent that for the set of 19 cellular structural monomers studied here at 298.15 K, values for the ratio of $\Delta_f Q^{\circ}_{298.15}/\sum Q^{\circ}_{298.15}$ (atoms) fall closely along a regression line, so that

$$\Delta_f Q^{\circ}_{298.15}\ (\text{Monomers}) = [–0.5969\sum Q^{\circ}_{298.15}\ (\text{atoms})–4.3375]\ \text{kJ mol}^{-1} \qquad (11)$$

indicating that this relationship could be a useful method for calculating values for the kinds of substances being studied.

However, although the *r*-values indicate a favorable relationship for purposes of calculation, the range of values for $\Delta_f Q^{\circ}_{298.15}/\sum Q^{\circ}_{298.15}$ (atoms) within this set is from –0.5833 for methionine to –0.6746 for glycine, indicating a considerable variation. This can create a large error depending on the substance. For example, using Eq. (11) the value of $\Delta_f Q^{\circ}_{298.15}$ for methionine is calculated to be –54.62 kJ mol^{-1}, as compared to –49.14 kJ mol^{-1} when calculated using Eq. (8) and data from Table 1, a difference of 11.15% from the experimental value. It is apparent that this method is too inaccurate to be used for small molecular mass substances, the heat capacities of which had better be determined by calorimetry.

On the other hand, in Fig. 1b the same plot is made with respect to biomass, where the biomass is represented by a UCF. For the biomass substances studied here, the regression line can be represented by the equation

$$\Delta_f Q^{\circ}_{298.15}\ (\text{biomass}) = [–0.6413\sum Q^{\circ}_{298.15}\ (\text{atoms})–0.0163]\ \text{kJ mol}^{-1} \qquad (12)$$

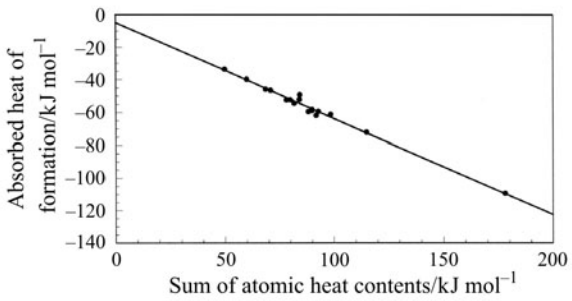

Fig. 1a Graph of the data for solid-state organic substances listed in column 3 of Table 1 plotted *vs.* those in column 4. The line is a linear regression using the sum of least squares method and calculated according to the equation $y=mx+b$, where m=–0.5969 and b=4.3375, r=–0.9920 and r^2=0.9840

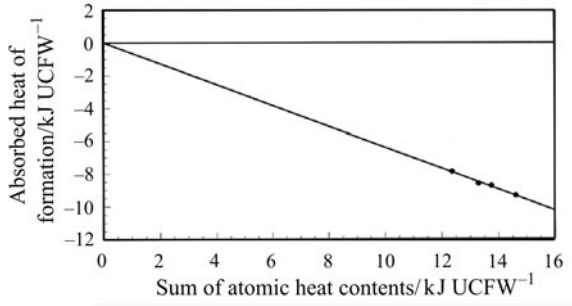

Fig. 1b Graph of the data for biomass listed in column 3 of Table 1 plotted *vs.* those in column 4. For the regression line m=–0.6413, b=0.0163, r=–0.9936, and r^2=0.9872

where –0.6413 is the slope and 0.0163 the y-intercept. In Fig. 1b, r=–0.994 and r^2=0.987, which is a better fit than that in Fig. 1a. There are no significant outliers on this graph. Using *S. cerevisiae* as an example, and with reference to Table 1.

$$\Delta_f Q^\circ_{298.15}\ \text{(biomass)}=[(-0.6413\cdot 14.600)-0.0163]=(-9.3630-0.0163)\ \text{kJ mol}^{-1}=$$

$$=-9.379\ \text{kJ UCFW}^{-1}$$

This compares well with the value of –9.351 kJ UCFW^{-1} determined using experimental, cellular heat capacity data from Table 1, a difference of only 0.30%.

Using the value for $\Delta_f Q^\circ_{298.15}$ (biomass) in Eq. (8), it is also possible to calculate individual values of $Q^\circ_{298.15}$ (biomass) by means of the following equation.

$$Q^\circ_{298.15}\ \text{(biomass)} = \Delta_f Q^\circ_{298.15}\ \text{(biomass)}+\sum Q^\circ_{298.15}\ \text{(atoms)} \qquad (13)$$

$$= [-0.6413\ \sum Q^\circ_{298.15}\ \text{(atoms)}+0.0163]\ \text{kJ mol}^{-1}+\sum Q^\circ_{298.15}\ \text{(atoms)}$$

As an example, for *S. cerevisiae* with reference to Table 1, and using Eq. (13)

$$Q^{\circ}_{298.15}\ (\text{Biomass}) = [(-0.6413 \cdot 14.600) + 0.0163] + 14.600$$
$$= [-9.3630 + 0.0163]\ \text{kJ mol}^{-1} + 14.600\ \text{kJ mol}^{-1}$$
$$= 5.253\ \text{kJ UCFW}^{-1}$$

This is in agreement by +0.08% with the experimental value of 5.249 kJ $UCFW^{-1}$ from Table 1. Equally good results should be obtained with the other biomass substances.

Discussion

In general

As with entropy, it is unfortunate that there is not a greater quantity of critically measured data with respect to the absorbed heat at 298.15 K and 0.1 MPa for polymeric substances of biological interest which form a part of the cellular fabric. Only two are listed for macromolecules because these are the only crystalline proteins for which the empirical composition is known based on a knowledge of the nature of the amino acid subunits, for which an empirical formula can be determined, and for which accurate heat capacity data are available. The two microbial species, *Escherichia coli* (*E. coli*) and *Saccharomyces cerevisiae* (*S. cerevisiae*) are the only ones for which heat capacity determinations have been made and for which an empirical composition is known containing all the elements (C, H, O, N, P, S and K) that together comprise about 99% of the structural fabric of the cells. Nevertheless, something can be learned by working with the available data.

It should be noted that the regression line in Fig. 1b is based on only on four points, which is hardly a good statistical number but is all the reliable data we have at the moment. Nevertheless, the data are strongly suggestive that Eq. (13) can be used to advantage in calculating values of $Q^{\circ}_{298.15}$ for biomass. The slope of the regression line in Fig. 1a is slightly different from that in Fig. 1b. This may be because with small molecular mass organic substances, the side groups on the carbon chains have a much greater effect on the absorbed heat than if these molecules were to be polymerized into macromolecules using covalent bonds that incorporate these side groups, as in peptides or esters, whereupon the effect of the side groups would tend to even out. Also, the slope of the line in Fig. 1a will change as a function of the data set. According to the idea of 'unity in biochemistry' introduced in 1926 by Kluyver and Donker [14], the biochemistry of all cells is effectively the same. This can be extended to assert that the polymeric cellular fabric of all cells, exclusive of storage substances, is comprised of very nearly the same proportions of elements and is essentially the same. And, the influence of the various monomers comprising the polymers will tend to average out. This may be responsible for the greater accuracy with which $\Delta_f Q^{\circ}_{298.15}$ (biomass) and $Q^{\circ}_{298.15}$ (biomass) can be calculated, as compared to that with respect to small molecular mass organic molecules of biological interest.

It must be emphasized that the empirical method described here, just as with entropy, is useful only within the physiological temperature ranges at which cells can be

grown, with an average being ca. 298.15 K. Also, values of $\Delta_f Q^o_{298.15}$ (biomass) and $Q^o_{298.15}$ (biomass) are for anhydrous substances, whereas biomass in its natural condition is to a certain extent hydrated. The addition of water to biomass to whatever extent this happens in nature would change all values of presently determined quantities. On the other hand, as a first approximation, cells can be considered to have many of the physical characteristics of unhydrated precipitates. It has yet to be determined to what extent a small degree of hydration of the fabric would affect the value of thermodynamic properties such as $\Delta_f Q^o_{298.15}$ (biomass) and $Q^o_{298.15}$ (biomass) or to what extent the addition of the water necessary to hydrate (left side) and the water of hydration (right side) would appreciably affect the thermodynamics of the growth process represented by Eq. (1).

On the concept of heat

Heat can be defined as thermal energy that is exchanged, or is exchangeable, between two masses because of a temperature difference between them, and is a part of the internal energy of a mass. The word 'heat' represents the random, undirected kinetic energies of translation, vibration, and rotation of the molecules or atoms that comprise mass (solid, liquid or gas). These can be referred to as random or undirected 'energies of motion', and as these energies increase or decrease in magnitude, the temperature of a mass rises or falls, respectively, as indicated by some temperature sensing instrument calibrated for a given temperature scale. The temperature of a mass is therefore a function of the magnitude of the random energies of motion of the molecules or atoms comprising it. A mass at a higher temperature would 'contain' more molecules and atoms having higher random energies of motion than the same mass at a lower temperature, and thus has a higher 'heat content' at the higher temperature. Just as everything has an entropy, everything has a heat content in the sense described above. Heat content is taken here to be the same as total absorbed thermal energy.

Heat is different from work, which latter can be defined as non-thermal energy which is exchanged between two masses because of a force exerted between them. Thus, a mass containing atoms or molecules having undirected energies of motion (at a given temperature) can be subject to a force that directs the totality of these randomly moving atoms or molecules in a given manner as the result of an exchange of non-thermal energy. Examples are bullets, rotating wheels, or falling objects.

Why use 'Q^o_T' instead of (H^o_T–H^o_0) to represent absorbed heat (heat content)?

The reason for this is simply to be able to distinguish between the exchange of absorbed heat resulting from the conversion of reactants into products during a reaction, from that resulting from the conversion of chemical energy into thermal energy during the same reaction. In modern terminology, the symbol (H^o_T–H^o_0), and sometimes only 'H^o_T' since 'H^o_0'=0, is called 'enthalpy'. For the reaction A+B→C+D taking place at 298.15 K, the total thermal exchange due to changes in the absorbed heat of the system would be:

$$\Delta_r H^\circ_{298.15} = [(H^\circ_{298.15} - H^\circ_0)_C + (H^\circ_{298.15} - H^\circ_0)_D] - [(H^\circ_{298.15} - H^\circ_0)_A + (H^\circ_{298.15} - H^\circ_0)_B] \quad (14)$$

For the same reaction, the heat of reaction (also an enthalpy change) would be:

$$\Delta_r H^\circ_{298.15} = (\Delta_f H^\circ_{298.15;C} + \Delta_f H^\circ_{298.15;D}) - (\Delta_f H^\circ_{298.15;A} + \Delta_f H^\circ_{298.15;B}) \quad (15)$$

It is apparent that although the term on the left is the same for Eqs (14) and (15), the equations are not the same. It is more simple if the symbol $(H^\circ_T - H^\circ_0)$ could be changed to Q°_T to recognize that it represents absorbed heat, as opposed to the heat of reaction which includes the absorbed heat exchange. Equation (14) would then become

$$\Delta_r Q^\circ_{298.15} = (Q^\circ_{298.15;C} + Q^\circ_{298.15;D}) - (Q^\circ_{298.15;A} + Q^\circ_{298.15;B}) \quad (16)$$

and Eq. (15) becomes

$$\Delta_r H^\circ_{298.15} = \Delta_r Q^\circ_{298.15} + \text{(a heat exchange due to the conversion of chemical energy into heat)} \quad (17)$$

This last term is the free energy (free enthalpy), which should be represented by an appropriate symbol which cannot be $\Delta_r G^\circ_{298.15}$ because it does not represent Gibbs free energy. A suggested symbol is $\Delta_r X^\circ_{298.15}$ because the letter '*X*' cannot be confused with any presently existing symbols in chemical or biological thermodynamics [28]. Other symbols are also possible.

Conclusions

The equations described for the calculation of values of $\Delta_f Q^\circ_{298.15}$ and $Q^\circ_{298.15}$ for small molecular mass monomers forming part of the structural material in cells are useful to within only about 10%. They are thus of little use in calculating values for these thermodynamic quantities. On the other hand, using the same methods the equations described for calculating values of $\Delta_f Q^\circ_{298.15}$ (biomass) and $Q^\circ_{298.15}$ (biomass) are highly accurate (for biological substances) with the caveat that these have been constructed using only 4 substances. On the basis of the data provided here, for most practical purposes it is not necessary to carry out low-temperature calorimetry for the purpose of determining $\Delta_f Q^\circ_{298.15}$ (biomass) and $Q^\circ_{298.15}$ (biomass), provided that an accurate UCF representing the biomass can be constructed.

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