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THE SOURCES OF THERMAL ENERGY EXCHANGE ACCOMPANYING MICROBIAL ANABOLISM

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Calculations are made of the thermal energy exchanges accompanying the anabolism of *Saccharomyces cerevisiae* of four substrates using the equations $\Delta_r H_B^{0'} = \Delta_r X_B^{0'} + \Delta_f Q_{ab,B}^{0'}$ and $\Delta_r H_B^{0'} = \Delta_f G_B^{0'} + T\Delta_r S_B^{0'}$. Contrary to a previous postulate cited in the Discussion, the free-energy changes accompanying anabolism are not zero, but can be either positive or negative. However, their magnitude with either sign is small compared to that of catabolism of the same substrates, so that even with free energy changes that are negative it is unlikely anabolism can be considered a spontaneous process.

Keywords: *enthalpy, entropy, free energy, heat of growth, microbial anabolism, thermodynamics*

Introduction

The anabolism of a given microorganism can be represented by a balanced equation, which is comprised of an initial state represented by the substrate and nutrients entering into the formation of a microbial biomass and a final state comprising the products of anabolism including the cells. The systems investigated here are those of *Saccharomyces cerevisiae* growing in batch culture anaerobically on glucose, and aerobically on glucose, ethanol, and acetic acid. These are useful in that they represent growth of the same microorganism anaerobically and aerobically on the same anabolic substrate (glucose), and aerobically on anabolic substrates that are more (ethanol) or less (acetic acid) reduced than the biomass, all on the same defined nutrient medium [1]. This makes possible a comparison of the thermodynamics of growth of the same microorganism on several substrates using batch cultures growing in the exponential phase at μ_{max}, under which condition storage materials are not found within the cells [2, 3]. This is important in that, as pointed out as early as 1900, storage materials are effectively internal substrates that have not been utilized for growth [4]. They thus contribute to the mass of cellular yield without being a part of the fabric of the cells, which leads to an erroneous value for the cellular yield [5a, 6a].

Methods

Methods for constructing anabolic equations are found in [6b]. The anabolic equations studied here are shown in Table 1.

Representation of cells

The following unit-carbon formula (UCF) representing yeast cells accounts for about 99.97% of the cellular dry mass, the rest being accounted for as trace elements [6c].

$$CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}(\text{cells}) \quad (1)$$

This formula represents an ion-containing unit carbon formula weight (ICUCFW) of 26.202 g. However, although the elemental analysis of microorganisms will always result in similar cellular formulae, this does not mean that all these elements participate significantly in the energetics or thermodynamics of cellular growth. The elements K, Mg and Ca function principally as electrolytic or osmotic balancers, and exist as ions that are not strictly a part of the cellular fabric. In the analysis of cells to determine their heat of combustion, the ions K^{1+}, Mg^{2+} and Ca^{2+} are already in their highest oxidation states and are not bound covalently to the substances forming the cellular fabric. Thus, although they form a part of the mass of microbial cells, they may not contribute significantly to the heat of combustion. The same applies to determining the heat capacities of dried cells using low-temperature calorimetry, where values of C_p for K, Mg and Ca are included but can also be considered separate from that of the fabric of the cells. For these reasons, the more accurate formula for 'cells' for the purpose of calculating energy exchanges would be one not including these elements, as follows.

$$CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(\text{cells}) \quad (2)$$

Formula (2) represents an ion-free unit carbon formula weight (IFUCFW) of 25.230 g. The yeast cells used in the present investigation were grown at

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Table 1 Equations representing anabolism of *Saccharomyces cerevisiae* anaerobically and aerobically on glucose, ethanol and acetic acid, per mol of metabolic substrate

Anaerobic growth on glucose $0.100C_6H_{12}O_6(aq)+0.093NH_3(aq)+0.007H_3PO_4(aq)+0.002H_2SO_4(aq)\rightarrow 0.010CO_2(aq)+0.282H_2O(l)$ $+0.590CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells)$
Aerobic growth on glucose $0.325C_6H_{12}O_6(aq)+0.302NH_3(aq)+0.023H_3PO_4(aq)+0.006H_2SO_4(aq)\rightarrow 0.036CO_2(aq)+0.916H_2O(l)$ $+1.914CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells)$
Aerobic growth on ethanol $0.350C_2H_6O(aq)+0.163NH_3(aq)+0.330CO_2(aq)+0.012H_3PO_4(aq)+0.003H_2SO_4(aq)\rightarrow 0.492H_2O(l)$ $+1.030CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells)$
Aerobic growth on acetic acid $0.316C_2H_4O_2(aq)+0.098NH_3(aq)+0.007H_3PO_4(aq)+0.002H_2SO_4(aq)\rightarrow 0.012CO_2(aq)+0.296H_2O(l)$ $+0.620CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells)$

μ_{max} in the same defined medium at the same pH, temperature, and pressure, any differences in the equations representing growth then being whether the systems are aerobic or anaerobic, or make use of different substrates. The only thing limiting the growth of the cells is the low concentration of substrate, which becomes exhausted during growth in batch culture. Under these conditions, no storage substances are formed within the cells, as shown by the lack of heat production after growth has ceased [2, 3]. In the systems studied, the composition of the cells has been taken as being the same for growth on all three substrates. This has not been conclusively proven. On the other hand, the enzymes metabolizing these substrates are all proteins having very similar compositions. The composition of the rest of the yeast cellular fabric in the absence of storage products would be expected to be the same for any substrate.

The sources of thermal energy exchange accompanying anabolism

These sources are represented by the following equation, or its modifications [7, 8].

$$\Delta_r H^0 = \Delta_r X^0 + \Delta_r Q^0_{ab} \quad (3)$$

where $\Delta_r H^0$ represents the heat of reaction, $\Delta_r X^0$ represents the thermal equivalent of a free-energy change resulting from the conversion of chemical potential energy into thermal energy during anabolism and $\Delta_r Q^0_{ab}$ represents a thermal exchange in that the sum of the directly measurable absorbed thermal energies of the reactants is different from that of the products. It should be emphasized that Eq. (3) is controversial in that it is in the form of the Gibbs free energy equation, but $\Delta_r G^0$ has been replaced by $\Delta_r X^0$, and $T\Delta_r S^0$ has been replaced by $\Delta_r Q^0_{ab}$, or their respective derivatives. Methods for calculating $\Delta_r H^0$, $\Delta_r X^0$, $\Delta_r Q^0_{ab}$, $\Delta_r H^{0'}$, $\Delta_r X^{0'}$, $\Delta_r Q^{0'}_{ab}$, $\Delta_r H^{0'}_B$, $\Delta_r X^{0'}_B$, and $\Delta_r Q^{0'}_{ab,B}$ are described in [8]. These last three quantities are thermal exchanges calculated for 0.001 concentrations of reactants and products. This concentration more nearly approaches the natural, 'biological' concentration of substances within cells and has been adopted as a thermodynamic 'biological' standard state [5b, 6d, 9]. At 0.001 *M*, concentrations can be taken as equal to activities for the calculation of values of $\Delta_r X^{0'}_B$; $\Delta_r H^{0'}_B$ can be taken as equal to $\Delta_r H^{0'}$; and $\Delta_r Q^{0'}_{ab,B}$ can be calculated using Eq. (15).

The thermodynamic properties of cells

The thermodynamic properties of cells are not found in conventional tabulations, and must be calculated. They would be expected to vary, depending on the cell type and culture conditions. The above methods have been developed to calculate the thermodynamic properties of small molecular mass substances in solution at the biological standard state of 0.001 M, 0.1 MPa and 298.15 K. These can be used for all of the components of an equation representing anabolism except for the cells, which are 'impure' substances not having a conventional standard state. Nevertheless, everything, including cells, possesses thermodynamic properties which can be determined and used in calculations.

Substances stored in microbial cells such as starch, β-hydroxy butyric acid, oil, glycogen, etc., are not really a part of the cellular fabric. Stored substances are assimilated internal substrates which are utilized when external substrates become exhausted [4]. For this reason, cells to be analyzed for their thermodynamic properties should be grown in batch culture at μ_{max} in media containing dilute substrate concentrations. Under these conditions microbial cells, in general, do not contain storage substances, and these are the conditions under which the cells used in this study were grown [2, 3]. Cells are particles (you can see them) containing highly polymeric substances and their thermodynamic properties are not those of substances in solution.

Determining $\Delta_c H^0_{cells}$

The mass used in describing cellular thermodynamic properties is the UCFW ('Representation of cells'), this being equivalent to the representation of one mol of a small molecular mass substance. The oxidation of one ICUCFW of *S. cerevisiae* cells in a bomb calorimeter can be represented as follows.

$$\begin{aligned}CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}(cells)\\ +1.151O_2(g)\rightarrow 1.000CO_2(g)+0.806H_2O(l)\\ +0.079N_2(g)+0.003P_4O_{10}(cr)+0.003SO_3(g)\\ +0.011K_2O(cr)+0.003MgO(cr)+0.001CaO(cr)\end{aligned} \quad (4)$$

The product of $SO_3(g)$ for this oxidation was established by Duboc *et al.* [10].

i.) The heat of combustion of dried *S. cerevisiae* cells ($\Delta_c H_{cells}$) can be measured directly in a bomb calorimeter and was determined to be –19.44±0.17 (n=7) kJ (g of dried whole cells)$^{-1}$ [11]. Multiplying this by 26.202 g ICUCFW^{-1} gives –509.37 kJ ICUCFW^{-1} as the value for $\Delta_c H_{cells}$. However, even though the ions K^+, Mg^{2+} and Ca^{2+} are already in their highest oxidation state, the formation of their oxides may still contribute a small quantity to the heat of combustion of the cells. The value of –509.37 kJ ICUCFW^{-1} may not apply equally to the IFUCFW (25.230 g), which does not contain K, Mg and Ca (see iii., below).

ii.) A second method is to use Thornton's rule, which states that for the combustion of organic substances the value of $\Delta_c H$ eq^{-1} transferred to $O_2(g)$ is a constant [12, 13]. This was reviewed and calculations were made of 488 substances of all kinds containing C, H, O and N for which $\Delta_c H^0$ is known [14]. Their data gave an average of –111.40 kJ eq^{-1} and a linear regression value of –110.88 kJ eq^{-1}. There is no reason to believe that one of these values is 'better' than the other, and these were later averaged to give –111.14 kJ eq^{-1} as a general value that could be used for organic substances of all kinds [15]. The number of equivalents transferred to $O_2(g)$ during the bomb calorimetry of cellular substance is calculated as follows.

$$eq=4nC+nH-2nO-0nN+5nP+6nS \quad (5)$$

where 'n' represents the quantity of each element in a UCF. For the yeast cells represented by Formula (2) the number of eq transferred to $O_2(g)$ during bomb calorimetry is 4.577 eq IFUCFW^{-1}. The value of $\Delta_c H_{cells}$ using this method is then 4.577 eq IFUCFW^{-1} X –111.14 kJ eq^{-1}=–508.69 kJ IFUCFW^{-1}.

iii.) A third method is to use the oxycaloric equivalent [16]. Here Eq. (4) must be modified so as to exclude the oxygen consumed in forming the oxides of K, Mg and Ca during bomb calorimetry.

$$\begin{aligned}CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells)+1.144O_2(g)\rightarrow\\ 1.000CO_2(g)+0.806H_2O(l)+0.079N_2(g)+\\ +0.003P_4O_{10}(cr)+0.003SO_3(g)\end{aligned} \quad (6)$$

Multiplying the value of –111.14 kJ eq^{-1} by the four electrons required to reduce $O_2(g)$ to $H_2O(l)$ gives –444.56 kJ mol^{-1} of $O_2(g)$ consumed during bomb calorimetric combustion. Multiplying –444.56 kJ mol^{-1} X 1.144$O_2(g)$ IFUCFW^{-1}(from Eq. (6)) gives –508.57 kJ IFUCFW^{-1} as $\Delta_c H^0_{cells}$.

iv.) Determining $\Delta_f H^0_{cells}$

The above three values for $\Delta_c H^0_{cells}$, i.e., –509.37, –508.69 and –508.57 kJ IFUCFW^{-1} are nearly identical. The last two are from indirect calorimetry. Averaging these last gives –508.63 kJ UCFW^{-1} which, when subtracted from –509.37 IFUCFW^{-1} (direct calorimetry) from Section *i*.) gives an estimate of –0.74 kJ ICUCFW^{-1} as the heat lost in forming the oxides of K, Mg and Ca. The total average for $\Delta_c H^0_{cells}$ then becomes –508.63 kJ IFUCFW^{-1}. A value for $\Delta_f H^0_{cells}$ is calculated using the following equation.

$$\Delta_c H^0 = \sum \Delta_f H^0_{prod} - \sum \Delta_f H^0_{react} \quad (7)$$

where $\Delta_c H^0$ represents the heat of combustion and the subscripts 'prod' and 'react' refer to the reactants and products, respectively. Using Eqs (6) and (7), the value for $\Delta_c H^0$ of –508.63 kJ IFUCFW^{-1}, and the appropriate $\Delta_f H^0$ values from Table 2, the value of $\Delta_f H^0_{cells}$ is calculated to be –125.40 kJ IFUCFW^{-1}.

v.) Determining Q^0_{ab}

The symbol $(H^0_T - H^0_0)$ represents a thermodynamic function of state, just as do $\Delta_f H^0$, $\Delta_f G^0$ and S^0. For the purposes of this presentation, the symbol Q^0_{ab} is taken to have the identical meaning as $(H^0_T - H^0_0)$ and is also a function of state [8]. It represents the quantity of heat that is required to be absorbed by a given substance for it to attain a given temperature provided that the reference temperature is 0 K. The reason for making this identification is that the symbol Q classically represents 'reversibly' absorbed heat (after Clausius). This is not immediately evident with respect to the symbol H, which is also identified with the heat of reaction, $\Delta_r H$, and the heat of formation, $\Delta_f H$. By using low-temperature calorimetry the quantity Q^0_{ab} can be determined directly for substances in a condensed phase, as represented by the following equation.

$$Q^0_{ab} = (H^0_T - H^0_0) = \int_0^{298.15} C_p dT + \sum \Delta_{trs} H^0_T \quad [17a] \quad (8)$$

Statistical mechanics rather than calorimetric measurement is usually used to determine values of $C_p dT$ for substances in the gas phase. At the present time there is only one determination of $Q^0_{ab,\,cells}$ for *S. cerevisiae*, resulting in a value of 201.21 J g^{-1} dry mass [18]. Multiplying this by 26.202 g ('Representa-

Table 2 Thermodynamic properties of substances of anabolic importance at 298.15 K and 0.1 MPa[a]. The postscripts in parentheses indicate conventional standard states

Substance		$\Delta_f H^0$	$\Delta_f H^{0'}$	$\Delta_f H_B^{0'}$	$\Delta_f X^0$	$\Delta_f X^{0'}$	$\Delta_f X_B^{0'}$	Q_{ab}^0	$\Delta_f Q_{ab}^0$	$\Delta_f Q_{ab}^{0'}$	$\Delta_f Q_{ab,B}^{0'}$	$\Delta_f G^0$	$\Delta_f G^{0'}$	$\Delta_f G_B^{0'}$
		kJ mol^{-1}												
Elements[b]														
Calcium (cr)	Ca	0	0	0	0	0	0	5.71	0	0	0	0	0	0
Carbon (s)	C	0	0	0	0	0	0	1.05	0	0	0	0	0	0
Hydrogen (g)	H_2	0	–4.18	–4.18	0	17.57	0.45	8.99	0	–21.75	–4.63	0	17.57	0.45
Magnesium (cr)	Mg	0	0	0	0	0	0	5.00	0	0	0	0	0	0
Nitrogen (g)	N_2	0	–10.54	–10.54	0	18.07	0.95	15.51	0	–28.61	–11.49	0	18.07	0.95
Oxygen (g)	O_2	0	–12.09	–12.09	0	16.32	–0.80	17.39	0	–28.41	–11.29	0	16.32	–0.80
Phosphorous (cr)	P	0	0	0	0	0	0	5.36	0	0	0	0	0	0
Potassium (cr)	K	0	0	0	0	0	0	7.09	0	0	0	0	0	0
Sulfur (cr)	S	0	0	0	0	0	0	4.41	0	0	0	0	0	0
Inorganic[b]														
Ammonia (g)	NH_3	–46.11	–80.29	–80.29	–64.05	–74.05	–91.17	39.18	17.94	–6.24	10.88	–16.57	–26.57	–43.69
Carbon dioxide (g)	CO_2	–393.51	–413.80	–413.80	–411.31	–402.94	–420.06	36.24	17.80	–10.86	6.26	–394.37	–386.01	–3.13
Potassium oxide (cr)	K_2O	–363.17						14.03	–10.44			–321.84		
Phosphoric acid (cr)	H_3PO_4	–1279.0	–1288.34	–1288.34	–1214.56	–1240.0	–1257.12	16.98	–64.44	–48.34	–31.22	–1119.1	–1142.54	–1159.66
Phosphorous decoxide (cr)	P_4O_{10}	–2984.0			–2906.21			33.96				–2697.7		
Sulfur trioxide (g)	SO_3	–395.72			–376.93			11.70	–18.79			–371.06		
Sulfuric acid (l)	H_2SO_4	–813.99	–909.27	–909.27	–785.05	–730.52	–747.64	28.23	–28.94	–178.75	–161.63	–690.00	–744.53	–761.65
Water (l)	H_2O	–285.83	–285.83	–285.83	–281.42	–281.42	–281.42	13.27	–4.41	–4.41	–4.41	–237.18	–237.18	–237.18
Organic[b]														
Acetic acid (l)	$C_2H_4O_2$	–484.21	–485.26	–485.26	–474.89	–489.53	–506.65	28.15	–9.32	4.27	21.39	–389.45	–404.09	–421.21
Ethanol (l)	C_2H_6O	–276.98	–287.02	–287.02	–263.50	–270.28	–287.40	24.28	–13.48	–16.74	0.38	–174.18	–180.96	–198.08
α-*D*-Glucose (cr)	$C_6H_{12}O_6$	–1274.45	–1263.07	–1263.07	–1195.11	–1199.08	–1216.20	33.07	–79.34	–63.99	–46.85	–910.56	–914.54	–931.66

[a]Values for entropic thermodynamic properties can be calculated using the appropriate values for $\Delta_f H^0$, $\Delta_f H^{0'}$, $\Delta_f H_B^{0'}$, $\Delta_f G^0$, $\Delta_f G^{0'}$ and $\Delta_f G_B^{0'}$, above, and appropriate modifications of the equation $T\Delta_f S^0 = \Delta_f H^0 - \Delta_f G^0$

[b]For gaseous elements and inorganic substances, the values of Q^0 for CO_2, CH_4, H_2, N_2, NH_3 and O_2, at 298.15 K and 0.1 MPa, are calculated from the original literature and include data for the solid and liquid states of these gases [22]. Those for $\Delta_f H^0$, $\Delta_f H^{0'}$, $\Delta_f G^0$ and $\Delta_f G^{0'}$ were taken from [17, 24]. Values for $\Delta_f H_B^{0'}$, $\Delta_f X_B^{0'}$, $\Delta_f Q_{ab,B}^{0'}$ and $\Delta_r G_B^{0'}$ were calculated as described in (8). For organic substances, values for $\Delta_f H^0$, $\Delta_f H^{0'}$, $\Delta_f G^0$ and $\Delta_f G^{0'}$ were taken from [17, 24] except for glucose, where a value for Q^0 was taken from [25].

tion of cells') gives 5.272 kJ ICUCFW^{-1} as the value of $Q^0_{ab,\,cells}$. However, the Q^0_{ab} values for the elements K, Mg and Ca contribute to this value, and these must be subtracted from the value obtained with whole cells if Formula (2) is to be used. These are 7.09, 5.00 and 5.71 kJ g atom^{-1} respectively [17]. The appropriate equation for calculating Q^0_{ab} for ion-free cells is then

$$\begin{aligned} Q^0_{ab,\,cells\,(Formula\,2)} &= Q^0_{ab,\,cells\,(Formula\,1)} - \sum Q^0_{ab}\,(K+Mg+Ca) \\ &= 5.272\ kJ - [(0.022\cdot 7.09) + \\ &\quad + (0.003\cdot 5.00) + (0.001\cdot 5.71)]\ kJ = \\ &= 5.272\ kJ - 0.177\ kJ = \\ &= 5.095\ kJ\ IFUCFW^{-1} \end{aligned} \tag{9}$$

vi.) Calculating $\Delta_f Q^0_{ab,\,cells}$

$$\Delta_f Q^0_{ab,\,cells} = Q^0_{ab,\,cells} - \sum Q^0_{ab,\,component\,atoms} \tag{10}$$

In Eq. (10), $\Delta_f Q^0_{ab,\,cells}$ represents the 'absorbed thermal energy of formation', analogous, but not equal to the 'entropy of formation' of a substance. A value for $\Delta_f Q^0_{ab,\,cells}$ is obtained with Eq. (10), Formula (2), and data from Table 2, as follows.

$$\begin{aligned} \Delta_f Q^0_{ab,\,cells} &= 5.09 - [(1.05) + (1.613\cdot 4.50) + \\ &\quad + (0.557\cdot 8.69) + (0.158\cdot 7.75) + \\ &\quad + (0.012\cdot 5.36) + (0.003\cdot 4.41)] = \\ &= 5.09 - 14.44 = \\ &= -9.35\ kJ\ IFUCFW^{-1} \end{aligned}$$

vii.) Calculating $\Delta_f X^0_{cells}$

$$\begin{aligned} \Delta_f X^0_{cells} &= \Delta_f H^0_{cells} - \Delta_f Q^0_{ab,\,cells} \\ &= -125.40 - (-9.35) \\ &= -116.05\ kJ\ IFUCFW^{-1} \end{aligned} \tag{11}$$

Anabolic, thermodynamic changes under biological conditions

$$\Delta_r H^{0'}_B = \sum \Delta_f H^{0'}_{B,\,prod} - \sum \Delta_f H^{0'}_{B,\,react} \tag{12}$$

$$\Delta_r X^{0'}_B = \sum \Delta_f X^{0'}_{B,\,prod} - \sum \Delta_f X^{0'}_{B,\,react} \tag{13}$$

$$\Delta_r Q^{0'}_{ab,\,B} = \sum \Delta_f Q^{0'}_{ab,\,B,\,prod} - \sum \Delta_f Q^{0'}_{ab,\,B,\,react} \tag{14}$$

to give

$$\Delta_r H^{0'}_B = \Delta_r X^{0'}_B + \Delta_r Q^{0'}_{ab,\,B} \tag{15}$$

Methods for calculating values of $\Delta_r H^{0'}_B$, $\Delta_r X^{0'}_B$, and $\Delta_r Q^{0'}_{ab,\,B}$ for substances other than cells under biological conditions are described and data provided in [8].

Analogies with the Gibbs free energy equation or its modifications

Descriptions of calculations using this equation are, in general, identical to those in 'anabolic, thermodynamic changes under biological conditions', except that the symbols $\Delta_r X^0$ and $\Delta_r Q^0_{ab}$ are replaced by $\Delta_r G^0$ and $T\Delta_r S^0$, respectively, as appropriately modified, and using the same references. Thus,

$$\Delta_r H^{0'}_B = \sum \Delta_f H^{0'}_{B,\,prod} - \sum \Delta_f H^{0'}_{B,\,react} \tag{12}$$

$$\Delta_r G^{0'}_B = \sum \Delta_f G^{0'}_{B,\,prod} - \sum \Delta_f G^{0'}_{B,\,react} \tag{16}$$

$$T\Delta_r S^{0'}_B = \sum T\Delta_f S^{0'}_{B,\,prod} - \sum T\Delta_f S^{0'}_{B,\,react} \tag{17}$$

to give

$$\Delta_r H^{0'}_B = \Delta_r G^{0'}_B + T\Delta_r S^{0'}_B \tag{18}$$

Values of S^0 are calculated as follows

$$S^0 = \int_0^{298.15} C_p \mathrm{d}\ln T + \sum \Delta_{trs} H^{0'}_T / T \quad [17b] \tag{19}$$

The entropy of *Saccharomyces cerevisiae* cells has been determined to be 34.167 J K^{-1} ICUCFW^{-1} [18]. However, the S^0 values for the elements K, Mg and Ca contribute to this value, and these must be subtracted from the value obtained with whole cells if Formula (2) is to be used. S^0 values for K, Mg and Ca are 64.18, 41.42 and 32.68 J K^{-1} g atom^{-1}, respectively [17]. The appropriate equation is then

$$\begin{aligned} S^0_{cells\,(Formula\,2)} &= S^0_{cells\,(Formula\,1)} - \sum S^0\,(K+Mg+Ca) \\ &= \{34.17 - [(0.022\cdot 64.18) + \\ &\quad (0.003\cdot 32.68) + (0.001\cdot 41.42)]\} \\ &= (34.17 - 1.55)\ J\ K^{-1} \\ &= 32.62\ J\ K^{-1}\ IFUCFW^{-1} \end{aligned} \tag{20}$$

Calculating $\Delta_f S^0_{cells}$

$$\begin{aligned} \Delta_f S^0_{cells} &= S^0_{cells} - \sum S^0_{component\,atoms} \\ &= 32.62 - [(5.74) + (1.613\cdot 65.34) + \\ &\quad + (0.557\cdot 102.57) + (0.158\cdot 95.80) + \\ &\quad + (0.012\cdot 41.09) + (0.003\cdot 32.06)] \\ &= 32.62 - 183.99 \\ &= -151.37\ J\ K^{-1}\ IFUCFW^{-1} \end{aligned} \tag{21}$$

With regard to Q^0_{ab} and S^0

It is important to note that although for a given substance the same C_p data are used to calculate both Q^0_{ab} and S^0 using Eqs (8) and (19), respectively, these quantities represent two, different, mathematical

functions. If S^0 is multiplied by 298.15 K, TS^0 will not equal Q^0_{ab} at the same temperature.

Results and discussion

Table 3 summarizes the thermodynamic properties of *S. cerevisiae* cells. Table 1 shows the four anabolic equations studied in this paper representing the growth of *S. cerevisiae* anaerobically on glucose, and aerobically on glucose, ethanol, and acetic acid. Table 2 gives the thermodynamic properties of the substances used in the calculations for Table 4, except for those of the cells, which are in Table 3. Table 4 shows the results of calculations of the changes in thermodynamic properties accompanying the anabolic reactions represented in Table 1. All data necessary for making these calculations are found in Tables 1, 2 and 3.

The data in Table 4 show that the free energy changes ($\Delta_r X^{0'}_B$ and $\Delta_r G^{0'}_B$) accompanying the anabolic reactions represented in Table 1 can be either positive or negative. For glucose anabolism, the aerobic/anaerobic ratios of the thermodynamic changes are the same for all five thermodynamic properties. This is to be expected since anabolism does not require gaseous oxygen provided that, if the microorganisms are eukaryotic, a suitable steroid is provided as a 'vitamin' in the culture medium, as was done with *S. cerevisiae* in this study [1]. As seen in Table 1, per mol of glucose consumed metabolically, the anabolic consumption of glucose is 3.25 times greater aerobically than anaerobically. From Table 4 the ratios of aerobic values/anaerobic values for $\Delta_r H^{0'}_B$, $\Delta_r X^{0'}_B$ and $\Delta_r Q^{0'}_B$, using Eq. (15) are all 3.25, respectively, as are the same ratios for $\Delta_r H^{0'}_B$, $\Delta_r G^{0'}_B$ and $T\Delta_r S^{0'}_B$, using Eq. (18). Values of $\Delta_r Q^{0'}_{ab,\,B}$ and $T\Delta_r S^{0'}_B$ are all negative, indicating a loss of absorbed thermal energy from all systems. In this respect there is no difference between Eqs (15) and (18), but $\Delta_r Q^{0'}_{ab,\,B}$ is less negative than $T\Delta_r S^{0'}_B$ in all four anabolic reactions in Table 1. The anabolism of acetic acid is endothermic.

From Table 4, three values of $\Delta_r H^{0'}_B$ accompanying the anabolic processes represented in Table 1 are negative and one is positive. They are not zero. On the other hand, it was at one time postulated that the free energy changes accompanying anabolism as represented in Table 1 should be zero [5c]. As shown in Table 4, using the above systems and methods this is not correct. Values of $\Delta_r X^{0'}_B$ and $\Delta_r G^{0'}_B$ are positive for the anabolism of ethanol and acetic acid, but negative for glucose. This is because the 'electron energy charge' for glucose is larger than that for either ethanol or acetic acid [19]. Even if anabolic $\Delta_r X^{0'}_B$ or $\Delta_r G^{0'}_B$ values are negative, these values are so small that anabolism is unlikely to be spontaneous. When coupled to the utilization of the ATP available from catabolism the coupled reactions would all become so.

All values of $\Delta_r Q^{0'}_{ab,\,B}$ and $T\Delta_r S^{0'}_B$ are negative. However, this tells us nothing about whether the cells are more 'probable' than the single substrates which provide the carbon and energy sources for cellular syn-

Table 3 Thermodynamic properties of one IFUCFW of *Saccharomyces cerevisiae* cells at 298.15 K and 0.1 MPa[a]

$\Delta_f H^0_{cells}$/kJ	$\Delta_f X^0_{cells}$/kJ	$\Delta_f Q^0_{cells}$/kJ	$\Delta_f G^0_{cells}$/kJ	$\Delta_f S^0_{cells}$/J K^{-1}	$T\Delta_f S^0_{cells}$/kJ
–125.40	–116.05	–9.35	–82.68	–151.37	–45.13

[a] Methods for calculating these energies of formation are given in the text

Table 4 Thermodynamic exchanges for the anabolic processes represented in Table 1, in which, other than the cells, the products and reactants are in the 'biological aqueous standard state' at a concentration of 0.001 M at 298.15 K and 0.1 MPa. Except for Columns E and F, negative values represent heat produced; positive values represent heat absorbed. Values in Column E represent percentages that $\Delta_r X^{0'}_B$ is more negative than $\Delta_r G^{0'}_B$. [a]Values in Column F represent percentages that $\Delta_r Q^{0'}_{ab,\,B}$ is more positive than $T\Delta_r S^{0'}_B$

Growth process		A	B	C	D	E	F
	$\Delta_r H^{0'}_B$	$\Delta_r X^{0'}_B$	$\Delta_r Q^{0'}_{ab,\,B}$	$\Delta_r G^{0'}_B$	$T\Delta_r S^{0'}_B$		
Anaerobic growth on glucose	–14.12	–11.64	–2.48	–11.40	–2.72		
Percent source of thermal energy	100.00	82.44	17.56	80.74	19.26	–2.10	8.83
Aerobic growth on glucose	–45.89	–37.83	–8.06	–37.05	–8.84		
Percent source of thermal energy	100.00	82.43	17.56	80.74	19.26	–2.10	8.83
Aerobic growth on ethanol	–1.51	13.41	–14.92	26.31	–27.82		
Percent source of thermal energy	100.00	988.08	–888.08	1842.38	–1742.38	–46.37	49.03
Aerobic growth on acetic acid	4.73	19.04	–14.31	22.22	–17.49		
Percent source of thermal energy	100.00	402.54	–302.54	469.77	–369.77	–14.31	18.18

[a]Data on values of $\Delta_r H^{0'}_B$, $\Delta_r X^{0'}_B$, $\Delta_r Q^{0'}_{ab,\,B}$, $\Delta_r X^{0'}_B$ and $T\Delta_r S^{0'}_B$ for the reactants and products participating in the processes shown in Table 1, except for those of the cells which are found in Table 3, can be found in [8]

thesis. Values of $\Delta_r Q^{0'}_{ab,B}$ in all four anabolic processes are numerically less negative than $T\Delta_r S^{0'}_B$. The comparative percentages with respect to the difference of this negativity between the two for a given process can be considerable, which can have an equal effect on the free energy exchanges as calculated using Eqs (15) or (18). The differences in the values of $\Delta_r X^{0'}_B$ or $\Delta_r G^{0'}_B$ are not trivial when either is compared to their respective catabolic values [8] and raise the question as to whether Eq. (15) or (18) is more accurate for the purpose of calculating free energy changes. Certainly Eq. (15) is easier to understand, since it involves the calculation of the function Q^0_{ab} using Eq. (8) or, theoretically the direct measurement of the quantity of thermal energy necessary to raise the temperature of a substance from 0 to 298.15 K if this is done calorimetrically in small increments and with sufficient relaxation times. The meaning of Eq. (18) is not easy to understand since it involves the calculation of the function *S*, using Eq. (19). Entropy is essentially a mathematical function [20, 21], has the dimensions of J unit-mass^{-1} K^{-1}, does not represent energy, and has often been identified with 'probability', 'randomness', and 'information' even though these have no dimensions. When multiplied by *T*, the term TS^0 acquires the dimensions of energy (J unit-mass^{-1}). The present author has always argued that this is absorbed thermal energy, since it is by the addition of heat that C_p measurements are made to use in Eqs (8) and (19). If this is true, the magnitude of TS^0 is always greater than that of Q^0 for the same substance [22, 23]. Because there cannot be two different quantities of thermal energy required to be absorbed to raise the *T* of this substance from 0 to 298.15 K, it is reasonable to believe that the directly measurable quantity, Q^0 is a better function to use, and that Eq. (15) is more correct.

Conclusions

With respect to the anabolic systems studied here, no changes in thermodynamic properties are zero. Values of $\Delta_r X^{0'}_B$ may or may not be numerically larger that those of $\Delta_r G^{0'}_B$ but in either case probably do not contribute significantly to the spontaneity of anabolism, which can occur only using the activation energy of ATP. Values of $\Delta_r Q^{0'}_{ab,B}$ and $T\Delta_r S^{0'}_B$ are all negative with respect to the four systems studied in Table 1, and represent a passive exchange of absorbed thermal energy into heat. There are no differences in the form of equations $\Delta_r H^{0'}_B = \Delta_r X^{0'}_B + \Delta_r Q^{0'}_{ab,B}$ (Eq. (15)) and $\Delta_r H^{0'}_B = \Delta_r G^{0'}_B + T\Delta_r S^{0'}_B$ (Eq. (18)). However, there is a difference in the interpretation of what is meant by Q^0 and S^0. Both are well-defined mathematical functions. The meaning of Q^0 is very easy to understand, but the meaning of S^0 has defied description for decades [8].

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