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The development of direct and indirect methods for the study of the thermodynamics of microbial growth[1]

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Abstract

Equations are constructed representing the growth of *Saccharomyces cerevisiae* in batch culture anaerobically on glucose, and aerobically on glucose, ethanol, and acetic acid. The mass of cells formed during a growth process is defined in terms of an ion-containing carbon mol (ICC-mol) of cells, the thermodynamic properties of which have been determined. Because the process of growth is completely irreversible, the free energy change accompanying a growth process cannot be measured or calculated directly. It was, therefore, necessary to obtain data from direct calorimetry for the heat of combustion of 1 ICC-mol of whole dried cells (not ash free) and from this to calculate its heat of formation. It was also necessary to determine, by direct calorimetry, the entropy of this mass of cells, and from this to calculate its entropy of formation. With these two values, the free energy of formation of 1 ICC-mol of cells was calculated with the Gibbs free-energy equation. These thermodynamic properties were then used together with those of the reactants and products of a given growth process to calculate, by indirect calorimetry, the thermodynamic changes accompanying the growth of *S. cerevisiae* on the substrates used. Considerations are made as to what growth is most closely related. It is concluded that the quantity of electrons conserved within the biomass produced during growth is the most accurate indication of the efficiency of energy conservation, all other energy changes being calculable from such data. © 1998 Elsevier Science B.V.

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1. Introduction

The study of the thermodynamics of microbial growth involves an interesting combination of biology, chemistry and physics, and the incorporation of many ideas from these separate disciplines. It is necessary to establish some form of limiting boundary that separates a working growth-process system from its environment. The energy changes that occur as the system passes from a thermochemically defined initial state to a thermochemically defined final state can be determined, either instantaneously or as the result of the consumption of a given quantity of substrate. Initially, it is best to use systems involving the lowest number of reactants and products, and this implicates the use of microorganisms as tools for such a study. Individual microbial cells are open systems, and impractical to study individually. It is easier to deal with them in the aggregate as products of a growth process. Open

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systems in the form of continuous cultures can be used. However, because of the way in which they work, continuous cultures are usually rate-limited, and may (or may not) accumulate storage substances that are not a part of the fabric of the cells. This may increase markedly the apparent cellular yield on a given substrate. Closed systems offer many advantages. The limiting boundary can be the wall of a closed batch culture vessel containing a thermochemically defined culture medium that constitutes the initial state of a growth-process system. There must be sufficient head space above the culture medium to allow gas exchange. For aerobic cultures, the initial state must contain O_2(aq) supplied by O_2(g) in the head space. For anaerobic cultures, the head space is filled with an inert gas. Provided that a small amount of substrate is used, the amount of PV work done by any change in gas pressure is minimal, and usually can be neglected relative to the total energy changes that take place. A few cells must be inoculated into the medium, but their mass is so small that this can be neglected as a part of the initial state. The cells act as self-reproducing catalysts during the process of growth, and increase in number to become the mass of one of the products of the final state that exists after the substrate has been completely consumed. The other products of the final state usually comprise CO_2(aq), H_2O(l), and any organic products other than the cells. These latter are always produced during fermentative growth processes. The initial and final states can be represented by a growth-process equation which satisfies the Law of the Conservation of Mass. Then, the object of the thermodynamicist is to determine the energy changes that occur during the process of growth as this passes from an initial to a final state in a closed system, in a manner that satisfies the Law of the Conservation of Energy.

Theoretically, any microorganism can be used as a biological tool for thermodynamic studies, provided that it can be grown on a single substrate and that thermochemically definable products are formed during the growth process. However, the use of facultative yeasts is particularly advantageous. Any study of the thermodynamics of microbial growth should not be restricted only to aerobic growth-process systems, but should also include anaerobic growth. *Saccharomyces cerevisiae* is a yeast that can grow aerobically or anaerobically on glucose. It can also grow aerobically on ethanol and on acetic acid as substrates that are more highly reduced or oxidized. Thus, an interesting spread of environmental conditions can be studied with the same microorganism growing in the same thermochemically-defined culture medium, except for the substrate. In theory, the same thermodynamic approach should apply to *all* of these conditions.

The development of ideas with respect to studying the thermodynamics of growth of microorganisms is described in Ref. [1]. A more practical and succinct description of the methods is given in Ref. [2].

1.1. Direct and indirect calorimetry

Any study of thermodynamics involves thermal measurements. In direct calorimetry, a measurement is made directly of the heat exchange accompanying a reaction or process. This is the 'observed' thermal measurement. It is, perhaps, the observed thermal measurement that is required. However, one or more corrections must be frequently applied with respect to the side reactions or processes that take place within the calorimeter vessel at the same time. Once this is done, the corrected value becomes the required 'true' thermal measurement. The corrections are made using data not obtained at the time of direct measurement, and are therefore made indirectly. Thus, even direct calorimetry may require indirect calorimetry to correct for side reactions or processes. This is certainly true of the direct measurement of the heat of microbial growth. However, it is not true of the direct calorimetric measurement of cellular entropy.

Indirect calorimetry involves the calculation, using previously obtained thermal data, of what the heat exchange accompanying a given reaction or process 'ought' to be. No direct thermal measurements are made. What is described here is the calculation of the thermal changes (enthalpy and entropy changes), and from these the non-thermal energy changes (chemical free energy changes), accompanying the growth of *S. cerevisiae* anaerobically on glucose, and aerobically on glucose, ethanol, and acetic acid.

Only one of the thermodynamic changes that can be calculated with respect to the microbial growth is subject to direct calorimetric verification, and this is the heat of growth. Ideally, it should be possible to calculate the heat of growth indirectly, and then verify this with a direct measurement of the heat of growth.

Practically, an agreement within ±2% is unlikely. This is because of the large number of indirectly measured variables that are involved, and the necessity of dealing with fractional coefficients for the chemical terms in the growth-process equations that are constructed. Often, it is also because of not knowing what choices to make in deciding the physical and chemical natures of the reactants and products. For example, how do CO_2 species interact with a cell? Is it as CO_2(aq), $H_2CO_3^-$(aq), or HCO_3^{2-}(aq)? Which of these should be included as a product or a reactant in growth-process equations? It must be recognized that whereas studies on the thermodynamics of microbial growth may indeed indicate something about the manner in which non-thermal and thermal energy is exchanged in biological systems involving cellular growth, the kinds of accuracy cannot be expected that thermodynamicists can expect when dealing only with pure, small molecular weight substances.

2. Experimental

2.1. Composition of yeast cells

The principal problem of using indirect calorimetry to study the thermodynamics of microbial growth is the determination of the elemental composition and the thermodynamic properties of the cells, those of the reactants and the other products in microbial growth processes being, for the most part, already known and available from the literature. Initial experiments on the composition of yeast cells grown anaerobically on glucose, and aerobically on glucose, ethanol, and acetic acid appeared initially to indicate that the composition of the cells varied with the substrate on which they were grown [3]. However, these analyses were performed in 1956, when microchemical determinations were just beginning to be performed, and possibly the small differences observed were the result of experimental error. Although different enzymes are involved in a metabolic sequence of reactions, depending on the substrate, these are all proteins. At the present time, it seems completely reasonable to believe that the fabric of the cells of a given microbial species, grown exponentially on one substrate (exclusive of storage products), has the same composition as that of the same species grown exponentially on a different substrate (again, exclusive of storage products). On the other hand, the cells of a given species would not be expected to have the same composition when grown under limiting conditions (as in continuous culture), nor would the cells of one species be expected to have the same composition as those of another species. Compressed Red Star 'universal' bakers yeast (*S. cerevisiae*, manufactured by General Foods Corporation, Milwaukee, Wisconsin 53218, USA) is grown on a solution of clarified molasses and nutrients, and washed twice by being passed through a separator (effectively a zonal centrifuge), after which it is compressed. This commercial preparation was suspended in distilled water in a 1 : 1, wt : vol ratio and incubated at 30°C to ferment away any stored inositol or glycogen. Aliquots of this suspension were tested for residual carbohydrate at intervals using the Durham tube technique [4]. When no further fermentation was observed, the cells were centrifuged from suspension and lyophilized [5]. An empirical analysis of these lyophilized yeast cells is given in Table 1. It is necessary to determine O directly, rather than by difference [6]. Conventionally the percentage of O in the cellular fabric is found by determining the percentages of C, H, N, and the ash remaining from complete combustion and subtracting the sum of these values from 100%. In Table 1, the weight of the ash is calculated to be 8.62%, making the sum of the percentages of C, H, N, and ash 69.18%. When this is subtracted from 100% the percentage of O as determined by difference becomes 30.82%, whereas this percentage as determined by direct chemical analysis is 34.03%. Determining O by difference will result in a different value for the UCFW, and in too high a value for the number of available electrons (AE) contained in one UCFW. From Table 1 this yeast has the following empirical formula.

$$C_{3.820}H_{6.161}O_{2.127}N_{0.605}P_{0.045}S_{0.012}K_{0.083}Mg_{0.010}Ca_{0.003} \quad (A)$$

However, this is not a particularly convenient formula to work with, and the use of a unit-carbon formula has been adopted [3], the concept of which has come into general use. Dividing the subscript for carbon, formula (A) becomes

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

Table 1
Analysis of the elements in 100 g dry wt of *Saccharomyces cerevisiae* cells [a]

Elements	g % dry weight	average (g %)	g % as ash
Structural			
Carbon [b]	45.78, 45.68, 45.93, 46.12	45.88	
Hydrogen [b]	6.42, 6.26, 6.03, 6.14	6.21	
Oxygen [b]	34.55, 34.86, 33.11, 33.61	34.03	
Nitrogen [b]	8.48, 8.45, 8.65, 8.28	8.47	
Phosphorous [b]	1.27, 1.44, 1.41, 1.39	1.38	3.16 as P_4O_{10}(cr)
Sulfur [c]		0.39	2.12 as K_2SO_4(cr)
Subtotal structural elements:		96.36	
Ionic:			
Potassium [d]		0.95	as K_2SO_4(cr)
		2.29	2.76 as K_2O(cr)
Magnesium [d]		0.25	0.41 as MgO(cr)
Calcium [d]		0.12	0.17 as CaO(cr)
Subtotal cellular ions:		3.61	
Total:		99.97	8.62
Empirical formula: $C_{3.820}H_{6.161}O_{2.127}N_{0.605}P_{0.045}S_{0.012}K_{0.083}Mg_{0.010}Ca_{0.003}$			
Ion-containing unit carbon formula: $CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}$			
Ion-containing unit carbon formula weight: 26.202 g; AE=4.079; eq=4.577			

[a] Red Star baker's yeast (General Foods Corp., Milwaukee, Wisconsin 53218, USA).
[b] These values were determined by MHW labs, P.O.Box 15149, Phoenix, AZ 85018, with oxygen being determined directly using the method of Aluise, et al. [7].
[c] This value was taken from Reed and Nagodawithana [8].
[d] The values for K^+, Mg^{++} and Ca^{++} add up to the difference between 96.36% structural material and 99.97%. According to the data of Reed and Nagodawithana [8], the trace elements amount to about 0.03 g% dry weight and about 0.83% of the total ion content. The ratios of $K^+ : Mg^{2+} : Ca^{2+}$ are 1.000 : 0.079 : 0.036. The product of *bomb calorimetric* sulfur oxidation is taken here as K_2SO_4(cr). This may not be entirely correct if some of the sulfur remains as SO_2(g) or SO_3(g). The products K_2O(cr), MgO(cr) and CaO(cr) are assumed from the data of Gurakan et al. [9], which showed that at 600°C there was no carbon in the ash. On the other hand, when plant-like products are burned in air the mineral products of combustion are carbonates. Thus, if part or all of the combustion inside a bomb calorimeter were to take place at a temperature of less than 600°C (for example, at 400°C), the products would be more correctly K_2CO_3(cr), $MgCO_3$(cr), and $CaCO_3$(cr). The ash weight calculated here is similar to the 8.12% dry wt obtained for *S. cerevisiae* by Gurakan et al. [9] and might not be expected to be exactly the same in that the ash content of cells depends on the strain of cells used and how they are grown.

Formula (B) is more complete than the usual representations of cellular biomass, which are only in terms of C, H, O and N (e.g., [10,11]) and would better be referred to as an 'ion-containing, unit-carbon formula' (ICUCF). In this example, it represents 99.97% of the dry biomass, the difference from 100% being due to trace elements, the quantities of which are too small to be represented conveniently. Formula (B) represents a biomass having an ion-containing unit carbon formula weight (ICUCFW), analogous to a conventional formula weight, of 26.202 Da. Although the term 'unit-carbon formula weight', or its derivatives, has historical precedence, it has become customary in the European literature to refer to this mass as a 'carbon mol' (C-mol). This acronym is, in fact, easier to say or use, and will be adopted here as an 'ion-containing carbon mol' (ICC-mol). However, the acronym ICUCF remains the same since it does not represent a mass.

Growth processes are oxidation–reduction (OR) processes involving the transfer of electrons, and it is important to know the quantities of electrons that are available for a transfer within a given growth-process system. The number of equivalents (eq) in an organic substance is defined as that number of electrons transferred to O during a complete combustion (as in a bomb calorimeter) to CO_2(g), H_2O(lq), N_2(g), P_4O_{10}(cr), and SO_3(g) [12]. This number can be calculated by means of the following equation [13].

$$\mathrm{eq} = 4n\mathrm{C} + n\mathrm{H} - 2n\mathrm{O} - 0n\mathrm{N} + 5n\mathrm{P} + 6n\mathrm{S} \quad (1)$$

where nC, nH, nO, nN, nP and nS are, respectively, the numbers of C, H, O, N, P and S atoms in the formula representing an organic substance. If a particular atom is not present in the substance, it is ignored. Inorganic ions are not included since they are not involved in the transfer of electrons as represented by growth-process equations. As examples, using Eq. (1) glucose, $C_6H_{12}O_6$, contains 24 eq, and yeast cells, represented by formula B, contain 4.577 eq. Biologically, organic substances are oxidized in aqueous solution, and nitrogen and sulfur remain reduced. The electrons from these oxidations, because a lesser number is 'available', are called 'available electrons' (AE) [14]. They can be defined as that number of electrons transferred to O or other oxidant (as in fermentative dismutation or anaerobic respiration) during the oxidation of an organic substance to CO_2(aq), H_2O(lq), NH_3(aq), $H_2PO_4^-$(aq) and H_2S(aq). Usually, the oxidizing agent is O_2(aq), but in anaerobic respiration processes the oxidizing agent can be SO_4^{2-}(aq), NO_3^-(aq), etc. If a substance does not contain nitrogen or sulfur, then eq=AE. The number of AE can be calculated by means of the following equation [15],

$$\mathrm{AE} = 4n\mathrm{C} + n\mathrm{H} - 2n\mathrm{O} - 3n\mathrm{N} + 5n\mathrm{P} - 2n\mathrm{S} \quad (2)$$

where the terms have the same significance as for Eq. (1). As before, if certain elements are not present, they are ignored. Using Eq. (2) glucose has 24 AE, but with yeast cells the number of AE becomes 4.079 because the nitrogen and sulfur are not oxidized. Phosphorous is always in its highest oxidation state in biological substances. Eqs. (1) and (2) explain the values for the eq and AE of yeasts in Table 1.

2.2. *Construction of growth-process equations*

Here, formula (B) is used to represent the biomass in all growth-process equations involving the yeast *S. cerevisiae*. These are shown in Table 2, and have been constructed using the methods described in Refs. [1,2] and the carbon balances from Ref. [3]. In Table 2, a 'non-conservative' (NC) process is one in which there is a maximum conversion of available chemical free energy into heat, and no available free energy remains conserved within the system. Examples of this are the complete biological oxidation or fermentation of glucose. A 'conservative' (C) process is one in which, *relative to its respective non-conservative process*, some of the chemical free energy that would otherwise be dissipated as heat becomes conserved within the system. This is because the non-conservative process becomes coupled to another process so that, in effect, the system is changed. Examples of this are the fermentative or oxidative formation of ATP from glucose, or an anaerobic or aerobic growth process. In conservative processes, chemical free energy becomes conserved within the system in the form of organic substances not formed in the respective non-conservative processes. When these organic substances are fermented or oxidized, respectively, the chemical free energy that has been conserved within them will be converted into heat. This is discussed in greater detail in Refs. [1,2].

Considerable data are needed to construct growth-process equations, and one of the problems associated with doing this is in knowing whether they are accurate. Here, this can be tested with respect to three of these equations in the following manner. Under the environmental conditions used to establish the carbon balances [3] aerobic growth on glucose first occurred fermentatively, even in the presence of pure O_2(g). Following fermentative growth and a subsequent adaptation, growth then occurred using as a substrate the ethanol formed during the fermentative growth process. The glycerol formed during this latter process did not serve as a substrate, but was simply oxidized. Theoretically, it should be possible to add the growth-process equation representing anaerobic growth on 1 mol of glucose, one representing aerobic growth on the ethanol produced during the anaerobic growth, and the other representing the oxidation of the glycerol produced during the anaerobic growth, to give an equation representing aerobic growth on glucose. The resultant equation should be the same as that obtained experimentally for aerobic growth on glucose. Table 3 shows that this can be done with excellent agreement between the aerobic glucose growth-process equation obtained by summation, and that obtained experimentally. This demonstrates an internal consistency indicating that the equations representing growth anaerobically on glucose, and aerobically on ethanol and glucose are accurate within

Table 2
Process equations representing the growth of *Saccharomyces cerevisiae* anaerobically on glucose and aerobically on glucose, ethanol, and acetic acid, with ammonia as the nitrogen source [a]

Anaerobic growth on glucose
Non-conservative process:
Alcoholic glucose fermentation: $C_6H_{12}O_6(aq) \rightarrow 2CO_2(aq) + 2C_2H_6O(aq)$, A_E=8.000
Conservative process:
Anabolism: $0.100C_6H_{12}O_6(aq) + 0.093NH_3(aq) + 0.007H_2PO_4^-(aq) + 0.002SO_4^{2-}(aq) + 0.013K^+(aq) + 0.002Mg^{2+}(aq) + 0.001Ca^{2+}(aq) + 0.008OH^-(aq) \rightarrow$
$0.010CO_2(aq) + 0.285H_2O(l) + 0.590CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}$(cells)
Catabolism:
Glycerol formation: $0.252C_6H_{12}O_6(aq) + 0.216H_2O(l) \rightarrow 0.432C_3H_8O_3(aq) + 0.216CO_2(aq)$
Ethanol formation: $0.650C_6H_{12}O_6(aq) \rightarrow 1.300C_2H_6O(aq) + 1.300CO_2(aq)$
Metabolism: $C_6H_{12}O_6(aq) + 0.093NH_3(aq) + 0.007H_2PO_4^-(aq) + 0.002SO_4^{2-}(aq) + 0.013K^+(aq) + 0.002Mg^{2+}(aq) + 0.001Ca^{2+}(aq) \rightarrow$
$0.432C_3H_8O_3(aq) + 1.300C_3H_6O(aq) + 1.526CO_2(aq) + 0.069H_2O(l) + 0.008OH^-(aq) + 0.590CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}$(cells)

Aerobic growth on glucose
Non-conservative process:
Glucose oxidation: $C_6H_{12}O_6(aq) + 6O_2(aq) \rightarrow 6CO_2(aq) + 6H_2O(l)$, AE=24.000
Conservative process:
Anabolism: $0.325C_6H_{12}O_6(aq) + 0.302NH_3(aq) + 0.023H_2PO_4^-(aq) + 0.006SO_4^{2-}(aq) + 0.042K^+(aq) + 0.006Mg^{2+} + 0.002Ca^{2+}(aq) + 0.023OH^-(aq) \rightarrow$
$0.036CO_2(aq) + 0.925H_2O(l) + 1.914CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}$(cells)
Catabolism: $0.675C_6H_{12}O_6(aq) + 4.050O_2(aq) \rightarrow 4.050CO_2(aq) + 4.050H_2O(l)$
Metabolism: $C_6H_{12}O_6(aq) + 0.302NH_3(aq) + 4.050O_2(aq) + 0.023H_2PO_4^-(aq) + 0.006SO_4^{2-}(aq) + 0.042K^+(aq) + 0.006Mg^{2+} + 0.002Ca^{2+}(aq) + 0.023OH^-(aq) \rightarrow$
$4.086CO_2(aq) + 4.975H_2O(l) + 1.914CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}$(cells)

Aerobic growth on ethanol
Non-conservative process:
Ethanol oxidation: $C_2H_6O(aq) + 3O_2(aq) \rightarrow 2CO_2(aq) + 3H_2O(l)$, AE=12.000
Conservative process
Anabolism: $0.350C_2H_6O(aq) + 0.163NH_3(aq) + 0.330CO_2(aq) + 0.012H_2PO_4^-(aq) + 0.003SO_4^{2-}(aq) + 0.022K^+(aq) + 0.002Mg^{2+}(aq) + 0.001Ca^{2+}(aq) + 0.010OH^-(aq) \rightarrow$
$0.496H_2O(l) + 1.030CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}$
Catabolism: $0.650C_2H_6O(aq) + 1.950O_2(aq) \rightarrow 1.300CO_2(aq) + 1.950H_2O(l)$
Metabolism: $C_2H_6O(aq) + 0.163NH_3(aq) + 1.950O_2(aq) + 0.012H_2PO_4^-(aq) + 0.003SO_4^{2-}(aq) + 0.022K^+(aq) + 0.002Mg^{2+}(aq) + 0.001Ca^{2+}(aq) + 0.010OH^-(aq) \rightarrow$
$0.970CO_2(aq) + 2.446H_2O(l) + 1.030CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}$(cells)

Aerobic growth on acetic acid
Non-conservative process:
Acetic acid oxidation: $C_2H_4O_2(aq) + 2O_2(aq) \rightarrow 2CO_2(aq) + 2H_2O(l)$, AE=8.000
Conservative process:
Anabolism: $0.316C_2H_4O_2(aq) + 0.098NH_3(aq) + 0.007H_2PO_4^-(aq) + 0.002SO_4^{2-}(aq) + 0.014K^+(aq) + 0.001Mg^{2+}(aq) + 0.001Ca^{2+}(aq) + 0.007OH^-(aq) \rightarrow$
$0.012CO_2(aq) + 0.297H_2O(l) + 0.620CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}$(cells)
Catabolism: $0.684C_2H_4O_2(aq) + 1.368O_2(aq) \rightarrow 1.368CO_2(aq) + 1.368H_2O(l)$
Metabolism: $C_2H_4O_2(aq) + 0.098NH_3(aq) + 1.368O_2(aq) + 0.007H_2PO_4^-(aq) + 0.002SO_4^{2-}(aq) + 0.014K^+(aq) + 0.001Mg^{2+}(aq) + 0.001Ca^{2+}(aq) + 0.007OH^-(aq) \rightarrow$
$1.380CO_2(aq) + 1.665H_2O(l) + 0.620CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}$(cells)

[a] These equations have been constructed by replacing the original UCFs from Battley [3] with that from Table 1. This does not change the carbon balance of these former, and includes a formula representing cells for which both $\Delta_f H'$ and $\Delta_f S'$ values have been determined (please see Sections 2.3 and 2.4.)

Table 3
Addition of the equation representing the growth of *Saccharomyces cerevisiae* anaerobically on 1 mol of glucose, that representing growth aerobically on the 1.300 mol of ethanol produced during anaerobic growth, and that representing the complete oxidation of the glycerol produced during anaerobic growth, to equal the equation representing aerobic growth on glucose. This latter is then compared to the experimentally-obtained equation representing aerobic growth on glucose

Anaerobic growth on 1 mol of glucose

$$C_6H_{12}O_6(aq) + 0.093NH_3(aq) + 0.007H_2PO_4^-(aq) + 0.002SO_4^{2-}(aq) + 0.013K^+(aq) + 0.002Mg^{2+}(aq) + 0.001Ca^{2+}(aq) + 0.008OH^-(aq) \rightarrow 0.432C_3H_8O_3(aq) + 1.300C_2H_6O(aq) + 1.528CO_2(aq) + 0.069H_2O(l) + 0.590CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}(cells)$$

Aerobic growth on the 1.300 mol of ethanol produced during anaerobic growth

$$1.300C_2H_6O(aq) + 0.212NH_3(aq) + 2.535O_2(aq) + 0.016H_2PO_4^-(aq) + 0.004SO_4^{2-}(aq) + 0.029K^+(aq) + 0.004Mg^{2+}(aq) + 0.001Ca^{2+}(aq) + 0.016OH^-(aq) \rightarrow 1.261CO_2(aq) + 3.172H_2O(l) + 1.339CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}(cells)$$

Aerobic oxidation of the glycerol produced during anaerobic growth

$$0.432C_3H_8O_3(aq) + 1.512O_2(aq) \rightarrow 1.296CO_2(aq) + 1.728H_2O(l)$$

Addition of the above equations to equal one representing aerobic growth on 1 mol of glucose

$$C_6H_{12}O_6(aq) + 0.305NH_3(aq) + 4.047O_2(aq) + 0.023H_2PO_4^-(aq) + 0.006SO_4^{2-}(aq) + 0.042K^+(aq) + 0.006Mg^{2+}(aq) + 0.002Ca^{2+}(aq) + 0.024OH^-(aq) \rightarrow 4.085CO_2(aq) + 4.969H_2O(l) + 1.929CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}(cells)$$

Experimentally determined equation

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

the limits of experimental error. There is no equivalent way of demonstrating this with the equation representing aerobic growth on acetic acid.

In Table 2, no O_2 enters into any equation representing anabolism. Facultative yeasts are eukaryotic cells and synthesize steroids, for which oxygen is required. They can be grown strictly anaerobically only if steroids are supplied in trace quantities in the medium [3,16]. Aerobically, the quantity of oxygen needed for steroid synthesis is extremely small and can be ignored. Bacteria do not synthesize steroids and no oxygen is needed at all for their anabolism. The main function of oxygen in microbial growth is that of a final electron acceptor.

2.3. Determination of the enthalpy of formation of lyophilized yeast cells

The enthalpy of formation of lyophilized yeast cells ($\Delta_f H_{cells}$) can be obtained by measuring the heat of combustion of a known mass of these cells, and by constructing an equation representing the reaction that takes place inside the calorimeter vessel. An appropriate combustion equation for the cells represented by formula (B), is as follows.

$$CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}(cells) + 1.151O_2(g) \rightarrow 1.000CO_2(g) + 0.806H_2O(l) + 0.079N_2(g) + 0.003P_4O_{10}(cr) + 0.003SO_3(g) + 0.011K_2O(cr) + 0.003MgO(cr) + 0.001CaO(cr) \quad (3)$$

It should be noted that Eq. (3) may not be a perfect representation of what goes on in a bomb calorimeter. There appear to be no data in the literature as to the actual temperature at which the bomb calorimetric combustion of the cells takes place. Ashing cells with a muffle furnace at different temperatures indicate that at 400–500°C some carbonates are formed, whereas at 600°C and above these are not present [9]. It is assumed in Eq. (3) that no carbonates of K, Mg, or Ca are formed, but this may not be strictly true. In addition, P_4O_{10}(cr) is highly deliquescent and may absorb water during the time that the calorimeter comes to a temperature equilibrium after a combustion experiment with lyophilized cells, forming H_3PO_4(cr) or H_3PO_4(lq), or a salt of potassium phosphate, all involving a heat exchange. Using a rotating bomb calorimeter would be preferable for measuring the heat of combustion of lyophilized cells, but has not been used for this purpose as yet.

Table 4
Thermodynamic properties at 298.15 K and 1 atm. [a]

Substance	Formula	State	$\Delta_f G^0$ (kJ mol^{-1})	$\Delta_f G'$ [b] (kJ mol^{-1})	$\Delta_f H^{0\prime}$ (kJ mol^{-1})	$\Delta_f S'$ (J K^{-1} mol^{-1})
Acetic acid	$C_2H_4O_2$	aq		−421.20	−485.26	
Ammonia	NH_3	aq		−43.68	−80.29	
Biphosphate ion	$H_2PO_4^-$	aq		−1105.66	−1296.29	
Bisulfide ion	HS^-	aq		12.05	−17.57	
Calcium ion	Ca^{2+}	aq		−570.65	−542.83	
Calcium oxide	CaO	cr	−604.04		−635.09	
Carbon dioxide	CO_2	aq		−403.13	−413.80	
		g	−394.37		−393.51	
Yeast cells	ICUCF	s		−87.96	−133.09	−151.37
	IFUCF	s		−82.16	−126.83	−149.82
Ethanol	C_2H_6O	aq		−198.07	−287.02	
Glucose	$C_6H_{12}O_6$	aq		−931.65	−1263.07	
Glycerol	$C_3H_8O_3$	aq		−514.59	−676.55	
Hydrogen ion	H^+	aq			−17.11	
Hydroxyl ion	OH^-	aq		−174.40	−229.99	
Magnesium ion	Mg^{2+}	aq		−471.91	−466.85	
Magnesium oxide	MgO	cr	−568.94		−601.24	
Nitrogen	N_2	g	0		0	
Oxygen	O_2	g	0		0	
		aq		−0.79	−12.09	
Potassium ion	K^+	aq		−300.37	−252.38	
Potassium oxide	K_2O	cr	−321.84		−363.15	
Phosphorous decoxide	P_4O_{10}	cr	−2697.84		−2984.03	
Sulfate ion	SO_4^{2-}	aq		−761.74	−909.27	
Sulfur trioxide	SO_3	g	−371.08		−395.72	
Water	H_2O	l	−237.18		−285.83	

[a] The data for inorganic substances except for oxygen were taken from Ref. [18], that for glucose from Ref. [19], those for organic substances and for oxygen from Ref. [20], and for K_2O from Ref. [21]. The data for yeast cells are calculated in the text in Sections 2.3–2.5.
[b] It has been recommended [22] that $\Delta_f G'$ values calculated on the basis of a 0.001 m real concentration are more closely representative of the thermodynamic properties of biological systems than conventional $\Delta_f G^{0\prime}$ values. This has been adopted here and the values in this column are therefore 17.11 kJ more negative than those of the aqueous standard state.

The heat of combustion ($\Delta_c H$) of *lyophilized S. cerevisiae* cells has been measured at −19.44±0.17 kJ (n=7) (g of *whole cells*) [17]. By 'whole cells' is meant living cells that have been lyophilized, and not cells represented by their 'ash-free' dry weight and a formula containing only C, H, O and N. Multiplying this value by the weight of 1 ICC-mol gives −509.37 kJ ICC-mol^{-1} as the heat of the combustion, represented by Eq. (3). Using this value, Eq. (3), and the appropriate values from Table 4, the value of $\Delta_f H_{cells}$ is calculated to be −133.13 kJ ICC-mol^{-1}.

Lyophilized yeast cells are not living yeast cells, although when properly prepared they can be rehydrated in an appropriate medium and once again become capable of reproduction. The heat of hydration has been determined to be less than 90 J g^{-1} of dried cells [23], or about 2.36 kJ ICC-mol^{-1} of yeast cells as represented by formula (B). This is only 0.46% of the enthalpy change accompanying combustion and well within the standard error of the combustion analysis [17]. Because of this, and as a first approximation, the enthalpy change equivalent to cellular hydration will be ignored, although the idea that energies are involved in cellular hydration should be borne in mind.

2.4. Determination of the entropy of formation of lyophilized yeast cells

It is only recently that the entropy of yeast cells has been measured experimentally [24], the value

obtained being 1.304 J K^{-1} g^{-1}. It should be emphasized that the value of 1.304 J K^{-1} g^{-1} for yeast cells must be considered as a lower bound. The quantity of residual entropy that may exist because yeast cells are not pure crystalline substances is unknown. Here, the assumption is made that it is very small compared to the total entropy. The additional assumption is made that the entropy change accompanying hydration is also small. Thus, when the entropy per gram of the lyophilized cells is multiplied by the weight of 1 ICC-mol, the total entropy of 1 ICC-mol becomes 34.167 J K^{-1} ICC-mol^{-1}, and this is taken to be the entropy of 1 ICC-mol of hydrated cells. This value, however, is that of the 'absolute' entropy of this mass of cells, and not the entropy of formation of the cells ($\Delta_f S_{cells}$). This latter can be calculated by means of the following equation.

$$\Delta_f S_{cells} = S_{cells} - 5.740nC - 65.34nH - 102.57nO - 95.81nN - 41.09nP - 31.80nS - 64.18nK - 32.68nMg - 41.42nCa \quad (4)$$

where S is the entropy of 1 ICC-mol of lyophilized *S. cerevisiae* cells, and n the subscript for each atom represented by formula (B) for the cells. The constants 65.34, 102.57 and 95.81 are one half of the standard entropies of $H_2(g)$, $O_2(g)$ and $N_2(g)$, respectively. The other constants are the standard entropies of solid graphite, white phosphorous, rhombic sulfur, potassium, magnesium and calcium. All constants have the dimensions of J K^{-1} g $atom^{-1}$. The terms $\Delta_f S_{cells}$ and S_{cells} have no superscripts as they represent an impure, solid substance. Using Eq. (4), $\Delta_f S_{cells}$ is calculated to be -151.37 J K^{-1} ICC-mol^{-1}.

2.5. *Calculating the free energy of formation of lyophilized yeast cells*

The free energy of formation ($\Delta_f G_{cells}$) of lyophilized yeast cells is calculated by means of the following form of the Gibbs free-energy equation,

$$\Delta_f G_{cells} = \Delta_f H_{cells} - T\Delta_f S_{cells} \quad (5)$$

where T is the standard temperature. Using the values for $\Delta_f H_{cells}$ and $\Delta_f S_{cells}$, from Sections 2.3 and 2.4, respectively, the value for $\Delta_f G_{cells}$ is calculated to be -88.00 kJ ICC-mol^{-1}. The cautions applicable for $\Delta_f H_{cells}$ and $\Delta_f S_{cells}$ will also be applicable for $\Delta_f G_{cells}$ with respect to hydration.

3. Results

3.1. *Energy changes accompanying the growth of S. cerevisiae*

The process of microbial growth is completely irreversible. No microorganism has ever been known to 'ungrow' itself, or to dissociate into the small molecular weight substances from which it was synthesized, as if the initial and final states represented in Table 2 were connected by a double arrow indicating reversibility. And, in a healthy, batch culture of microorganisms growing exponentially at μ_{max}, when the *only* limitation is a small quantity of substrate, no detectable substrate remains after growth has ceased. Under these conditions, which are those with which the equations representing the growth processes in Table 2 have been constructed, it is impossible to calculate a thermodynamic equilibrium constant that has any meaning. It is, therefore, questionable that classical equilibrium thermodynamics, or the more recent 'near-equilibrium' thermodynamics, can be utilized to calculate the changes in energy accompanying microbial growth. The only practical approach, at the present time, appears to be the subtraction of the sum of the energies of formation of the products from that of the reactants of a growth process. The thermodynamic changes accompanying the growth of *S. cerevisiae* anaerobically on glucose and aerobically on glucose, ethanol, and acetic acid can be calculated using the growth-process equations in Table 2 and the appropriate thermodynamic properties in Table 4. These are listed in Table 5 and indicate *only* the energy changes taking place with respect to the conversion of the anabolic, catabolic, and metabolic substrates to the substances produced during the various growth processes. It should be mentioned that protozoans, or animal cells in culture, are capable of the autolysis of some of their constituents in order to provide substrates with which to maintain themselves under conditions of food deprivation, and hence becoming smaller. However, this does not represent a true reversibility of the growth process.

Table 5
Thermodynamic changes *accompanying Saccharomyces cerevisiae* growth processes [a]

Growth process	Δ_pG'	Δ_pH'	Δ_pS'
	(kJ mol^{-1} substrate consumed during metabolism)		
Anaerobic glucose			
Non-conservative	−270.75	−138.57	132.18
Anabolism	−11.05	−13.71	−2.66
Glycerol formation	−23.37	−1.62	21.75
Catabolism	−175.99	−90.07	85.92
Metabolism	−210.41	−105.40	105.01
Aerobic glucose			
Non-conservative	−2905.47	−2862.17	43.30
Anabolism	−35.76	−44.31	−8.55
Catabolism	−1961.19	−1931.96	29.23
Metabolism	−1996.95	−1976.27	20.68
Ethanol			
Non-conservative	−1317.36	−1361.80	−44.44
Anabolism	28.86	1.45	−27.41
Catabolism	−856.28	−885.17	−28.89
Metabolism	−827.42	−883.72	−56.30
Acetic acid			
Non-conservative	−857.84	−889.82	−31.98
Anabolism	24.10	6.78	−17.32
Catabolism	−586.76	−608.64	−21.88
Metabolism	−562.66	−601.86	−39.20

[a] The values for $T\Delta_pS'$ were calculated using the Gibbs free energy equation and the values above for Δ_pG' and Δ_pH' for the respective processes. Slightly different values would be obtained if the Δ_pS' values were to be calculated directly, because of carrying the fractional values of Δ_pG' and Δ_pH' to only two decimal places.

4. Discussion

4.1. Electron energy content and its importance

In Table 5, the values of Δ_pG' accompanying the anabolism of glucose anaerobically and aerobically are both negative, whereas those accompanying the anabolism of ethanol and acetic acid are both positive. The reason for this is the difference between the electron energy content (EEC) of the substrates and that of the cellular substance. Practically, EEC can be expressed in terms of free energy (EEC_G), or enthalpy (EEC_H), and represents the energy change accompanying the non-conservative, *biological* oxidation of a substance divided by the number of AE transferred to O_2(aq). Because of the method of constructing the anabolic equations in Table 2, the quantity of substrate consumed in anabolism and the quantity of cells produced are AE-equivalent. If the EEC of the substrate is different from that of the cells, this will be reflected in the sign and the quantity of the energy changes accompanying anabolism. From Table 5, the $\Delta_cG'_{NC}$ of glucose is −2905.47 kJ mol^{-1} and 24 AE mol^{-1} are transferred to O_2(aq). The EEC_G for glucose is therefore −2905.47 kJ mol^{-1}/24 AE mol^{-1} = −121.06 kJ AE^{-1}. The non-conservative biological oxidation of 1 ICC-mol of cells can be represented by the following equation.

$$CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}(\text{cells}) + 1.026O_2(aq) \rightarrow 1.000CO_2(aq) + 0.547H_2O(l) + 0.158NH_3(aq) + 0.012H_2PO_4^-(aq) + 0.003HS^-(aq) + 0.022K^+(aq) + 0.003Mg^{2+}(aq) + 0.001Ca^{2+}(aq) + 0.015OH^-(aq) \quad (6)$$

Using the appropriate data from Table 4, the free energy change accompanying the biological combustion of 1 ICC-mol of yeast cells (Δ_cG_{cells}) as represented by Eq. (6), is calculated to be −475.44 kJ. From Table 1, the number of AE in this quantity of cells is 4.079. The EEC_G for cells is then

Table 6
Differences in energy changes per AE transferred to O_2(aq) during non-conservative biological oxidation

Substance	$\Delta_c G'_{NC}$ (kJ mol^{-1} AE^{-1})	$\Delta_c H'_{NC}$ (kJ mol^{-1} AE^{-1})
Glucose oxidation	−121.06	−119.26
Yeast cell oxidation [a]	−116.56	−113.72
Ethanol oxidation	−109.78	−113.48
Acetic acid oxidation	−107.23	−111.22

[a] These values were calculated using Eq. (6) and the appropriate data in Table 4.

−475.44 kJ ICC-mol^{-1}/4.079 AE mol^{-1}=−116.56 kJ AE^{-1}. This value is included in Table 6 along with similar values for glucose, ethanol, and acetic acid that were calculated using data from Table 5. The same method can be used to calculate EEC_H values.

As an example of the importance of EEC values, for aerobic anabolism on glucose in Table 2, the anabolic substrate contains 0.325 mol glucose×24 AE mol^{-1} =7.80 AE. Multiplying this by the EEC_G for glucose of −121.06 kJ AE^{-1} gives −944.27 kJ as the total energy content for the anabolic substrate. For the cells, −1.914 ICC-mol×4.079 AE ICC-mol^{-1}=7.80 AE, which when multiplied by −116.56 kJ AE^{-1} as the EEC_G for the cells, gives −909.17 kJ as the energy content of the cells. Subtracting the EEC_G value for the cells from that of the anabolic substrate gives −944.27 kJ−(−909.17 kJ)=−35.10 kJ as the value of $\Delta_p G'_{an}$ for aerobic glucose anabolism, in satisfactory agreement with the value of −35.76 kJ from Table 5. A similar calculation gives a value of −45.09 kJ as the value of $\Delta_p H'_{an}$ for aerobic glucose anabolism, which is also in satisfactory agreement with the value of −44.31 kJ from Table 5. These same procedures apply equally well to both aerobic and anaerobic anabolic processes, and demonstrate the origin of the anabolic energy changes.

4.2. Free energy changes accompanying growth processes

Table 5 shows that the free energy changes of all four growth processes are negative, as they would be expected to be for a process that occurs spontaneously. On the other hand, the anabolic free energy changes are negative for growth on glucose, and positive for growth on ethanol and acetic acid. This is because of the difference in the EEC_G values of the substrates and the cells. The positive values for $\Delta_p G'_{an}$ are made up by coupling anabolism to ATP hydrolysis, as discussed in Section 4.5.

4.3. Enthalpy changes accompanying growth processes

These are the only energy exchanges that can be practically measured, and this is done by culturing the cells in a calorimeter vessel. This has been done for the systems described here. The average agreement of the values for $\Delta_p H'_{met}$ in Table 2 for the four growth processes is 1.17% lower than those that were measured experimentally [25]. However, these latter data showed a wide variation, possibly because of the primitive calorimetric equipment used for the measurements, and further verification would be appropriate.

4.4. Entropy changes accompanying growth processes

It is apparent from Table 5 that the entropy changes accompanying anabolism are all negative, whereas those accompanying metabolism are positive for growth aerobically or anaerobically on glucose, and negative for growth on ethanol and acetic acid. This latter is due to the negative entropy change accompanying catabolism in these last two growth processes. It is also an example of the fact that within closed systems entropy changes may be positive, negative, or zero.

4.5. The inclusion of coupled ATP in growth process thermodynamics

The energy changes involved in any of the anabolic process equations represented in Table 2, as shown in Table 5, would in all probability not enable anabolism, per se, to take place. They are too small. However, when all of these anabolic processes are coupled to the expenditure of the ATP generated by catabolism, the sum of the $\Delta_p G'$ values of the coupled processes (i.e., anabolism as represented in Table 2 and the expenditure of ATP) will give a sufficiently negative free energy change to accomplish anabolism. The most accurate example of this is the substrate

level phosphorylation accompanying alcoholic fermentation, which yields a net 2 ATP molecules per mol of glucose fermented. As shown in Table 2, 0.650 mol of glucose is catabolized during anaerobic growth on glucose, giving 1.300 mol of ATP available for anabolism. The value for the free energy change accompanying the hydrolysis of ATP ($\Delta_r G'_{hyd}$) at a concentration of about 0.001 m, taken to be the value found in cells, is about -55 kJ mol^{-1} [26]. Multiplying this by 1.300 mol of ATP produced during catabolism, gives the value of -71.50 kJ for $\Delta_p G'_{hyd}$ generally available from ATP for anabolic reactions. Part of this would be utilized in the synthesis of α-glycerol phosphate, which would then become hydrolyzed to glycerol. Adding to the -71.50 kJ from ATP the values of -11.05 from anabolism and -23.37 kJ from the production of glycerol gives -105.92 kJ of free energy expended in processes other than catabolism. The corresponding $\Delta_r G'$ accompanying catabolism would then be -104.49 kJ mol^{-1} of glucose consumed (-175.99 kJ available from catabolism from Table 5 $-$ 71.50 kJ conserved in ATP). The free energy changes accompanying anaerobic catabolism, and anabolism plus glycerol formation are nearly equal, when regarded in this manner. These figures also indicate that during exponential growth, very little of the net energy in ATP becomes incorporated in the fabric of the cells. ATP acts primarily as an activating agent, the activating chemical free energy of which eventually becomes dissipated as heat [27].

4.6. General thermodynamic relationships

What it is that drives microbial (cellular) growth? Is it caused by a change in free energy, enthalpy, or entropy? Is it related to the total potential energy changes available from the non-conservative oxidation or fermentation of the substrate, to the energy changes accompanying metabolism, or to those accompanying catabolism? Some insight into providing answers to these questions is given in Fig. 1. In Fig. 1(A), the free energy and enthalpy changes accompanying the non-conservative processes are plotted against those of metabolism; in Fig. 1(B), the same NC energy changes are plotted against those of catabolism, and in Fig. 1(C), the free energy and enthalpy changes of metabolism are plotted against those of catabolism, all as linear regressions. These show a remarkably close relationship between the parameters plotted, with all correlation coefficients being effectively 1. But, the data in Fig. 1 do not indicate that any relationship in any of these graphs is the most important in driving growth processes, and hence the above questions remain unanswered.

4.7. Calculating efficiencies of microbial growth

By efficiency is meant the quantity of some parameter that becomes conserved within the substance of the cells, or cells plus other organic substances produced during a growth process, divided by the total quantity of that same parameter that is available for expenditure from the initial state or from metabolism or catabolism. Efficiencies can be expressed in terms of dry weight, carbon content, free energy, enthalpy, entropy, available electrons, etc. It all depends on the purpose for which the efficiency determinations are intended. With respect to the efficiencies of various types of energy conservation, those of entropy conservation have not been dealt with largely because the units are three orders of magnitude smaller than those of free energy or enthalpy, and small inaccuracies in the latter can lead to large relative errors in the calculation of entropy changes. Various types of efficiencies have been discussed recently in considerable detail [2]. Here, only those will be treated that deal with free-energy or enthalpy conservation. The first of these compares the quantity of energy conserved with that potentially available from the relative NC processes, as represented by the following equations,

$$\eta_G = \frac{\Delta_p G'_{NC} - \Delta_p G'_{met}}{\Delta_p G'_{NC}} \tag{7}$$

$$\eta_H = \frac{\Delta_p H'_{NC} - \Delta_p H'_{met}}{\Delta_p H'_{NC}} \tag{8}$$

where η_G and η_H, respectively, represent the efficiencies of free energy and enthalpy conservation within a growth-process system, and where the other terms have been already defined. With anaerobic growth, part of the conserved energy will also be found in the organic products of the growth process, other than the cells, and in this case the numerators of Eqs. (7) and (8) represent total conserved energy, not just that in the cells. These efficiencies are plotted in Fig. 2(A).

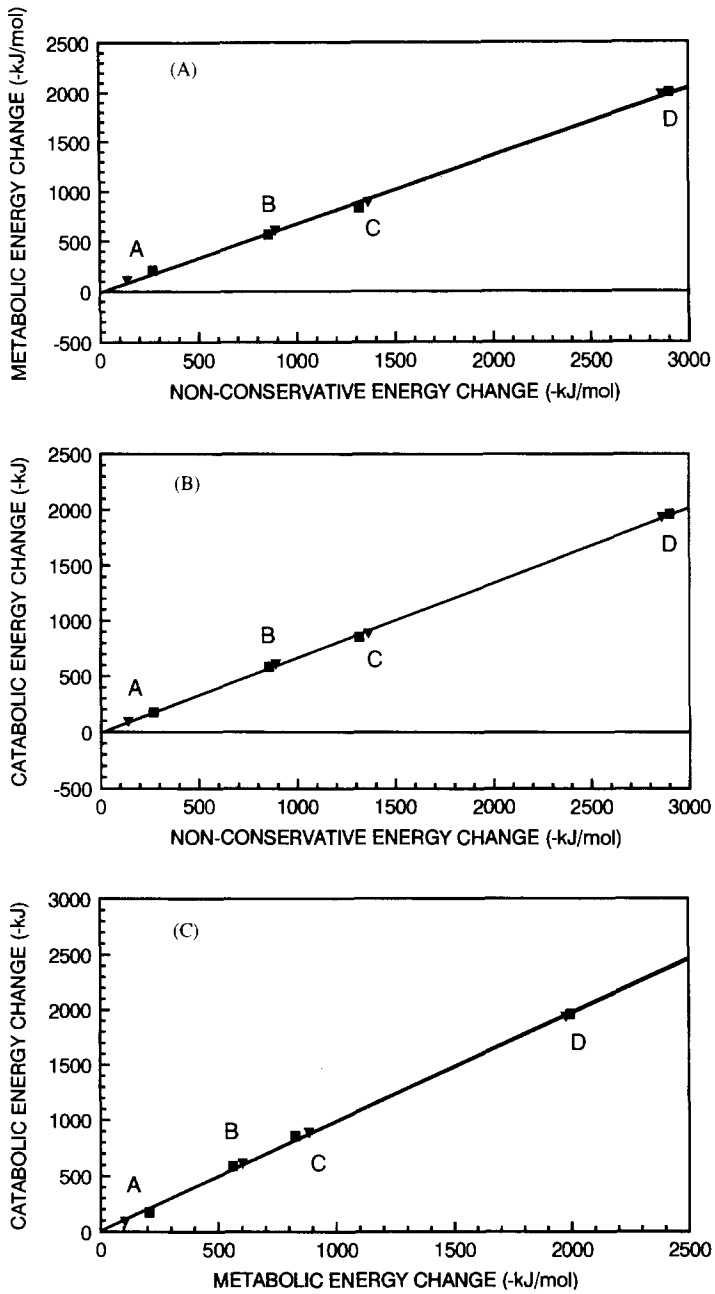

Fig. 1. For all of the graphs in Fig. 1, data relative to anaerobic growth on glucose, and aerobic growth on acetic acid, ethanol, and glucose are labeled A, B, C and D, respectively. Free energy data from Table 5 are indicated by ■, and enthalpy data by ▲. The data for the regression lines in all graphs are represented by the equation, $y=mx+b$., where x and y are the axes, m is the slope, and b is the y-intercept. In Fig. 1(A), the metabolic energy changes are plotted against those of the respective NC processes. For the free energy graph, $r=0.998$, $m=0.684$ and $b=-16.014$. For the enthalpy graph, $r=0.997$, $m=0.688$ and $b=-11.910$. In Fig. 1(B), the catabolic energy changes are plotted against those of the respective NC processes. For the free energy graph, $r=0.999$, $m=0.676$ and $b=-9.461$. For the enthalpy graph, $r=0.999$, $m=0.675$ and $b=-7.048$. In Fig. 1(C), the catabolic energy changes are plotted against the metabolic energy changes. For the free energy graph $r=0.999$, $m=0.986$ and $b=8.564$. For the enthalpy graph $r=0.999$, $m=0.980$ and $b=5.174$. Modified, with permission, from Ref. [2].

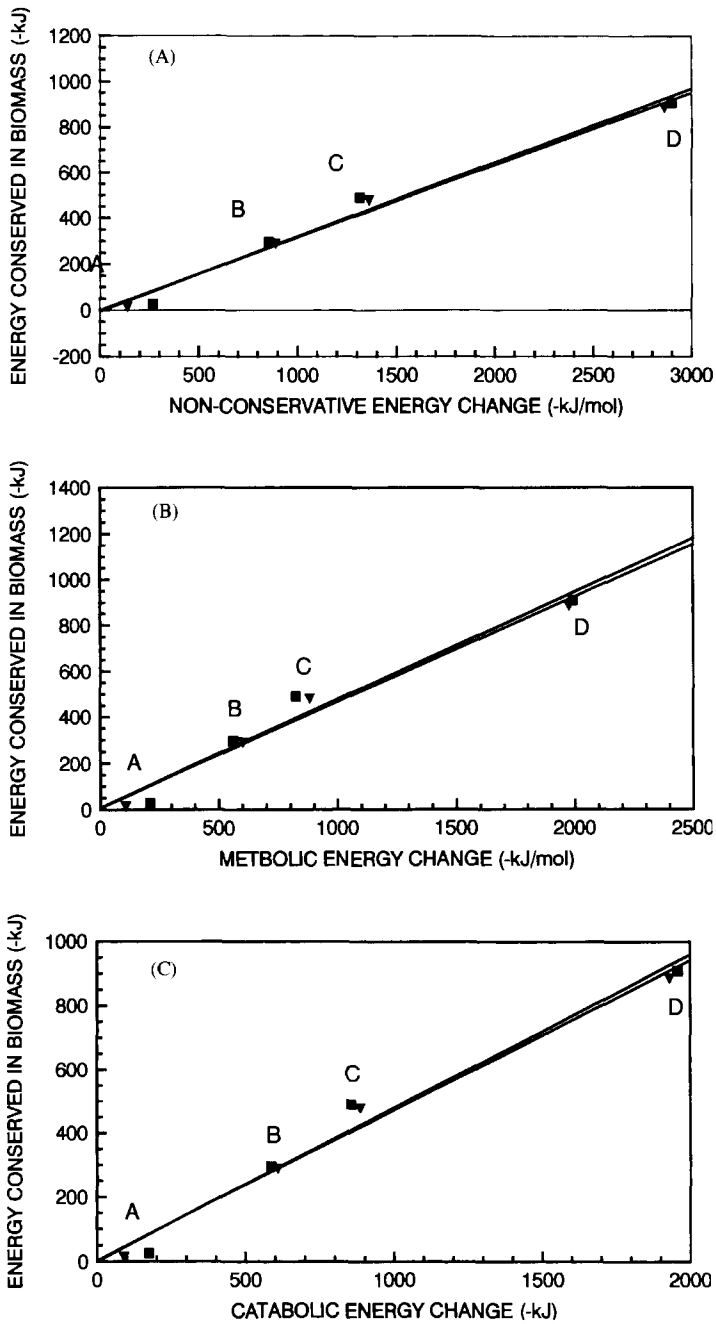

Fig. 2. For all of the graphs in Fig. 2 the data are represented as in Fig. 1. In Fig. 2(A), the energies conserved in the biomass are plotted against those of the relative NC processes. For the free energy graph r=0.898, m=0.325 and b=−5.070. For the enthalpy graph, r=0.995, m=0.318 and b=−0.883. In Fig. 2(B), the energies conserved in biomass are plotted against the respective metabolic energy changes. For the free energy graph r=0.979, m=0.470 and b=7.553. For the enthalpy graph r=0.991, m=0.459 and b=6.592. In Fig. 2(C), the energies conserved in biomass are plotted against the respective catabolic energy changes. For the free energy change r=0.987, m=0.480 and b=0.551. For the enthalpy graph r=0.993, m=0.470 and b=3.362. Modified, with permission, from Ref. [2].

Another type of efficiency considers a comparison of the quantity of energy conserved with that expended during metabolism, according to the following equations.

$$\eta_G = \frac{\Delta_p G'_{NC} - \Delta_p G'_{met}}{\Delta_p G'_{met}} \quad (9)$$

$$\eta_H = \frac{\Delta_p H'_{NC} - \Delta_p H'_{met}}{\Delta_p H'_{met}} \quad (10)$$

These efficiencies are plotted in Fig. 2(B).

A third efficiency of interest to engineers considers the energy expended during catabolism to be 'energy in', and that conserved within the system to be 'energy out' [28]. This is represented as follows

$$\eta_G = \frac{\Delta_p G'_{NC} - \Delta_p G'_{met}}{\Delta_p G'_{cat}} \quad (11)$$

$$\eta_H = \frac{\Delta_p H'_{NC} - \Delta_p H'_{met}}{\Delta_p H'_{cat}} \quad (12)$$

These efficiencies are plotted in Fig. 2(C).

It is evident upon inspection of the graphs in Fig. 2 that Fig. 2(A–C) all exhibit relationships for which the correlation coefficients are close to 1, and therefore that would be considered equally valid. Because the energy conserved in biomass is the same for the same points on all graphs, the spread of the points will increase as the values on the X-axis decrease. Thus, it cannot be claimed rigorously that the quantity of energy conserved within the substance of the biomass is best related to any one of the other parameters (i.e. the non-conservative processes, metabolism, or catabolism). It is important to note that for anaerobic growth, the values in all three graphs are relative to the energy conserved in the biomass only, and not the total energy conserved in the biomass plus the glycerol. If this latter were included, the correlation coefficients would be higher.

4.8. Available electron efficiencies

A kind of efficiency not considered in Ref. [28] is the available electron efficiency [29–33], represented by the following equation.

$$\eta_{AEcells} = \frac{MYC_{cells}AE_{cells}}{AE_{sub}} \quad (13)$$

As an example, for the aerobic growth of *S. cerevisiae* on glucose as represented in Table 2, the MYC is 1.914 and the AE values are calculated using Eq. (2). Using Eq. (13),

$$\eta_{AEcells} = \frac{MYC_{cells}AE_{cells}}{AE_{sub}} = \frac{1.914(4.079AE)}{24} = 0.325 \quad (14)$$

Fig. 3 shows that AE conservation is a function of the number of AE initially present within a growth-process system, and is independent of the nature and the

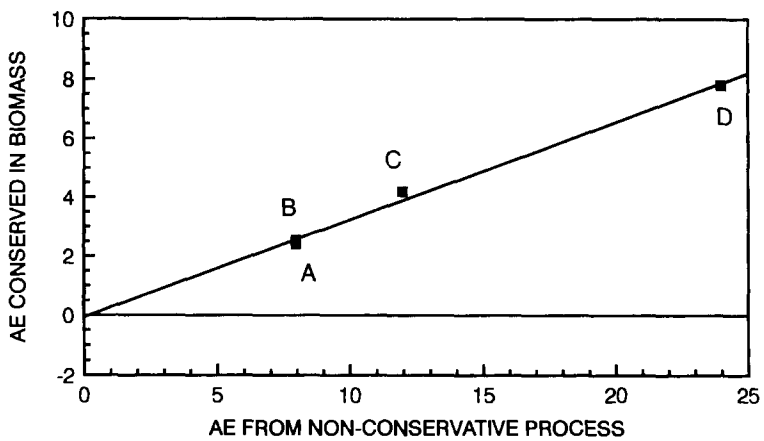

Fig. 3. A plot of the available electrons conserved in biomass against those potentially available from the respective NC processes, with the data being represented as in Fig. 1. Here, r=0.993, m=0.331 and b=−0.073. Reprinted, with permission, from Ref. [2].

overall metabolism of the electron donor. Both alcoholic fermentation and acetic acid oxidation, which are completely different metabolic processes, both transfer eight AE during the non-conservative process, and virtually the same number of AE becomes conserved within the substance of the cells during the respective growth processes. This is true even during autotrophic growth, when there is no substrate in the usual sense, and the electron donor and the carbon source are different substances. An example of this is the growth of *Pseudomonas saccharophila* on H_2(aq) as the electron donor and CO_2(aq) as the carbon source [34]. The advantage of using AE efficiencies is that they are common to both free energy and enthalpy efficiencies, and can be used to calculate these latter.

4.9. *Calculating free energy and enthalpy efficiencies and the corresponding metabolic free energy and enthalpy changes for aerobic growth processes using AE efficiencies*

Eq. (14) does not by itself permit calculations to be made of the free energies and enthalpies conserved during microbial growth processes because it does not take into account the EEC of different electron donors. The quantities Δ_cG' AE^{-1} and Δ_cH' AE^{-1} are constants for any given substance. Calculating η_G can be done using a derivation of Eq. (14) if AE_{cells} is multiplied by $\Delta_cG'_{cells}$ AE^{-1} and if AE_{sub} is multiplied by $\Delta_cG'_{sub}$ AE^{-1}. For the aerobic growth of *S. cerevisiae* on glucose as shown in Table 2,

$$\eta_{Gcells} = \frac{MYC_{cells}AE_{cells}}{AE_{sub}} \frac{(\Delta_cG'_{cells}AE^{-1}_{cells})}{(\Delta_cG'_{sub}AE^{-1}_{sub})} \tag{15}$$

This reduces to

$$\eta_{Gcells} = \frac{MYC_{cells}\Delta_cG'_{cells}}{\Delta_cG'_{sub}} \tag{16}$$

Using Eq. (6) and the appropriate data in Table 4, $\Delta_cG'_{cells}$ is calculated to be −475.44 kJ. From Table 2 MYC_{cells} is 1.914, and from Table 5$\Delta_cG'_{sub}$ ($= \Delta_pG'_{NC}$) is −2905.47 kJ mol^{-1}. Using these data,

$$\eta_{Gcells} = \frac{1.914(-475.44\,\text{kJ ICC-mol}^{-1})}{-2905.47\,\text{kJ mol}^{-1}} = 0.313 \tag{16a}$$

From Eq. (16a), η_{Gcells} represents the fraction of free energy from 1 mol of substrate that becomes conserved within the substance of the cells during a growth process. If there are no other organic products of the growth process, the fraction $(1-\eta_{Gcells})$ must be that of the free energy change accompanying metabolism. Thus,

$$\begin{aligned}\Delta_pG'_{met} &= (1-\eta_G)\Delta_pG'_{NC}\\ &= (1-0.313)(-2905.47\,\text{kJ mol}^{-1})\\ &= -1996.06\,\text{kJ mol}^{-1}\\ &\quad \text{of glucose consumed.}\end{aligned} \tag{17}$$

This value is nearly identical to that of −1996.95 kJ mol^{-1} in Table 5.

The same methods can be used to calculate enthalpy conservation efficiencies with respect to aerobic growth. Using Eq. (6) and the appropriate data in Table 4, $\Delta_cH'_{cells}$ is calculated to be −463.89 kJ ICCmol^{-1}. From Table 2 MYC_{cells} is 1.914, and from Table 5$\Delta_cH'_{sub}$ ($= \Delta_pH'_{NC}$) is −2862.17 kJ mol^{-1}. For the growth of *S. cerevisiae* aerobically on glucose as represented in Table 2,

$$\begin{aligned}\eta_{Hcells} &= \frac{MYC_{cells}\Delta_cH'_{cells}}{\Delta_cH'_{sub}}\\ &= \frac{1.914(-463.89\,\text{kJ ICC-mol}^{-1})}{-2862.17\,\text{kJ mol}^{-1}}\\ &= 0.310;\end{aligned} \tag{18}$$

and

$$\begin{aligned}\Delta_pH'_{met} &= (1-\eta_H)\Delta_pH'_{NC}\\ &= (1-0.310)(-2862.17\,\text{kJ mol}^{-1})\\ &= -1974.89\,\text{kJ mol}^{-1}\text{ of glucose consumed.}\end{aligned} \tag{19}$$

This value agrees well with that of −1976.27 kJ mol^{-1} in Table 5.

It is apparent from Eqs. (14),(16) and (18) that there are three different types of efficiency relative to the aerobic growth of *S. cerevisiae* on glucose. However, since both η_G and η_H can be derived from η_{AE} through the use of appropriate constants, η_{AE} must be considered the most basic efficiency of the three [35].

4.10. Calculating electron conservation efficiencies for anaerobic growth processes

Anaerobic growth processes take place in the absence of $O_2(aq)$, and no AE can be transferred to $O_2(aq)$ as a result of a biological oxidation, even though a biological oxidation does occur as an internal dismutation. Nevertheless, AE can still be used to indicate the *relative* energies in organic substances. The anaerobic growth of *S. cerevisiae* as represented in Table 2 can be used as an example. Here, all organic products must be considered in the calculations, including those produced during catabolism. For anaerobic growth processes the efficiencies of electron conservation are calculated just as for aerobic growth processes. The MYC data are taken from the anaerobic glucose growth-process equations in Table 2, and the AE values are calculated using Eq. (2). A separate efficiency must be calculated for each of the organic products formed during anaerobic growth, including those of catabolism. These must then be added to give the total AE conservation efficiency. Theoretically, the total electron conservation efficiency should equal 100% because no AE have been transferred to $O_2(aq)$ within the system. Thus,

$$\eta_{\mathrm{AEcells}} = \frac{\mathrm{MYC_{cells}AE_{cells}}}{\mathrm{AE_{sub}}} = \frac{0.590(4.079\,\mathrm{kJ\,ICC\text{-}mol^{-1}})}{24} = 0.100 \tag{20}$$

$$\eta_{\mathrm{AEgly}} = \frac{\mathrm{MYC_{gly}AE_{gly}}}{\mathrm{AE_{sub}}} = \frac{0.432(14.000\,\mathrm{kJ\,ICC\text{-}mol^{-1}})}{24} = 0.252 \tag{21}$$

$$\eta_{\mathrm{AEeth}} = \frac{\mathrm{MYC_{eth}AE_{eth}}}{\mathrm{AE_{sub}}} = \frac{1.300(12.000\,\mathrm{kJ\,ICC\text{-}mol^{-1}})}{24} = 0.650 \tag{22}$$

$$\text{Total } \eta_{\mathrm{AE}} = 1.002$$

The efficiency, comparable to that for the aerobic growth of *S. cerevisiae* on glucose, would be expected to comprise the electrons in the substances of those organic products of the growth process which are different from that produced in the anaerobic, catabolic process; in this case it is ethanol. Thus, the sum of the efficiencies calculated with Eqs. (20) and (21) gives 0.352, which is reasonably close to that of 0.325 calculated with Eq. (14). Because the same substrate is being used to form cells of the same composition, both aerobically and anaerobically, it might be expected that the two efficiencies would have the same value. That they do not may be because only a part of the electrons are conserved within the substance of the cells, the rest being conserved within the substance of the glycerol that is also produced. This latter is much more reduced than the cells (γ=4.666), which raises the value of the total electron conservation efficiency.

4.11. Calculating free-energy conservation efficiencies for anaerobic growth processes

Free energy and enthalpy conservation efficiencies accompanying anaerobic growth can be calculated in the same way as for aerobic growth. The value for $\Delta_c G'_{\mathrm{cells}}$ is calculated in Section 4.6. The values of $\Delta_c G'_{\mathrm{sub}}$ and $\Delta_c G'_{\mathrm{eth}} (= \Delta_p G'_{\mathrm{NC}})$ for glucose and ethanol were taken from Table 5. The value for $\Delta_c G'_{\mathrm{gly}}$ is calculated using the equation

$$C_3H_8O_3(aq) + 3.5O_2(aq) \rightarrow 3CO_2(aq) + 4H_2O(l) \tag{23}$$

and the appropriate data in Table 4 to be $-1640.76\,\mathrm{kJ\,mol^{-1}}$. With respect to anaerobic growth as represented in Table 2, for the cells produced during growth,

$$\eta_{G\mathrm{cells}} = \frac{\mathrm{MYC_{cells}}\Delta_c G'_{\mathrm{cells}}}{\Delta_c G'_{\mathrm{sub}}} = \frac{0.590(-475.44\,\mathrm{kJ\,ICC\text{-}mol^{1}})}{-2905.47\,\mathrm{kJ\,mol^{-1}}} = 0.097 \tag{24}$$

For the glycerol produced during growth,

$$\eta_{G\mathrm{gly}} = \frac{\mathrm{MYC_{gly}}\Delta_c G'_{\mathrm{gly}}}{\Delta_c G'_{\mathrm{sub}}} = \frac{0.432(-1640.76\,\mathrm{kJ\,mol^{-1}})}{-2905.47\,\mathrm{kJ\,mol^{-1}}} = 0.244 \tag{25}$$

For the ethanol produced during growth,

$$\eta_{G\text{eth}} = \frac{\text{MYC}_{\text{eth}}\Delta_c G'_{\text{eth}}}{\Delta_c G'_{\text{sub}}} = \frac{1.300(-1317.36\,\text{kJ mol}^{-1})}{-2905.47\,\text{kJ mol}^{-1}} = 0.589 \quad (26)$$

Total $\eta_G = 0.930$

The sum of the above values represents the total free-energy conservation efficiency. The fraction of the free energy that is converted into heat during metabolism must then be (1−0.930).

$$\Delta_p G'_{\text{met}} = (1 - 0.930)\Delta_c G'_{\text{NC}} = 0.070(-2905.47\,\text{kJ mol}^{-1}) = -203.38\,\text{kJ mol}^{-1}\text{ of glucose consumed.} \quad (27)$$

This value agrees satisfactorily with that for $\Delta_p G'_{\text{met}}$ of -210.41 kJ mol^{-1} for anaerobic growth on glucose in Table 2, although the agreement is not as good as with the aerobic glucose values.

4.12. Calculating enthalpy conservation efficiencies for anaerobic growth processes

The value for $\Delta_c H'_{\text{cells}}$ is calculated in Section 4.6. The values for $\Delta_c H'_{\text{sub}}$ and $\Delta_c H_{\text{eth}} (= \Delta_p H'_{\text{NC}})$ for glucose and ethanol were taken from Table 5. The value for $\Delta_c H'_{\text{gly}}$ is calculated using Eq. (23) and the appropriate values in Table 4 to be -1665.85 kJ mol^{-1}. For the cells produced during growth,

$$\eta_{H\text{cells}} = \frac{\text{MYC}_{\text{cells}}\Delta_c H'_{\text{cells}}}{\Delta_c H'_{\text{sub}}} = \frac{0.590(-463.89\,\text{kJ ICC-mol}^{-1})}{-2862.17\,\text{kJ mol}^{-1}} = 0.095 \quad (28)$$

For the glycerol produced during growth

$$\eta_{H\text{gly}} = \frac{\text{MYC}_{\text{gly}}\Delta_c H'_{\text{gly}}}{\Delta_c H'_{\text{sub}}} = \frac{0.432(-1665.85\,\text{kJ mol}^{-1})}{-2862.17\,\text{kJ mol}^{-1}} = 0.251 \quad (29)$$

For the ethanol produced during growth

$$\eta_{H\text{eth}} = \frac{\text{MYC}_{\text{eth}}\Delta_c H'_{\text{eth}}}{\Delta_c H'_{\text{sub}}} = \frac{1.300(-1361.80\,\text{kJ mol}^{-1})}{-2862.17\,\text{kJ mol}^{-1}} = 0.618 \quad (30)$$

Total $\eta_H = 0.964$

The sum of the above mentioned efficiency values represents the total enthalpy conservation efficiency. The fraction of the enthalpy that is lost during metabolism must then be (1−0.964), from which

$$\Delta_p H'_{\text{met}} = (1 - 0.964)\Delta_c H'_{\text{NC}} = 0.036(-2862.17\,\text{kJ mol}^{-1}) = -103.04\,\text{kJ mol}^{-1}\text{ of glucose consumed.} \quad (31)$$

This value agrees satisfactorily with that of -105.40 kJ mol^{-1} from Table 2.

From the above discussion, as well as from Ref. [35], it is apparent that η_{AEcells} is a more basic form of efficiency measurement, from which $\eta_{G\text{cells}}$ and $\eta_{G\text{cells}}$ can be calculated, and from these, the values for $\Delta_p G'_{\text{met}}$ and $\Delta_p H'_{\text{met}}$. This is not surprising, considering the statement of Minkevich and Eroshin [36] in 1975, that "...the equivalent of available electrons is the measure of substance quantity which gives the best reflection of its chemical store." (see also Ref. [37]).

4.13. The Gibbs free-energy equation and microbial growth

The data in Table 5 indicate that the entropy changes accompanying a growth process (metabolism) can be either positive or negative. Metabolism is the sum of all the processes occurring during a given growth process, and a reasonable interpretation of these positive or negative entropy changes is that a growth-process system, by itself, is not driven by a change in the physical entropy of the system. The passage from its initial to its final state occurs *only* because the free energy change of the system is negative, whatever this implies. The interpretation can also be made that the entropy change is a purely passive phenomenon that occurs because the individual entropies of the products of a growth process are

different from those of the reactants. As products are formed, entropy is acquired by the absorption of thermal energy at a constant temperature. As reactants are consumed, entropy becomes dissipated at constant temperature in the form of thermal energy. The difference between these two phenomena is an overall positive or negative entropy change of the system that results in a thermal change. The change in entropy of a growth-process system is related to the changes in the interatomic or intermolecular vibrations, internal rotations, external rotations, and translations of the component substances as these latter change in the passage from the initial to the final state, all being a function of the temperature. The change in Gibbs free energy of a growth-process system is related to the movement of electrons from the electron donor to the electron acceptor in a typical, chemical OR process. This spontaneous process results in the conversion of non-thermal chemical energy into thermal energy, as represented by a minus sign, the magnitude of which depends on the nature of the system. The minus sign of the free energy change accompanying spontaneous processes represents the loss of free energy from the system. However, according to the 1st Law, energy cannot be 'lost'. What happens is that the loss of non-thermal, chemical free energy becomes a gain in thermal energy released into the system. The sum of two process of entropy change and free energy change, both of which result in a thermal exchange within the system, is the total heat of reaction (process), represented by Δ_pH'. This can be visualized better in Table 7. Eq. (1) in Table 7 represents a process in which at the constant temperature of the system all components are in a state of thermal equilibrium. Therefore, there is no accompanying change in free energy, and only a change in entropy. What appears to drive the growth process is represented by Eq. (2) in Table 7. This involves the spontaneous movement of electrons from the electron donor to the electron acceptor(s) and is represented thermodynamically by the change in non-thermal, chemical, free energy to an equivalent quantity of thermal energy. This process changes the physical nature of the final state from that of the initial state, resulting, incidentally, in the change in entropy.

5. Conclusions

It is possible to calculate the values for Δ_pG', Δ_pH', $T\Delta_pS'$ and Δ_pS', with respect to anabolism, catabolism and metabolism for both anaerobic and aerobic growth processes, using methods involving both direct and indirect calorimetry.

The conservation of AE within the substance of the cells is a better measure of the efficiency of growth than is the conservation of cellular free energy or enthalpy, to both of which it is in common.

It is not changes in entropy that drive a growth process (metabolism).

6. List of symbols

$'$	superscript indicating a biological process taking place in an aqueous environment under non-standard conditions at 298.15 K and 1 atm (1 atm=101.325 kPa)
AE	available electrons transferred to O_2(aq) during the biological oxidation of an organic substance
AE_{NC}	available electrons transferred to O_2(aq) during a non-conservative process
AE_{cells}	available electrons contained in 1 Cmol of cells

Table 7
Relationships of the terms of the Gibbs free-energy equation as applied to microbial growth processes

	Initial state→Final state	Thermodynamic result
1.	$\sum\Delta_fS_{reactants}\rightarrow\sum\Delta_fS_{products}$	ΔS or $T\Delta S$ at T=thermal energy
2.	$\sum\Delta_fG_{reactants}\rightarrow\sum\Delta_fG_{products}$	ΔG=non-thermal chemical energy converted to thermal energy
Sum	$\sum\Delta_fH_{reactants}\rightarrow\sum\Delta_fH_{products}$	ΔH=thermal energy=$\Delta G+T\Delta S$

AE_{prod} available electrons contained in 1 mol of organic product

AE_{sub} available electrons contained in 1 mol of substrate

EEC_G electron energy content with respect to free energy

EEC_H electron energy content with respect to enthalpy

η_{AE} efficiency of available electron conservation in a growth process

η_G efficiency of free-energy conservation in a growth process

η_H efficiency of enthalpy conservation in a growth process

eq electron equivalents transferred to $O_2(g)$ during a bomb calorimetric oxidation

γ the number of AE transferred to $O_2(aq)$ per atom of carbon during the biological oxidation of an organic substance to $CO_2(aq)$, $H_2O(l)$, $NH_3(aq)$, and $HS^-(aq)$. This is referred to as the degree of reduction

Δ_fG^0, Δ_fH^0, Δ_fS^0 free energy, enthalpy, or entropy of formation, respectively, of a specified quantity of a pure substance in its standard state at 298.15 K and 1 atm

$\Delta_fG^{0\prime}$, $\Delta_fH^{0\prime}$, $\Delta_fS^{0\prime}$ free energy, enthalpy, or entropy of formation, respectively, of a specified quantity of a pure substance in the aqueous standard state at 298.15 K and 1 atm

Δ_fG, Δ_fH, Δ_fS free energy, enthalpy, or entropy of formation, respectively, of a specified quantity of an impure, condensed substance not having a standard state at 298.15 K and 1 atm. An example of this would be dried biomass of any kind

Δ_fG', Δ_fH', Δ_fS' free energy, enthalpy, or entropy of formation, respectively, of a specified quantity of an impure substance in aqueous solution or suspension, not having a standard state at 298.15 K and 1 atm. An example of this would be living cells, or dissolved substances at a concentration of other than 1 m (such as 0.001 m)

Δ_fG_{cells}, Δ_fH_{cells}, Δ_fS_{cells} free energy, enthalpy, or entropy of formation, respectively, of 1 ICC-mol of cells

Δ_cG^0, Δ_cH^0, Δ_cS^0 free energy, enthalpy, or entropy change, respectively, accompanying the bomb calorimetric oxidation of a pure substance at 298.15 K and 1 atm

Δ_cG, Δ_cH, Δ_cS free energy, enthalpy, or entropy change, respectively, accompanying the bomb calorimetric oxidation of an impure substance at 298.15 K and 1 atm

Δ_cG', Δ_cH', Δ_cS' free energy, enthalpy, or entropy change, respectively, accompanying biological oxidation at 298.15 K and 1 atm

$\Delta_pG^{0\prime}_{NC}$, $\Delta_pH^{0\prime}_{NC}$, $\Delta_pS^{0\prime}_{NC}$ free energy, enthalpy, or entropy change, respectively, accompanying a non-conservative process

$\Delta_pG'_{an}$, $\Delta_pH'_{an}$, $\Delta_pS'_{an}$ free energy, enthalpy, or entropy change, respectively, accompanying anabolism

$\Delta_pG'_{cat}$, $\Delta_pH'_{cat}$, $\Delta_pS'_{cat}$ free energy, enthalpy, or entropy change, respectively, accompanying catabolism

$\Delta_pG'_{met}$, $\Delta_pH'_{met}$, $\Delta_pS'_{met}$ free energy, enthalpy, or entropy change, respectively, accompanying metabolism

$\Delta_rG'_{hyd}$ free energy change accompanying hydrolysis

ICUCF ion-containing unit carbon formula

ICUCFW ion-containing unit carbon formula weight (equivalent to 1 ICC-mol)

ICC-mol	ion-containing carbon mol
MYC_{cells}	molar yield coefficient for the cells
MYC_{prod}	molar yield coefficient for an organic product
O–R	oxidation–reduction
r	coefficient of correlation
μ_{max}	maximum specific rate of growth

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