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A comparison of energy changes accompanying growth processes by *Saccharomyces cerevisiae*

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Abstract Calculations are made using the equations $\Delta_r G = \Delta_r H - T\Delta_r S$ and $\Delta_r X = \Delta_r H - \Delta_r Q$ where $\Delta_r X$ represents the free energy change when the exchange of absorbed thermal energy with the environment is represented by $\Delta_r Q$. The symbol Q has traditionally represented absorbed heat. However, here it is used specifically to represent the enthalpy listed in tabulations of thermodynamic properties as $(H_T - H_0)$ at $T = 298.15$ K, the reason being that for a given substance TS equals 2.0 Q for solid substances, with the difference being greater for liquids, and especially gases. Since $\Delta_r H$ can be measured, and is tangibly the same no matter what thermodynamics are used to describe a reaction equation, a change in the absorbed heat of a biochemical growth process system as represented by either $\Delta_r Q$ or $T\Delta_r S$ would be expected to result in a different calculated value for the free energy change. Calculations of changes in thermodynamic properties are made which accompany anabolism; the formation of anabolic, organic by-products; catabolism; metabolism; and their respective non-conservative reactions; for the growth of *Saccharomyces cerevisiae* using four growth process systems. The result is that there is only about a 1% difference in the average quantity of free energy conserved during growth using either Eq. 1 or 2. This is because although values of $T\Delta_r S$ and $\Delta_r Q$ can be markedly different when compared to one another, these differences are small when compared to the value for $\Delta_r G$ or $\Delta_r X$.

Keywords Microbial growth process equations · Free energy changes accompanying growth · Changes in the absorbed heat of growth · Changes in entropy during growth · Free energy efficiency of growth

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Introduction

Saccharomyces cerevisiae is a convenient microorganism with which to study the thermodynamics of microbial growth. It is easy to detect contamination in its cultures. It can be grown on a defined medium containing minerals, trace elements, a single source of carbon and energy, and a few vitamins which do not significantly contribute to the mass of cells that are grown. And, it can be grown aerobically and anaerobically, both of these conditions being found in the natural environment. The basic thermodynamics of all microorganisms (cells) can be expected to be similar, although the chemical requirements for growth can range from very simple to very complex. These latter are difficult to study, and at least initially, experimental inferences are better drawn from simpler systems. This applies to the growth of *S. cerevisiae* anaerobically on glucose, and aerobically on glucose and on acetic acid, both substances of which are slightly less reduced, and to its growth on ethanol, which is more reduced, than the fabric of the cells. Provided that appropriate methods for growth and analysis of the cells can be devised, growth process equations can be written. As with any oxidation–reduction reaction, for these equations the empirical composition and quantities of all available-electron containing products must be known. For heterotrophic microorganisms the initial state includes a source of organic carbon and hydrogen, nitrogen, phosphorous, sulfur, and oxygen (for aerobic cultures) plus a few living cells. These elements plus potassium, which is not a part of the covalent structures of cells, comprise about 99% of the dry mass of

most cells. The final state includes the cells that have been grown; any organic products of the growth process, and inorganic substances such as carbon dioxide and water that are also products of metabolism. To exclude the formation of storage products the cells must be grown at μ_{max} using low concentrations of substrate. Storage products are actually internal substrates, and not a true part of the fabric of the cells [1]. If the substrate is provided in small quantities and has become completely consumed, the quantity of this used for metabolism is already known. Then, knowing the composition of the cells with respect to C, H, O, N, P, and S, expressed as a unit-carbon formula, the composition of any other organic products of the growth process, and whether growth is aerobic or anaerobic, the coefficients for all the terms in a growth process equation can be determined by difference (i.e., anything on the right side of the equation has to have originated from something on the left side). It is convenient to represent the composition of the cells with a "unit-carbon" formula (UCF), in which the quantities of all the constituent elements is relative to that of carbon, which is taken as 1 [2]. Far more detailed descriptions related to constructing growth process equations can be found in [3, Chapters 8 and 10; 4, Chapter 5; 5, 6].

Microorganisms such as *S. cerevisiae* are also convenient to work with experimentally because they grow well at μ_{max} at temperatures between 25 and 30 K. This temperature range is commonly found in temperate zones under natural conditions, and is close to 25 °C (298.15 K) at which tables of thermodynamic properties are conventionally tabulated. In experimental work of this kind, we are lucky to make biological measurements that have a standard error of less than ±2%, and temperatures of 5 °C greater than 25 are not likely to make it inadvisable to use thermodynamic property values determined for 25 °C. This is purely a matter of convenience.

One of the problems attending work with growth process equations is achieving accurate analyses. The coefficients of growth process equations are not small whole numbers, and errors in analyses cannot be easily adjusted unless you know what they are, which you often do not. According to Hess's Law, it should be possible to add the growth process equation representing the growth of *S. cerevisiae* anaerobically on glucose and similar equations representing growth aerobically on the organic products of anaerobic growth on glucose, to equal the growth process equation representing aerobic growth on glucose. When this addition is made, the sum of the various reactions and processes results in an equation that is very nearly exactly the same as that directly determined for aerobic growth on glucose [3, p. 264]. The quantity of electrons conserved in biomass is nearly the same for both anaerobic growth on glucose and aerobic growth on acetic acid. This is because the number of AE available from the respective substrate is the same for both growth processes. For whatever reason, the glycerol formed during anaerobic growth on glucose is not used for aerobic growth, but instead becomes completely oxidized. There is no similar test that can be used to assess the accuracy of the process equation representing the growth of *S. cerevisiae* on acetic acid. However, it can be reasonably assumed that the composition of the cellular fabric is the same when *S. cerevisiae* is grown exponentially under all four substrate conditions (i.e., glucose anaerobically and aerobically, acetic acid, and ethanol) using the precautions described above. When a graph of the number of available electrons (AE) conserved in the biomass of the cells grown during these processes is plotted against the number of AE per mol of substrate used during growth, a straight line is obtained represented by the equation [4, Fig. 2, p. 255]

$$\mathrm{AE_{cells}} = 0.331\mathrm{AE_{substrate}} - 0.072 \quad (1)$$

The correlation coefficient for the points in Eq. 1 is 0.997 ($n = 4$). Although four data points is not a statistical number, observations such as those above give credence to the accuracy of these particular growth process equations.

Until the present time all studies on the thermodynamics of microbial growth, whether from an academic or an engineering orientation, have made use of the Gibbs free energy equation

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

There has recently been one exception to this practice. This is a series of studies by the present author investigating whether $T\Delta S$ is the correct value representing the exchange of absorbed thermal energy taking place during a growth process [5–10]. After an initial error in identifying Q with TS [7, 9] the conclusion has been that it is not the correct value, resulting in a proposal that a more correct equation should be written as [9]

$$\Delta_r X = \Delta_r H - \Delta_r Q \quad (3)$$

where $\Delta_r X$ represents the free energy change when the exchange of absorbed thermal energy with the environment is represented by $\Delta_r Q$. The symbol Q has traditionally represented absorbed heat. However, here it is used specifically to represent the enthalpy listed in tabulations of thermodynamic properties as $(H_T - H_0)$ at $T = 298.15$ K. The reason for using Eq. 3 is that for a given substance TS equals $2Q$ for solid substances, with the difference being greater for liquids, and especially gases.

Entropy is a function of state. The entropy of a given substance can be determined using the following equation [11, p. 2–10],

$$(S_{298.15} - S_0) = S_T(S_0 = 0) = \int_0^{298.15} C_p \mathrm{d}\ \log T + \sum \Delta_{trs} H_T / T_{trs} \quad (4)$$

where the second term on the right represents transitions and phase changes, if any. The practical units of S are J K^{-1} unit-mass^{-1}. These are not the units of energy, which are J unit-mass^{-1}. Entropy is not a physical entity. As pointed out by Klotz in 1963, S is essentially a mathematical function [12]. It is only when S is multiplied by the T for which S is determined, that TS acquires the units of energy. Even though the symbol S remains in the symbol TS, its function as a mathematical function vanishes and S no longer exists as such. TS represents absorbed thermal energy. This is because of the way in which S is determined using Eq. 4 involving C_p data. TS also 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 is also absorbed heat, since it is no longer in transit across a boundary). But, although we can make this calculation, is the quantity of absorbed thermal energy calculated to be absorbed, that which is truly absorbed by a given substance for it to exist at the T at which S was determined?

There is another function of state called the "enthalpy", represented by the symbol $(H_T - H_0)$, which can be determined for a given substance using the following equation [11, p. 2–11].

$$(H_{298.15} - H_0) = H_T(H_0 = 0) = Q_T = \int_0^{298.15} C_p \mathrm{d}T + \sum \Delta_{trs} H_T \quad (5)$$

The second term on the right in Eq. 5 represents transitions and phase changes, if any. Equations 4 and 5 are identical in that the same C_p data are used with respect to a given substance. However, in Eq. 4 these are integrated against log T. Since logarithms have no units, those of S remain those of C_p (J K^{-1} unit-mass^{-1}), which are not those of energy. S must be multiplied by the T at which S was determined, arriving at the units of energy. Equation 5 represents a non-logarithmic integration of the same C_p data as in Eq. 4, whereupon the T units cancel out and the result is units of energy directly.

Equation 4 times the T for which S was determined (i.e., TS) and Eq. 5 both purport to yield a number representing the quantity of thermal energy required for the temperature of a given substance to be raised from 0/K to T/K. The problem is that each equation provides a different answer. For solid substances, $TS = 2Q$. For liquid and gaseous substances, the difference is varied and greater.

Studies have been made recently of the sources of thermal energy exchange accompanying both the anabolism [5] and the catabolism [6] of *S. cerevisiae* anaerobically on glucose, and aerobically on glucose, ethanol, and acetic acid. Because living cells are comprised mostly of water, the thermodynamic properties of cellular ions would be expected to be nearly the same whether inside or outside the cell wall or cell membrane, and would thus cancel out of a growth process equation. The purpose of this study is to combine the catabolic and anabolic equations comprising the above growth processes and to compare the thermodynamics of the resulting metabolic processes using Eqs. 2 and 3.

Methods

Methods of construction of growth process equations

These can be found in [2–6]. The anabolic and catabolic equations for growth anaerobically on glucose, and aerobically on glucose, ethanol, and acetic acid to form metabolic equations were taken from [5] and [6]. All reactants and products of these processes except for the cells and for water are represented as being dissolved in aqueous solution in the "biological" standard state at a concentration of 0.001 M [4, Table 2, p. 236]. Cells can be considered to be slightly hydrated but otherwise insoluble precipitates, designated by the suffix "cells." All polyvalent substances and ammonia are represented in a non-ionic form because of making the vastly simplifying assumption that these pass through a biological membrane most easily in an uncharged form. This may not always be true, but will not appreciably affect the thermodynamics.

Thermodynamic properties

All cells live in an aqueous environment so that, except for the cells, all thermodynamic properties used must be those relative to the aqueous standard state. The conventional concentration standard for thermodynamic calculations is that of a hypothetical 1 mol of solute dissolved in solution. For $\Delta_f H^{o\prime}$ it is a hypothetical 1 mol of substance in solution at infinite dilution. For $\Delta_f G^{o\prime}$ it is a hypothetical 1 mol of substance in solution at unit activity. For $\Delta_f S^{o\prime}$ it is a hypothetical 1 mol of substance in solution having a finite concentration that is not zero but also not at unit activity, i.e., somewhere in between (which does not provide a lot of information). It is apparent that these three thermodynamic properties apply to three different conditions for the same substance all at the same time, i.e.; infinite dilution, unit activity, and somewhere in between. This has always been something of a mystery.

In general, cells do not live in environments containing molal (or molar) quantities of solute, because of the

unfavorable osmotic conditions this would impose (although there are many exceptions). An infinitely dilute solution is an abstraction that does not practically exist. Because real $\Delta_f H^{o\prime}$ values asymptotically approach those at infinite dilution, it was suggested in Pitzer and Brewer's 2nd revision of Lewis and Randall's *Thermodynamics* [13] that at a concentration of about 0.001 molal, solute molecules or ions are sufficiently separated from one another that further dilution has little effect on intermolecule or interion interaction. It was proposed by the present author that this could well be the basis for a "biological standard state." as described more completely in [4, p. 241], which would more closely describe the natural conditions in which microorganisms find themselves (with exceptions). This practice will be used in this present study, and indicated in Table 1 using the subscript "$_B$". The methods of calculating values for this biological standard state are as follows.

Calculating values of thermodynamic properties used in this study as found in Table 1

The symbol "*M*" (for 'molar') will be used instead of "*m*" (for 'molal') to designate concentrations because at 0.001 M the two concentrations are nearly identical.

For a given substance

A. $\Delta_f H_B^{o\prime}$: This value is identical to that of $\Delta_f H^{o\prime}$ as found in the literature. It has the same value in both Eqs. 2 and 3.

B. $\Delta_f G_B^{o\prime}$: Look up the value for $\Delta_f G^{o\prime}$ as found in the literature with respect to an aqueous solution. Add to this -17.12 kJ mol^{-1} because of the dilution of the substance from hypothetical 1 mol at unit activity to 0.001 m, at which dilution concentrations closely approach activities [4, p. 241]. This is $\Delta_f G_B^{o\prime}$.

C.
$$T\Delta_f S_B^{o\prime} = \Delta_f H_B^{o\prime} - \Delta_f G_B^{o\prime} \quad (6)$$

D. $\Delta_f Q_B^{o\prime}$: Look up the value for the enthalpy ($H_T - H_0$) as found in the literature. Change this designation to Q_{ab}^{o} to represent more clearly that this is absorbed heat. Then calculate $\Delta_f Q_{ab}^{o}$ using the following equation.
$$\Delta_f Q_{ab}^{o} = Q_{ab}^{o} - \sum Q_{ab,atoms}^{o} \quad (7)$$
where $Q_{ab,atoms}^{o}$ represents the enthalpies of the individual atoms comprising a substance. Calculate $\Delta_f Q_{ab,B}^{o\prime}$ using F below.

E. $\Delta_f X_B^{o\prime}$: Look up $\Delta_f H^{o}$ as found in the literature. Then use the equation:

Table 1 Thermodynamic properties of substances of biological importance at 298.15 K and 0.1 MPa

Substance		$\Delta_f H_B^{o\prime}$/kJ mol^{-1}	$\Delta_f X_B^{o\prime}$/kJ mol^{-1}	$\Delta_f G_B^{o\prime}$/kJ mol^{-1}	$\Delta_f Q_{ab,B}^{o\prime}$/kJ mol^{-1}	$T\Delta_f S_B^{o\prime}$/kJ mol^{-1}
Elements[a]						
Oxygen (g)	O_2	−12.09	−0.80	−0.80	−11.29	−11.29
Inorganic[a]						
Ammonia (g)	NH_3	−80.29	−91.17	−43.69	10.88	−36.60
Carbon dioxide (g)	CO_2	−413.80	−420.06	−403.13	6.26	−10.67
Phosphoric acid (cr)	H_3PO_4	−1288.34	−1257.12	−1159.66	−31.22	−128.68
Sulfuric acid (l)	H_2SO_4	−909.27	−747.64	−761.65	−161.63	−147.62
Water (l)	H_2O	−285.83	−281.42	−237.18	−4.41	−48.65
Organic[a]						
Acetic acid (l)	$C_2H_4O_2$	−485.26	−506.65	−421.21	21.39	−64.05
Ethanol (l)	C_2H_6O	−287.02	−287.40	−198.08	0.38	−88.94
α-D-Glucose (cr)	$C_6H_{12}O_6$	−1263.07	−1216.22	−931.66	−46.85	−331.41
Glycerol (cr)	$C_3H_8O_3$	−676.55	−681.40	−514.60	4.85	−161.95
Biological[b]						
Yeast cells (dried)	UCF	−125.40	−116.05	−80.27	−9.35	−43.13

The values are calculated for a quantity of one mol at a concentration of 0.001 M (please see text)

[a] Thermodynamic properties except for those of $\Delta_f S_B^{o\prime}$ were taken from [6], from which values of $\Delta_f S_B^{o\prime}$ were calculated. Thermodynamic properties of glycerol except for $\Delta_f S_B^{o\prime}$ were taken from [14], from which $\Delta_f S_B^{o\prime}$ was calculated. The value $\Delta_f Q_{ab}^{o\prime}$ for glycerol was taken from [15], from which the value of $\Delta_f Q_{ab,B}^{o\prime}$ was calculated

[b] The structure of yeast cells is considered to be that of a slightly hydrated precipitate, so they do not have an aqueous concentration, nor do they have a standard state. The data presented above were taken from Table 3 in [6], with the correction that $\Delta_f G_{cells}^{o}$ should be −80.27 kJ rather than −82.68 kJ

$$\Delta_f X^o = \Delta_f H^o - \Delta_f Q^o_{ab} \quad (8)$$

Make the assumption that the difference between $\Delta_f X^o$ and $\Delta_f X^{o\prime}$ is the same as that between $\Delta_f G^o$ and $\Delta_f G^{o\prime}$. These last values can usually be found in the literature. Add this difference to the value for $\Delta_f X^o$ to give $\Delta_f X^{o\prime}$.

Then, as for B, above, add −17.12 kJ to $\Delta_f X^{o\prime}$ to give $\Delta_f X^{o\prime}_B$.

F.

$$\Delta_f Q^{o\prime}_{ab,B} = \Delta_f H^{o\prime}_B - \Delta_f X^{o\prime}_B \quad (9)$$

G. as compared with

$$T\Delta_f S^{o\prime}_B = \Delta_f H^{o\prime}_B - \Delta_f G^{o\prime}_B \quad (6)$$

A comparison of the changes in thermodynamic properties accompanying the growth process equations shown in Table 2 is shown in Table 3.

Results

The total quantity of free energy initially available to a growth process is that theoretically available from the complete conversion of a substrate to the products of a growth process other than the cells plus other organic substances, as shown in Table 2. Such reactions are said to be "non-conservative [3, p. 269]." The difference between the quantity of free energy change accompanying a non-conservative reaction and that accompanying its respective metabolism represents the quantity of free energy remaining within the system, bound in cellular substance or organic products of the growth process *other* than those that are products of the non-conservative reaction. Thus, free energy conservation during anaerobic growth would be confined to the cells and the glycerol (here a mixture of glycerol and α-glycerophosphoric acid, but represented as glycerol), but not the ethanol, since this is a product of the non-conservative reaction. For the three systems represented here growing aerobically, the conserved free energy resides only within the cells.

The data in Table 3 indicate that the $\Delta_r G^{o\prime}_B$ and $\Delta_r X^{o\prime}_B$ efficiencies of total free energy conservation during metabolism ($\Delta_r G^{o\prime}_B$ eff. or $\Delta_r X^{o\prime}_B$ eff.) for the four growth process systems studied differ by an average of only 1.12%. On average, this value is probably less than the standard errors of making the measurements required for this kind of research. This indicates that there is little difference in using either Eq. 2 or 3 to calculate free energy changes accompanying growth processes. Table 3 shows that in all four systems the $\Delta_r G^{o\prime}_B$ eff. is larger or equal to the $\Delta_r X^{o\prime}_B$ eff., but what this means is uncertain in that the mass of cellular substance formed during a given growth process is the same whether Eq. 2 or 3 is used to calculate the accompanying free energy change. Therefore, the free energy conservation efficiency differences observed can be due only to the methods of calculation. One might not have expected these efficiencies to be so close. Metabolic differences between $\Delta_r X^{o\prime}_B$ and $\Delta_r G^{o\prime}_B$ can be calculated as follows.

$$\text{Percent difference of } \Delta_r X^{o\prime}_B \text{ with respect to } \Delta_r G^{o\prime}_B = [(\Delta_r X^{o\prime}_B - \Delta_r G^{o\prime}_B) / \Delta_r X^{o\prime}_B] \times 100 \quad (10)$$

For aerobic growth on glucose, ethanol, and acetic acid these differences are 2.74, 7.05, and 4.79%. For anaerobic growth on glucose this difference is −32.86%, i.e., the loss of free energy appears to be −32.86% greater for $\Delta_r G^{o\prime}_B$ than it is for $\Delta_r X^{o\prime}_B$ and to represent a relatively large loss of free energy compared to aerobic growth.

It should be possible to add individually the values of $\Delta_r G^{o\prime}_B$ and $\Delta_r X^{o\prime}_B$ for the anaerobic growth of *S. cerevisiae* to that for the aerobic growth on the ethanol produced during the anaerobic growth process, and that for the total oxidation of the glycerol produced during anaerobic growth, to equal that for the aerobic growth on glucose. That this can be done is shown in Table 4. The results are remarkably good, and far easier to understand than those in Table 3.

Discussion

From Table 3, it is interesting to note the considerable differences in the values of $\Delta_r G^{o\prime}_B$ and $\Delta_r X^{o\prime}_B$ with respect to anabolism and catabolism in the aerobic glucose, ethanol, and acetic acid growth process systems, and in these plus the formation of glycerol during anaerobic growth. Biologically, reaction systems like catabolism and anabolism physically do not exist separately, but they do exist conceptually. Why these calculable differences exist can only be due to the mathematics involved in calculating them, in that it is physically impossible for different changes in free energy to apply to the same reaction system.

Such differences are even more pronounced with respect to $T\Delta_r S^{o\prime}_B$ and $\Delta_r Q^{o\prime}_{ab,B}$, both of which represent a change in the absorbed thermal energy of their respective reaction systems. Changes in absorbed thermal energy are more easily visualized than free energy changes, and in the case of $\Delta_r Q^{o\prime}_{ab,B}$ can be measured directly. But again, it is impossible for a given reaction system to have two values for changes in physically absorbed thermal energy. On the other hand, the ability to make a direct measurement of the heat absorbed (Q, see Eq. 5) by the components of a reaction system gives some credence to the changes in Q (i.e., $\Delta_r Q^{o\prime}_{ab,B}$) and to any calculations using them.

Table 2 Equations representing the growth of *S. 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

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

Non-conservative

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

Aerobic growth on glucose

Anabolism

$0.325\ C_6H_{12}O_6(aq) + 0.302NH_3(aq) + 0.023\ H_3PO_4(aq) + 0.006\ H_2SO_4(aq) \rightarrow 1.914\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) + 0.036\ CO_2(aq) + 0.926\ H_2O(l)$

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

$C_6H_{12}O_6(aq) + 0.302NH_3(aq) + 4.050\ O_2(aq) + 0.023\ H_3PO_4(aq) + 0.006\ H_2SO_4(aq) \rightarrow 1.914\ CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}(cells) + 4.086\ CO_2(aq) + 4.976\ H_2O(l)$

Non-conservative

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

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

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

Non-conservative

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

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

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

Non-conservative

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

All anabolic equations are from Table 1 in [5]; all catabolic equations are from Table 2 in [6]; the equation representing the formation of glycerol was taken from Table 2 in [4, Table 2, p. 236]

In spite of the differences in values for the changes in thermodynamic properties of the systems shown in Table 3 calculated using Eqs. 2 and 3, the values for conserved free energy ($\Delta_f G_B^{o\prime}$ eff. and $\Delta_f X_B^{o\prime}$ eff.) in Table 3, are remarkably close with an average difference of 1.12% for the four growth processes studied and an outside range of about 2%.

Table 3 Energy changes accompanying the growth of *S. cerevisiae* anaerobically on glucose and aerobically on glucose, ethanol, and acetic acid, as represented by the equations in Table 1 and the free energy conserved in anabolism as a percentage of potential non-conservative free energy available

System	$\Delta_r H_B^{o\prime a}$	$\Delta_r G_B^{o\prime a}$	$T\Delta_r S_B^{o\prime a}$	$\Delta_r X_B^{o\prime a}$	$\Delta_r Q_{ab,B}^{o\prime\ a}$	$\Delta_r S_B^{o\prime a}$	Free energy conservation efficiency (%)	
							$\Delta_r G_B^{o\prime}$ eff. [(NC − Met)/NC] × 100	$\Delta_r X_B^{o\prime}$ eff.
Anaerobic growth on glucose								
A. Anabolism	−14.12	−11.40	−2.72	−11.64	−2.48	−0.0009	22.16	20.16
B. Formation of glycerol	−1.62	−23.38	21.75	−17.81	16.20	0.073		
C. Catabolism	−90.07	−175.99	85.92	−129.15	39.08	0.288		
D. Metabolism (Met)	−105.82	−210.77	104.96	−158.64	52.80	0.352		
E. Non-conservative (NC)	− 138.57	−270.76	132.19	−198.70	60.13	0.443		
Aerobic growth on glucose								
A. Anabolism	−45.89	−37.05	−8.84	−37.83	−8.06	−0.030	31.23	31.23
B. Catabolism	−1956.64	−1962.76	6.32	−2018.43	61.98	0.021		
C. Metabolism (Met)	−2002.53	−1999.81	−2.52	−2056.26	53.92	−0.008		
D. Non-conservative (NC)	−2898.44	−2907.80	9.36	−2990.26	91.92	0.031		
Aerobic growth on ethanol								
A. Anabolism	−1.51	26.31	−27.82	13.41	−14.92	−0.127	37.98	35.96
B. Catabolism	−885.17	−856.45	−29.91	−906.48	21.31	−0.100		
C. Metabolism (Met)	−886.68	−830.14	−57.73	−893.07	6.39	−0.194		
D. Non-conservative (NC)	− 1361.80	−1317.32	−44.48	−1394.58	32.78	−0.149		
Aerobic growth on acetic acid								
A. Anabolism	4.73	22.22	−17.49	19.04	−14.31	−0.059	34.19	33.73
B. Catabolism	−608.64	−586.74	−21.89	−611.98	3.34	−0.132		
C. Metabolism (Met)	−603.90	−564.52	−39.38	−592.93	−10.97	−0.264		
D. Non-conservative (NC)	−889.82	−857.81	−32.01	−894.71	−4.89	−0.107		

Basic data for the above Table were taken from [5, 6]

[a] kJ mol^{-1} of substrate consumed

Table 4 Addition of the free energy change accompanying the growth of *S. cerevisiae* anaerobically on glucose; that accompanying growth on the ethanol produced during anaerobic growth on glucose; and that accompanying the total oxidation of the glycerol produced during anaerobic growth on glucose, to equal that accompanying aerobic growth on glucose; with respect to both $\Delta_r G_B^{o\prime}$ and $\Delta_r X_B^{o\prime}$

Growth process metabolism	$\Delta_r G_B^{o\prime}$/kJ	$\Delta_r X_B^{o\prime}$/kJ
Anaerobic growth on glucose	−210.77	−158.64
Aerobic growth on ethanol (×1.3, see Table 1)	−1079.18	−1160.99
Total oxidation of glycerol (×0.432, see Table 1)	−708.79	−733.91
Total free energy change	−1998.74	−2053.84
Aerobic growth on glucose (see Table 3)	−1999.81	−2056.26

Data taken from Table 3, except for the oxidation of glycerol, which was calculated from data in Table 2

These differences may lie within the errors of the experimental methods, although statistics using only four growth process systems is questionable. The data suggest that either Eq. 2 or 3 is equally appropriate for use in studying the thermodynamics of the metabolism of growth process systems. The data in Table 3 also suggest that entropy changes do not always "tend towards a Maximum" (Clausius). The NC $T\Delta_r S_B^{o\prime}$ changes for growth on ethanol and acetic acid are negative, whereas those for glucose are positive. It is true that the statement of Clausius is generally taken to apply to the "universe", but it is apparent that it need not apply to small parts of the universe. Thinking about the "universe", just as thinking about "infinite dilution", is often not a good basis for practical thought.

It still remains that Eq. 3 is more desirable than Eq. 2 in that there is no entropy term involved and therefore Eq. 3 is more understandable (see also reference 18 in [16]). Entropy is not a physical entity that can be seen, sensed, or measured physically. It exists as a result of the application of Eq. 4, but becomes transformed into a quantity of thermal energy when multiplied by the temperature for which the entropy value is determined (*TS*). However, although the symbol *S* remains in the term *TS*, whatever

S represents vanishes completely in that *TS* becomes a quantity of absorbed thermal energy.

Conclusions

With respect to the four growth process systems studied here, the data show that Eqs. 2 and 3 can be used to equal advantage for all practical purposes when calculating metabolic, thermodynamic changes in that the free energy conservation efficiencies differ by an average of only about 1%. Two of the four non-conservative entropy changes are positive, whereas two are negative with respect to the four systems. It is thus difficult to agree with Clausius that, at least with respect to the systems studied here, the entropy of a system always increases as it proceeds spontaneously from its initial to its final state. Equation 3 is certainly more comprehensible than Eq. 2, and in this respect can be used to a greater advantage.

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