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A short review and an empirical method for estimating the absorbed enthalpy of formation and the absolute enthalpy of dried microbial biomass for use in studies on the thermodynamics of microbial growth

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Abstract The equation $\Delta_r X = \Delta_r H - \Delta_r Q$ represents a calculated free-energy change when the exchange of absorbed thermal energy in a chemical system represented by $T\Delta_r S$ in the Gibbs free-energy equation is replaced by $\Delta_r Q$. The symbol Q is used in place of H [enthalpy $= H_T - H_0 = H_T$] to represent absorbed thermal energy. Acquiring the experimental data for determining both S and Q requires the use of a low-temperature calorimeter to measure C_p as a function of T/K and these are not generally available. In a previous study it was demonstrated that for one unit-carbon formula dry weight of cells, $\Delta_f S_{biomass} = -0.813 \sum S_{atoms}$ and $S_{biomass} = 0.187 \sum S_{atoms}$, where $\sum S_{atoms}$ represents the sum of the entropies of the numbers and kinds of atoms in the biomass. Using similar techniques, it is shown here that $\Delta_f Q_{biomass} = -0.648 \sum Q_{atoms}$ and that $Q_{biomass} = 0.352 \sum Q_{atoms}$, where $\sum Q_{atoms}$ represents the sum of the absorbed thermal energies of the numbers and kinds of atoms in the biomass. Because mathematically the value of TS for solid substances is twice that of Q for the same T/K (usually referenced at 298.15 K), one of these values must be physically incorrect. There cannot be two different values for the quantity of thermal energy which must be absorbed to raise the temperature of the same quantity of the same substance from 0 K to a given temperature. The argument is made that the use of Q is preferable to the use of S in the calculation of free-energy changes.

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Introduction

The natural environment of free-living microorganisms is one in which a population in a small, localized space increases exponentially until it exceeds its carrying capacity or encounters a limiting factor. The population then collapses, with most cells dying off, reverting to a very low metabolic rate, or sporulating. The time period of these cycles can be short, measured in hours. All cells live in an aqueous environment, and the high heat capacity of water resists a rapid temperature change. For purposes of thermodynamic studies the generalization can then be made that the temperature of growing microbial cells is constant during a growth cycle, as is the pressure at which this growth takes place.

Biological growth is a chemical event that is no different from any other organic chemical reaction taking place in an aqueous environment except in that it is extremely complex, and would better be called a "process." It is impossible to express in any concise manner all the intermediate reactions that go on within a cell during growth. It is far less difficult to write a growth-process equation in terms of its initial state comprised of the substances that enter into the composition of the cells that are formed, and its final state comprised of the cells and other products of a growth-process. As an example, for the yeast *Saccharomyces cerevisiae*, the process of microbial growth aerobically on glucose, excluding Ca^{++}(aq) and Mg^{++}(aq), can be written as follows [1].

$$\begin{aligned} &C_6H_{12}O_6(aq) + 0.302NH_3(aq) + 4.050\ O_2(aq) \\ &+ 0.023\ H_2PO_4^-(aq) + 0.006\ SO_4^{2-}(aq) + 0.042\ K^+(aq) \\ &\rightarrow 1.914\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}(cells) \\ &+ 4.086\ CO_2(aq) + 4.975\ H_2O(l) \end{aligned} \quad (1)$$

Here the cells are represented by a unit-carbon formula (UCF) followed by the suffix "cells" and have a unit-carbon formula weight (UCFW) in which the quantities of all the other atoms in the formula are relative to that of carbon, which is set at unity [2]. The quantities of inorganic ions other than K^+ are usually not included because of being insignificantly small. Similar equations can be written representing growth of other microorganisms on other substrates [3–6].

The energy exchanges accompanying biological reactions or processes, including microbial growth, are usually represented by the Gibbs free-energy equation or variations thereof.

$$\Delta_r G = \Delta_r H - T\Delta_r S \quad (2)$$

In Eq. 2 $\Delta_r G$ (free-energy) represents an exchange of some or all of the available chemical potential energy of a system into work, or into heat which becomes exchanged with the environment; $\Delta_r H$ (enthalpy) represents the total heat exchange of the system with the environment; and $T\Delta_r S$ represents an exchange of heat with the environment because the sum of the absorbed thermal energies of the reactants in the system is different from that of the products, all at a constant temperature and pressure. The units of all terms in Eq. 2 are those of energy divided by mass. Thermodynamic exchanges with the environment involving microbial growth such as those represented in Eq. 2 and preceded by a Δ sign are not represented with the superscript "o" because there is no standard state for the cells that participate as products in microbial growth-processes.

The enthalpy change, $\Delta_r H$, accompanying microbial growth

Enthalpy, H, is a function of state. The meaning of $\Delta_r H$ is easy to understand in a non-mathematical sense as the total quantity of heat exchanged with the environment as the growth of a population of microorganisms proceeds from its initial to its final state at constant temperature and pressure, as represented by a growth-process equation. The heat of growth can be measured directly by means of a sensitive calorimeter which, after corrections, gives a quantity of heat which is the result of a growth-process such as represented in Eq. 1. Microbial growth is not a reversible process. Cells cannot be "ungrown", and their state is that of an insoluble, slightly hydrated substance represented by the suffix "(cells)". The heat released to the environment during microbial growth originates from two sources, as shown by rearranging Eq. 2.

$$\Delta_r H = \Delta_r G + T\Delta_r S \quad (3)$$

$\Delta_r H$ can be measured directly, but $\Delta_r G$ and $T\Delta_r S$ cannot. $\Delta_r H$ can be determined also with the following equation:

$$\Delta_r H = \sum \Delta_f H_{prod} - \sum \Delta_f H_{react} \quad (4)$$

where $\Delta_f H$ represents the enthalpies of formation at 298.15 K and 0.1 MPa of the reactants and products of a growth-process.

The entropy exchange, $\Delta_r S$, accompanying microbial growth

Entropy, S, is a function of state. Although it cannot be measured directly, it can be determined indirectly by a measurement of the heat capacities of a given mass of substance with respect to a series of incremental rises in temperature. Briefly, in this procedure, a sample of dried cells is placed in a low-temperature calorimeter, after which the temperature of the sample is lowered to 5–10 K. The sample is heated with a known quantity of thermal energy from a calibrated, electrical resistance wire, so as to raise the temperature of the sample by an increment which theoretically should be infinitely small, but since this is impossible these increments are kept practically small. For each increment, the temperature of the sample is allowed to equilibrate; the heat capacity, C_p, of the substance is determined for that temperature increment, and another small quantity of heat is provided to the sample. This is done many times, each increment requiring a measurable quantity of heat from which a heat capacity can be calculated. During this procedure, the temperature of the sample slowly rises and the sample itself may pass through one or more phase changes. The final result of a sequence of experimental runs is a table of incremental heat capacity values at constant pressure having the dimensions of J K^{-1} unit-mass^{-1} listed as a function of T/K up to 298.15 K. Heat capacities between 8 and 0 K are usually calculated using the Debye T^3 equation. The absolute entropy, S, is the integral of these incremental heat capacities plotted against log T/K, as follows [7b]:

$$(S_{298.15} - S_0) = S^o = \int_0^{298.15} C_p d\log T + \sum \Delta_{trs} H_T / T_{trs} \quad (5)$$

where the second term on the right represents the heat exchange accompanying transitions or phase changes, if any. An example of the determination of the entropy of microbial cells can be found in Battley et al. [8]. All entropy values are

positive. Entropy has the units J K^{-1} unit-mass^{-1}, which are not those of energy, the latter being J unit-mass^{-1}. A problem may exist as to what the word "entropy" means. Entropy is claimed to be related to *thermodynamic* probability, randomness, complexity, information, etc. Entropy is not related to the sort of *numerical* probability commonly in use, which has no units. Entropy has been said to be "produced" or "consumed" as if it were a physical entity. Entropy is not a physical entity in this sense. It is essentially a mathematical function [9]. The value for this function cannot be transported across the limiting boundaries of a closed system unless the mass associated with it can also be transported. On a dry weight basis, at 298.15 K the entropy of *S. cerevisiae* cells has a value of 1.304 J K^{-1} g^{-1}, which falls about midway between those of a number of small molecular weight substances of biological importance such as sugars or amino acids but of very little complexity [8]. From this it was concluded that although cells have been thought to be extremely improbable substances because of their complexity compared with simple, organic molecules, the thermodynamic effect of cellular organization is negligible. This experimental proof was predated by the opinion of Morrison that, "Nearly all the manifest visual and mechanical intricacy of organisms, like their apt behavior, turns out to be without quantitative thermodynamic importance. Morphology and ecology are …… only small, secondary properties of a fundamentally thermodynamic system" [10].

The units of a physical quantity can be obtained if S becomes multiplied by the value of T/K for which the entropy value has been determined, which is the temperature of the reaction or process. The temperature unit cancels out, and the units of TS then become those of energy. But, is this thermal or non-thermal energy? Surely it must be thermal energy. Entropy must be related to the absorption of thermal energy because this is what appears in the two terms on the right in Eq. 5 as C_p measurements or as $\sum \Delta_{trs} H_T/T_{trs}$. Therefore, TS can only represent that quantity of thermal energy required to be absorbed by a given mass of substance for it to attain a temperature of 298.15 K at which most reference data of this kind are arbitrarily established. For *S. cerevisiae* cells,

$$S = 1.304\text{J K}^{-1}\ \text{g}^{-1} \times 26.202\ \text{g UCFW}^{-1} = 34.165\ \text{J K}^{-1}\ \text{UCFW}^{-1} \quad (6)$$

from which [8]

$$TS = 298.15\ \text{K} \times 34.17\ \text{J K}^{-1}\text{UCFW}^{-1} = 10.19\ \text{kJ UCFW}^{-1} \quad (7)$$

Because every substance has an entropy and a value for TS, the value of $T\Delta_r S$ accompanying microbial growth as represented by Eq. 1 is then:

$$T\Delta_r S = T\left[\sum S_{prod} - \sum S_{react}\right] \quad (8)$$

This quantity can also be calculated using the following equation:

$$T\Delta_r S = T\left[\sum \Delta_f S_{prod} - \sum \Delta_f S_{react}\right] \quad (9)$$

where $\Delta_f S_{prod}$ and $\Delta_f S_{react}$ represent the entropies of formation of the products and reactants, represented by the following equation:

$$\Delta_f S = S - \sum S_{atoms} \quad (10)$$

and where $\sum S_{atoms}$ represents the sum of the kinds and quantities of atoms in the substance being considered. As emphasized above, entropy is not energy, but acquires the units of energy when multiplied by T. $T\Delta_r S$ is a term in Eqs. 2 and 3 which has long been described as a quantity of energy that is unavailable for work. This is understandable with the realization that this is thermal energy being exchanged with an environment having a constant temperature and pressure.

The absorbed thermal energy exchange,
$\Delta_r(H_T - H_0) = \Delta_r Q$, accompanying microbial growth

Absorbed heat, $(H_T - H_0)$, or enthalpy, is a function of state. For single substances it is also represented simply as H_T in that H_0 has a value of zero. It represents a use of the same C_p data used to calculate S using Eq. 5 except that rather than being integrated as a function of log T, these data are integrated as a function of T. This integration is represented by the following equation [7a]:

$$(H_{298.15} - H_0) = H_T = Q_T = \int_0^{298.15} C_p\, dT + \sum \Delta_{trs} H_T \quad (11)$$

It is *theoretically* possible to accomplish what is represented in Eq. 11 by a single, practical physical experiment from, say, 10–298.15 K, using the same technique of raising the temperature in small increments each followed by a temperature equilibration. The data can be extended to 0 K with the Debye T^3 equation. Then, the final quantity of thermal energy delivered to the sample to achieve a temperature of 298.15 K would be the same as that obtained using Eq. 11, except that it would be obtained by a direct measurement, rather than by points in a series of experimental runs that are fitted to a smooth curve and integrated against T/K. Such a direct measurement also provides incontrovertible evidence that this quantity of absorbed heat is in fact that required to raise the temperature of the sample to 298.15 K, as represented by

($H_{298.15} - H_0$). On the other hand, the symbol H is also used to represent reaction enthalpy changes which, from Eq. 3, include more than absorbed heat exchanges. For this reason, the symbol H might better be replaced by the traditional symbol for absorbed thermal energy, which is Q, so that ($H_{298.15} - H_0$) becomes ($Q_{298.15} - Q_0$) = Q_T, and this will be done in the present article. An experimental determination of the enthalpy (supposedly this could also be called "absolute enthalpy" by analogy with "absolute entropy") gave a value for Q_{298} of 5.27 kJ UCFW^{-1} beginning at 10 K for the cells in Eq. 1 [8]. If the value of 10.19 kJ UCFW^{-1} obtained for TS in Eq. 7 represents the quantity of thermal energy required to raise the temperature of one UCFW of cells from 10 to 298.25 K, and if the value of 5.27 kJ UCFW^{-1} represents the same quantity as Q, then TS ought *not* to be 10.19/5.27 = 1.93 times greater than Q. There cannot be *two* quantities of thermal energy representing the value required to raise the temperature of a given mass of substance from 0 to 298.15 K.

The reason for this seems to be that the same C_p data can be used to make two different calculations, one of TS and one of Q, but they provide different answers both with the same units of thermal energy. An investigation confirmed this to be true mathematically [11]. The relationship is summarized by the equation

$$Q = S(T_2 - T_1)/2 = S(T_{avg}) \tag{12}$$

where $T_1 = 0$ K and where T_2 is the T/K temperature for which S was determined. In addition, using data from the literature, for 23 solid, crystalline, small MW substances of biological importance the average TS at 298.15 K and 0.1 MPa, was 1.993 times greater than Q. The fact that $S = 2Q$ (for *solid* organic substances) *is not new*, but it does not seem to have been much considered, given that Q is theoretically a directly measurable quantity of thermal energy required to bring a mass from 0 K to T/K. Just as with Eq. 8 for $T\Delta_rS$,

$$\Delta_rQ = \sum Q_{prod} - \sum Q_{react} \tag{13}$$

or

$$\Delta_rQ = \sum \Delta_fQ_{prod} - \sum \Delta_fQ_{react} \tag{14}$$

where Δ_fQ_{prod} and Δ_fQ_{react} represent the absorbed thermal energies of formation of the products and reactants, represented by the following equation:

$$\Delta_fQ = Q - \sum Q_{atoms} \tag{15}$$

and where $\sum Q_{atoms}$ represents the sum of the kinds and quantities of atoms in the substance being considered. The idea of the absorbed thermal energy of formation is completely analogous to the entropy of formation, but it has a different value.

The free-energy change accompanying microbial growth

The free-energy change calculated using Δ_rG

Free energy, however it is calculated, is a function of state. Using the Gibbs free-energy equation, conventional free-energy exchanges with the environment are calculated with Eq. 2, using values for Δ_rH from direct measurement or Eq. 4, and values for $T\Delta_rS$ using Eqs. 8 or 9. Free energy is often described as the amount of energy in a system that is available for doing work. If $T\Delta_rS$ represents thermal energy that is unavailable at constant temperature and pressure for doing work, and if Δ_rH represents the total heat of reaction, then Δ_rG must represent *non-thermal* energy that becomes partly or completely converted into thermal energy during the course of a reaction, and becomes lost to the environment as heat, any residual non-thermal energy becoming *conserved* in the substance of the cells or other non-thermal energy containing products of microbial growth [12]. If gases are consumed or produced during growth, they are in solution at the moment of consumption or production. It is incorrect to consider energy conserved during growth as "work." No "force × distance" work is done during growth except what very small amount is involved in cyclosis, movement of chromosomes, or flagellar or ciliary action. Very little or no pressure–volume work is done in that all activities take place at low concentrations of reactants and products in solution at a constant temperature and pressure, at least under most natural conditions. The exceptions can be those of the net catabolic or photosynthetic exchange of gases with the gaseous external environment.

The free-energy change calculated using Δ_rQ

A different free-energy equation in the same form as the Gibbs free-energy equation has been proposed by Battley using Q rather than TS [13]. This is

$$\Delta_rX = \Delta_rH - \Delta_rQ \tag{16}$$

where Δ_rX is a free-energy exchange involving Δ_rQ rather than $T\Delta_rS$, Δ_rH is the total heat of reaction, and Δ_rQ is the absorbed heat exchange that takes place as reactants become products. Equations 16 and 2 have the same form, except that one uses X and Q, and the other G and S. They are thus different equations. Δ_rH is the same for both equations. The total heat of reaction exchanged by any microbial growth-process is what it is, quite apart from any equational description. However, because TS for any given substance participating in a growth-process can be nearly twice or more than that of Q for the same substance, the free-energy calculated using Eq. 2 can be expected to be

different, although perhaps not greatly different, from that calculated using Eq. 16.

Is there a practical difference in the free-energy change calculated using Eq. 2 or 16?

This was specifically addressed in a comparison of the $\Delta_r G$ and $\Delta_r X$ changes accompanying the bomb calorimetric oxidations in their standard states of organic substances of biological importance [13]. $\Delta_r X$ is represented initially in the tables of this early paper by $\Delta_c G_{\Delta Q}$, but the suggestion is made at the end of this paper to change this designation to $\Delta_r X$. This showed that for 17 reactions the ratios of $\Delta_c X/\Delta_c G$ averaged 1.03 ± 0.01 for 17 solid, crystalline substances and 1.04 ± 0.01 for 5 pure, liquid substances. $\Delta_c X$ is far easier to understand because of the identification of $\Delta_c Q$ as an easily visualized change in absorbed thermal energy. However, when the same computations were made for 6 common microbial fermentations, rather than oxidations, the ratios of $\Delta_r X/\Delta_r G$ ranged from 0.20 to 1.14. These differences are not trivial and are of great importance when considering the thermodynamics accompanying the "origin of life" as it is thought to occur under anaerobic conditions.

Calculating values of Q for high polymers and cells

Whereas values of Q for many small molecular weight substances of biological importance can be found in the literature, this is not true for pure proteins or for cells, for which there are presently values only for two crystalline proteins and a preparation of *S. cerevisiae* cells especially grown so as not to contain any storage substances, these not being a part of the fabric of the cells [14]. The purpose of this present study is to develop a method of calculating Q or $\Delta_f Q$ for complex biological substances based on the absorbed thermal energies of their constituent atoms.

Methods

A method for estimating S within $\pm 2\%$ of the values obtained from experimental data has been published [15] and the same technique can be used with respect to Q or $\Delta_f Q$. Experimentally, Eq. 11 is used to determine $(H_{298.15} - H_0) = H_T$. However, it has been pointed out that $(Q_{298.15} - Q_0) = Q_T$ is probably a clearer terminology because of its classical identification with *absorbed* heat, and this practice will be used here [13]. Also, for a given reaction at a set temperature, the symbol $\Delta_r H$ representing the total enthalpy change for the reaction would be the same as the symbol $\Delta_r H$ representing the change in absorbed thermal energy. Just as there is an entropy of formation, $\Delta_f S$, there is an "absorbed heat of formation", $\Delta_f Q$ at constant temperature and pressure, calculated using Eq. 15. A plot of $\Delta_f Q$ against $\sum Q_{\text{atoms}}$ for many substances of biological importance will give the slope of a line which, if constant, will ideally enable a value of Q to be calculated for complex biological substances for which this value is not known and difficult to determine experimentally. The appropriate equation for biomass is

$$\Delta_f Q_{\text{biomass}} = c \sum Q_{\text{atoms}} \tag{17}$$

where "c" is a constant equal to $\Delta_f Q/\sum Q_{\text{atoms}}$. The appropriate data for many small molecular weight substances of biological importance are shown in Table 1. A value of Q has been determined for only two crystalline proteins and one microorganism. These are also listed in Table 1.

Values for Q_{biomass} or Q_{cells} are only needed for calculations using Eq. 15. On the other hand, a comparison of these calculated values with those obtained experimentally can serve as a check for either. Q_{biomass} can be calculated using Eq. 16 expressed in terms of biomass and substitution in Eq. 14, as follows:

$$\begin{aligned} Q_{\text{biomass}} &= c \sum Q_{\text{atoms}} + \sum Q_{\text{atoms}} \\ &= \Delta_f Q_{\text{biomass}} + \sum Q_{\text{atoms}} \end{aligned} \tag{18}$$

Results

From Table 1, the average slope of a line in which $\Delta_f Q$ for various small molecular weight organic solids of biological importance can be plotted on the y-axis against $\sum Q_{\text{atoms}}$ on the x-axis is -0.648. For all practical purposes, it is apparent that the range of values listed for $\Delta_f Q/\sum Q_{\text{atoms}}$ in Table 1 is far too wide to provide any confidence in the accuracy of calculation *for a given substance*. On the other hand, the *average* may be significant in that it eliminates these variations with respect to the mass of substances as a whole. This is illustrated with two crystalline proteins and one dried cell preparation at the bottom of Table 1. It is unfortunate that there appear to be only three good examples of complex biomass for which the empirical formula is accurately known from a direct analysis of the C, H, O, N, P, S, and metals in biological samples. In addition, any samples of cells used for analysis would have to be free of storage substances, which are actually internal substrates and not a part of the fabric of the cells [14]. The only example of this stricture that has been used for low temperature calorimetry is the one given. In high-polymeric substances such as proteins and cells the thermal energy absorbing effects of all of the monomers comprising the polymers would tend to average out. Since the fabric of all cells, exclusive of any storage products, is comprised for

Table 1 Enthalpy data at 298.15 and 0.1 MPa[a]

Substance	Formula	Q	$\Delta_f Q^o$	$\sum Q^o_{atoms}$	$\Delta_f Q^o / \sum Q^o_{atoms}$/kJ mol^{-1}
Elements[b]					
Carbon	C	1.050	0	1.050	0
Hydrogen	H_2	8.988	0	8.988	0
Nitrogen	N_2	15.512	0	15.512	0
Oxygen	O_2	17.390	0	17.390	0
Phosphorous	P	5.360	0	5.360	0
Sulfur	S	4.412	0	4.412	0
Zinc	Zn	5.657	0	5.657	0
Organic solids					
L-Alanine[c]	$C_3H_7O_2N$	20.034	−39.720	59.754	−0.665
L-Aspartic acid[d]	$C_4H_7O_4N$	25.810	−52.384	78.194	−0.670
α-D-Glucose[e]	$C_6H_{12}O_6$	32.996	−79.402	112.398	−0.706
Glycine[c]	$C_2H_5O_2N$	16.179	−33.537	49.716	−0.675
Glutamic acid[d]	$C_5H_9O_4N$	28.766	−52.384	88.232	−0.594
L-Glutamine[d]	$C_5H_{10}O_3N_2$	30.051	−61.736	91.787	−0.673
Glycylglycine[f]	$C_4H_5O_2N$	27.394	−24.422	51.816	−0.471
L-Leucine[g]	$C_6H_{13}O_2N$	31.623	−58.215	89.868	−0.648
Palmitic acid[h]	$C_{16}H_{32}O_2$	68.621	−109.377	177.998	−0.614
L-Phenylalanine[i]	$C_9H_{11}O_2N$	31.447	−52.583	84.030	−0.626
L-Proline[i]	$C_5H_9O_2N$	24.437	−46.405	70.851	−0.655
L-Serine[j]	$C_3H_7O_3N$	22.653	−45.796	68.449	−0.669
Succinic acid[k]	$C_4H_6O_4$	25.233	−40.711	65.944	−0.617
Sucrose[l]	$C_{12}H_{22}O_{11}$	62.340	−144.773	207.113	−0.699
L-Tryptophan[i]	$C_{11}H_{12}O_2N$	37.211	−61.169	90.624	−0.675
L-Tyrosine[i]	$C_9H_{11}O_3N$	33.369	−59.356	92.725	−0.640
L-Valine[g]	$C_5H_{11}O_2N$	27.251	−52.249	79.830	−0.658
Average = −0.648					Range = −0.471 to 0.710

Table 1 continued

Substance	Formula	Q	$\Delta_f Q^o$	$\sum Q^o_{atoms}$	$\Delta_f Q^o / \sum Q^o_{atoms}$/kJ mol^{-1}
Polymeric organic substances					
Anhyd chymotrypsinogen[m,o]	$CH_{1.612}O_{0.318}N_{0.282}S0.011$	4.676	−8.619	13.295	−0.648
Anhyd zinc insulin[m,o]	$CH_{1.480}O_{0.295}N_{0.256}\ S_{0.024}Zn_{0.002}$	4.489	−7.882	12.271	−0.642
S. cerevisiae[n]	$CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}$	5.09	−9.35	14.44	−0.648
Average = −0.646					Range = −0.642 to −0.648

[a] $Q^o = (H^o_T - H^o_0)$ as explained in "The absorbed thermal energy exchange, $\Delta_r(H_T - H_0) = \Delta_r Q$, accompanying microbial growth" section of the text

[b] Values of Q^o for C, H_2, O_2, N_2, NH_3, and CO_2 were taken from Battley [13]. Values of Q^o for the other inorganic substances were taken from Cox et al. [19]

[c] Hutchens et al. [20]

[d] Hutchens et al. [21]

[e] Boerio-Goates [22]

[f] Hutchens et al. [23]

[g] Hutchens et al. [24]

[h] Wirth et al. [25]

[i] Cole et al. [26]

[J] Hutchens et al. [27]

[k] Vanderzee and Westrum [28]

[l] Putnam and Boerio-goates [29]

[m] Hutchens et al. [16]

[n] Battley et al. [8]

[o] These unit carbon formulas were calculated from the empirical formulas for anhydrous chymotrypsinogen A and bovine zinc insulin from Hutchens et al. [16]. The original values of $\left(H^o_T - H^o_0\right) = Q^o$ are 20.309 and 19.772 kJ 100 g^{-1} for chymotrypsinogen and bovine zinc insulin, respectively. The values given above have been calculated as the UCFW of 23.026 and 22.709 for chymotrypsinogen and bovine zinc insulin, respectively

the most part of the same sorts of polymers, it is possible that the constant "c" in Eq. 18 applies closely to many high molecular weight biopolymers containing amino acids and to cells in general. The values of $\Delta_f Q/\sum Q_{atoms}$ for chymotrypsinogen and bovine zinc insulin are 0.648 and 0.642, respectively, and that for *S. cerevisiae* is 0.648 as results of experimental determinations. The three values are close, averaging 0.646 within a narrow range. As an example, using Eq. 17, and the data from Table 1 for *S. cerevisiae* cells

$$\begin{aligned} Q_{cells} &= -0.648 \sum Q_{atoms} + \sum Q_{atoms} \\ &= (-0.648 \times 14.44) + 14.44 \\ &= -9.357 + 14.44 \\ &= 5.08 \text{ kJ UCFW}^{-1} \end{aligned}$$

From Eq. 7, the experimentally determined value of TS for *S. cerevisiae* is 10.19 kJ UCFW^{-1} [8]. The ratio of TS/Q is therefore 10.19/5.08 = 2.01, as predicted mathematically. Similar TS/Q ratios for chymotrypsin A and zinc insulin are 9.27/4.68 = 1.98 and 8.90/4.49 = 1.99, respectively, using experimental S values from Hutchens et al. [16]. For all practical purposes, the value of Q could also be calculated accurately using entropy data, and the reverse.

Discussion

Two free-energy equations are being considered here: $\Delta_r G = \Delta_r H - T\Delta S$ (Eq. 2) and $\Delta_r X = \Delta_r H - \Delta_r Q$ (Eq. 16). For any given reaction, the quantity $\Delta_r H$ is the same for Eqs. 2 and 16, as are the quantities $\int_0^{298} C_p$ and $\sum \Delta_{trs} H_T^o$ in Eqs. 5 and 11. The differences are that in Eq. 5 C_p is integrated with respect to log T in Eq. 5 whereas it is not in Eq. 11, and that in Eq. 5 $\sum \Delta_{trs} H_T$ is divided by T whereas in Eq. 11 it is not. For the solid substances considered in this study there are no phase changes or transitions associated with the rise in temperature inside a low-temperature calorimeter, and the $\sum \Delta_{trs} H_T$ term can be ignored. Thus, the difference between TS and Q is due to the mathematical operation of integration. *S is a mathematical function, not a physical quantity*, but when multiplied by T the resulting term, TS, acquires the units of absorbed thermal energy per unit mass. These units also apply to Q. Both symbols supposedly represent the quantity of thermal energy required to raise a given mass from 0 K to a working temperature, usually 298.15 K. However, the quantity $TS = 2Q$, and it is impossible to have two different quantities of absorbed thermal energy (TS and Q) raising the temperature of the same solid mass to the same degree. One of these values has to be incorrect; but which one? Surely it must be TS, if only because Q is obtained directly as a function of T.

The measurement of C_p values in a low-temperature calorimeter to determine values of both S (Eq. 5) and Q (Eq. 11) is done with dried cells, whereas the cells in Eq. 2 are living and hydrated. Would this hydration have an appreciable effect on the C_p values obtained and how would this affect the values of S and Q? An actual experiment has not been done, However, the enthalpy of hydration has been measured by Von Stockar et al. [17] to be within the range of about $1 - 2$ kJ/UCFW. Their opinion is that this value is sufficiently small compared to the enthalpy of combustion that the latter can be used without further correction to calculate enthalpy balances for wet biomass.

The simplicity of Q

The heat absorbed by a substance can be experimentally measured and calculated from a series of incremental measurements of C_p as a function of T/K or *theoretically* measured directly provided that the heat application in the low temperature calorimeter is carried out in small, sequential increments with sufficient relaxation time between increments (see Eq. 11). Heat capacity has the units of J K^{-1} unit-mass^{-1}, and when it is integrated as a function of T/K, the temperature unit cancels out, the result being Q which then acquires the units of energy (J unit-mass^{-1}). It is easily seen that Q is nothing more than the quantity of thermal energy required for a given mass of substance to exist at a given temperature above 0 K.

The non-simplicity of S

On the other hand, S is not a quantity of thermal energy. S has the units J K^{-1} unit-mass^{-1} which are not the units of energy, these being J unit-mass^{-1}. These units are acquired when C_p, having the units J K^{-1} unit-mass^{-1}, is integrated with respect to log T, rather than simply T, as for Q. A logarithm has no units, and therefore T does not cancel out, as it does when calculating Q, and the units of C_p become those of S. A question exists as to why C_p was integrated with respect to log T in the development of the Gibbs free-energy equation. A possible reason is simply that doing so shortens the abscissa, something often done in graphic representations. But, with the multiplication by T to provide the units of energy required for all of the terms in the Gibbs free-energy equation, a value is acquired that, for solid substances, is very close to twice that of the actual thermal energy experimentally required to raise the temperature of a substance from 0 K to any given temperature.

It is apparent that the concept of entropy has been difficult for many people. In an article on entropy and information, Tribus and McIrvine [18] related a conversation with Shannon in which Tribus, "……asked Shannon what

he had thought about when he had finally confirmed his famous measure. Shannon replied: 'my greatest concern was what to call it. I thought of calling it 'information,' but the word was overly used, so I decided to call it 'uncertainty.' When I discussed it with John von Neumann, he had a better idea. Von Neumann told me, 'You should call it entropy for two reasons. In the first place your uncertainty function has been used in statistical mechanics under that name, so it already has a name. In the second place, and more important, no one knows what entropy really is, so in a debate you will always have the advantage." Von Neumann's comment refers to Clausius' entropy, not to one of the several other defined entropies, and not all physicists would agree with it. Actually, Clausius's entropy *is* a well-defined mathematical function (Eq. 5). The problem may be that no one knows what it really *means*. Physically, it is not energy. It has been used for years in physical chemistry as a term which, when multiplied by T, becomes a *different* term that does have the units of energy which could not be other than thermal energy if we acknowledge the basic data from which S is derived as being C_p and $\sum \Delta_{trs} H_T$. It should be emphasized that although the term TS still retains the symbol, S, the entropy function has totally disappeared and what is left is energy. On the other hand, TS is always nearly two times greater than Q for solid organic substances, and can be more so in the case of liquids and gases. TS does not appear to be the correct function describing the quantity of heat required for an organic substance of biological interest to exist at a given T/K. Tribus and McIrvine [18] went on to state that, "Simple physical arguments lead one to believe in the correctness of most quantities in physics. Surrounding Clausius' entropy there has always been an extra mystery." And there still is!

The mathematical relationship between S and Q

A description of the mathematical relation between S and Q can be found in [11].

A better idea

If what is stated above is correct, the energy changes accompanying microbial growth would be better described by Eq. 16. This is written in the same form as Eq. 2, but incorporates a different function as the second term on the right. For a given reaction, the value of $\Delta_r H$ would be the same for both representations, but because $T\Delta_r S$ has a different value from $\Delta_r Q$, $\Delta_r G$ is going to have a different value from $\Delta_r X$. The ratio between these last two is not great for most aerobic, microbial reactions or processes because the energy exchanges involving $T\Delta_r S$ and $\Delta_r Q$ are usually quite small. The ratio can be much different for anaerobic microbial reactions or processes. However, the best reason for becoming interested in Eq. 16 is that, lacking S, it is eminently comprehensible.

Just as with entropy studies requiring the use of a low-temperature calorimeter [8] which led to a method whereby the entropy of cellular substance could be calculated based on the number and kind of atoms it contains [15], it is useful to devise a similar method with respect to absorbed thermal energy, Q. Equally as with the entropy studies, it is unfortunate that only three critical, low-calorimetric studies have been made, two on crystalline proteins, and one on a preparation of *S. cerevisiae* cells that were grown so as not to contain storage substances, these last being internal substrates rather than a part of the fabric of the cells [14]. However, the result is the same as with entropy studies [15]. The methods do not work well with small molecular weight substances because the range of agreement between experimentally measured and calculated values is often too large. This range disappears with complex substances like proteins and cells, probably because the individual differences of the individual monomers average out when there is a variety of these present in the substance. The values of Q are satisfying close to what was obtained with a low-temperature calorimeter. The methods described here can therefore be used to some advantage in future studies.

Conclusions

A satisfactory method has been devised whereby the absorbed heat, and the absorbed heat of formation of dried microbial cells and crystalline proteins can be calculated, based on the kinds and numbers of atoms these substances contain. The data show that for high biopolymers and for cells $\Delta_f Q = -0.648 \sum Q_{atoms}$ and $Q = 0.352 \sum Q_{atoms}$.

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