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Source: *The Quarterly Review of Biology*, Vol. 88, No. 2 (June 2013), pp. 69-96
Published by: The University of Chicago Press
Stable URL: http://www.jstor.org/stable/10.1086/670529

Volume 88, No. 2 June 2013

The Quarterly Review of Biology

A THEORETICAL STUDY OF THE THERMODYNAMICS OF MICROBIAL GROWTH USING *SACCHAROMYCES CEREVISIAE* AND A DIFFERENT FREE ENERGY EQUATION

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keywords
thermodynamics of microbial growth, free energy, enthalpy, entropy, absorbed thermal energy, heat

abstract

Microbial growth is a biological process that has been previously treated as a chemical reaction operating in accord with the Gibbs free energy equation, $\Delta G=\Delta H-T\Delta S$. *The heat of yeast growth was the first to be measured, in 1856, by direct calorimetry of a large wine vat. Until then there was a tendency for biologists to continue with the old notion that the energy change accompanying the growth of microorganisms was reflected in the amount of heat that was produced during this process. The application of chemical thermodynamics to systems involving microbial growth did not occur until much later. The full application of the Gibbs equation to microbial growth did not take place until the experimental measurement of yeast cell entropy was made in 1997. Further investigations then showed that the quantity of thermal energy for solid substances represented by* TS *was twice that of the quantity of thermal energy represented by* Q_{ab} *that is experimentally necessary to raise* T *of a substance from* 0/K *to* T/K. *Since there can only be one value for this, the use of the equation* $\Delta X=\Delta H-\Delta Q_{ab}$ *was investigated with respect to microbial growth, and is described in this review.*

Introduction

THE STUDY of the thermodynamics of microbial growth has never really acquired much general interest. This is surprising. Many pure cultures of microorganisms are easy to work with. They have short life cycles, and if treated appropriately can provide good material for reproducible experi-

The Quarterly Review of Biology, June 2013, Vol. 88, No. 2

0033-5770/2013/8802-0001$15.00

ments. Much been studied and learned about them, but not about their thermodynamics. Dubrunfaut (1856) was the first to measure the heat produced by microorganisms, using an indigenous culture of yeast growing in a large wine vat during a fermentation. The concept of free energy was developed by Gibbs (1873). It was systematically applied to chemical reaction systems by Lewis (1913) and by Lewis and Gibson (1917). However, it was a whole new advance for biologists of that time to adopt the teachings of chemists, and to accept the idea that it was not the heat of a reaction but its free energy change at constant temperature and pressure that was the true indication of the energy change of a reaction. It was not until the classic volume published by Lewis and Randall (1923) that mostly biochemists studying enzyme reactions began to take advantage of the principles of chemical thermodynamics. The first person to have investigated free energy changes accompanying microbial activity was Linhart (1920), who studied the fixation of nitrogen gas by *Azotobacter*. Prior to this time, most biologists had little or no knowledge of thermodynamics. It should have been possible for them to approach physical chemists with their problems related to biological energetics. Unfortunately, when this happened, chemists often answered that, "[b]iological processes are so highly complex that they are not susceptible to thermodynamic treatment" (Linhart 1920:248). The same is largely true today with respect to cellular thermodynamics. Cells are not pure, crystalline substances and therefore not susceptible to rigorous, physical description. The free energy change accompanying cellular growth cannot be directly measured, but it can be calculated using the Gibbs equation if the heat of growth can be measured and the entropy change accompanying growth can be calculated, both using experimental techniques. An understanding of what entropy is, or means, has proven elusive since it was described by Clausius (1865). On the other hand, everything has an entropy. The first measurement of cellular entropy was made by Battley et al. (1997) using dried yeast cells. With growth-process equations it became possible, using experimentally determined measurements rather than theories, to calculate the free energy changes accompanying the growth of *Saccharomyces cerevisiae* (*S. cerevisiae*) anaerobically on glucose, and aerobically on glucose, ethanol, and acetic acid and to combine these with heat and entropy changes. This review describes the work that has been done with these systems up to the present time.

Growth-Process Systems

A growth-process system is a combination of two subsystems. One is a biochemical subsystem comprising the biochemical and physical aspects of microbial growth, called anabolism. The other comprises the chemical, energy exchanging aspects of microbial growth, called catabolism. Microbial growth comprises an initial and a final state of the whole system, including the energy exchanges accompanying the progression of one to the other. Although catabolism can be demonstrated experimentally to occur independently of anabolism, the latter cannot exist without the former, and the separate existence of anabolism is a theoretical construct. It is important to note that the phenomenon of microbial growth is not a reversible process. Cells cannot "ungrow" themselves, although some cells undergo autolysis and death when their internal and external substrates have become exhausted.

BEGINNINGS

The studies described here began with the observation of what seemed to be a discrepancy between the amounts of growth obtained aerobically or anaerobically when *S. cerevisiae* was grown on glucose in a defined medium (Battley 1960a). The equation representing the total calorimetric oxidation of glucose at 298.15 K and 0.1MPa is

$$C_6H_{12}O_6(aq) + 6\,O_2(aq) \rightarrow 6\,CO_2(aq) + 6\,H_2O(aq)\ \Delta G^{o'} = -2824\ \text{kJ mol}^{-1} \quad (1)$$

and that for total alcoholic fermentation is

$$C_6H_{12}O_6(aq) \rightarrow 2\ CO_2(aq) + 2\ C_2H_6O(aq)\ \Delta G^{o'} = -220\ kJ\ mol^{-1} \quad (2)$$

where the postscript (aq) and the "pip" indicate that the substances involved are in their aqueous standard states, and where $\Delta G^{o'}$ represents the standard Gibbs energy change taking place in aqueous solution. Equations (1) and (2) represent "nonconservative" processes, during which the maximum quantity of chemical, potential energy within the system becomes converted into heat, and nothing becomes "conserved" in cells or other products of a growth process. Assuming it is the change in the Gibbs energy that "drives" both reactions, it might be expected that per mol of glucose utilized, the amount of growth would be proportional to the amount of nonthermal energy initially available, and that there would be -2824 kJ/-220 kJ = 12.8 times more growth aerobically than anaerobically. However, when the growth for these two systems was measured turbidometrically, it was found that there was only 3.4 times more growth aerobically than anaerobically (Battley 1960a). Attempting to understand this fact has taken many years.

The Biochemistry of Microbial Growth Processes

initial and final states of growth-process systems

One of the problems with experimentation involving microbial growth is that, for duplication and consideration by other investigators, the conditions of experimentation have to be exactly repeated. For decades, studies were conducted using culture media made of extracts of natural products such as yeast ash, yeast juice, grape juice, beer wort, and meat extract, often supplemented with sugar and with little consistency of preparation (Battley 1987:71–95; 96–122). In contrast, to get good results it must always be asked what the microorganisms require, not what is convenient for the experimenter. It was not until single sources of carbon and energy and defined culture media came into use that this situation began to improve (Tamiya 1935; Hoover and Allison 1940; Battley 1960a). Even so, for some unexplainable reason, growth experiments with microorganisms are still not exact and we are lucky to be accurate within 1 or 2 percent.

To study the thermodynamics of microbial growth it is first necessary to establish a defined "system," separated from its environment and having an initial and a final state, in the transition between which there is a transfer of material accompanied by a transfer of energy. Isolated microbial growth processes are irreversible and do not have a state of equilibrium. The system most closely related to what takes place biologically is a "closed" system within which growth occurs, with respect to which there is no transfer of material from the system, but there can be a transfer of energy between the system and its environment. The most convenient system to use and one offering a free-living, natural condition is represented by a closed vessel containing a nutrient solution wherein the process of growth can take place. The word "process" is used because growth is far more complex than any simple reaction. This "growth process" can be considered a "system" represented by an equation comprising an initial state (left side) that proceeds to a final state (right side), called a "growth-process equation." If the concentrations of all reactants and products as represented in a growth-process equation are low and in an aqueous solution or suspension, the volume changes are low, and there are no significant thermodynamic problems with pressure/volume (PV) energy changes. The generation time for most microorganisms under optimum conditions is usually minutes to hours during which, because of the heat capacity of the aqueous medium in which they grow, the temperature remains nearly constant, as does the gas pressure above the medium. Experimentally, the temperature is artificially kept constant and the pressure is atmospheric.

To obtain growth, the culture medium must be inoculated with a small quantity of cells which, together with the utilized components of the medium, comprise the initial state. Battley (1960a) defined this latter as

one in which all of the potentialities for growth are present with respect to materials and energy. This was provided in the form of a solution containing a limiting quantity of a single substrate as a source of carbon and energy, and nonlimiting but not large quantities of all the minerals, trace elements, vitamins, and cofactors necessary for the growth of a chosen microorganism, here *S. cerevisiae*. Except for the substrate, all of these nutrients are not completely used by the cells, and simply remain in solution within the system after growth has ceased. He further described the initial state as the nutrient medium plus a small quantity of living cells, the initial mass of which can be ignored in calculating mass balances. A loopful or a known, small quantity of culture medium containing exponentially growing cells was commonly used as an inoculum. The total quantity of substances in solution in the culture medium must be such that there is no osmotic inhibition of cellular growth. The amount of substrate used in the initial state is an arbitrary decision. There is good reason to keep its concentration as small as convenient, in order to shorten the duration of an experiment. This confers the additional advantage that the values of its thermodynamic properties in a defined, aqueous state can be very closely approximated. Other than the sources of carbon, hydrogen, nitrogen, phosphorous, sulfur, and potassium, the minerals and trace elements that also form a part of the initial state of the nutrient medium, while necessary for cellular growth, usually do not enter significantly into the material exchange of a growth process.

The final state was defined as one in which the potentiality for growth no longer exists because of the complete utilization of the limited quantity of substrate. All of the products of the growth process are included in the final state and comprise the cells and other organic or inorganic products. The initial nutrient solution has to be balanced so that no precipitates occur during sterilization. Otherwise, the offending substances have to be sterilized separately and then added to the nutrient solution when cold using precautions to ensure sterility. This is because any precipitates will increase the apparent weight and yield of the cell mass when this is centrifuged from suspension.

The system under consideration here comprises the substances and quantities of both the initial and final states of a growth-process equation, and the environment is the aqueous fluid in which the system is suspended.

THE NUTRIENT MEDIUM USED FOR GROWTH STUDIES

The nutrient culture medium was comprised largely of the substrate plus the phosphate salts that buffered the pH of the medium and provided a source of K, N, and P. The remainder of the defined medium for growing *S. cerevisiae* cells consisted in small excess of minerals, trace elements, and vitamins required for growth both aerobically and anaerobically (Battley 1960a). This yeast is a eukaryote that requires a steroid for strict anaerobic growth (Andraesen and Stier 1953), although it was not found necessary also to include oleic acid for anaerobic growth, as claimed by Andraesen and Stier (1954). A trace of $O_2(g)$ in the system will replace the steroid (White and Munns 1951; Andraesen and Stier 1953). The steroid requirement applies only to anaerobic growth, as is evident from the studies of Maguigan and Walker (1940), indicating that steroid synthesis in yeast takes place only under aerobic conditions. However, in Battley's (1960a) studies, the steroid was also included for aerobic growth. Sterility should be considered for all nutrient media.

PHYSICAL CONDITIONS FOR GROWTH

pH

Ideally, the physical environment within the growth-process system ought not to change during the course of an experiment. With nonionic substances this is not a problem, but pH changes can occur when culture media are used comprising the salts of organic acids or bases. For Battley's (1960a) experiments, no pH-stat was available and a pH buffer had to be used. This comprised a mixture of 0.20% $(NH_4)_2HPO_4$ and 0.20% KH_2PO_4, which also provided sources of N, P, and K, and which, when adjusted, buffered the nutrient medium at pH 6.5. In

addition, the pH had to be maximally appropriate for growth. It should not change during growth such that the cells would be inhibited or a precipitate formed.

Anaerobic and Aerobic Growth

Anaerobiosis was usually accomplished by flushing a culture system with pure $N_2(g)$. By itself, this procedure can remove even traces of $O_2(g)$, but can also have a deleterious effect in that if done rigorously, it also removes carbon dioxide gas from the nutrient medium, without which *S. cerevisiae* will not grow. To avoid this, the inoculum should be comprised of exponentially growing cells in sufficient quantity to generate $CO_2(g)$ after flushing. This quantity does not have to be large, and depends on the volume of the culture. For aerobic growth, the O_2 in the air was sufficient for the experiments described here.

Only the Quantity of Substrate Should Limit Growth

Constructing an appropriate nutrient medium for experimentation requires that it can be shown to be nonlimiting except for the quantity of the substrate. This was accomplished by growing the cells with increasing concentrations of substrate from zero, and by plotting the cell density after growth had ceased against each concentration of substrate. If it is only the substrate that is limiting, a straight line should be observed passing through the origin, and the concentrations used in the experimentation should be within that range (Battley 1960a).

Temperature and Pressure

To get maximum growth, the temperature should be that at which the microorganism grows at or close to its maximum specific growth rate, μ_{max}, which can be kept constant by growing the cultures in a thermostat. Atmospheric pressure is a natural condition, and is acceptable. For aerobic experiments, this can usually be accomplished by aseptically opening the system to the atmosphere.

Absence of Storage Products in the Cells

It was pointed out originally by Duclaux (1900:378) and more recently emphasized by Battley (1987:254) that microbial storage products are not really a part of the fabric of the cells, but are internal substrates that become used when an external substrate becomes exhausted. Cells must be free of storage products to obtain a correct yield of cellular mass and an accurate elemental analysis of cellular structure. This can happen when cells are grown at μ_{max} in batch culture, as shown by the complete cessation of heat production when the substrate disappears (Battley 1960b; Forest et al. 1961; Dermoun and Belaich 1979), as well as a cessation of gas exchange (Battley 1960a). The same applies to other microorganisms (Battley 1987:325; 339). Seemingly, *S. cerevisiae* cells growing at μ_{max} do not form storage products. This may not be true for all kinds of cells, in which case the total quantity of storage products in the cells must be determined separately and subtracted from the total mass of cells that have grown. Storage products in microorganisms appear to be formed when the rate of primary assimilation is greater than the rate of growth, and also occur when there is some limitation on the growth process.

Growth-Process Equations

initial studies

The information in the sections on growth-process systems and the biochemistry of microbial growth processes describe a number of important conditions that should be met to obtain satisfactory results in constructing growth-process equations representing microbial growth. These are necessary to determine both the mass and the energy balance of a growth-process system. However, the mass balance has to be constructed in an equation form before the energy balance can be calculated. In Battley's (1960a) experiments, a decision had to be made as to which substrates should be used for the growth of *S. cerevisiae*. One obvious choice was glucose, which could be metabolized both anaerobically and aerobically. Two other choices were aerobic growth on ethanol, which is more reduced than the cellular material, and acetic acid, which is less reduced. These four substrates cover most

circumstances of microbial growth as it occurs in nature on single substrates and is the only set of studies of its kind. In Battley's (1960a) studies, the cells were inoculated in small amounts into defined growth-process systems containing one of these substrates, so that the initial cellular mass was negligible. In some experiments, cells were grown in Warburg vessels in order to measure CO_2(g) and O_2(g) exchange manometrically. Dry cell yields were determined gravimetrically in separate experiments and used to calculate the quantity of substrate used to form the cells on a molar basis. Dried cells grown from each substrate were subjected to analyses for C, H, O, N, and water-soluble, inorganic residue (ash), from which an empirical formula representing the cells could be constructed. Following cessation of growth, the supernatant liquid was analyzed for residual carbon-containing substances. For anaerobic growth on glucose, organic carbon other than that in the cells was found to be present in the form of ethanol, glycerol, and α-glycerophosphoric acid, with only trace quantities of carbon-containing substances other than the cells remaining after anaerobic growth. Because it had been shown (Meyerhof and Green 1949; Sevag et al. 1954) that α-glycerophosphatase is present on the surface of *S. cerevisiae* cells, these two substances were combined into an equivalent quantity of glycerol for convenience in writing an equation. The presence of glycerol plus α-glycerophosphoric acid in the quantity found is highly unusual, but may have been due to using a large quantity of phosphate salts as a buffer. On the other hand, the same medium was used for all four systems, and in this respect all media were identical. With the above information, equations could be constructed in which the quantity of ammonia nitrogen used in cell synthesis could be determined for the initial state. The nitrogen in the cells in the final state could only have come from the ammonia in solution in the initial state. The equation representing anaerobic growth on glucose could then be written where the last term represents the dry mass of cells that have grown and the postscripts represent the aqueous state:

$$\begin{aligned} &C_6H_{12}O_6(aq) \\ &+ 0.12\ NH_3(aq) \rightarrow 1.54\ CO_2(aq) \\ &+ 1.30\ CH_3CH_2OH(aq) \\ &+ 0.43\ C_3H_8O_3(aq) \\ &+ 0.59\ (CH_{1.75}O_{0.45}N_{0.20})(\text{cells}). \end{aligned} \quad (3)$$

Cells have no standard state and were simply represented as "cells." Other equations were written to represent the aerobic growth of *S. cerevisiae* aerobically on glucose, ethanol, and acetic acid. These are similar to Equation (3) except that there are no organic products of growth other than the cells (Battley 1960a).

When constructing equations such as Equation (3), some choices had to be made with respect to the chemical nature of each substance represented by the terms in the reaction. It seemed reasonable to use a representation of the latter as of the instant they entered or left the cell membrane. Thermodynamic changes associated with the passage of CO_2(g) from the aqueous phase or O_2(g) into the aqueous phase within the culture vessel are not directly related to the form in which they enter the cell. There is no problem with nonionic substances. Ionic substances can take more than one form. For example, nitrogen can be written as NH_3(aq) or as NH_4^{+1} (aq), and CO_2(aq) can be written as H_2CO_3(aq), HCO_3^{-1} (aq), or CO_3^{-2} (aq). Not knowing which form actually enters the cell and becomes incorporated into the structure, as opposed to the principal form in the nutrient medium at a given pH, the practice was adopted to represent all substances as being uncharged. The great advantage of this assumption is that no corrections have to be made regarding related thermal effects taking place in the nutrient medium. These latter can be changes accompanying equilibrium reactions in the nutrient solution, and movement of gases into and out of the aqueous suspending fluid.

UNIT-CARBON FORMULAS

In the early literature it was common to represent the product of primary assimila-

tion, or cells in general, with the formula CH_2O as a first approximation (Battley 1987: 152–179). The first to depart seriously from this was Tamiya and his group who studied the growth of *Aspergillus niger* (*A. niger*) in a remarkable series of papers summarized by Battley (1987:180–218). The initial formula they used was derived from one published by Mazé (1902) plus the ash composition published by Takata (1929). Because the weight of ash was thought by Tamiya to be negligible compared to the weight of the organic components, this was eliminated to give a tentative formula of $C_{86}H_{160}O_{45}N_7$. More accurate formulas were obtained by Yamagata (1934) from *Aspergillus oryzae* grown on four different substrates with ammonia or nitrate as N sources. Each of the eight formulas was slightly different, although the formula weights were nearly identical. The formula $C_{409}H_{717}O_{233}N_{46}$ represented *A. niger* cells grown on glucose and ammonia (Battley 1987:191). Hoover and Allison (1940), working independently, described an analogous method of representing *Rhizobium meliloti* as $C_{19}H_{32}N_2O_9$. Battley (1960a) obtained empirical formulas for the growth of *S. cerevisiae* anaerobically on glucose and aerobically on glucose, ethanol, and acetic acid. As with Yamagata's observations, formulas for cells grown on different substrates were all slightly different, the one for glucose and ammonia being $C_{4.20}H_{7.36}O_{1.90}N_{0.84}$. To facilitate the writing of growth-process equations, Battley divided the subscripts of all atoms in the empirical formula by the subscript for carbon to obtain what he called a unit-carbon formula (UCF), the one for growth on glucose and ammonia becoming $CH_{1.75}O_{0.45}N_{0.20}$. Each UCF has a "unit-carbon formula weight" (UCFW). The use of unit-carbon formulas has been generally adopted since that time (for example, see von Stockar and Marison 1993). The acronyms UCF and UCFW have been generally accepted, but little used by anyone except Battley. Apparently beginning with a paper by Roels (1983:23), the acronym UCF has been nearly universally replaced by the term "C-mol," and the acronym UCFW by "C-molecular weight." The use of UCFs (or C-mols) greatly facilitates the writing of growth-process equations.

In the evolution of constructing unit-carbon formulas as represented in Equation (3), it became apparent that the formulas for all cells grown using different substrates and nitrogen sources were slightly different (Tamiya 1935; Hoover and Allison 1940; Battley 1960a). However, this may have been due to experimental error, and not because of an actual difference. Provided that no storage products were formed (an experimental necessity), the empirical composition of the same cells metabolizing any given substrate would be expected to be the same. For this reason, one average UCF became used for the four conditions of growth. It was then investigated whether the inclusion of other elements would result in more accurate experimental answers. These additions were initially P, S, K, Ca, and Mg (Battley 1998), with values for these elements being taken from the literature and resulting in a UCF of $CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}$ (cells), the last three elements being ions. However, when calculations were made using a UCF containing or not containing these ions, there appeared to be less than an average 2% difference in $\Delta G^{o'}$ values for the growth of this yeast (Battley 1999a:250). Ions are not structural elements and do not significantly contribute to the covalent mass of the cells. It was finally decided to remove K, Ca, and Mg, leaving P and S in place, to give $CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}$ (cells), good to about ± 1–2% accuracy (Battley 2007, 2011a,b). This latter type of formula was previously recommended by Duboc et al. (1995). However, to get the covalent mass of the cells these must be ashed. Most of the ash is comprised of P_2O_5 and K_2O. The quantity of P in the ash can be determined and converted to an equivalent quantity of P_2O_5, which can be subtracted from the weight of the ash, leaving the weight of K_2O. From this, the weight of K can be obtained that should then be subtracted from the equivalent dry weight of cells to leave a calculated mass comprised of C, H, O, N, P, and S, which is close to the true weight of covalently bound atoms in the cells. This means that, although no K is included in the UCFs, its quantity must be known.

THE CONSTRUCTION OF GROWTH-PROCESS EQUATIONS

With the above considerations in place, it becomes possible to construct growth-process equations. This is covered in greater detail in Battley (1999a:219–266).

Oxidation and Reduction (OR)

The process of microbial growth is a very complex oxidation/reduction (OR) reaction. To construct an equation representing any OR reaction, the nature and composition of all reactants and products must be known. This is not usually difficult here since we are dealing with only six elements. In a defined medium, the C source is a single organic substance or CO_2(aq); the H sources can be organic substances or water; the O source is water, O_2(aq), or combined oxygen; the N source is ammonia, nitrate, or organic nitrogen; the P source is a phosphate; and the S source is hydrogen sulfide or sulfate. These substances plus a very small cellular inoculum comprise the initial state since trace elements, vitamins, or other minerals comprise too small a mass to be considered a part of the cells, although they have to be present in the culture medium. For aerobic, growth-process equations, the final state comprises the cells, CO_2(aq) and H_2O(l), at least for the simplest systems. For anaerobic growth processes, the final state usually includes organic fermentation products in addition to cells. The main problem here is to be able to calculate the quantities of the reactants and products.

Available Electrons

The original methods of directly measuring manometrically the quantities of CO_2 produced and O_2 consumed during growth using Warburg manometry are difficult and tedious (Battley 1960a). These methods have since been completely replaced by the use of available electrons (AE). The concept of the "available electron" came into use through the studies of Mayberry et al. (1967). These were directed toward finding significant parameters that could be used to relate the quantity of microorganisms produced during growth to the quantity of a single, organic substance that was used as a source of carbon and energy. An available electron (AE) is one that is available either for transfer to oxygen in respiration, for an internal or external dismutation in fermentation, or for activities involved in synthesis.

Using Available Electrons

Biologically important substances such as cells contain C, H, N, O, P, and S, and the products of their *biological* oxidation are CO_2(aq), H_2O(l), NH_3(aq), H_3PO_4 (aq), and H_2S(aq). The number of AE present in *S. cerevisiae* cells can be calculated using the UCF $CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}$ (cells) (Battley 1999a:230) as follows,

$$\begin{aligned} \mathrm{AE} = {} & 4n\mathrm{C} + n\mathrm{H} - 2n\mathrm{O} \\ & - 3n\mathrm{N} + 5n\mathrm{P} - 2n\mathrm{S} \end{aligned} \qquad (4)$$

where the lower case "*n*" represents the proportions of atoms relative to one carbon atom, respectively, of a given substance. Using the above UCF (Battley 1999a:230),

$$\begin{aligned} \mathrm{AE\ UCFW^{-1}} &= (4 \times 1\mathrm{C}) + (1.613\ \mathrm{H}) \\ &\quad - (2 \times 0.557\ \mathrm{O}) - (3 \times 0.158\ \mathrm{N}) \\ &\quad + (5 \times 0.012\ \mathrm{P}) - (2 \times 0.003\ \mathrm{S}) \\ &= 4 + 1.613 - 1.114 - 0.474 \\ &\quad + 0.060 - 0.006 = 4.079. \end{aligned} \qquad (5)$$

In a culture medium containing glucose ($C_6H_{12}O_6$) as the substrate, AE = 24 AE mol^{-1}. Referring to Table 1, during aerobic growth on glucose 1.914 unit carbon formula weights (UCFW) of dried *S. cerevisiae* cells are produced for every mol of glucose consumed. This is called the Molar Yield Coefficient (MYC), having the units UCFW mol^{-1} of substrate consumed. With only a single source of carbon and energy the electrons in the biomass can only come from that portion of the substrate that is used to construct the biomass. The quantity of glucose required to form this quantity of cells is then determined as follows,

$$\begin{aligned} \mathrm{sub_{an}} &= (\mathrm{MYC_{cells}} \times \mathrm{AE_{cells}})/\mathrm{AE_{sub}} \\ &= (1.914 \times 4.079)/24 \\ &= 0.325\ \mathrm{mol\ glucose} \end{aligned} \qquad (6)$$

TABLE 1

Equations representing the growth of Saccharomyces cerevisiae *anaerobically on glucose, and aerobically on glucose, ethanol, and acetic acid*

Anaerobic growth on glucose

Anabolism

$$0.100\ C_6H_{12}O_6(aq) + 0.093\ NH_3(aq) + 0.007\ H_3PO_4(aq) + 0.002\ H_2SO_4(aq) \rightarrow 0.590\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) + 0.010\ CO_2(aq) + 0.282\ H_2O(l)$$

Formation of glycerol

$$0.252\ C_6H_{12}O_6(aq) + 0.216\ H_2O(l) \rightarrow 0.432\ C_3H_8O_3(aq) + 0.216\ CO_2(aq)$$

Catabolism

$$0.650\ C_6H_{12}O_6(aq) \rightarrow 1.300\ C_2H_6O(aq) + 1.300\ CO_2(aq)$$

Metabolism

$$C_6H_{12}O_6(aq) + 0.093\ NH_3(aq) + 0.007\ H_3PO_4(aq) + 0.002\ H_2SO_4(aq) \rightarrow 0.590\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) + 1.300\ C_2H_6O(aq) + 0.432\ C_3H_8O_3(aq) + 1.526\ CO_2(aq) + 0.066\ H_2O(l)$$

Nonconservative

$$C_6H_{12}O_6(aq) \rightarrow 2\ CO_2(aq) + 2\ C_2H_6O(aq)$$

Aerobic growth on glucose

Anabolism

$$0.325\ C_6H_{12}O_6(aq) + 0.302NH_3(aq) + 0.023\ H_3PO_4(aq) + 0.006\ H_2SO_4(aq) \rightarrow 1.914\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) + 0.036\ CO_2(aq) + 0.900\ H_2O(l)$$

Catabolism

$$0.675\ C_6H_{12}O_6(aq) + 4.050\ O_2(aq) \rightarrow 4.050\ CO_2(aq) + 4.050\ H_2O(l)$$

Metabolism

$$C_6H_{12}O_6(aq) + 0.302NH_3(aq) + 4.050\ O_2(aq) + 0.023\ H_3PO_4(aq) + 0.006\ H_2SO_4(aq) \rightarrow 1.914\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) + 4.086\ CO_2(aq) + 4.950\ H_2O(l)$$

Nonconservative

$$C_6H_{12}O_6(aq) + 6\ O_2(aq) \rightarrow 6\ CO_2(aq) + 6\ H_2O(l)$$

Aerobic growth on ethanol

Anabolism

$$0.350\ C_2H_6O(aq) + 0.163\ NH_3(aq) + 0.330\ CO_2(aq) + 0.012\ H_3PO_4(aq) + 0.003\ H_2SO_4(aq) \rightarrow 1.030\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) + 0.492\ H_2O(l)$$

Catabolism

$$0.650\ C_2H_6O(aq) + 1.950\ O_2(aq) \rightarrow 1.300\ CO_2(aq) + 1.950\ H_2O(l)$$

Metabolism

$$C_2H_6O(aq) + 0.163\ NH_3(aq) + 1.950\ O_2(aq) + 0.012\ H_3PO_4(aq) + 0.003\ H_2SO_4(aq) \rightarrow 1.030\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) + 0.970\ CO_2(aq) + 2.440\ H_2O(l)$$

Nonconservative

$$C_2H_6O(aq) + 3\ O_2(aq) \rightarrow 2\ CO_2(aq) + 3\ H_2O(l)$$

Aerobic growth on acetic acid

Anabolism

$$0.316\ C_2H_4O_2(aq) + 0.098\ NH_3(aq) + 0.007\ H_3PO_4(aq) + 0.002\ H_2SO_4(aq) \rightarrow 0.620\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) + 0.012\ CO_2(aq) + 0.296\ H_2O(l)$$

Catabolism

$$0.684\ C_2H_6O(aq) + 1.368\ O_2(aq) \rightarrow 1.368\ CO_2(aq) + 1.368\ H_2O(l)$$

Metabolism

$$C_2H_4O_2(aq) + 0.098\ NH_3(aq) + 0.007\ H_3PO_4(aq) + 0.002\ H_2SO_4(aq) \rightarrow 0.620\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) + 1.368\ CO_2(aq) + 1.664\ H_2O(l)$$

Nonconservative

$$C_2H_4O_2(aq) + 2\ O_2(aq) \rightarrow 2\ CO_2(aq) + 2\ H_2O(l)$$

(Table from Battley 2011b; reproduced with permission from Springer Science + Business Media.)

where sub_{an} (anabolic substrate) represents the quantity of substrate, here glucose, required to form the cells. Everything else can now be filled in by the difference between the initial and final states. As an example, for aerobic growth on glucose we can start with the following,

$$0.325\ C_6H_{12}O_6 + n\ NH_3 + p\ H_3PO_4 + s\ H_2SO_4 \rightarrow 1.914\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(\text{cells}) + c\ CO_2 + w\ H_2O. \quad (7)$$

The remaining initial state coefficients can now be filled in by multiplying the MYC by the subscript for a given atom in the cells (i.e., $1.914 \times 0.158\ N = 0.302\ NH_3$) to give

$$0.325\ C_6H_{12}O_6 + 0.302\ NH_3 + 0.023\ H_3PO_4 + 0.006\ H_2SO_4 \rightarrow 1.914\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(\text{cells}) + c\ CO_2 + w\ H_2O. \quad (8)$$

The remaining final state coefficients can be filled in by subtracting (1.914×1) from $(0.325 \times 6) = 0.036$ for CO_2 and by subtracting (1.914×1.613) from $(0.325 \times 12) + (0.302 \times 3) + (0.023 \times 3) + (0.006 \times 2) = 1.800$ for hydrogen. This last should be divided by 2 to give $0.900\ H_2O$. The elemental balance should always be checked, because the coefficients to the terms in the equation are not small, whole numbers. This can be done by adding the individual elements on both sides of the equation. When necessary, small adjustments can be made to H and O on either side of the equation to even the equality. The completed equation is listed in Table 1 under "anabolism" for aerobic glucose. For the catabolic equation, the MYC for glucose must be subtracted from 1.000, and an equation written for its aerobic oxidation. Anabolic and catabolic equations can then be combined (as in Table 1) to give the full, metabolic growth-process equation. Metabolic equations can also be calculated directly in the same manner. For anaerobic, anabolic, growth-process equations, there may be additional fermentation products other than those of catabolism, one example of which is glycerol. The same method is used as for the cells to determine the coefficients of other anabolic, organic products in addition to cells. The full set of equations describing the growth of *S. cerevisiae* anaerobically on glucose, and aerobically on glucose, ethanol, and acetic acid, is shown in Table 1; see also Battley (2011b).

The use of coefficients expressed to three places in the growth-process equations described here does not imply this degree of accuracy. This simply makes it somewhat easier to balance the equations.

It is important to note that no O_2 appears in any anabolic equation in Table 1. This is characteristic of establishing an equality between the quantities of electrons in the substrate and in the cells, as described above.

On CO_2 in Growth-Process Equations

It was shown by Müller (1933) that when photosynthetic bacteria were grown anaerobically in a medium containing only minerals and low concentrations of a single source of organic carbon, the organic carbon disappeared completely from the medium. When he used acetic or lactic acid as the substrate, there was always a production of carbon dioxide gas during the growth of these bacteria, but when he used substrates more reduced than these, carbon dioxide gas was always absorbed. These phenomena were explained by Van Niel (1941) in a consideration of the relative degrees of reduction of the substrate and the cellular material being grown. If a substrate more oxidized than cellular material becomes incorporated into cellular substance, there will be some carbon remaining that must be eliminated as carbon dioxide. Conversely, with the anaerobic conditions under which many photosynthetic bacteria live, if the substrate is more reduced than the cells, there will have to be an uptake of carbon dioxide so that the average degree of reduction of these substances taken together will be equivalent to that of the cellular substance. Battley adopted Van Niel's interpretation of Müller's experiments with respect to constructing equations when the substrate is more reduced than the cells, whether or not they are photosynthetic. Table 1 shows that anabolism is always "anaer-

obic" whether *S. cerevisiae* is grown in the absence or presence of oxygen in the sense that O_2(aq) is not required (Battley 2007, 2009, 2011b). This suggests that fermentative microbial growth may be one of the most conservative of all biological processes, in that the lack of a necessity for oxygen made it possible for progenotes to be the first, self-reproducing, biological catalysts. The result of most microbial fermentations is the production of organic substances that are too reduced to be further fermented. Had the photosynthetic bacteria not evolved, metabolism and growth of progenotes would have stopped for lack of a carbon and energy source. Here, aided by the energy provided by light, these reduced, residual substances could be combined with CO_2 to photosynthesize organic substances at about the same average level of reduction as the cells.

The experiments of Müller and their interpretation by Van Niel, described above, introduced the idea of the importance of the reduction of substrates, and cells and organic products. This came to be known as the "reductance degree." The reductance degree (γ) of an organic substance, including cells, was defined by Minkevich and Eroshin (1973a) as the number of gram-equivalents of oxygen required for the complete combustion of one gram atom of carbon in the substance, or the number of available electrons per unit carbon atom of the substance. The equation used by the present author is

$$\gamma = (4n\mathrm{C} + n\mathrm{H} - 2n\mathrm{O} - 3n\mathrm{N} + 5n\mathrm{P} - 2n\mathrm{S})/n\mathrm{C} \quad (9)$$

where γ represents the reductance degree on scale of 8, and n represents the number of atoms of elements in the formula for an organic substance. If an element is not present, it is removed from the equation. Here CO_2 has a reductance degree of 0, glucose of 4, and methane of 8. For substances with only one carbon atom, the reductance degree is the same as the number of AE. Using the UCF for *S. cerevisiae* and Equation (9) gives a γ of 4.079 (Battley 1999a:227). Thus, for glucose as a substrate that is less reduced than the cells CO_2(aq) will have to be a product of anabolism, and for ethanol as a substrate that is more reduced than the cells, it will have to be a reactant in anabolism. This is evident from Table 1. Whether CO_2(aq) is considered to be a product or a reactant, it must be included in an anabolic equation. This happens whether the total growth process is anaerobic or aerobic. No substrate has a reductance degree that is exactly that of the cells. Effectively, glucose is becoming reduced to the level of the cells by forming CO_2 from the glucose molecule, and ethanol is becoming oxidized to the level of the cells by becoming combined with CO_2, as suggested by Van Niel and described above.

Results from the Study of Growth-Process Equations

Growth-process equations representing the growth of *S. cerevisiae* anaerobically on glucose and aerobically on glucose, ethanol, and acetic acid are shown in Table 1 as a result of attention to the details as described in the section, Growth-Process Equations, above. They are organized into anabolic equations representing the production of organic substances, including cells, from some of the substrate during a given growth process, and a catabolic equation representing the consumption of the remaining part as the result of oxidative reactions, including fermentations. Anabolic and catabolic processes do not operate separately during actual growth, and are theoretical constructs that can be added to give an equation representing metabolism. This latter can also be constructed directly. It is the metabolic equation that represents what is called a "growth process." During anabolism, that portion of the substrate used for the construction of cells and other organic products results in the conservation in chemical form of nonthermal energy contained within this portion of the substrate. With *S. cerevisiae*, another portion of the substrate is used to produce glycerol. The remaining portion of the substrate is reacted catabolically to provide energy for anabolism. Anabolism plus additional organic product formation thus saves, or conserves, a part of the substrate energy in the form of cells and other anabolic, organic products. Metabolism is therefore said to be

TABLE 2
Demonstration of Hess's Law with respect to microbial growth[a,b]

Reactions	$\Delta_r G_B^{o'}$	$\Delta_r X_B^{o'}$
	——kJ——	
1. Anaerobic growth on glucose as determined experimentally		
$C_6H_{12}O_6(aq) + 0.093\ NH_3(aq) + 0.007\ H_3PO_4(aq) + 0.002\ H_2SO_4(aq) \rightarrow$		
$0.059\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) + 0.432\ C_3H_8O_3(aq) + 1.300\ C_2H_6O(aq)$		
$+ 1.528\ CO_2 + 0.066\ H_2O(l)$	−210.77	−158.64
2. Aerobic growth on ethanol produced during anaerobic growth on glucose as determined experimentally		
$1.300\ C_2H_6O(aq) + 0.212\ NH_3(aq) + 2.525\ O_2(aq) + 0.016\ H_3PO_4(aq) + 0.004\ H_2SO_4\ (aq) \rightarrow$		
$1.339\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) + 1.261\ CO_2(aq) + 3.166\ H_2O(l)$	−1079.18	−1160.99
3. Complete oxidation of glycerol produced during anaerobic growth on glucose (calculated)		
$0.432\ C_3H_8O_3(aq) + 1.512\ O_2(aq) \rightarrow 1.296\ CO_2(aq) + 1.728\ H_2O(aq)$	−708.79	−733.91
4. Addition of 1 + 2 + 3 above to equal aerobic growth on glucose as determined using Hess's Law		
$C_6H_{12}O_6(aq) + 0.305\ NH_3(aq) + 4.047\ O_2(aq) + 0.023\ H_3PO_4(aq) + 0.006\ H_2SO_4\ (aq) \rightarrow$		
$1.929\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) + 4.085\ CO_2(aq) + 4.960\ H_2O(aq)$	−1998.74	−2053.54
5. Aerobic growth on glucose as determined experimentally		
$C_6H_{12}O_6(aq) + 0.302\ NH_3(aq) + 4.050\ O_2(aq) + 0.023\ H_3PO_4\ (aq) + 0.006\ H_2SO_4\ (aq) \rightarrow$		
$1.914\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) + 4.086\ CO_2(aq) + 4.975\ H_2O(l)$	−1999.81	−2056.26

[a] Data taken from Tables 1 and 3.
[b] Note the close agreement with respect to mass and energy between the coefficiencts of the last two equations.

"conservative" in that not all of the nonthermal, chemical potential energy of the substrate is lost as heat to the environment, but remains in the substance of the organic products of the growth process that are different from those formed during catabolism (Battley 1960b). The complete aerobic oxidation or anaerobic fermentation (also an oxidation) of a substrate is a "nonconservative" process in that no nonthermal energy becomes conserved in cellular substance or other organic product as a result of anabolism. The ratio (AE conserved in cells/AE transferred during a nonconservative reaction) is one measure of the efficiency of growth. It was the opinion of Minkevich and Eroshin (1973a,b, 1975) that the equivalent of AE provides a measure of substance quantity that best reflects its chemical store. It is this relationship of the quantity of AE to the quantity of substance in which they are incorporated that makes it possible to construct with the greatest simplicity equations representing microbial growth.

Growth-process equations, for all their complexity, are not different in many ways from representations of simple reactions. The principal difference is that cells as a product are self-reproducing, biological catalysts, and are unique in this respect. The use of AE in constructing growth-process equations reinforces the idea that "*life is an ordered movement of electrons into and through a self-reproducing biological catalyst.*"

Growth processes obey Hess's Law and in this respect are not different from all biochemical reactions or processes. For whatever reason, the *S. cerevisiae* strain used in these investigations will not grow on glycerol; accordingly, it ought to be possible to add the equation representing anaerobic growth on glucose, that representing the complete oxidation of the glycerol formed during anaerobic growth, and that representing aerobic growth on the ethanol formed during anaerobic growth, to give the equation representing aerobic growth on glucose. Table 2 shows that this can be done with high accuracy, using the equations from Table 1.

Oxygen does not enter into any of the anabolic equations in Table 1. This is the result of using electron equivalencies rather than C-mol equivalencies in constructing anabolic equations. Surely this must make anabolism one of the most conservative biological processes of all time. It is generally

believed that before and during the origin of life, no $O_2(g)$ was present on the Earth's surface; therefore, it could not be used for growth. The progenotes were precursors to bacteria, which do not presently have steroids, and could presumably live without oxygen before the evolution of cyanobacteria. If anabolism is truly represented by the anabolic equations in Table 1, then the lack of a need for oxygen in cell anabolism has survived since life began (Battley 2009).

On the other hand, modern *S. cerevisiae* will not grow totally anaerobically without the presence of a steroid, the synthesis of which requires $O_2(g)$. Using electron equivalency between the substrate used to form the cells and the cells, there is no $O_2(aq)$ in any equation representing anabolism. Using C-mol equivalency $O_2(aq)$ appears as an anabolic product in anaerobic growth of yeast on glucose and in aerobic growth on glucose and acetic acid because these substrates are less reduced than the cells. Also, using C-mol equivalency $O_2(aq)$ appears as an anabolic reactant in aerobic growth on ethanol, which is more reduced then the cells. The formation of $O_2(aq)$ either as a reactant or a product of anabolism has not yet been demonstrated biochemically, even though using C-mol equivalencies is formally correct and is routinely used.

The quantity of growth of *S. cerevisiae* cells is strictly a function of the ratio of AE conserved in biomass to the AE per mol of substrate. This is shown in Figure 1. The conservation of AE in the biomass is therefore independent of the nature and overall metabolism of the electron donor. This applies even during autotrophic growth, an example of which is *Pseudomonas saccharophila* growing on $H_2(aq)$ and $CO_2(aq)$, so that there is no substrate simultaneously performing the functions of electron donor and carbon source in the usual heterotrophic sense (Battley 1996).

The equations in Table 1 are all that are needed chemically for determinations of the energy transformations that accompany them. The same equations, but using ions instead of undissociated molecules, are found in Battley (2009). A complete comparison is also made here between the use of electron equivalencies and C-mol equivalencies in describing growth anaerobically on glucose and anaerobically on glucose and aerobically on glucose, ethanol, and acetic acid.

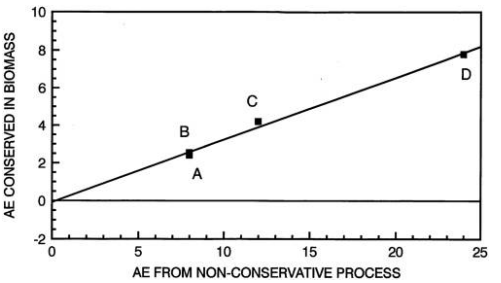

Figure 1. The Relation of AE Per Mol of Substrate to AE Conserved in Biomass

A linear regression of the available electrons (AE) in *Saccharomyces cerevisiae* cells grown anaerobically on glucose (A) and aerobically on acetic acid (B), ethanol (C), and glucose (D) plotted against the number of AE per mol of substrate. The line has a slope of 0.331, a Y-intercept of −0.072, and a correlation coefficient of 0.997 (Battley 1999a:255). Points (A) and (B) are nearly identical because the AE from alcoholic fermentation and from the oxidation of acetic acid are identical at 8 AE. (Figure from Battley 1999a; reproduced with permission from Elsevier.)

The intent of the previous three sections has been to emphasize the material exchange that takes place during microbial growth, as represented by growth-process equations. It is absolutely imperative that these be accurately constructed and tested, and that what are thought to be appropriate states and concentrations of the reactants and products are determined and indicated. Otherwise, the energy exchanges accompanying the microbial growth represented by the growth-process equations cannot be correctly calculated. This will also depend on the assumptions and methods used.

The Thermodynamics of Growth Processes

two equations for the study of the thermodynamics of growth processes

The equation conventionally used to describe the chemical thermodynamics of a reaction is the Gibbs equation,

$$\Delta_r G^\circ = \Delta_r H^\circ - T\Delta_r S^\circ \qquad (10)$$

where $\Delta_r G^o$ represents the change in Gibbs energy of the system, $\Delta_r H^o$ represents the change in enthalpy, and $T\Delta_r S^o$ the change in absorbed thermal energy comprising the change in entropy, $\Delta_r S^o$, multiplied by the absolute temperature (T). All terms in Equation (9) have the units of energy, which are J mol^{-1}. Battley has proposed a completely different thermodynamic system for estimating energy changes accompanying microbial growth, represented by a new equation analogous to the Gibbs equation,

$$\Delta_r X^o = \Delta_r H^o - \Delta_r Q^o_{ab} \qquad (11)$$

where $\Delta_r X^o$ represents the change in free energy of the system, $\Delta_r H^o$ represents the change in enthalpy, and $\Delta_r Q^o_{ab}$ the change in absorbed thermal energy, which is not the same as $T\Delta_r S^o$. The remainder of this presentation will examine the differences between Equations (10) and (11).

THE MICROBIAL CELL ENVIRONMENT

All cells live in an aqueous environment. In general, free-living, microbial cells live in a dilute aqueous solution of salts and substrates, although there are exceptions, such as some that can live in hypertonic solutions of sugar from fruit exudates, or others that can live in saturated sea salt solutions. The life cycles of microorganisms are minutes to hours, and because the aqueous environment in which they live has a high heat capacity, the environmental temperature during their life cycle is nearly constant, as is the atmospheric pressure over the aqueous solution. Thus, since T and P are constant, the changes in thermodynamic properties of the system comprising the growth of microorganisms are described conventionally by the Gibbs equation. To avoid the formation of storage products in the cells, the temperature of growth should be that at μ_{max}. Otherwise the quantity of storage products may have to be determined and subtracted from the dry weight of the cells.

THERMODYNAMIC PROPERTIES IN GENERAL

Thermodynamic properties are symbols that represent the quantity of nonthermal or thermal energy in a given substance as a function of its mass, expressed as J mol^{-1} or as J mol^{-1} K^{-1}. The ones presently in use for biologists are $\Delta_f G^o$, $\Delta_f H^o$, and $\Delta_f S^o$, representing Gibbs energies, enthalpies, and entropies of formation from the elements at a given T and P, respectively. These are called "thermodynamic functions of state" or "thermodynamic properties." Lists of thermodynamic properties are available for pure substances of biological importance in their standard states under environmental conditions of constant T and P (Wilhoit 1969; Wagman et al.1982:2–10).

Gibbs Reaction Energy: $\Delta_r G^o$

For the growth of microorganisms, $\Delta_r G^o$ represents a change of nonthermal, chemical potential energy of the system into heat as a growth process proceeds spontaneously from its initial to its final state. Gibbs energy cannot be directly measured, but changes in energy can be calculated using Equation (10), where the subscript "r" represents a reaction, and the superscript "o" indicates that the changes involve substances in their standard states. The environmental conditions are those of constant T and P and G, H, S, T, and P are all considered to be functions of state. $\Delta_r G^o$ has the units of energy (kJ mol^{-1}) and its sign has always been negative when growth takes place, indicating that nonthermal chemical-potential energy becomes transformed into heat, which becomes transferred to the environment. A negative free energy change indicating a "spontaneous reaction" is what "drives" a growth process, utilizing a portion of the substrate to do so. This is usually accompanied by a transfer of electrons. $\Delta_r H^o$ represents the enthalpy change or heat of reaction. It is a thermal quantity having the units of energy (kJ/mol), and usually has a negative sign for a spontaneous reaction, indicating that heat becomes lost to the system. The Gibbs energy equation is more understandable when written as:

$$\Delta_r H^o = \Delta_r G^o + T\Delta_r S^o. \qquad (10)$$

Equation (10) shows more clearly that the heat of reaction originates in two sources: the conversion of nonthermal, chemical po-

tential Gibbs energy into heat ($\Delta_r G^o$) and another term, $T\Delta S^o$. This latter is related to the physical absorption or loss of heat by the system, but both symbols have the units kJ/mol. The observation that reactions give off heat as they proceed from an initial to a final state is a common experience. Equation (10) shows this in that $\Delta_r H^o$ has a negative sign for a spontaneous reaction in that the value of the $T\Delta_r S^o$ term is usually much smaller than that of $\Delta_r G^o$. The value of $\Delta_r G^o$ can also be calculated using the equation,

$$\Delta_r G^o = \Sigma \Delta_f G^o_{prod} - \Sigma \Delta_f G^o_{react} \quad (12)$$

where the subscripts represent that the terms on the right are the Gibbs energies of formation of the products and reactants, respectively. $\Delta_f G^o$ values are zero for any element at 298.15 K. No energy is required to form an element in a given phase from itself.

Enthalpy: $\Delta_r H^o$

For organic, biological substances, $\Delta_c H^o$ is usually determined by combustion calorimetry. For *S. cerevisiae* cells, an appropriate equation representing an oxygen bomb, calorimetric combustion, would be

$$\begin{aligned} &CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) \\ &+ 1.151\ O_2(g) \rightarrow 1.000\ CO_2(g) \\ &+ 0.079\ N_2(g) + 0.003\ P_4O_{10}(cr) \\ &+ 0.003\ SO_3(g) + 0.806\ H_2O(l). \end{aligned} \quad (13)$$

The cells should be appropriately dried (Battley and DiBiase 1980) and pelleted (see also Lamprecht 1999). The analyses of Duboc et al. (1995) indicate that sulfate is not a part of the ash resulting from the combustion of cells, but is most likely $SO_3(g)$. Having measured the value for the heat of combustion of the cells, $\Delta_c H^o$ (cells), and using $\Delta_f H^o$ values from the literature for the other terms in the equation, the value of $\Delta_f H^o$ (cells) can be calculated by difference using the following equation,

$$\Delta_c H^o(cells) = \Sigma \Delta_f H^o_{prod} - \Sigma \Delta_f H^o_{react}. \quad (14)$$

Entropy and $T\Delta_r S$

Entropy has had a long history that will not be rigorously treated here. It has been related to probability, information, randomness, organization, and disorder. There are presently several different functions called "entropy," of which only one, Clausius entropy, is of practical importance in biological thermodynamics. It was introduced into thermodynamics at the phenomenological level by Clausius in 1865, and is referred to as Clausius entropy. The idea of entropy being a probability comes from statistical Boltzmann-Gibbs entropy, which is related to probability density (Kurzyński 2006:40). However, everything has a Clausius (absolute) entropy, which can be determined using the following equation and low-temperature calorimetry,

$$\begin{aligned} (S_{298.15} - S_0) &= S_T\ [S_0 = 0] \\ &= \int_0^{298.15} C_P\ d\ ln\ T + \Sigma \Delta_{trs}\ H_T/T_{trs}. \end{aligned} \quad (15)$$

Here the symbol C_P represents the heat capacity of a sample, the integral is for a temperature range between 0 K and 298.15 K, and the second term on the right represents transitions and phase changes, if any (Wagman et al. 1982: 2–11). Because S_0 usually equals zero, entropy is often expressed simply as S_T, or S if the T is stated elsewhere. If there are no transitions or phase changes, as in determining the entropy of dried cells, this second term is ignored. The practical units of S are J mol^{-1} K^{-1}, which are not the units of energy, these latter being J mol^{-1}. Clausius entropy, per se, is not a physical entity. Mathematically, it is not related to ordinary probability in that this latter has no units, whereas entropy does. In an article by Tribus and McIrvine (1971), Tribus cites a conversation with Claude Shannon, who related a conversation with John von Neumann in 1961 in which von Neumann stated that no one knows what entropy really is. It was pointed out by Klotz (1964) and Klotz and Rosenberg (2000:145) that Clausius entropy is essentially a mathematical function. Multiplying S by the T for which S is determined gives TS, which has the units of energy. However, although the

symbol *S* remains in the symbol *TS*, it is no longer just a mathematical function. *TS* or $T\Delta S$ represent absorbed thermal energy having the units J mol^{-1}. Because the lower bound to the integration in Equation (15) is 0 *K* (a special case), *TS* theoretically represents the quantity of thermal energy required to raise the temperature of a substance from 0 *K* to that *T* for which *S* was determined. This is absorbed thermal energy. It was well described by Linhart (1920). *TS* is quite different and far more simple than considering entropy to be randomness, probability, organization, information, or disorder. Passynsky (1961) was of the opinion that thermodynamic entropy in its physical sense is not the same as the information function and that any connection between entropy and disorder in biological systems must be very carefully considered. This was amplified by Morrison in the following profound statement: "Nearly all the manifest visual and mechanical intricacy of organisms, like their apt behavior, turns out to be without quantitative thermodynamic importance. Morphology and ecology are . . . only small secondary properties of a fundamentally thermodynamic system" (Morrison 1964:520). Whatever entropy is with respect to microbial cells, its quantity is related largely to the lowest level of cellular integration, which is that of the atomic building blocks of which cells are composed. This has been shown to be true both for entropy (Battley 1999a:255) and absorbed thermal energy (Battley 2011a). For dried cells, these properties can be closely calculated by multiplying the sums of the absolute entropies of the individual constituent atoms by a constant. An empirical method has been described for making a close estimate of the entropy and the entropy of formation of dried *S. cerevisiae* cells using the equations,

$$S_{biomass} = 0.187\ \Sigma S^{o}_{atoms} \qquad (16)$$

and

$$\Delta_f S_{biomass} = -0.813\ \Sigma S^{o}_{atoms} \qquad (17)$$

where ΣS^{o}_{atoms} represents the sum of the absolute entropies of the kinds and quantities of atoms in one unit mass of solid substance (Battley 1999b). The entropy of dried yeast cells is 1.304 J g^{-1} K^{-1}, which falls about midway on a long list of entropies of small molecular weight substances such as amino acids and sugars, all of which are very simple and completely lacking organization compared to cells (Battley et al. 1997). This is the only measurement so far made of dried cells using low temperature calorimetry according to Equation (15).

The entropy of formation of a substance can also be calculated according to the following equation,

$$\Delta_f S^{o} = S^{o} - \Sigma S^{o}_{atoms} \qquad (18)$$

where $\Delta_f S^{o}$ represents the entropy of formation, and ΣS^{o}_{atoms} the sum of the entropies of the kinds and quantities of atoms in a substance (Battley 1999b). The change in the entropy of a system that takes place during a reaction is calculated using the equation,

$$\Delta_r S = \Sigma \Delta_f S^{o}_{prod} - \Sigma\ \Delta_f S^{o}_{react} \qquad (19)$$

where the subscripts "prod" and "react" represent products and reactants of the system, respectively. Because the source of the energy used to calculate *S* is thermal energy, this does not imply that *S* must also have the units of energy. The units of energy appear when *S* is multiplied by *T*. But is the quantity of absorbed thermal energy calculated theoretically to be absorbed as *TS* that which is truly absorbed by a given substance for it to exist at the *T* at which *S* was determined? The following arguments show that it is not.

Q^{o}_{ab} and $\Delta_r Q^{o}_{ab}$

There is another function that is similar to entropy in that for a given substance the same physical data are initially used in the calculation. This is the "enthalpy," having the symbol $(H_T - H_0)$, and it appears in most older tables of thermodynamic properties. It is a function of state and also uses the symbol *H*, but has been modified here from a similar equation from Wagman et al. (1982:2–11):

$$(H_{298.15} - H_0) = H_{ab,T}\ (H_0 = 0) = [Q_{ab,T}] = \int_0^{298.15} C_p\ \mathrm{d}\ T + [\Sigma \Delta_{trs}\ Q_T]. \qquad (20)$$

Here, as with Equation (15), the integral is for values between 0 K and 298.15 K, and the last term on the right represents transitions and phase changes, if any. If there are no transitions or phase changes, this latter term is ignored. One source of confusion in general is that the symbol H is used in more than one context. As an example, with respect to any given reaction

$$\Delta_r H^o = \Delta_r G^o + T\Delta_r S^o \qquad (10)$$

$$\Delta_r H^o = \Sigma\Delta_f H^o_{prod} - \Sigma\Delta_f H^o_{react} \qquad (14)$$

$$\Delta_r H^o_{ab,T} = \Sigma H^o_{ab,T,\,prod} - \Sigma H^o_{ab,T,\,react}. \qquad (21)$$

The symbol $\Delta_r H^o$ is the same for all three equations. However, Equations (10) and (14) represent the change comprising the total heat of reaction, which is also the total amount of thermal energy that the system can exchange with the environment as heat. Equation (21) represents only the change in the quantity of absorbed thermal energy exchanged by the system with the environment in order for the system to exist at T/K as it passes from an initial to a final state, which is something quite different. In Equation (20), the symbol $(H_{298.15} - H_0)$ becomes equated with $(Q_{ab,T})$ to better represent that it is absorbed thermal energy that is being considered, and not heat, which is thermal energy in motion that crosses system boundaries. In writing Equation (20), $Q_{ab,T}$ becomes a state function.

In 1999, Battley published an article on what he considered to be a biologist's (his own) perspective on entropy and absorbed thermal energy (Battley 1999c), agreeing with Klotz (1964) that S is essentially a mathematical function, and concluding that TS equals absorbed thermal energy, and nothing else. He also came to the erroneous conclusion that $\Delta Q_{ab} = T\Delta S$. However, eventually he came to realize that his biologist's perspective was in error, and that TS was not equal to Q_{ab}, which was also absorbed thermal energy. He then correctly equated the term $(H_{298.15} - H_0)$ in Equation (20) with the symbol $Q_{ab,\,T}$, or just Q_{ab} if the T is stated elsewhere, representing *only* absorbed thermal energy. This does not include the thermal energy generated from non-thermal, free energy exchanges, and is represented as follows,

$$\Delta_r Q^o_{ab} = \Sigma\Delta_f Q^o_{ab,\,prod} - \Sigma\Delta_f Q^o_{ab,\,react}. \qquad (22)$$

The symbol Q^o_{ab} here represents "enthalpy," in that it is thermal energy required to be absorbed by a single substance in order for it to exist at a given temperature. Just as for entropy, for any given substance,

$$\Delta_f Q^o_{ab} = Q^o_{ab} - \Sigma Q^o_{ab,\,atoms} \qquad (23)$$

where $\Delta_f Q^o_{ab}$ represents the absorbed thermal energy of formation, and $\Sigma Q^o_{ab,\,atoms}$ the sum of the absorbed thermal energies of the kinds and quantities of atoms in a substance. The former is the change in absorbed thermal energy accompanying the formation from its constituent elements of a substance in its standard state at a given temperature. Q^o_{ab} is not the same as "Q" representing heat (i.e., Q with no subscript), which is a process function. Rather than representing absorbed thermal energy, Q represents thermal energy in motion across a system boundary, and is commonly called "heat."

Similar to entropy,

$$Q^o_{ab} = 0.352\,\Sigma Q^o_{ab,\,atoms} \qquad (24)$$

and

$$\Delta_f Q^o_{ab} = -0.648\,\Sigma Q^o_{ab,\,atoms} \qquad (25)$$

where $\Sigma Q^o_{ab,\,atoms}$ represents the sum of the absolute enthalpies of the kinds and quantities of atoms in 1 mol of substance (Battley 2011a). The change in the absorbed thermal energy of a system during a reaction is calculated using Equation (22), where $\Delta_r Q^o_{ab}$ represents the overall change in the absorbed thermal energy and where the subscripts "prod" and "react" represent reactants and products of a system, respectively.

The Difference Between S and Q_{ab}

With respect to thermodynamics as applied to growth-process equations, the thermodynamic properties of all the terms except that for cells can be found in standard tables, from which the properties of the biological standard states can be calculated (see the

TABLE 3

Thermodynamic properties for substances of importance in this review, in kJ mol^{-1} at 298.15 K and 0.1 MPa[a,b]

Properties	$\Delta_f G^o$	$\Delta_f G^{o'}$	$\Delta_f G^{o'}_B$	$\Delta_f X^o$	$\Delta_f X^{o'}$	$\Delta_f X^{o'}_B$	$\Delta_f H^o$
Substance							
Inorganic							
O_2 (g)	0.00	16.32	−0.79	0.00	16.32	−0.80	0.00
NH_3 (g)	−16.57	−26.57	−43.69	−64.05	−74.05	−91.17	−46.11
CO_2 (g)	−394.37	−386.01	−403.13	−411.31	−402.94	−420.06	−393.51
H_3PO_4 (cr)	−1119.10	−1142.54	−1159.66	−1214.56	−1240.00	1257.12	−1279.00
H_2SO_4 (l)	−690.90	−744.53	−761.75	−785.05	−730.52	−747.64	−813.99
H_2O (l)	−237.18	−237.18	−237.18	−281.42	−281.42	−281.42	−285.83
Organic							
Acetic acid (l)	−389.45	−404.09	−421.21	−474.89	−489.53	−506.65	−484.21
Ethanol (l)	−174.18	−180.96	−198.08	−263.50	−270.28	−287.40	−276.98
Glucose (cr)	−910.56	−914.54	−931.66	−1195.11	−1199.08	−1216.20	−1274.45
Glycerol (cr)	−479.48	−497.47	−514.60	−646.40	−664.28	−681.40	−670.69
Biological							
Yeast cells[c]	−80.27	−80.27	−80.27	−116.05	−116.05	−116.05	−125.40

[a] Values of the "biological standard state" are calculated for a quantity of 1 mol at a concentration of 0.001 *M* (please see text).

[b] Thermodynamic properties except for those of $\Delta_f S^{o'}_B$ were taken from Table 2 in Battley (2007) from which values of $\Delta_f S^{o'}_B$ were calculated. The thermodynamic properties of glycerol were taken from Wilhoit (1969) and from Wilhoit et al. (1985) from which the value of $\Delta_f Q^{o'}_{ab,B}$ was calculated.

[c] The structure of yeast cells is considered to be that of a slightly hydrated precipitate, so that they do not have an aqueous concentration, nor do they have a standard state. The yeast cell data shown above were taken from Table 1 in Battley (2011b).

section entitled The "Biological" Standard States). Data exist in the literature on $\Delta_c H^o$ for the combustion of cells, from which values of $\Delta_f H^o$ can be calculated. For dried cells, the last term in Equations (15) and (20) is ignored (no phase changes) and the integrated thermal data are identical. However, in Equation (20), these data are integrated against T, and not $ln\ T$, as they are in Equation (15). Logarithms have no units, and in Equation (15), the units of S remain the same as those of C_P (i.e., J unit-mass^{-1} K^{-1}), which are not those of energy. Entropy is not energy. Entropy is not a physical quantity that can be exported or imported. In Equation (20), the T units cancel out of the integration, leaving the units of energy with a value that represents directly the quantity of absorbed thermal energy required to raise the T of a calorimetric sample from $0/K$ to T/K. To get the units of energy, S must be multiplied by T to give kJ/unit mass. Although the same calorimetric data are used in the calculations involving Equations (15) and (20) and for the same purpose, it was shown by Battley and Stone (2000) and Battley (2002) that the value for the absorbed heat (TS) is twice that of Q_{ab} for the same solid substance, calculated using Equation (20). They made no claim to be the first to emphasize this; it is just not generally recognized. In older thermodynamic tables where the enthalpy ($H_{298.15} - H_0$) and the entropy (S) are both listed at 298.15 K and 0.1 MPa, if S and T for the same pure, solid substances are multiplied, the result for organic substances of biological importance will be two times that for ($H_{298.15} - H_0$) (i.e., $Q_{ab,T}$). Both TS and Q_{ab} represent the quantity of thermal energy required to raise the temperature of a given mass of substance from 0 K to $T/$K, for which there can be only one value. If TS and Q_{ab} each represent a different quantity of thermal energy, which they do, then one of them is incorrect. The use of incorrect values of absorbed thermal energy will change the quantity of the calculated free energy change

TABLE 3
Continued

$\Delta_f H^{o\prime}$	$\Delta_f H^{o\prime}_B$	$T\Delta_f S^o$	$T\Delta_f S^{o\prime}$	$T\Delta_f S^{o\prime}_B$	$\Delta_f Q^o_{ab}$	$\Delta_f Q^{o\prime}_{ab}$	$\Delta_f Q^{o\prime}_{ab,B}$
−12.09	−12.09	0.00	−28.41	−11.30	0.00	16.32	−0.80
−80.29	−80.29	−29.54	−53.72	−36.60	17.94	−6.24	10.88
−413.80	−413.80	0.86	−27.79	−10.67	17.8	−10.86	6.26
−1288.34	−1288.34	−159.90	−145.80	−128.68	−64.44	−48.34	−31.22
−909.27	−909.27	−123.09	−164.74	−147.52	−28.94	−178.75	−161.63
−285.83	−285.83	−46.65	−46.65	−46.65	−4.41	−4.41	−4.41
−485.26	−485.26	−94.76	−81.17	−64.05	−9.32	4.27	21.39
−287.02	−287.02	−102.80	−106.06	−88.94	−13.48	−16.74	0.38
−1263.07	−1263.07	−363.89	−348.53	−331.41	−79.34	−63.99	−46.85
−676.55	−676.55	−173.22	−179.08	161.95	−24.29	−12.27	−4.85
−125.40	−125.40	−45.13	−45.13	−45.13	−9.35	−9.35	−9.35

accompanying microbial growth, although usually not by a lot.

Nonthermal Energies, $\Delta_r G^o$ and $\Delta_r X^o$

Values for $\Delta_r G^o$ accompanying microbial growth cannot be determined directly, but can be calculated using the common form of the Gibbs equation,

$$\Delta_r G^o = \Delta_r H^o - T\Delta_r S^o. \qquad (10)$$

However, because of the difference in the values of TS^b and Q^o_{ab}, Battley (2002) proposed a different free energy equation as follows,

$$\Delta_r X^o = \Delta_r H^o - \Delta_r Q^o_{ab} \qquad (11)$$

where the form is that of the Gibbs equation, but where $\Delta_r X^o$ represents a nonthermal, energy change when the change in absorbed thermal energy of the system is calculated as $\Delta_r Q^o_{ab}$. The value for $\Delta_r H^o$ remains the same both for Equations (10) and (11). It is what it is measured to be. But, because the values of $T\Delta_r S^o$ and $\Delta_r Q^o_{ab}$ are not the same, neither will be the values for $\Delta_r G^o$ and $\Delta_r X^o$. There cannot be two values representing the same nonthermal energy change for a given growth process, and the question arises as to which is more correct.

Thermodynamic Properties in Aqueous Solution

All that has been stated above applies to the conventional standard states of the substances involved in growth processes, with the exception of the cells. These latter have no real standard state, but a case can be made for a pseudo-standard state comprising cells not containing storage substances and occurring naturally as a slightly hydrated precipitate. The water inside a cell serves as a matrix and as a vehicle for the transport of soluble substances that eventually become metabolized or polymerized into cellular structure. It is not a part of the fabric of the cells. The fabric of all cells is insoluble or slightly hydrated, but all cells live in an aque-

ous environment and all of the other reactants and nonstorage products of a growth process are soluble. All substances in their nonaqueous standard states at 298.15 K and 0.1 MPa have thermodynamic properties with definite values as solids, liquids, or gases. When these are dissolved in water, represented by the suffix (aq), the values of these properties change, some more than others, and often not a lot. The process of solution involves the spontaneous separation of molecules from their standard state to become dispersed in an aqueous matrix, much as gas molecules or atoms can become dispersed in a void. For solutes in aqueous solution, this is usually accompanied by a slight change in the values of their thermodynamic properties. A quantity of substance in solution is specified by adding a "pip" (′) to the superscript of a thermodynamic property. Examples are found in Table 3, where comparisons can be made of the values representing different physical conditions. Conventionally, the concentration standard for thermodynamic calculations is that of 1 mol of solute dissolved in 1 liter of solution. For $\Delta_f G^{o'}$, it is a hypothetical 1 mol of solute in solution at unit activity. For $\Delta_f H^{o'}$, it is a hypothetical 1 mol of solute in solution at infinite dilution. For $\Delta_f S^{o'}$, it is a hypothetical 1 mol of solute in solution having a finite concentration that is not 0, but also not at unit activity, i.e., "somewhere in between" (Klotz 1964; Klotz and Rosenberg 2000:376). Thus, these three thermodynamic properties apply to three different conditions for the same solute (i.e., unit activity, infinite dilution, and "somewhere in between"). This has always been difficult to understand. Concentrations of 1 mol at unit activity would pose hyperosmotic problems for many microorganisms. It is uncertain at what concentration a substrate actually enters a cell in a growth-process system if, in such small spaces, the idea of concentration has any meaning. This would not be expected to be proportional to its concentration in the nutrient solution, but to what a permease channel would permit. As noncellular products of a growth process are formed, their concentrations in the nutrient solution increase and their $\Delta_f G^{o'}$ values become more positive. As noncellular reactants of a growth process are consumed, their concentrations in the nutrient solution decrease and their $\Delta_f G^{o'}$ values become more negative. Lacking knowledge about this, the only possibility is to set up the system so that for the substances participating in a growth process, the difference between the standard 1 mol at unit activity for $\Delta_f G^{o'}$ and 1 mol at hypothetical infinite dilution for $\Delta_f H^{o'}$ is eliminated. This can be done by working at low concentrations of solutes. This is more the natural situation for microorganisms, where the value of the activity approaches closely that of the physical concentration.

The "Biological" Standard States

A solution that is infinitely dilute does not practically exist. However, decreasing real $\Delta_f H^{o'}$ values asymptotically approach those at hypothetical infinite dilution. Pitzer and Brewer (1961) suggested in their second revision of *Lewis and Randall's Thermodynamics* that solute molecules or ions at a concentration of about 0.001 *m* (or 0.001 *M*) are sufficiently separated that further dilution has little effect on intermolecule or interion activity. Probably concentrations of 0.05 *m* (or 0.05 *M*) would not be much different. It was proposed by Battley (1987:374–422) that this could well be the basis for a practical, "microbiological" standard state that would more closely describe the natural conditions experienced by microbial cells, and which could be designated by the subscript "$_B$." If this is done, the value of $\Delta_f H^{o'}$ for a given solute at hypothetical infinite dilution becomes a real value of $\Delta_f H^{o'}_B$ of a solute at, say, 0.001 *M*, for purposes of calculation. Nonthermal energy is lost (i.e., becomes more negative) when a solute becomes more diluted, and there is a three orders of magnitude difference between the value of $\Delta_f G^{o'}$ or $\Delta_f X^{o'}$ in the standard state for 1 mol of solute at a concentration of unit activity and that of 1 mol of solute at a concentration of 0.001 *m*. The free energy change, $\Delta G'_{dil}$, accompanying the process of dilution of a solute from the aqueous standard state to a lower concentration, here 0.001 *m*, can be calculated using the following equation, applicable to both $\Delta G'_{dil}$ and $\Delta X'_{dil}$:

$$\Delta G'_{\text{dil}}\ (\Delta X'_{\text{dil}}) = \frac{-RT}{1000} \ln \frac{\text{conc. in std. st.} \cdot \text{activity coeff. in std. st.}}{\text{lower conc.} \cdot \text{activity coeff. at lower conc.}} \quad (26)$$

where $\Delta G'_{\text{dil}}$ ($\Delta X'_{\text{dil}}$) are in kJ mol^{-1}. The standard state is a hypothetical 1 (m or M) solution that corresponds to the limiting condition implied by Henry's Law, in which the solute has an activity of one (unity). The numerator inside the logarithm in Equation (26) thus equals 1. If the concentration of the solute in the denominator is sufficiently low, its activity coefficient also approaches 1 and the activity can be taken to be equal to the concentration. At 0.001 m(M),

$$\Delta G'_{\text{dil}}\ (\Delta X'_{\text{dil}}) = -2.479 \ln \frac{1}{0.001} = -17.12 \text{ kJ mol}^{-1} \quad (27)$$

$$\Delta_f G^{o'}_B\ (\Delta_f X^{o'}_B) = [\Delta_f G^{o'}\ (\Delta_f X^{o'}) + (-17.12)] \text{ kJ mol}^{-1}. \quad (28)$$

Values of thermodynamic properties relative to this review are given in Table 3. For $\Delta_f G^{o'}$ and $\Delta_f X^{o'}$, these are listed as a hypothetical 1 mol quantity at unit activity, and for $\Delta_f H^{o'}$, a hypothetical 1 mol quantity at infinite dilution. The value of $\Delta_f S^{o'}$, per se, has no physical meaning, but when multiplied by the T for which it was determined, it becomes a quantity of energy. This last quantity is not listed in tables of thermodynamic properties, but can be calculated using the Gibbs equation and values for $\Delta_f G^{o'}$ and $\Delta_f H^{o'}$. Values for the biological standard states of $\Delta_f G^{o'}_B$ and $\Delta_f H^{o'}_B$ are for 1 mol at a practical concentration of 0.001 m. This means that values for $\Delta_f S^{o'}_B$ are also at that same quantity and concentration. These values are not found in standard tables, but only in the following publications by Battley (2006, 2011b). As seen in Table 3, values for $\Delta_f G^{o'}_B$ and $\Delta_f S^{o'}_B$ are different from those for $\Delta_f G^{o'}$ and $\Delta_f S^{o'}$. However, this is not true for $\Delta_f H^{o'}_B$ and $\Delta_f H^{o'}$, which have the same values because of equating a real concentration of 0.001 M with that of infinite dilution (Pitzer and Brewer 1961; Battley 1987:374–422).

With respect to chemical thermodynamics as applied to the growth of microorganisms, the standard $\Delta_r G^{o'}_B$ and $\Delta_r X^{o'}_B$ values represent nonthermal, chemical potential energy that becomes converted into heat during the course of a reaction or growth process. They cannot be directly measured, but their values can be calculated using energy changes for which values for the term in the center of Equation (28) can be determined from standard state values from the literature. That $\Delta_r G^{o'}_B$ and $\Delta_r X^{o'}_B$ each represent one of two sources of heat during a growth process is illustrated by the following two equations:

$$\Delta_r H^{o'}_B = \Delta_r G^{o'}_B + T\Delta_r S^{o'}_B \quad (29)$$

$$\Delta_r H^{o'}_B = \Delta_r X^{o'}_B + \Delta_r Q^{o'}_{\text{ab, B}}. \quad (30)$$

The symbol $\Delta_r H^{o'}_B$ is common to both equations. It is the total heat of reaction, of which there is only one for a given reaction or process, regardless of whether the Gibbs energy equation or the Battley free energy equation is used to calculate nonthermal energy changes. Empirically, for a growth process to proceed, the signs of $\Delta_r G^{o'}_B$ and $\Delta_r X^{o'}_B$ must be negative, showing that nonthermal energy is lost to the system (i.e., the aqueous, nutrient environment) at constant T and P. It is this energy change that actually "drives" a growth process. Changes in $T\Delta_r S^{o'}_B$ or $\Delta_r Q^{o'}_{\text{ab,B}}$ have nothing directly to do with "driving" a reaction or process. Except for the cells, values of $\Delta_f G^{o}_B$ and $\Delta_r X^{o'}_B$ can be obtained by adding −17.11 kJ to values of $\Delta_r G^{o'}$ and $\Delta_r X^{o'}$ obtained from the literature—see Equation (28)—as reactants and products of a growth process.

Calculating Values for Energies of Formation

Proceeding from left to right, Table 3 shows the results of all calculations necessary to go from standard state values for the thermodynamic properties of substances appearing in Table 1 to those in the biological standard state of a concentration of 0.001 mol at 298.15 K and 0.1 MPa. The thermodynamic properties of the biological standard state (Battley 2011b) used for calculating changes ac-

TABLE 4

Energy changes accompanying the growth of Saccharoymyces cerevisiae *anaerobically on glucose and aerobically on glucose, ethanol, and acetic acid, as represented by the equations in Table 1 and calculated using the data in Table 3*

System	$\Delta_r H_B^{o'}$	$\Delta_r G_B^{o'}$	$\Delta_r X_B^{o'}$	$\Delta_r Q_{ab,B}^{o'}$	$T\Delta_r S_B^{o'}$	$\Delta_r S_B^{o'}$	Free energy conservation efficiency	
							$\Delta_r G_B^{o'}$ eff.	$\Delta_r X_B^{o'}$ eff.
	(———— kJ mol^{-1} of substrate consumed————)						[(NC − Met)/NC] × 100	
Anaerobic growth on glucose								
A. Anabolism	−14.12	−11.40	−11.64	−2.48	−2.72	−0.0009	22.16%	20.16%
B. Formation of glycerol	−1.62	−23.38	−17.81	16.20	21.75	0.073		
C. Catabolism	−90.07	−175.99	−129.15	39.08	85.92	0.288		
D. Metabolism (Met)	−105.82	−210.77	−158.64	52.80	104.96	0.352		
E. Nonconservative (NC)	−138.57	−270.76	−198.70	60.13	132.19	0.443		
Aerobic growth on glucose								
A. Anabolism	−45.89	−37.05	−37.83	−8.06	−8.84	−0.030	31.23%	31.23%
B. Catabolism	−1956.64	−1962.76	−2018.43	61.98	6.32	0.021		
C. Metabolism (Met)	−2002.53	−1999.81	−2056.26	53.92	−2.52	−0.008		
D. Nonconservative (NC)	−2898.44	−2907.80	−2990.26	91.92	9.36	0.031		
Aerobic growth on ethanol								
A. Anabolism	−1.51	26.31	13.41	−14.92	−27.32	−0.127	37.98%	35.96%
B. Catabolism	−885.17	−856.45	−906.48	21.31	−29.91	−0.100		
C. Metabolism (Met)	−886.68	−830.14	−893.07	6.39	−57.73	−0.194		
D. Nonconservative (NC)	−1361.80	−1317.32	−1394.58	32.78	−44.48	−0.149		
Aerobic growth on acetic acid								
A. Anabolism	4.73	22.22	19.04	−14.31	−17.49	−0.059	34.19%	33.73%
B. Catabolism	−608.64	−586.74	−611.98	3.34	−21.89	−0.132		
C. Metabolism (Met)	−603.90	−564.52	−592.93	−10.97	−39.38	−0.264		
D. Nonconservative (NC)	−889.82	−857.81	−894.71	−4.89	−32.01	−0.107		

(Table from Battley 2011b; reproduced with permission from Springer Science + Business Media.)

companying microbial growth are $\Delta_f H_B^{o'}$, $\Delta_f X_B^{o'}$, $\Delta_f G_B^{o'}$, $\Delta_f Q_{ab,B}^{o'}$, and $T\Delta_f S_B^{o'}$.

Calculations of Changes in Thermodynamic Properties Accompanying Microbial Growth

enthalpy changes, $\Delta_r H_B^{o'}$

Battley's (1960b) original measurements of the heat of growth of *S. cerevisiae* were conducted in a calorimeter that was primitive by present-day standards. It was necessary to build a microcalorimeter, since none was commercially available. After measuring the observed heat of growth, this was corrected with respect to side reactions represented by seven parameters related to the reactants and products of the growth process. The end result was −96 kJ mol^{-1} of glucose consumed anaerobically, and − 2004, −854, and −677 kJ mol^{-1} of glucose, ethanol, and acetic acid consumed aerobically, respectively. It eventually became evident that all the tedium and difficulty of manometry and direct calorimetry could be largely avoided. This was by using available electrons with which, using growth-process equations and the known thermodynamic properties of the reactants and products, the changes in thermodynamic properties (including $\Delta_r H_B^{o'}$) could be calculated rather than measured. This involves writing growth-process equations, just as with direct calorimetry, but also assuming,

for whatever reason, the working status of all the substances entering or leaving growing cells. As described here, the assumption is that all substances enter and leave the cells in an uncharged state which, for dissociable reactants and products, exists in a state of equilibrium at a pH of 6.5. This procedure may arouse criticism. On the other hand, it simplifies the writing of growth-process equations and the calculation of changes in thermodynamic properties. The same equations written with the P and S sources represented as ions can be found in Battley (2009). Doing this does not appreciably change the thermodynamic values of metabolism for the growth processes. Values for the indirect calorimetry of *S. cerevisiae* using growth-process equations from Table 1 are given in Table 4. The calculated values for the heats of growth are −105.82 kJ mol^{-1} of glucose fermented, and − 2002.53, −886.68, and −603.90 kJ mol^{-1} of glucose, ethanol, and acetic acid oxidized, respectively (Battley 2011b). Compared to the values obtained with direct calorimetry (Battley 1960b), the differences are 9.28% for growth anaerobically on glucose, and −0.07%, 3.68%, and −12.10% for growth aerobically on glucose, ethanol, and acetic acid. The comparison with aerobic glucose growth is excellent, but that with the other three substrates is not. However, the *average* comparison gives only a 0.8% difference, and a plot of the heat of growth against the calculated, nonconservative enthalpy change for all four growth processes gives a slope of 0.712 for direct calorimetry as compared with 0.696 for indirect calorimetry as shown in Table 4. The opportunity has never returned for the author to repeat the direct calorimetry experiments.

Nonthermal energy changes, $\Delta_r G_B^{o'}$ and $\Delta_r X_B^{o'}$

These are described in Table 4, and represent the quantity of nonthermal, chemical energy that is converted into heat during the process of microbial growth, as represented by the metabolic growth-process equations in Table 1. These values cannot be measured directly and, as seen in Table 4, differ depending on whether the Gibbs or the Battley equation is used. This is because $\Delta_r H_B^{o'}$ has the same value for both equations.

Entropy and absorbed thermal energy changes

Absorbed thermal energy, Q_{ab}^{o}, is a lot easier to understand than S^o because it involves a simple integration of C_p data as a function of T using Equation (20), rather than as a function of $ln\ T$ using Equation (15). It is thus possible to visualize Q_{ab}^{o} directly as a quantity of thermal energy required to be absorbed to raise the T of a mass of substance from $0K$ to T/K. For solid substances, when calculated as Q_{ab}^{o} directly, the value for the absorbed thermal energy is half of that calculated as TS^o (Battley 2002). These values are different for liquids and gases because of phase changes. But, there cannot be more than one value for the quantity of thermal energy required to raise the temperature of a given substance from $0K$ to 298.15 K at constant P. If there are two values, such as $TS^{o'}{}_B$ and $Q_{ab,B}^{o'}$, the question then becomes which value is correct and does this matter?

Equations (15) and (20) are both theoretically correct, but the present author has always been puzzled as to why the C_p data are integrated against $ln\ T$ using Equation (15) followed by multiplication by the T at which S^o was determined, rather than a direct integration against T using Equation (20). Equation (20) is so much more direct and parsimonious.

From what has been written previously, there are two final equations that must be considered further. These are:

$$\Delta_r G_B^{o'} = \Delta_r H_B^{o'} - T\Delta_r S_B^{o'} \text{ (Gibbs equation)} \quad (29)$$

$$\Delta_r X_B^{o'} = \Delta_r H_B^{o'} - \Delta_r Q_{ab,B}^{o'} \text{ (Battley equation).} \quad (30)$$

From Table 4, it is apparent that except for values of $\Delta_r H_B^{o'}$, the calculated changes in thermodynamic properties using Equation (29) are different from those using Equation (30). This does not imply, per se, that one equation is more correct than the other.

There is a choice as to whether to integrate the C_p data in Equations (15) and (20) directly or using a logarithm.

SUMMARY

A number of precautions and details have been presented during the research reviewed here. These are factual or procedural, are related to acquiring reproducible growth-process equations, and in general work as described. These precautions are essential, but most are not amenable to discussion. One procedure does represent an important development that may or may not be correct. This is the establishment of a so-called "biological standard state." The conventional thermodynamic properties for substances in solution have the units of a hypothetical 1 mol at unit activity for $\Delta_f G^{o'}$, a hypothetical 1 mol at infinite dilution for $\Delta_f H^{o'}$, and for $T\Delta_f S^{o'}$, a concentration, not zero, that is somewhere in between. To the present author, the situation of having these three thermodynamic properties for a given substance represented by three different concentrations is incorrect. Data are available for substances at infinite dilution, and these values are not appreciably different from those at 0.001 *m* (Pitzer and Brewer 1961). Values of $\Delta_f H^{o'}$ at 0.001 M can be taken to be the same as those at 0.001 *m*. Values of $\Delta_f G^{o'}$ can be calculated for 0.001 M. Values of $T\Delta_f S^{o'}$ will then be related to the same concentration, making all three thermodynamic properties equivalent in this respect, and can be represented by the subscript "$_B$" (for biological). Is doing this a correct procedure? It may be, but it can still be incorrect. Chemical free energy and the kinetic free energy of dilution are two different kinds of nonthermal, free energy. One unanswered question is still, to what extent does the kinetic, free energy of dilution of a solute have anything to do with its chemical free energy? The present author has made the assumption that these two free energies are physically separate.

Anabolic equations represent the formation of cells (growth) or other organic products of anabolism. The aerobic/anaerobic ratio of the quantity of glucose used for cellular synthesis as shown by the two anabolic equations in Table 1 is (0.325/0.100) = 3.25. The ($\Delta_r G_B^{o'}$ aerobic/$\Delta_r G_B^{o'}$ anaerobic) ratio is (−37.05/−11.40) = 3.25. The same ratio for $\Delta_r X_B^{o'}$ is (−37.83/11.64) = 3.25. This is an excellent agreement of chemistry and energy for both $\Delta_r G_B^{o'}$ and $\Delta_r X_B^{o'}$ values in the two systems where the substrate, glucose, is the same, but where catabolism of the same substrate is completely different (i.e., aerobic versus anaerobic).

Table 1 shows that except for the nature of the substrates, the equations for anabolism are identical for all four systems in that the number of AE in the anabolic substrate is the same as the number in the cellular mass that is grown. This shows clearly that what determines the efficiency of growth is the number of AE transferred from the substrate to the cells. It is this relationship of available electrons to the quantity of substance in which they are incorporated that provides the simplest means for constructing equations representing microbial growth (Battley 1987: 322).

Battley (2002) calculated the ratio of $\Delta_r X^o/\Delta_r G^o$ for the combustion of organic solids of biological importance to get an average value of 1.03 ± 0.01, n = 17. For the combustion of liquids of biological importance, the average value of this ratio was 1.04 ± 0.01, n = 5. However, for six bacterial fermentations, averages were meaningless, because the range of $\Delta_r X^o/\Delta_r G^o$ values was 0.20 to 1.14. These values are all for substances in their standard states. Values obtained with substances in their biological standard states are better illustrated in Table 4. This shows that the values of $\Delta_r X_B^{o'}$ and $\Delta_r G_B^{o'}$ for any given process are all different from one another, and can often vary widely. The largest of these differences is with the anaerobic metabolism of glucose, where $\Delta_r G_B^{o'}$ is 24.73 percent greater than $\Delta_r X_B^{o'}$, relative to $\Delta_r G_B^{o'}$. Nevertheless, using Hess's Law and data from Tables 2, 3, and 4, the addition of the metabolic values for $\Delta_r X_B^{o'}$ and $\Delta_r G_B^{o'}$ in Equations 1, 2, and 3 in Table 2 give values for Equation 4 of − 2053.54 and − 1998.74 kJ mol^{-1} for $\Delta_r X_B^{o'}$ and $\Delta_r G_B^{o'}$, respectively. These can be compared with the values for the experimental aerobic

growth process represented by Equation 5 in Table 2 which, from Table 4, are − 2056.26 and − 1999.81 kJ mol^{-1} for $\Delta_r X_B^{o'}$ and $\Delta_r G_B^{o'}$, respectively. The agreement between the chemical representations of the growth processes shown in Table 2 and the energy exchanges shown in Table 4 are a good indication that these two methods can provide data that are internally consistent.

Table 4 also shows the free energy conservation efficiencies within cells and other organic products. For anaerobic growth, the ratio of these efficiencies is $\Delta_r G_B^{o'} / \Delta_r X_B^{o'} =$ 22.16%/20.16%, including glycerol. The efficiency of energy conservation with respect to $\Delta_r X_B^{o'}$ is thus = 9.02% less than that of $\Delta_r G_B^{o'}$. For aerobic growth on glucose, ethanol, and acetic acid, these values are 0.00%, 5.32%, and 1.34%. All of the above indicates that with respect to several comparisons, the values for free energy changes accompanying the growth (metabolism) of *S. cerevisiae* anaerobically on glucose, and aerobically on glucose, ethanol, and acetic acid do not differ on average more than 4% (but with a wide range) when calculated using the Gibbs (Equation 29) or Battley (Equation 30) free energy equations. Further, the heat exchanges represented by $T\Delta_r S_B^{o'}$ and $\Delta_r Q_{ab,B}^{o'}$ are only a small part of $\Delta_r H_B^{o'}$. The question then arises as to whether there is any particular advantage to using either of these. Percent differences with respect to the free energy exchanges accompanying the Gibbs and the Battley equations are shown in Table 2.

The solution to the question of whether to prefer Equation (29) or Equation (30) is as much logical as thermodynamic. Both equations purport to calculate the same thing. But even though the two equations give different results, a comparison of the data alone does not tell us whether one is more "correct" than the other. Even if Battley's equation could be shown to be more correct, is there any point in changing from the use of the Gibbs equation to the Battley equation when the former has been considered so satisfactory for so many people for so many decades?

As described above, although the form of Equations (29) and (30) is the same, the value for the free energy changes is calculated in two different ways, giving two different values. The most direct and simple method is to use Equation (30). This is likely to be more correct, according to the principle of parsimony. Equation (30) is far easier to comprehend in that the symbol S is not present in the equation and its interpretation is not necessary. The existence and an exchange of absorbed thermal energy as a part of $\Delta_r H_B^{o'}$ is made apparent by the symbol $\Delta_r Q_{ab,B}^{o'}$, as shown in Equation (30).

At constant T and P, an exchange of absorbed thermal energy is completely passive, and does not accomplish anything. It does not do work and does not drive a reaction or process. There is no transfer of electrons accompanying the gain or loss of thermal energy, per se. All of this is not apparent in Equation (29) where the symbol $\Delta_r S_B^{o'}$ appears, since it is inapparent what S represents other than being a mathematical function. Multiplying $\Delta_r S_B^{o'}$ by the temperature, T, at which S was determined gives a value, $T\Delta_r S_B^{o'}$, with the units of energy, but which as Table 4 shows, can have a value significantly different from $\Delta_r Q_{ab,B}^{o'}$.

Entropy has a numerical value, and the statement by Clausius asserts that during a passage from an initial to a final state this value tends to increase. This may happen with a change in the entropy of the world or the universe (it would be hard to measure). However, with respect to a localized entropy change such as that in Table 4, the sign of the entropy change ($\Delta_r S_B^{o'}$) can be either positive or negative.

Perhaps the most important aspect of these differences is that with Equation (30) the classic concept of Clausius entropy (whatever that is) disappears from consideration, leaving a greatly simplified idea as to the energy exchange accompanying microbial growth processes and by chemical reactions in general.

What is described in this review suggests that the Battley free energy equation, Equation (30), appears to be more accurate and

understandable than the Gibbs free energy equation, Equation (29), for the calculation of free energy changes and for the thermodynamic description of microbial growth. What is most convincing is that Equation (29) has no true physical meaning, whereas Equation (30) does because Equation (20) is used to calculate correctly the quantity of absorbed thermal energy required for a given substance to achieve a given temperature.

ACKNOWLEDGMENTS

I wish to express my deep appreciation and thanks to the friends, colleagues, members of the International Society for Biological Calorimetry, and especially Richard Kemp, Ingolf Lamprecht, Urs von Stockar, and Nieves Barros for their interest in the work described in this review. I also wish to thank Daniel Dykhuizen for his concern with the elements of style in writing a review article and his enthusiasm during its construction.

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Handling Editor: Daniel E. Dykhuizen