

ORIGINAL ARTICLE

The energetics of anabolism in natural settings

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The environmental conditions that describe an ecosystem define the amount of energy available to the resident organisms and the amount of energy required to build biomass. Here, we quantify the amount of energy required to make biomass as a function of temperature, pressure, redox state, the sources of C, N and S, cell mass and the time that an organism requires to double or replace its biomass. Specifically, these energetics are calculated from 0 to 125 °C, 0.1 to 500 MPa and –0.38 to +0.86 V using CO₂, acetate or CH₄ for C, NO₃⁻ or NH₄⁺ for N and SO₄²⁻ or HS⁻ for S. The amounts of energy associated with synthesizing the biomolecules that make up a cell, which varies over 39 kJ (g cell)⁻¹, are then used to compute energy-based yield coefficients for a vast range of environmental conditions. Taken together, environmental variables and the range of cell sizes leads to a ~4 orders of magnitude difference between the number of microbial cells that can be made from a Joule of Gibbs energy under the most (5.06 × 10¹¹ cells J⁻¹) and least (5.21 × 10⁷ cells J⁻¹) ideal conditions. When doubling/replacement time is taken into account, the range of anabolism energies can expand even further.

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Introduction

In an effort to better understand the origin, evolution and extent of life, significant effort has gone into finding the limits of temperature, pressure, pH, salinity and other compositional and physical variables that define habitability (Rothschild and Mancinelli, 2001; Pikuta *et al.*, 2007; Canganella and Wiegel, 2011; Colwell and D'Hondt, 2013; Picard and Daniel, 2013; Takai *et al.*, 2014). However, one variable in particular—energy—has received much less attention (Amend and Teske, 2005; Shock and Holland, 2007; Hoehler and Jørgensen, 2013; LaRowe and Amend, 2015a, b). Virtually every aspect of microbial behavior requires energy, including growth, nutrient uptake, motility, excretion of biomolecules, synthesis of external structures such as stalks, sheaths, tubes and extracellular polymeric saccharides, and changes in stored nutrients. Although some of these fall within the definition of maintenance functions (van Bodegom, 2007), many, such as growth, require the synthesis of biomolecules. Consequently, the energy required to make biomolecules is a fundamental limit to the habitability of an environment.

Because the Gibbs energy of all chemical reactions is a function of temperature, pressure and

composition, determining the energetic limits of anabolism will differ depending on these environmental variables (for example, Amend and Shock, 1998, 2000; McCollom and Amend, 2005; LaRowe and Regnier, 2008; Amend and McCollom, 2009; Amend *et al.*, 2011, 2013; LaRowe and Amend, 2014; Osburn *et al.*, 2014; Teske *et al.*, 2014; Price *et al.*, 2015). In addition, the amount of energy that it takes to produce and sustain a given number of microorganisms depends on the mass of cells and the relative proportion of biomolecule types that make up these cells. As a result, the energy boundary for life is a moving one that changes depending on a large number of factors. The purpose of this study is to quantify the influence of environmental variables on the amount of energy it takes to synthesize microbial biomass.

Our approach considers a great range of growth conditions to account for the fact that life has been found in hydrothermal systems, deep marine and freshwater sediment, hydrocarbon reservoirs, unconsolidated sedimentary rock, shale, sandstone, fractured granite, deep aquifers, caves, mines, paleosols, aquitards, permafrost and more (Amy and Haldeman, 1997; Fredrickson and Fletcher, 2001; Steven *et al.*, 2006; Orcutt *et al.*, 2011; Strapóć *et al.*, 2011; Edwards *et al.*, 2012). Other attempts to account for the energy required to make biomass rely upon laboratory experiments that are designed to promote growth, conditions that are rarely encountered in nature (for a review see, LaRowe and Amend, 2015a). In addition, numerous energy-based models developed to predict biomass yields have limited applicability because of being restricted

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to standard and/or reference states (for reviews see, Heijnen and van Dijken, 1992; LaRowe and Amend, 2015a). Most of these models fix biomass yield coefficients, which lead to predictions of equal biomass production (for the same synthesis reactions) under very different environmental conditions. The methods adopted in this study explicitly quantify the role that physiochemical variables have on biomass synthesis.

Materials and methods

Computational techniques

The Gibbs energy required to make a microbial cell, ΔG_{cell} , can be calculated with

$$\Delta G_{cell} = \Delta G_{synth} + \Delta G_{main} \quad (1)$$

where ΔG_{synth} represents the energy required to synthesize biomolecules and ΔG_{main} stands for the energy that is used for all other functions in support of viability, the so-called maintenance energy. ΔG_{synth} accounts for the reaction energetics of monomer biomolecules (for example, amino acids, nucleotides, saccharides) from simple starting materials (for example, CO_2 , acetate, CH_4 , SO_4^{2-} , HS^- , NH_4^+ , NO_3^-) and the energetics associated with polymerization of these monomers into biomacromolecules (for example, proteins, DNA, polysaccharides). Although the types and amounts of biomolecules found in microorganisms can vary depending on growth conditions (see below), the most detailed analysis of the biochemical composition of a microbial cell, *Escherichia coli* (Battley, 1991), is used here as a model for microbial composition. Briefly, Battley (1991) reports the proportions of individual amino acids, nucleotides, lipids, saccharides, amines and other compounds, mostly from *E. coli* B/r in exponential phase growth on glucose minimal media corrected for the presence of glycogen. These biomolecules and the proportions in which they are found in *E. coli*, as modified by McCollom and Amend (2005), are listed in Table 1. Values of ΔG_{synth} are calculated using

$$\Delta G_{synth} = \sum_i (\Delta G_{r,i}) \cdot \left(\frac{\text{mol } i}{\text{g cell}} \right) + \Delta G_{poly} \quad (2)$$

where $\Delta G_{r,i}$ stands for the Gibbs energy of the reaction describing the synthesis of the *i*th biomolecule, $\left(\frac{\text{mol } i}{\text{g cell}} \right)$ represents the number of moles of biomolecule *i* in a dry gram of bacterial cells (Table 1) and ΔG_{poly} denotes the Gibbs energy required to polymerize biomolecules into their respective biomacromolecules.

The energy calculations are normalized per dry gram of biomass, J (g cell)^{-1} , because cell masses and the proportions of biomacromolecules in them vary considerably (see below). The energy required to form all the biomacromolecules that constitute a dry gram of microbial cells, ΔG_{poly} , was calculated using the energy that it takes to form all of the polypeptides

in a dry gram of cells (Amend *et al.*, 2013) and the following assumptions. Since 55% of the dry weight of a bacterial cell (*E. coli*) is protein and nearly all of the biomolecules listed in Table 1 are thought to be in biopolymers such as RNA, DNA, lipopolysaccharides and peptidoglycan (Battley, 1991), it was assumed that the energy required to produce polypeptides from amino acids is proportional to that for polymerizing the other biomacromolecules. This is based on the fact that biopolymerization reactions are dehydration reactions, the energetics of which should not change much between one set of biomolecules and another. That is, if 191 J is required to polymerize all of the amino acids into protein in a dry gram of cells at 25 °C and 0.1 MPa (Amend *et al.*, 2013), protein comprises 55% of the dry weight of a single cell and 45% of the rest of the dry weight is other polymers, then $156 \text{ J (g cells)}^{-1} = (45/55) \times 191 \text{ J (g cells)}^{-1}$ is required to polymerize all the RNA, DNA, lipopolysaccharides and peptidoglycan in a cell.

Values of $\Delta G_{r,i}$ and ΔG_{poly} were calculated as a function of temperature and pressure using

$$\left\{ \begin{array}{l} \Delta G_{r,i} \\ \Delta G_{poly} \end{array} \right\} = \Delta G_r^0 + RT \ln Q_r \quad (3)$$

where ΔG_r^0 and Q_r refer to the standard molal Gibbs energy and the reaction quotient of the indicated reaction, respectively, *R* represents the gas constant and *T* denotes temperature in Kelvin. Values of ΔG_r^0 are calculated as a function of temperature and pressure using the revised-HKF equations of state (Helgeson *et al.*, 1981; Tanger and Helgeson, 1988; Shock *et al.*, 1992), the SUPCRT92 software package (Johnson *et al.*, 1992) and thermodynamic data taken from Shock and Helgeson (1988); Shock *et al.* (1989); Shock and Helgeson (1990); Shock (1995); Sverjensky *et al.* (1997); Amend and Helgeson (1997a, b); Amend and Plyasunov (2001); Schulte *et al.* (2001); Dick *et al.* (2006); LaRowe and Helgeson (2006) and LaRowe and Dick (2012). Values of Q_r are calculated using

$$Q_r = \prod_i a_i^{v_i} \quad (4)$$

where a_i stands for the activity of the *i*th species and v_i corresponds to the stoichiometric coefficient of the *i*th species in the reaction of interest. The thermodynamic properties and revised-HKF equation of state parameters for some of the biomolecules considered in this study were estimated using existing algorithms (McCollom and Amend, 2005; Amend and McCollom, 2009). These algorithms are reproduced in the Supplementary Information along with a table of the resulting standard molal thermodynamic properties of these compounds at 25 °C and 0.1 MPa and the revised-HKF equation of state parameters required to calculate these properties as a function of temperature and pressure (Supplementary Table S1).

Table 1 Concentrations of biomolecules used to represent biomass in the current study

Biomolecules considered	Formula	mol (g cell) ⁻¹	NOSC	MW
<i>Amino acids</i>				
Alanine	C ₃ H ₇ NO ₂	5.43E-04	0.00	89.0935
Arginine	C ₆ H ₁₃ N ₄ O ₂ ⁺	2.81E-04	0.33	174.2017
Asparagine	C ₄ H ₈ N ₂ O ₃	2.29E-04	1.00	132.1184
Aspartate	C ₄ H ₆ NO ₄ ⁻	2.29E-04	1.00	133.1032
Cysteine	C ₃ H ₇ NO ₂ S	8.70E-05	0.67	121.159
Glutamate	C ₅ H ₈ NO ₄ ⁻	2.78E-04	0.40	147.1299
Glutamine	C ₅ H ₁₀ N ₂ O ₃	2.50E-04	0.40	146.1451
Glycine	C ₂ H ₅ NO ₂	5.82E-04	1.00	75.0669
Histidine	C ₆ H ₉ N ₃ O ₂	9.00E-05	0.67	155.1552
Isoleucine	C ₆ H ₁₃ NO ₂	2.76E-04	-1.00	131.1736
Leucine	C ₆ H ₁₃ NO ₂	4.28E-04	-1.00	131.1736
Lysine	C ₆ H ₁₃ N ₂ O ₂ ⁺	3.26E-04	-0.67	146.1882
Methionine	C ₅ H ₁₁ NO ₂ S	1.46E-04	-0.40	149.2124
Phenylalanine	C ₉ H ₉ NO ₂	1.76E-04	-0.44	165.19
Proline	C ₅ H ₉ NO ₂	2.10E-04	-0.40	115.131
Serine	C ₃ H ₇ NO ₃	2.05E-04	0.67	105.093
Threonine	C ₄ H ₉ NO ₃	2.41E-04	0.00	119.1197
Tryptophan	C ₁₁ H ₁₂ N ₂ O ₂	5.40E-05	-0.18	204.2262
Tyrosine	C ₉ H ₁₁ NO ₃	1.31E-04	-0.22	181.1894
Valine	C ₅ H ₁₁ NO ₂	4.02E-04	-0.80	117.1469
<i>Amines</i>				
Ethanolamine	C ₂ H ₇ NO	1.31E-04	-1.00	61.08
Diaminopimelic acid	C ₇ H ₁₄ N ₂ O ₄	2.79E-05	0.00	190.2
Putrescine	C ₄ H ₁₂ N ₂	3.40E-05	-1.50	88.15
Spermidine	C ₇ H ₁₉ N ₃	6.88E-06	-1.43	145.25
<i>Nucleotides</i>				
AMP ²⁻	C ₁₀ H ₁₂ N ₅ O ₇ P ²⁻	1.65E-04	1.00	345.2055
GMP ²⁻	C ₁₀ H ₁₂ N ₅ O ₈ P ²⁻	1.26E-04	1.20	361.2049
CMP ²⁻	C ₉ H ₁₂ N ₃ O ₈ P ²⁻	2.03E-04	0.67	321.1808
UMP ²⁻	C ₉ H ₁₁ N ₂ O ₆ P ²⁻	1.36E-04	0.67	322.1655
dAMP ²⁻	C ₁₀ H ₁₂ N ₅ O ₆ P ²⁻	2.46E-05	0.80	329.2061
dGMP ²⁻	C ₁₀ H ₁₂ N ₅ O ₇ P ²⁻	2.54E-05	1.00	345.2055
dCMP ²⁻	C ₉ H ₁₂ N ₃ O ₇ P ²⁻	2.54E-05	0.44	305.1814
dTMP ²⁻	C ₁₀ H ₁₄ N ₂ O ₆ P ²⁻	2.46E-05	0.20	289.2018
<i>Fatty acids</i>				
Palmitate	C ₁₆ H ₃₁ O ₂ ⁻	1.12E-04	-1.75	255.4241
Oleate	C ₁₈ H ₃₅ O ₂ ⁻	6.22E-05	-1.78	281.4614
Palmitoleate	C ₁₆ H ₂₉ O ₂ ⁻	8.56E-05	-1.63	253.408
Myristate	C ₁₄ H ₂₇ O ₂ ⁻	1.67E-05	-1.71	227.37
β-Hydroxymyristate	C ₁₄ H ₂₇ O ₃ ⁻	3.37E-05	-1.57	243.3703
<i>Saccharides and more</i>				
Glycerol	C ₃ H ₈ O ₃	1.61E-04	-0.67	92.09382
Glucose	C ₆ H ₁₂ O ₆	2.50E-05	0.00	180.1559
Heptose	C ₇ H ₁₄ O ₇	2.52E-05	0.00	210.182
Galactose	C ₆ H ₁₂ O ₆	8.33E-06	0.00	180.1559
Rhamnose	C ₆ H ₁₂ O ₅	8.53E-06	-0.33	164.1565
Glucoseamine	C ₆ H ₁₃ NO ₅	1.67E-05	0.00	179.17
N-acetylglucosamine	C ₈ H ₁₅ NO ₆	3.62E-05	0.00	221.208
N-acetylmuramic acid	C ₁₁ H ₁₇ NO ₈	2.76E-05	0.18	293.2705

Abbreviations: NOSC, nominal oxidation state of carbon; MW, molecular weight.

The maintenance energy in Equation (1) is calculated using

$$\Delta G_{main} = t_{d/r} \cdot P_d \quad (5)$$

where $t_{d/r}$ refers to the amount of time that a cell or population takes to double or replace all of its biomass and P_d stands for the maintenance power of the cell or population in units of Watts (for example, W cell⁻¹ or W cm⁻³). To compare properly the

energetics of maintenance, ΔG_{main} , and biomass synthesis, ΔG_{synth} , the values of ΔG_{synth} must be converted from units of kJ per gram of cell biomass (kJ (g cell)⁻¹) into the same units of ΔG_{main} , J cell⁻¹. This requires knowing the number of cells in a dry gram of cellular biomass and the mass of an individual dry cell. In the literature, masses of microorganisms are typically given in units of fg C cell⁻¹, but to transform carbon masses into total cell

masses, the stoichiometry of the major elements in a cell must be known:

$$\text{cellmass} = \left(\frac{\text{g C}}{\text{cell}}\right) \left(\frac{\text{g bio}}{\text{mol bio}}\right) \left(\frac{\text{mol bio}}{\text{mol C}}\right) \left(\frac{\text{mol C}}{\text{g C}}\right) \quad (6)$$

It should be noted that the values of the mass of carbon in microbial cells, $\left(\frac{\text{g C}}{\text{cell}}\right)$, and the stoichiometry of biomass, $\left(\frac{\text{g bio}}{\text{mol bio}}\right)$ and $\left(\frac{\text{mol bio}}{\text{mol C}}\right)$, required to evaluate Equation (6) differ significantly as a function of several variables such as species type, growth stage, nutrient limitation and habitat (see Supplementary Information). A review of the literature reveals that cell carbon masses in bacteria range from at least 3 to 310 fg C cell⁻¹, while cellular stoichiometries result in average nominal carbon oxidation states, NOSC, from +0.89 to -0.45 (LaRowe and Van Cappellen, 2011). Using the lowest estimated cell carbon mass, 3 fg C cell⁻¹ (Vrede et al., 2002), the stoichiometry of *E. coli* given by Rittman and McCarty (2001) and Equation (6), the total dry mass of a very small cell would be 5.7 fg. On the other hand, using the largest estimated carbon mass, 310 fg C cell⁻¹ (Bratbak, 1985), the cellular stoichiometry given by Redfield *et al.* (1963) and Equation (6), the resulting dry mass of a large bacterial cell is 865 fg. Here, we use a dry cell mass of 122 fg cell⁻¹. This corresponds to a stoichiometry C₅H₇NO₂ (Rittman and McCarty, 2001) and a cell carbon mass of 65 fg C cell⁻¹ (Hoehler and Jørgensen, 2013), which reflect a weighted average of those reported in the literature.

In cases where values of ΔG_{synth} are negative, an infinite number of biomolecules could be made. To make sense of these negative values of ΔG_{synth} and the large range of cell masses that seem to exist, energy-based yield coefficients have been developed, which are consistent with a recently proposed bioenergetic model that posits that any power available above and beyond the power required for maintenance has the potential to become new biomass (LaRowe and Amend, 2015a). The yield coefficient, which relates this excess power to biomass production, is based on the energy required to synthesize biomolecules, and calculated using

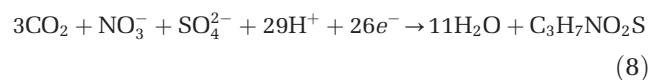
$$Y = \left(\frac{1}{\Delta G_{\text{synth}}}\right) \left(\frac{\text{cell}}{\text{g cell}}\right) \quad (7)$$

Range of environmental conditions

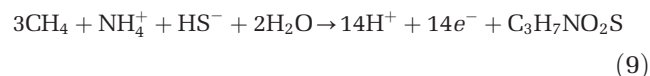
In this study, we evaluate the effects of temperature (0–125 °C), pressure (0.1–500 MPa) and composition on the energetics of biomolecular synthesis. We note that composition can influence the energetics of anabolism in a number of different ways, with the identities of the C, N and S sources having the greatest impact (see McCollom and Amend (2005) and below). We consider the full range of oxidation states in sources of C (CO₂, acetate, CH₄), N (NO₃⁻ and

NH₄⁺) and S (SO₄²⁻ and HS⁻). Because phosphorous is typically found only in the +5 oxidation state, we use only phosphate in the reactions describing biomass synthesis. To focus on the effects of differing redox states on the energetics of anabolism, we fixed the activities of the C-, N-, S- and P-source compounds (as well as all other reactants and products, see Table 2).

The prevailing oxidation state of the host environment also dictates anabolism energetics. For instance, in a relatively oxidizing environment, such as surface waters, any organism that fixes CO₂, NO₃⁻ and SO₄²⁻ into biomass must acquire a sufficient stream of electrons to reduce these compounds to the common oxidation states in biomolecules (-3 for N, -2 for S, between +2 and -2 for C). On the other end of the redox spectrum, organisms that fix CH₄, NH₄⁺ and HS⁻ into biomass would not have to reduce their N and S sources, and would have to partially oxidize their carbon source. Let us examine these two scenarios with the synthesis of the amino-acid cysteine (C₃H₇NO₂S), in Reaction (8) from the most oxidized and in Reaction (9) from the most reduced set of precursor compounds:



and



Note that the free electrons in these half-reactions are on opposite sides in Reactions (8) and (9).

The quantitative impact of the redox state of the environment on the energetics of electron transfer reactions (including biomass synthesis) is commonly assessed using the activity of electrons (a_{e^-}), even though there are no free, hydrated electrons in solution (Truesdell, 1968). Values of a_{e^-} are directly related to the redox potential of an environment, typically reported as E_h (mV), through a version of

Table 2 Activities of reactants and products used to calculate values of the reaction quotient, Q (Equation (4)), related to ΔG_{synth}

Species	$\log a$
CO ₂	-3
CH ₃ COO ⁻	-5
CH ₄	-6
NO ₃ ⁻	-5
NH ₄ ⁺	-6
SO ₄ ²⁻	-3
HS ⁻	-6
HPO ₄ ²⁻	-5
N ₂	-3.3
H ₂ O	0
H ⁺	-7
Biomolecule	-9

the Nernst equation,

$$pe = \frac{F \cdot E_h}{2.303RT} \quad (10)$$

where $pe = -\log a_{e^-}$, F stands for the Faraday constant, and R and T are as defined above. The range of E_h values considered in this study is +0.858 V to -0.384 V, corresponding to the most oxidizing and reducing natural settings in which microorganisms have been found—adjusted to pH 7 because the energetics of the biomolecule synthesis reactions have been calculated for pH 7. More specifically, +0.858 mV corresponds to O₂-saturated fresh water at 0.01 °C and pH 7. The most reducing value, -0.384 V, has been converted from an ORP measurement of -0.656 V at 25 °C and pH 11.6 taken at the Cedars alkaline spring in northern California, United States of America (Morrill *et al.*, 2013). It should be noted that for a given concentration of O_{2(aq)}, calculated values of E_h are a function of pH and temperature. For example, if the O_{2(aq)} concentration in freshwater is at the saturation limit but in a pH 1 fluid (instead of pH 7), then the value of E_h at 0.01 °C would be +1.18 V (instead of +0.858). Furthermore, it should be noted that other redox state scales, such as those using the fugacities of H₂ and O₂, can be easily converted to values of pe using Equation (10) and the thermodynamic properties of the appropriate half-reactions (see McCollom and Amend, 2005; Dick, 2009; Dick and Shock, 2011).

Results and discussion

Synthesis energy, ΔG_{synth}

Values of ΔG_{synth} associated with the synthesis of 1 g of microbial biomass are shown in Figure 1 as a function of temperature under very oxidizing (+0.858 V, red lines) and reducing (-0.384 V, blue lines) conditions. The different panels reflect different sources of C, N and S. It can be seen quantitatively how different combinations of redox state and biomolecular precursor compounds influence the energetics of growth. It should be emphasized that values of ΔG_{synth} are the sum of the 46 monomer synthesis reactions (Table 1) in the proportions that they exist in a bacterial cell. Values of $\Delta G_{r,i}$ are positive (endergonic) for some and negative (exergonic) for others. That is, just because the overall value of ΔG_{synth} is negative does not mean that no energy is required to make the biomolecules that constitute a cell. Additionally, it cannot be said with certainty whether or not some of the exergonic biosynthetic reactions considered here can be coupled to endergonic ones. To minimize our assumptions, we think of exergonic anabolic reactions simply as decreasing the cost of biosynthesis and freeing up energy for those that are endergonic.

Our results show that values of ΔG_{synth} depend strongly on the combination of the redox state of the precursor compounds and that of the environment,

and weakly on temperature. Overall, values of ΔG_{synth} vary by 39 kJ (g cell)⁻¹ with the most energy-demanding conditions occurring when the environment is oxidizing and CO₂, NO₃⁻ and SO₄²⁻ are the precursor compounds (+25 (g cell)⁻¹) and the most conducive when CH₄, NH₄⁺ and HS⁻ are coupled with oxidizing conditions (-14 kJ (g cell)⁻¹). When CO₂ is the carbon source, the amount of energy required to build a gram of cells is larger under oxidizing than under reducing conditions (Figures 1a and b). However, this difference in ΔG_{synth} in Figures 1a and b shrinks when NO₃⁻ and SO₄²⁻ are replaced by NH₄⁺ and HS⁻, respectively. When acetate provides C, the same trend is observed as when CO₂ is the carbon source, but the gap between the oxidized and reduced scenarios is much smaller, particularly when NH₄⁺ and HS⁻ supply N and S (Figures 1c and d). Opposite of CO₂ and acetate, biomass synthesis under reducing conditions requires more energy in reducing environments than in oxidizing ones when CH₄ is the carbon supply (Figures 1e and f).

The impact of pressure on ΔG_{synth} is negligible. This is illustrated in Figure 2, where acetate, NH₄⁺ and HS⁻ are the sources of C, N and S under oxidizing conditions (+0.858 V) at two pressures (P_{sat} for the red line and 0.5 GPa for the dashed black line). Note the very minor difference in ΔG_{synth} at these two pressures. It should be noted that pressure can influence other variables that in turn have an impact on ΔG_{synth} . For instance, at higher pressures, gases are generally more soluble, resulting in changes to the reaction quotient (Q) in the computation of Gibbs energies (see Equation (3)).

Although values of ΔG_{poly} are small (<0.4 kJ (g cell)⁻¹), the proportion of ΔG_{poly} to $\Delta G_{r,i}$ varies considerably. For instance, the energy required to synthesize biomolecules from CO₂, NO₃⁻ and SO₄²⁻ under oxidizing conditions at 0.01 °C (red line, Figure 1a) is about 64 times larger than the energetics of polymerizing these biomolecules at the same temperature. However, when instead, NH₄⁺ and HS⁻ are the sources of N and S and the environmental conditions are reducing (blue line, Figure 1b), values of ΔG_{poly} are essentially equal to those of $\Delta G_{r,i}$ between 25 and 40 °C. Although these examples represent the extremes of the $\Delta G_{r,i}$: ΔG_{poly} ratio considered in this study, it illustrates how variable the burden of polymerization energetics can be for biomass synthesis.

Maintenance energy, ΔG_{main}

Maintenance energy is generally defined as the energy consumed by organisms that does not result in new biomass. Values of maintenance power (P_d) vary by over five orders of magnitude depending on the metabolism, species and experimental procedure (LaRowe and Amend, 2015a)—values determined while the microorganisms of interest *were growing*. In natural settings, maintenance powers are likely

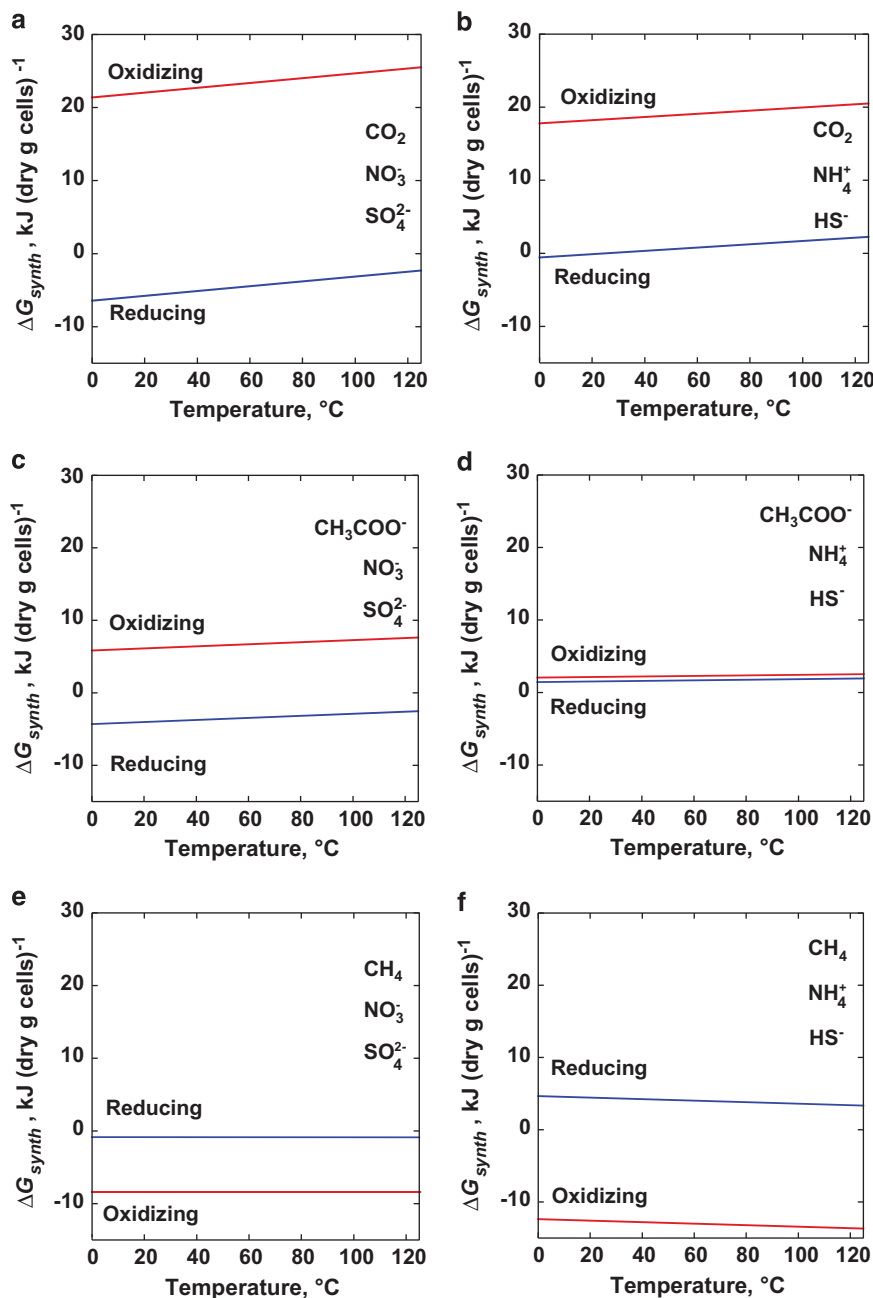


Figure 1 Gibbs energies of the synthesis, ΔG_{synth} , of all of the biomolecules listed in Table 1 in the proportions that they exist in *E. coli* from the sources of carbon, nitrogen and sulfur indicated in each panel, including the energetics of polymerization, as a function of temperature (see Equation (2)). For each combination of C, N and S sources, values of ΔG_{synth} were computed at two redox states, +0.858 V (oxidizing) and -0.384 V (reducing), as labeled in each panel.

much smaller than those measured in the laboratory, in part because laboratory growth experiments must be conducted at relatively high energy levels to be observable (Hoehler and Jørgensen, 2013). To better describe maintenance energy in natural, low-energy settings, Hoehler and Jørgensen (2013) coined the term 'basal maintenance power' for the power used by microorganisms to remain viable. Although basal maintenance power is difficult to measure directly, recent calculations suggest that it could be two orders of magnitude lower compared with the lowest

reported values of P_d in the literature (LaRowe and Amend, 2015a, b). Hence, we must first define whether organisms are growing in typical high-energy laboratory-like conditions or simply subsisting in natural low-power settings to correctly calculate values of ΔG_{main} .

The contribution of turnover/doubling time to laboratory-like and natural, low-energy maintenance powers is illustrated in Figure 3 by displaying values of ΔG_{main} , for two cases. The first case, indicated by

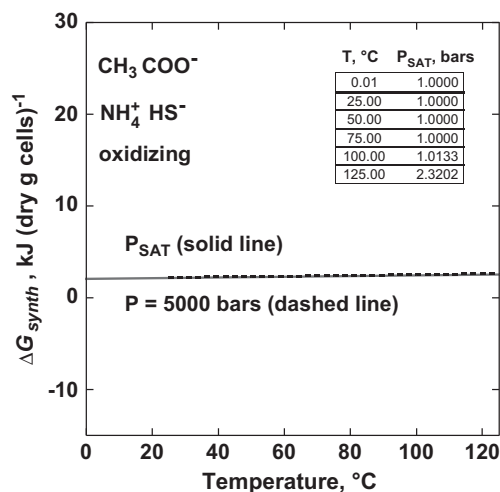


Figure 2 Comparison of the Gibbs energies of the synthesis, ΔG_{synth} , of all of the biomolecules listed in Table 1 in the proportions that they exist in *E. coli* from acetate (CH_3COO^-), NH_4^+ and HS^- at +0.858 V, including the energetics of polymerization, as a function of temperature at two extreme values of pressure, saturation pressure and 5000 bars (0.5 GPa). Saturation pressure corresponds to the minimum pressure required to prevent water from boiling. Values of ΔG_{synth} are not shown below 25 °C at 5000 bars because the equation of state for water used in these calculations is not valid under these conditions.

the solid line, refers to values of ΔG_{main} calculated using Equation (8) and a value of P_d for aerobic heterotrophy equal to $4.9 \times 10^{-14} \text{ J s}^{-1}$, the median value for this type of metabolism collected by LaRowe and Amend (2015a). As the doubling time for this aerobic heterotroph approaches 1 month, the amount of energy required for maintenance reaches $1.3 \times 10^{-7} \text{ J cell}^{-1}$, an amount of energy that exceeds the amount of energy required to synthesize all of its biomolecules from CO_2 or acetate serving as the carbon source for microorganism the size of *E. coli* (see below). For an organism that is not growing, or is doing so in a very low-energy environment, and thus subsisting at its basal P_d , far less energy goes into ΔG_{main} . This scenario is shown with the dashed line in Figure 1. A basal maintenance power of $1.9 \times 10^{-19} \text{ J s}^{-1}$ is used in conjunction with Equation (8) to generate this line, a value of P_d that is two order of magnitude lower compared with the lowest reported maintenance energy in the literature (Marschall *et al.*, 2010). An organism existing in this energy state would only use $6 \times 10^{-9} \text{ J cell}^{-1}$ over the course of 1000 years, more than two orders of magnitude less energy than the aerobic heterotroph uses for ΔG_{main} in 1 month.

Biomass yield

The number of cells that can be made per Joule of Gibbs energy, Y , are listed in Table 3 in 25 °C intervals. Values of Y were calculated using Equation (7), a cell mass of 122 fg cell^{-1} and the positive contributions to ΔG_{synth} . Only positive values of $\Delta G_{r,i}$ and ΔG_{poly} are used because at least some of the

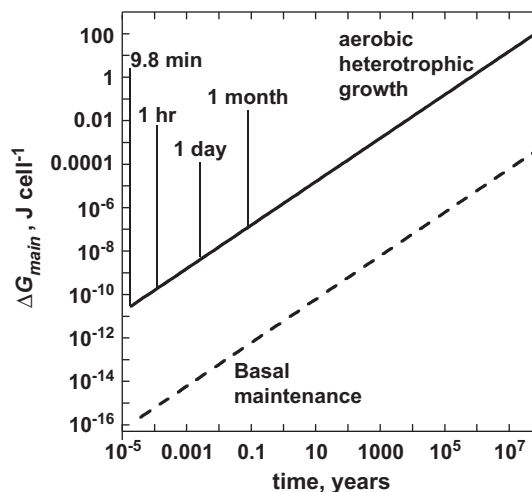


Figure 3 Maintenance energy, ΔG_{main} , as a function of time calculated using Equation (5) and a relatively high maintenance power, P_d (the median value for aerobic heterotrophy as collected by LaRowe and Amend, 2015a), of $4.9 \times 10^{-14} \text{ J s}^{-1}$ (solid line) and a basal maintenance power that is two orders of magnitude lower compared with the lowest P_d reported in the literature (Marschall *et al.*, 2010), $1.9 \times 10^{-19} \text{ J s}^{-1}$.

biomolecule synthesis reactions are exergonic, and should not be added to the positive ones since doing so effectively assumes that the exergonic synthesis reactions are coupled to the endergonic ones; there is no evidence that this is the case. The yield coefficients listed in Table 3, mirror the large-scale pattern seen in values of ΔG_{synth} in Figure 1 (see Supplementary Figure S1 for plots analogous to Figure 1). For combinations of redox states and molecular sources of C, N and S that favor biomolecule synthesis ($\Delta G_{\text{synth}} < 0$), yield coefficients are large (for example, $2.36 \times 10^{10} \text{ cells J}^{-1}$ for an oxidizing environment and CH_4 , NH_4^+ and HS^- are the sources of C, N and S), while those with positive values of ΔG_{synth} are considerably smaller (for example, $3.69 \times 10^8 \text{ cells J}^{-1}$ in an oxidizing system and CO_2 , NO_3^- and SO_4^{2-} as the sources of C, N and S). Values of Y under oxidizing conditions show little variation as a function of temperature, whereas those calculated at the lower redox state vary more. This variation is most pronounced for the two cases in which CO_2 is the source of carbon. The decreasing yield coefficients with temperature are due to the fact that many of the biomolecule synthesis reaction involving CO_2 switch from being exergonic at low temperatures to being endergonic at higher temperatures.

It should be kept in mind that the analysis presented above is based on a fixed cell size of 122 fg . In situations in which this mass does not represent the cell mass of interest, Equation (6) can be used to generate mass-appropriate yield coefficients. For instance, if the full range of cell masses computed above, 5.7 to 865 fg cell^{-1} , are combined with the largest ($22\,200 \text{ J (g cell}^{-1})$) and smallest ($347 \text{ J (g cell}^{-1})$) values of ΔG_{synth} at 25 °C, and

Table 3 Values of Y , yield coefficients for biomass synthesis (cell J^{-1}) at 0.01, 25, 50, 75, 100 and 125 °C for individual cell masses of 122 fg cell^{-1} ($65 \text{ fg C cell}^{-1}$, stoichiometry of $\text{C}_5\text{H}_7\text{NO}_2$)

	CO_2				CH_3COO^-			CH_4	
<i>Oxidizing</i>									
NO_3^- , SO_4^{2-}	3.84E+08	3.69E+08	3.56E+08	1.41E+09	1.32E+09	1.25E+09	2.46E+10	2.36E+10	2.29E+10
	3.44E+08	3.32E+08	3.22E+08	1.18E+09	1.13E+09	1.08E+09	2.22E+10	2.15E+10	2.09E+10
NH_4^+ , HS^-	4.62E+08	4.45E+08	4.36E+08	3.01E+09	2.84E+09	2.79E+09	2.46E+10	2.36E+10	2.29E+10
	4.23E+08	4.11E+08	3.99E+08	2.70E+09	2.62E+09	2.52E+09	2.22E+10	2.16E+10	2.11E+10
<i>Reducing</i>									
NO_3^- , SO_4^{2-}	2.45E+10	2.32E+10	2.18E+10	2.08E+10	1.98E+10	1.81E+10	7.13E+09	7.31E+09	7.70E+09
	2.05E+10	1.67E+10	1.34E+10	1.64E+10	1.50E+10	1.39E+10	7.98E+09	8.26E+09	8.53E+09
NH_4^+ , HS^-	1.59E+10	1.24E+10	9.64E+09	5.14E+09	4.91E+09	4.65E+09	1.75E+09	1.86E+09	1.99E+09
	6.96E+09	4.84E+09	3.53E+09	4.41E+09	4.20E+09	3.95E+09	2.13E+09	2.28E+09	2.44E+09

Oxidizing and reducing refer to +0.858 and -0.384 V .

The first three values of Y in each box correspond to yield coefficients at 0.01, 25 and 50 °C and the three values beneath these refer to 75, 100 and 125 °C.

Equation (7), values of Y would differ by a factor of nearly 9700, $5.21 \times 10^7 - 5.06 \times 10^{11} \text{ cell J}^{-1}$.

Time and energy

Although the calculations summarized above indicate that the number of microbial cells that can be made from per Joule could vary considerably, this is only the static contribution to ΔG_{cell} (Equation 1). For cells that have large maintenance powers and long doubling/replacement times, the resulting values of ΔG_{main} could dwarf those of ΔG_{synth} , which is what the yield coefficients are based on. The importance of doubling/replacement time on the energy budget of a microorganism can be illustrated by comparing values of $1/Y$ (J cell^{-1}) with ΔG_{main} . As an example, $1/Y$ for the most favorable biomolecule synthesis conditions considered in this study (CO_2 , NO_3^- , SO_4^{2-} , reducing at 25 °C) is $4.2 \times 10^{-11} \text{ J cell}^{-1}$ (assuming 122 fg cell^{-1}) and the lowest ΔG_{main} reported in the literature, $1.9 \times 10^{-17} \text{ J s}^{-1}$. If a cell characterized by these growth parameters turned over its biomass over the course of 26 days, the cumulative maintenance energy would surpass the energy required for making all of its biomolecules. For the largest maintenance power, P_d , collected in the review carried out by LaRowe and Amend (2015a), $4.7 \times 10^{-12} \text{ J s}^{-1}$, and the same value of $1/Y$, it would only take 9 s for the cumulative ΔG_{main} to surpass the energy required for making all of its biomolecules, far less time than the shortest doubling time reported in the literature (9.8 min; Eagon, 1962). If values of P_d in natural, low-energy setting are two orders of magnitude lower compared with the lowest reported in the literature, as suggested by Hoehler and Jørgensen (2013), then even a doubling/replacement time of 7.1 years would result in a value of ΔG_{main} equal to that of ΔG_{main} under the most favorable set of synthesis conditions considered in this study.

The cumulative amount of energy that would be needed to maintain a viable cell over increasingly long time spans makes the overall cost of doubling or

completely replacing cellular components a greater proportion of ΔG_{cell} . However, this assumes that the values of P_d are constant, which is unlikely. In low-energy environments, the basal maintenance power is likely quite low for long periods of time, and then during growth, it likely increases. An additional point should be made about Equation (1). Although it splits microbial energy usage into a simple dichotomy, it is not possible to completely separate the energies of biomolecule synthesis from maintenance. Not only are numerous so-called maintenance functions required to make biomolecules, such as the conversion of catabolic energy into electron donors such as NADP, and the active transport of metabolites, but some maintenance energy is expended on biomolecule synthesis and/or degradation via changes in stored polymeric carbon, extracellular secretions and the synthesis and turnover of macromolecules such as proteins and RNA (van Bodegom, 2007).

Concluding remarks

The results presented in this communication are intended to illustrate that a quantitative link can be made connecting the physiochemical properties of a natural setting (for example, temperature and composition) to the amount of biomass that exists in it. As noted above, the amount of energy available for microbial purposes is a critical component of this connection, and, as a result, the energy supply in a wide variety of settings has been determined (for example, Amend and Shock, 1998, 2000; McCollom and Amend, 2005; LaRowe and Regnier, 2008; Amend and McCollom, 2009; Amend *et al.*, 2011, 2013; LaRowe and Amend, 2014; Osburn *et al.*, 2014; Teske *et al.*, 2014; Price *et al.*, 2015). In this contribution, the microbial energy demand associated with building biomass under a broad range of conditions has been calculated. Because of the central role of oxidation–reduction processes in living organisms, this quantification is largely based

on the energy associated with changing the redox state of C, N and S source compounds into biomolecules as a function of the oxidation–reduction state of the environment. In addition, cell yield coefficients have been reported that take the same environmental conditions into account. Furthermore, the impact of doubling/replacement time on the energetics of making a cell are included in the analysis to show that this may be the largest component of the microbial energy budget in settings that are not characterized by vigorous growth. Taken together, the calculations summarized above can be used to quantify the energy limits for life, and shed light on the conditions that supported the origin of life.

Finally, the results reported in this study support the notion that, from a thermodynamic perspective, the abiotic origin of biomolecules, within the context of terrestrial planetary bodies, does not require high temperatures, but oxidation states that create favorable conditions to synthesize biomolecules from reduced and oxidized precursors depending on the redox state of precursor molecules. This notion, which is illustrated in most of the panels in Figure 1, has been a central tenet of some theories regarding the origin of life (for example, Russell and Hall, 1997; Martin and Russell 2003; Martin et al., 2008; Lane et al., 2010). The panels in this figure show that the sum of the Gibbs energies associated with synthesizing the core biomolecules that make up microorganisms can be negative, even at low temperature, for every combination of C, N and S sources except for those shown in Figure 1d. Although hydrothermal systems have been implicated in the origin of life, it is mostly the rapid mixing of fluids characterized by two very different redox states that promote the energetics of biomolecule synthesis rather than temperature, *per se*. For instance, Amend and Shock (1998) show that abiotic amino-acid synthesis is more thermodynamically favorable at 100 °C than at 2 °C, but it is the much more reducing conditions at the higher temperature that are the major driving force for biomass synthesis in this example. This is substantiated by Amend et al. (2011), who show that the optimal conditions for synthesizing biomolecules because of mixing of cold seawater and 12 hydrothermal fluids is between 11.2 and 32 °C. Similarly, LaRowe and Regnier (2008) showed that abiotic nucleotide synthesis is thermodynamically possible at low temperatures. Even the polymerization of amino acids into polypeptides is more favored at lower temperature (Amend et al. 2013 and Figure 1) compared with higher ones.

Conflict of Interest

The authors declare no conflict of interest.

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