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An empirical method for estimating the entropy of formation and the absolute entropy of dried microbial biomass for use in studies on the thermodynamics of microbial growth

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Abstract

An empirical method is described for calculating the entropy of formation, $\Delta_f S_{biomass}$, and the absolute entropy, $S_{biomass}$, of dried biomass for which the elemental composition is known. It is established that the ratio of $\Delta_f S_{biomass}$ to the sum of the atomic entropies of the atoms comprising the biomass multiplied by their respective coefficients is a dimensionless constant. This relationship can be used in the calculation of $S_{biomass}$, giving an accuracy comparable to experimentally determined values of 100.18±1.94% (n=5). The calculations appear to be valid for both anhydrous and hydrated biomass, provided that the quantity of water of hydration is known. The method is applicable to any solid, organic substance for which the elemental composition is known, with lesser accuracy at small molecular weights and greater accuracy as the molecular weight increases. It can be compared to Thornton's Rule, although completely different, in that it enables a thermal quantity to be calculated without the necessity of a direct thermal measurement.

Keywords: Entropy; Entropy of formation; Growth process; Indirect calorimetry; Microorganisms

1. Introduction

The phenomenon of microbial growth can be regarded as a process whereby various substances in solution are reacted by cells acting as autocatalysts to form more cells and other products of the growth process. This phenomenon can be represented by an equation representing an initial and a final state, called a 'growth process equation'. An example of one such equation is that representing the aerobic growth of *Saccharomyces cerevisiae* on glucose [1].

$$\begin{aligned} &C_6H_{12}O_6(aq) + 0.302NH_3(aq) + 4.050O_2(aq) \\ &\quad +0.023H_2PO_4^-(aq) + 0.006SO_4^{2-}(aq) \\ &\quad +0.042K^+(aq) + 0.006Mg^{2+}(aq) \\ &\quad +0.002Ca^{2+}(aq) + 0.023OH^-(aq) \\ &\quad \rightarrow 4.086CO_2(aq) + 4.975H_2O(l) \\ &\quad +1.914CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022} \\ &\quad \times Mg_{0.003}Ca_{0.001}(biomass) \end{aligned} \quad (1)$$

In Eq. (1), the last term represents the cells (biomass) in terms of their 'unit carbon formula' (UCF) from which a 'unit carbon formula weight' (UCFW) can be calculated [1–3]. This latter is more commonly referred to in the European literature as a 'carbon

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mol' (C mol) of cells, and this designation will be used here. It is essential that the UCF represents cells that have been grown exponentially and that do not contain storage substances [1–3]. These latter are internal substrates that are not a part of the fabric of the cells. Descriptions of how to construct growth-process equations can be found in Refs. [1–3].

Microbial growth processes, as represented by Eq. (1), are accompanied by changes in free energy ($\Delta_pG'_{met}$), enthalpy ($\Delta_pH'_{met}$), and entropy ($T\Delta_pS'_{met}$), where the prime indicates that the process for which the energy changes apply takes place in an aqueous environment and 'met' represents 'metabolism'. In studies of the thermodynamics of microbial growth, the problem is then how to calculate values for these changes in thermodynamic properties. Microbial growth takes place in an aqueous environment under conditions of temperature and pressure that can usually be considered constant, or can be made so experimentally. The thermodynamic changes accompanying growth can then be related by the Gibbs free energy equation, $\Delta G{=}\Delta H{-}T\Delta S$, or some variation of this. For a microbial growth process this becomes

$$\Delta_pG'_{met} = \Delta_pH'_{met} - T\Delta_pS'_{met} \quad (2)$$

where the subscript 'p' represents that microbial growth is a process rather than a simple reaction, which is usually represented by the subscript 'r'.

1.1. Determining values for $\Delta_pH'_{met}$

A value for $\Delta_pH'_{met}$ can be measured directly by growing the cells in a microcalorimeter, measuring the heat output, and correcting this for side reactions such as the heat of solution of O_2(g) from the gas phase to O_2(aq) in the culture medium, the evolution of CO_2(g) into the gas phase from the CO_2(aq) in the medium, the enthalpy change accompanying any change in pH, etc., all of which takes place as the culture grows and which can be measured chemically or physically. Or, the same value can be obtained with indirect calorimetry by using a growth process equation such as Eq. (1) and the following:

$$\Delta_pH'_{met} = \Sigma\Delta_fH'_{prod} - \Sigma\Delta_fH'_{react} \quad (3)$$

where $\Delta_fH'_{prod}$ and $\Delta_fH'_{react}$ represent the enthalpies of formation of the products and of the reactants, respectively. Values of $\Delta_fH^{0\prime}$ for the various substances represented in Eq. (1) can be found in Refs. [1–3] and in the literature, except for that of the cells, Δ_fH_{cells}. Values for $\Delta_fH_{biomass}$ can be calculated using an equation representing the combustion of the biomass (cells) in a bomb calorimeter, such as

$$\begin{aligned}\text{biomass} + n\mathrm{O_2(g)} &\rightarrow n\mathrm{CO_2(g)} + n\mathrm{N_2(g)} \\ &+ n\mathrm{H_2O(l)} + n\mathrm{P_4O_{10}(cr)} + n\mathrm{SO_3(g)} \\ &+ n\mathrm{K_2O(cr)} + n\mathrm{MgO(cr)} + n\mathrm{CaO(cr)} \quad (4)\end{aligned}$$

where n represents the appropriate coefficients for the respective substances participating in the combustion. For the biomass represented in Eq. (1), Eq. (4) becomes

$$\begin{aligned}&\mathrm{CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}} \\ &\times\mathrm{Ca_{0.001}(cells)} + 1.151\mathrm{O_2(g)} \rightarrow 1.000\mathrm{CO_2(g)} \\ &+0.806\mathrm{H_2O(l)} + 0.079\mathrm{N_2(g)} + 0.003\mathrm{P_4O_{10}(cr)} \\ &+0.003\mathrm{SO_3(g)} + 0.011\mathrm{K_2O(cr)} + 0.003\mathrm{MgO(cr)} \\ &+0.001\mathrm{CaO(cr)} \quad (5)\end{aligned}$$

The enthalpy change for the combustion represented by Eq. (5) has been determined experimentally to be $-19.44{\pm}0.17$ kJ (g of whole cells)$^{-1}$ or -509.37 kJ C mol^{-1} [1]. A value for Δ_fH_{cells} can then be calculated using Eq. (5) and the following:

$$\Delta_cH_{cells} = \Sigma\Delta_fH_{prod} - \Sigma\Delta_fH_{react} \quad (6)$$

In Eq. (6), $\Sigma\Delta_fH_{prod}$ and $\Sigma\Delta_fH_{react}$ represent the sums of the heats of formation of the products and of the reactants, respectively. Here, Δ_fH_{cells} is the only unknown quantity, the other quantities being available from the literature [1–3].

A slightly less accurate method of obtaining the heat of combustion of organic substances in general is to use Thornton's Rule [4], which states that Δ_cH^0 for most organic substances is directly proportional to the number of atoms of oxygen consumed during the combustion. Soon after its publication in 1917, the same idea was extended by others [5] to relate the heat of combustion of an organic substance to the number of electrons transferred to oxygen, as follows:

$$\begin{aligned}\Delta_cH = \text{constant X (eq. transferred to oxygen} \\ \text{during bomb calorimetric combustion)} \quad (7)\end{aligned}$$

Originally, the constant in Eq. (7) had a value of -108.99 kJ eq^{-1}. A more recent review [6] of Thornton's Rule, using data from 488 organic substances of

all kinds, gave an average value of $-111.40\ \text{kJ eq}^{-1}$ for this constant (as compared with $-108.99\ \text{kJ eq}^{-1}$ from Ref. [5]), and a linear regression value of $-110.88\ \text{kJ eq}^{-1}$. The authors point out that there is a slight spread of the points around the lower end of the regression line, indicating that calculations making use of Eq. (4) would give values slightly in error as compared to the experimentally determined values. However, the higher the molecular weight of the substance, the closer the points approached the regression line, so that with substances such as biomass the value obtained with Eq. (4) becomes quite accurate. An average of the values ($-111.40\ \text{kJ eq}^{-1}$ and $-110.88\ \text{kJ eq}^{-1}$), giving $-111.14\ \text{kJ eq}^{-1}$, has been used as a practical value [7], this value being within one per cent of whatever the 'best' value happens to be. Using this value, Thornton's Rule becomes

$$\Delta_c H = -111.14\ \text{kJ eq}^{-1}\ X\ (\text{eq. transferred to oxygen during bomb calorimetric combustion}) \quad (8)$$

Related to Thornton's Rule is the idea of the 'oxycaloric equivalent' $\Delta_c H_{O_2}$), where the subscript indicates that the value is obtained from bomb calorimetric combustion [8]. A close approximation to the generally accepted value for the oxycaloric equivalent can be obtained by multiplying the value of $-111.14\ \text{kJ eq}^{-1}$ (above) by the four electrons required to reduce O_2(aq) to H_2O(l) to equal $-444.56\ \text{kJ mol}^{-1}$ of O_2(g) consumed. A practical value for $\Delta_c H_{O_2}$ has been taken as $-450\ \text{kJ mol}^{-1}$ O_2(g) [8]. On the other hand, from Eq. (5) 4.577 eq are transferred to O_2(g) [1] and, using Eq. (8), $\Delta_c H_{\text{cells}}$ is calculated to be $-508.69\ \text{kJ C mol}^{-1}$, in excellent agreement with the experimentally determined value of $-509.37\ \text{kJ C mol}^{-1}$ (see above) [1]. Either of these values can then be used in to calculate the heat of formation of the cells. Using Eq. (6) and the data in Eq. Eq. (5), $\Delta_f H_{\text{cells}}$ for *S. cerevisiae* has been determined at $-133.13\ \text{kJ C mol}^{-1}$ [1]. Values for $\Delta_f H_{\text{cells}}$ obtained by Thornton's Rule apply to cells in the dry state. Living cells grown in an aqueous environment are hydrated. However, it has been shown [9] that the heat of hydration for yeast cells is less than $90\ \text{J g}^{-1}$ of dried cells, or about $2.36\ \text{kJ C mol}^{-1}$ of yeast cells as represented in Eq. (1). This is ca. 0.46% of the heat of combustion of one C mol of these cells, and well within the standard error of most combustion analyses. The value for $\Delta_f H_{\text{cells}}$ is thus equated with $\Delta_f H'_{\text{cells}}$ when using Eq. (3).

1.2. Determining values for $\Delta_p G'_{\text{met}}$

Growth processes as represented in Eq. (1) are irreversible, at least in that microorganisms have never been observed to 'ungrow' themselves during the process of growth, although internal storage substances, if they are present, can be metabolized after growth has ceased. In addition, microbial cells precipitate out of the liquid phase during growth, and cannot be represented as the product of an equilibrium reaction or process. Cells do not go back reversibly into solution. For this reason microbial growth processes cannot be considered to involve an equilibrium, and no true thermodynamic equilibrium constant can be calculated. This makes it impossible to calculate $\Delta_p G_{\text{met}}$ by means of the conventional equation $\Delta G = -RT\ln K$. It is then necessary to use equations, such as Eq. (1) and the following:

$$\Delta_p G'_{\text{met}} = \Sigma\Delta_f G'_{\text{prod}} - \Sigma\Delta_f G'_{\text{react}} \quad (9)$$

where $\Delta_f G'_{\text{prod}}$ and $\Delta_f G'_{\text{react}}$ represent the free energies of formation of the products and reactants, respectively. Values of $\Delta_f G^{0\prime}$ for the various substances, except for biomass represented in Eq. (1) can be found in Refs. [1–3], calculated for a concentration of $0.001m$. However, $\Delta_f G_{\text{cells}}$, unlike $\Delta_f H_{\text{cells}}$, cannot be determined calorimetrically. This is because $\Delta_f G_{\text{cells}}$ represents non-thermal, chemical energy, and is therefore not a thermal quantity that can be measured directly.

An attempt has been made [10] to calculate values for the free energy of combustion of cells, $\Delta_c G_{\text{cells}}$, using Thornton's Rule. Data for 253 organic substances of all kinds gave an average value for $\Delta_c G^0$ of $-107.06\ \text{kJ eq}^{-1}$ and a linear regression value of $108.74\ \text{kJ eq}^{-1}$, which have been averaged at $-107.90\ \text{kJ eq}^{-1}$ as a practical value [7]. Using the same techniques as described above for calculating $\Delta_f H_{\text{cells}}$, a value could be obtained for $\Delta_f G_{\text{cells}}$ which could then be used in the following equation:

$$\Delta_f G_{\text{cells}} = \Delta_f H_{\text{cells}} - T\Delta_f S_{\text{cells}} \quad (10)$$

In Eq. (10), $\Delta_f S_{\text{cells}}$ represents the entropy of formation of the cells. However, when this was done in

calculations of the entropy of one C mol of *Escherichia coli cells* [7], the resulting value was much too high as compared with that determined experimentally for *S. cerevisiae* [11]. It would be expected that cellular entropies would be reasonably close in value, despite minor differences with respect to cellular UCFs.

With respect to Eq. (10), methods of determining a value for $\Delta_f H_{cells}$ have been described above. To calculate a value for $\Delta_f G_{cells}$, it is necessary to determine a value for $T\Delta_f S_{cells}$, with T being the same temperature as that used in determining $\Delta_f H_{cells}$. The value for T is often taken as being that of the standard temperature, 298.15 K, this being the temperature at which most thermodynamic properties are listed in the literature. It is the determination of values for $\Delta_f S_{cells}$ that is the subject of this article.

1.3. Determining values of $\Delta_p S'$

Just as with $\Delta_p G'$ and $\Delta_p H'$, $\Delta_p S'$ can be calculated from an equation of the following form:

$$\Delta_p S' = \Sigma\Delta_f S'_{prod} - \Sigma\Delta_f S'_{react} \quad (11)$$

where $\Sigma\Delta_f S'_{prod}$ and $\Sigma\Delta_f S'_{react}$ represent the entropies of formation of the products and reactants, respectively. Values of $\Delta_f S^{0\prime}$ for all the substances except for the biomass represented in Eq. (1) can be found in the literature, or can be calculated from $\Delta_f G^{0\prime}$ and $\Delta_f H^{0\prime}$ data. However, as for any organic substance, $\Delta_f S_{biomass}$ can be calculated from the following:

$$\Delta_f S_{biomass} = S_{biomass} - \Sigma S^0_{atoms} \quad (12)$$

where $\Delta_f S_{biomass}$ is the entropy of formation, $S_{biomass}$ the absolute entropy, and ΣS^0_{atoms} the sum of the standard entropies of the individual atoms in an empirical formula for the biomass multiplied by their respective coefficients. The dimensions for $\Delta_f S_{biomass}$ and $S_{biomass}$ in Eq. (12) are J K^{-1} C mol^{-1} and for all the other terms are J K^{-1} g $atom^{-1}$. However, the summation of the entropies of all the atoms in the cellular fabric equals that for one C mol, and the dimensions of the last term in Eq. (12) are thus J K^{-1} C mol^{-1}. As biomass, $\Delta_f S_{cells}$ does not have a superscript in that it is not a pure substance in a standard state. As an example, the entropy of formation of cells (biomass) in Eq. (1) can be calculated as follows [1]:

$$\begin{aligned}\Delta_f S_{cells} = {} & S_{cells} - 5.74nC_{cells} - 65.34nH_{cells} \\ & - 102.57nO_{cells} - 95.81nN_{cells} - 41.09nP_{cells} \\ & - 31.80nS_{cells} - 64.18nK_{cells} - 32.68nMg_{cells} \\ & - 41.42nCa_{cells} \end{aligned} \quad (13)$$

where S_{cells} is the entropy of one C mol of lyophilized *S. cerevisiae* cells, and n represents the subscript for each atom in the formula for the cells in Eq. (1). The constants 65.34, 102.57, and 95.81 are one-half the standard entropies of $H_2(g)$, $O_2(g)$, and $N_2(g)$, respectively [12]. The other constants are the standard entropies of solid graphite, white phosphorous, rhombic sulfur, potassium, magnesium, and calcium [12]. For lyophilized *S. cerevisiae* cells, as represented in Eq. (1), the calculation is then as follows:

$$\begin{aligned}\Delta_f S_{cells} = {} & S_{cells} - 5.74(1) - 65.34(1.613) \\ & - 102.57(0.557) - 95.81(0.158) \\ & - 41.09(0.012) - 31.80(0.003) \\ & - 64.18(0.022) - 32.68(0.003) \\ & - 41.42(0.001) \\ = {} & S_{cells} - 5.74 - 105.39 - 57.13 \\ & - 15.14 - 0.49 - 0.09 - 1.41 \\ & - 0.10 - 0.04 \\ = {} & S_{cells} - 185.53\ \text{J K}^{-1}\ \text{C mol}^{-1} \end{aligned} \quad (14)$$

It is necessary to find the value of either $\Delta_f S_{cells}$ or S_{cells}. Conventionally, a value for $\Delta_f S_{cells}$ can only be calculated using Eq. (12). The remaining thermodynamic quantity that must be determined then becomes S_{cells}.

Entropies of organic substances in the condensed phase have been determined almost exclusively by the application of the Third Law, but there is a paucity of experimental data with respect to substances of biological importance, and especially with respect to the monomers making up the polymers comprising cellular fabric. An experimental determination of cellular entropy has only been made recently for the first time [11]. It would be extremely convenient if some method other than low-temperature calorimetry could be devised for the purposes of estimating the entropy of biomass.

2. Methods

Statistical mechanics has been remarkably successful in enabling calculations to be made of the entropies of small atomic and molecular weight gases. An interesting attempt has been made to apply statistical methods to the calculation of the entropy of *E. coli* cells, but without appreciable success [13,14]. It is unlikely that this discipline can be applied to the estimation of the entropy of substances like biomass. Conventional wisdom states (and teaches) that, with ideal gases, the entropy is a function only of the kinetic activity, and is independent of the structures of gaseous atoms or molecules. On the other hand, real gases are not ideal gases, and at least with respect to the translational entropy of gases, the atomic or molecular weight does enter into its calculation, as shown by the following equation [15].

$$S^0_{tr} = 3/2R \ln M + 5/2R \ln T - 2.311 \qquad (15)$$

where S^0_{tr} represents the molar translational entropy in the standard state in J K^{-1} mol^{-1} and M the atomic or molecular weight of the gas. R is independent of the nature of the gas. Monatomic gases do not possess any rotational or vibrational energy, only electronic and translational energy. The noble gases are monatomic and are probably as close to being as ideal as possible. Thus, Eq. (15) gives the entropy of a monatomic gas, except for possible electronic contributions. In case of the noble gases, the electronic partition function, Z_{el}, equals unity at 298.15 K, so that $S_{el}=0$, and the standard entropy is equal to the entropy of translation. The simplest possible example of the relationship of S^0 to atomic weight is shown in Fig. 1, graph A, in which S^0 is plotted against the atomic weights of the noble gases using the relationship, $y=a+b \ln x$. In graph B, the same plot is made against the atomic weights of three diatomic gases of biological importance. In both the graphs, there is an obvious correlation between the standard entropy and the atomic weight of the gases depicted. Such a relationship, even with gases, suggests that the entropies of the individual atoms in a biomass should be considered when looking for methods to calculate the entropy of biomass. The fabric of dried biomass has the physical qualities of a solid. Entropies of translation, and rotation can be expected to be minimal or non-existent, although entropies of vibration will certainly be present. But, as shown in Fig. 1, even in gases there seems to be some aspect of entropy that is associated with, or at least related to, or correlated with, the mass of the atoms of a substance.

As stated above, although standard entropy data are available for a large number of organic substances, very few of these are of the monomers or polymers comprising the fabric of cells. Of these few, only data

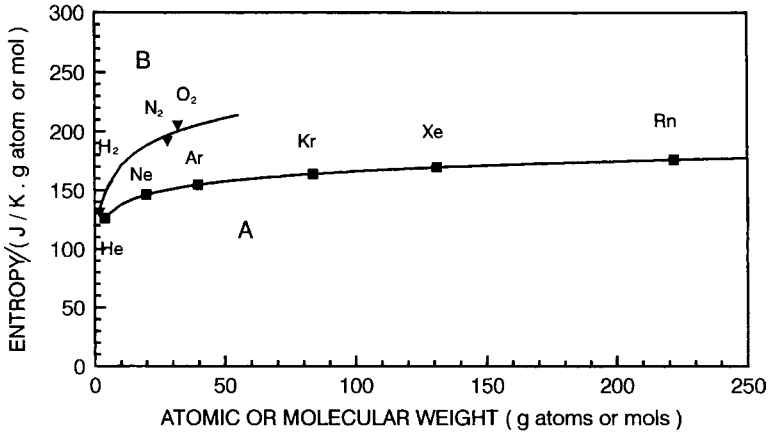

Fig. 1. Graph of the standard entropies of some monatomic or diatomic atoms or molecules against their atomic or molecular weights. The curves are log regressions using the sum of least-squares method and calculated according to the formula $y=a+b \ln x$. For Graph A, represented by ■, a=112.63 J K^{-1} mol^{-1}, b=25.23, r=0.999, and r^2=0.998. For Graph B, represented by ▼, a=108.75 J K^{-1} g $atom^{-1}$, b=12.47, r=0.871 and r^2=0.759. The low r and r^2 values are presumably due to the presence of the point for O_2(g) in the graph. The electron partition coefficient for O_2(g) is three at 298.15 K, rather than one, due to a triply degenerate ground state [17].

Table 1
Entropy data (in J K^{-1} mol^{-1}) relative to structural organic substances of biological interest in the solid state [a]

1 Substance	2 Formula	3 M.W. (D)	4 S^0 (JK^{-1} mol^{-1})	5 $\Delta_f S^0$ (JK^{-1} mol^{-1})	6 ΣS^0_{atoms} (JK^{-1} mol^{-1})	7 $\Delta_f S^0 / \Sigma S^0_{atoms}$
Glycine	$C_2H_5O_2N$	75.07	103.51	−535.62	639.13	−0.838
L-Alanine	$C_3H_7O_2N$	89.09	129.21	−646.34	775.55	−0.833
L-Serine	$C_3H_7O_3N$	105.09	149.16	−728.96	878.12	−0.830
L-Proline	$C_5H_9O_2N$	115.13	164.06	−753.65	917.71	−0.821
L-Valine	$C_5H_{11}O_2N$	117.15	178.87	−869.52	1048.39	−0.829
L-Leucine	$C_6H_{13}O_2N$	131.17	211.79	−973.02	1184.81	−0.821
L-Isoleucine	$C_6H_{13}O_2N$	131.17	207.99	−976.82	1184.81	−0.824
Glycyl glycine	$C_4H_8O_3N_2$	132.12	180.30	−864.71	1045.01	−0.827
L-Aspartic acid	$C_4H_7O_4N$	133.10	170.12	−816.31	986.43	−0.827
Adenine	$C_5H_5N_5$	135.13	151.01	−683.44	834.45	−0.819
L-Glutamine	$C_5H_{10}O_3N_2$	146.15	195.06	−986.37	1181.43	−0.835
L-Glutamic acid	$C_5H_9O_4N$	147.13	188.20	−934.65	1122.85	−0.832
Methionine	$C_5H_{11}O_2NS$	149.21	231.46	−848.73	1080.19	−0.786
Guanine	$C_5H_5ON_5$	151.13	160.98	−776.04	937.02	−0.828
L-Phenylalanine	$C_9H_{11}O_2N$	165.19	213.64	−857.71	1071.35	−0.800
L-Tyrosine	$C_9H_{11}O_3N$	181.19	214.01	−959.91	1173.92	−0.818
L-Cystine	$C_6H_{12}O_4N_2S$	240.29	280.58	1171.68	1452.26	−0.807
Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43	452.37	−1935.49	2387.86	−0.811
Anhydrous. bovine zinc insulin [b]	$CH_{1.480}O_{0.295}N_{0.256}S_{0.024}Zn_{0.002}$	22.69	29.85	−128.21	158.07	−0.811
Hydrated bovine zinc insulin [b]	$CH_{1.480}O_{0.295}N_{0.256}S_{0.024} \times Zn_{0.002}\cdot 0.052H_2O$	23.64	32.17	−138.02	170.19	−0.811
Anhydrous chymotrypsinogen A [c]	$CH_{1.612}O_{0.318}N_{0.282}S_{0.011}$	23.04	31.10	−139.96	171.06	−0.818
Hydrated chymotrypsinogen A [c]	$CH_{1.612}O_{0.318}N_{0.282} \times S_{0.011}\cdot 0.153H_2O$	25.80	39.24	−167.54	206.54	−0.811
Saccharomyces cerevisiae [d]	$CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}$	26.20	34.17	−151.36	185.53	−0.816

[a] All substances are in the crystalline state, except for *S. cerevisiae* which consists of lyophilized cells. All entropy data except for the cells have been taken from Ref. [16]. Except for adenine and guanine, which have been given a rating of 'B', the authors of Ref. [16] have given these substances an 'A' rating, meaning that the entropy was measured from within a range of 7 to 25 K, with extrapolation to 0 K using the Debye equation. The cellular data have been taken from Ref. [11].
[b] These unit carbon formulas were calculated from the empirical formulas, $C_{508}H_{752}O_{150}N_{130}S_{12}Zn$ and $C_{508}H_{752}O_{150}N_{130}S_{12}Zn\cdot 26.7H_2O$, taken from Ref. [16].
[c] These unit carbon formulas were calculated from the empirical formula, $C_{1077}H_{1736}O_{343}N_{304}S_{12}$ and $C_{1077}H_{1736}O_{343}N_{304}S_{12}\cdot 165H_2O$, taken from Ref. [16].
[d] This unit carbon formula was taken from Ref. [1].

in the 'A' category from Ref. [16], representing experimental determinations in which the lowest temperatures are in the 7–25 K range, have been used for inclusion in Table 1. A visual inspection of these data in columns 3 and 4 of Table 1 shows that, in general, S^0 increases with molecular weight. Using the data for the numbers and kinds of atoms in the substances listed in Column 2 of Table 1, and the experimentally determined data for S^0 in Column 4, the values of $\Delta_f S^0$ for these substances are calculated using Eq. (12) and are listed in Column 5 of Table 1. The values for ΣS^0_{atoms} are taken from these calculations and are listed in Column 6. As shown in Column 7 of Table 1, the ratio $\Delta_f S^0 / \Sigma S^0_{atoms}$ is surprisingly constant. These last data are graphed in Fig. 2 as a linear regression. In Fig. 2, the crystalline proteins and the yeast cells are all represented by C mol formulas, otherwise these points would be off the graph.

3. Results

Fig. 2 shows a straight line passing through the origin with a slope of −0.813, a value for r of −0.999,

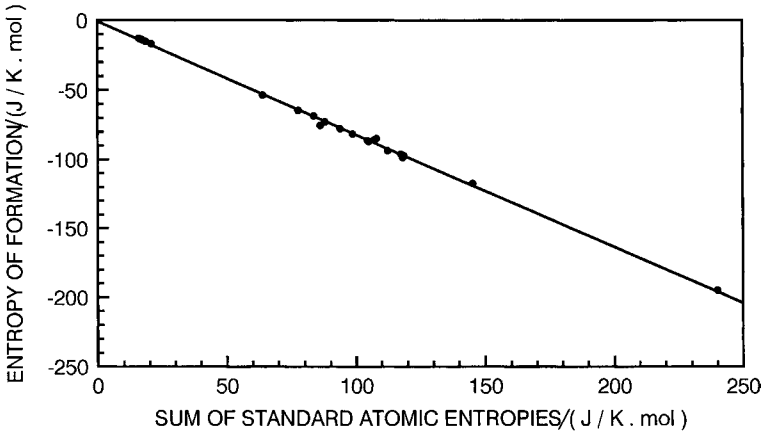

Fig. 2. Graph of the data listed in Column 5 of Table 1 plotted against those in Column 6. The line is a linear regression using the sum of least-squares method and calculated according to the equation, $y=a+bx$, where $a=0.385$ J K^{-1} mol^{-1}, $b=-0.813$, $r=-0.999$, and $r^2=0.998$. The regression line effectively passes through the origin.

and for r^2 of 0.998. Just as with values of $\Delta_c H/e^-$ [6] (Thornton's Rule), there is more of a spread of points for the small molecular substances, but as the molecular weight becomes larger, the points lie more closely on the regression line. The points representing the crystalline proteins and the yeast cells are lowest on the regression line because of being represented by a UCF, and are *not* small molecular weight substances. Otherwise, these points would have been far off the graph. The regression line in Fig. 2 can be represented by the following equation:

$$\Delta_f S^0 = -0.813 \Sigma S^0_{atoms} \quad (16)$$

Values for $\Delta_f S_{biomass}$ can be obtained from Eq. (16) and, subsequently, used in Eq. (10) to calculate values for $\Delta_f G_{biomass}$, provided that $\Delta_f H_{biomass}$ is known. Values of $\Delta_f G_{biomass}$, $\Delta_f H_{biomass}$, and $\Delta_f S_{biomass}$ can then be used, as in Eq. (1), together with appropriate values for the other thermodynamic quantities to calculate values for $\Delta_p G_{met}$, $\Delta_p H_{met}$, and $T\,\Delta_p S_{met}$. Values for $S_{biomass}$ or S_{cells} are only needed for calculations using Eq. (12). On the other hand, a comparison of these with those obtained experimentally can serve as a check of either. $S_{biomass}$ can be calculated using Eq. (16) expressed in terms of biomass and substitution in Eq. (12):

$$S_{biomass} = -0.813 \Sigma S^0_{atoms} + \Sigma S^0_{atoms} = 0.187 \Sigma S^0_{atoms} \quad (17)$$

As an example, for the dried *S. cerevisiae* cells in Column 6 of Table 1 $\Sigma S^0_{atoms} = 185.55$ J K^{-1} C mol^{-1}. Using Eq. (17), the calculated value of $S_{cells} = 34.69$ J K^{-1} C mol^{-1} compares well with the experimentally determined value of 34.17 J K^{-1} C mol^{-1}

Table 2
Accuracy of S^0 as calculated with the ratio for $\Delta_f S^0/\Sigma S^0_{atoms}$ of 0.813

Substance	S^0_{exp} (JK^{-1} C mol^{-1})	S^0_{calc} (JK^{-1} C mol^{-1})	$(S^0_{calc}/S^0_{exp})\times 100$ per cent
Anhydrous bovine zinc insulin [a]	29.84	29.56	99.06
Hydrated bovine zinc insulin [a]	32.17	31.82	98.91
Anhydrous chymotrypsinogen A [a]	31.08	31.99	102.93
Hydrated chymotrypsinogen A [a]	39.21	38.62	98.49
Anhydrous *S. cerevisiae* cells [a]	34.17	34.69	101.52
Mean			100.18±1.94 (n=5)

[a] The empirical formulas for these substances are given in Table 1.

[11]. Table 2 shows the accuracy of $S_{biomass}$ as calculated using Eq. (17) compared to that of the experimentally determined values using the Third Law. The mean agreement between the calculated and the experimentally determined values is 100.18±1.94% (n=5).

4. Discussion

It is unfortunate that a larger quantity of critically measured data is not available on the entropy of dried cells and crystalline proteins using Third Law determinations. No entropy data at all seem to be available for highly purified nucleic acids, lipoproteins, lipopolysaccharides, or other biopolymers that form a part of the cellular fabric. Nevertheless, the data in Fig. 2 are highly suggestive that Eqs. (16) and (17) would be valid for biopolymers and cells in general.

In making comparisons of values for $S_{biomass}$ as calculated using Eq. (17) with those obtained experimentally using the Third Law, it should be recognized that these latter are subject to experimental error.

If $\Delta_f H_{cells}$ is determined by the combustion calorimetry of dried cells or by the Thornton Rule, in order to use this value for the calculation of $\Delta_p H'$ the assumption has to be made that the value for $\Delta_f H_{cells}\,\Delta$ is not significantly different from that of $\Delta_f H'_{cells}$, representing the hydrated cellular fabric as it is found in living cells. It was pointed out in Section 1.1 that this assumption was valid. The same assumption with respect to values for $\Delta_f S_{cells}$ cannot be validated in the same manner as for $\Delta_f H_{cells}$ because of the complexities of carrying out Third Law measurements on wet cell material. It is not so much the entropy of hydration itself that is important as the effect it has on the total entropy change of a process. This can be illustrated by the synthesis of anhydrous and hydrated chymotrypsinogen A from glucose (as an arbitrarily chosen substrate), as represented by the following.

4.1. Anhydrous chymotrypsinogen A

$$0.175C_6H_{12}O_6(aq) + 0.282NH_3(aq) + 0.011SO_4^{2-}(aq) \rightarrow CH_{1.612}O_{0.318}N_{0.282}S_{0.011}(protein) + 0.048CO_2(aq) + 0.654H_2O(l) + 0.022OH^-(aq) \quad (18)$$

4.2. Hydrated chymotrypsinogen A

$$0.175C_6H_{12}O_6(aq) + 0.282NH_3(aq) + 0.011SO_4^{2-}(aq) \rightarrow CH_{1.612}O_{0.318}N_{0.282}S_{0.011} \cdot 0.153H_2O(protein) + 0.048CO_2(aq) + 0.501H_2O(l) + 0.022OH^-(aq) \quad (19)$$

In calculating $\Delta_p S$ for the processes represented by Eqs. (18) and (19), the cellular $\Delta_f S^0$ values in J K^{-1} C mol^{-1} were taken from Table 1. The $\Delta_f S^{0\prime}$ values for the other reactants and products were calculated from the appropriate values for $\Delta_f G^{0\prime}$ and $\Delta_f H^{0\prime}$ in Table 1 of Ref. [1] using Eq. (10) in this text, and T=298.15 K. These $\Delta_f S^{0\prime}$ values in J K^{-1} mol^{-1} are glucose(aq)=−1111.59, NH_3(aq)=−122.79, SO_4^{2-}(aq)=−494.82, CO_2(aq)=−35.79, H_2O(l)=−163.17, and OH^-(aq)=−186.45. Using these data and the appropriate data from Table 1, $\Delta_p S$ values for the processes represented by Eqs. (18) and (19) are calculated to be −17.89 and −20.51 J K^{-1} C mol, respectively. The difference, which is only 2.62 J K^{-1} C mol^{-1} more negative for the hydrated molecule, can be considered negligible when used in Eq. (2) and the same should apply to biomass in general. An additional reason for considering the effect of hydration to be negligible is that changes in entropy are usually expressed in J, whereas changes in free energy and enthalpy are usually expressed in kJ. The value of $\Delta_f S$ for hydrated, crystalline chymotrypsinogen A is 19.70% greater than that of the anhydrous molecule. Provided that the degree of hydration of cells is no greater than that of this molecule, $\Delta_f S_{biomass}$ can be used in place of $\Delta_f S'_{biomass}$ in calculations of $\Delta_p S'$ with minimal error. Most of the water within a cell serves as a fluid matrix, and not as water of hydration.

5. Conclusion

The equation, $\Delta_f S_{biomass} = -0.813\Sigma S^0_{atoms}$, can be considered an empirical 'rule', much as Thornton's Rule, in that it enables a thermal quantity to be calculated without the necessity of making a direct thermal measurement. The equation $S_{biomass} = -0.187\Sigma S^0_{atoms}$ is equally a 'rule' in that it accomplishes the same thing. Entropy values calculated with this latter equation agree with those determined

experimentally with thermal measurements by about ±2%. The dimensionless constant, −0.813, is similar to the oxycaloric equivalent in that it is inexact, but useful. Values for $\Delta_f H_{biomass}$ can be obtained either by a relatively simple thermal measurement (compared to Third Law entropy determinations that require low-temperature calorimetry) or by using Thornton's Rule. With this latter, and also the present 'rule' (law?), it becomes possible to calculate values for $\Delta_f G_{biomass}$, $\Delta_f H_{biomass}$, and $\Delta_f S_{biomass}$ at 298.15 K with an accuracy of at least ±2% without the necessity of making any thermal measurements, provided that the empirical composition of the biomass is accurately known. With growth processes such as that represented by Eq. (1) it then becomes possible to calculate values for $\Delta_p G'_{met}$, $\Delta_p H'_{met}$, and $T\Delta_p S'_{met}$, also without the necessity of making any direct thermal measurements.

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