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Thermodynamics of Microbial Growth Coupled to Metabolism of Glucose, Ethanol, Short-Chain Organic Acids, and Hydrogen▽†

Eric E. Roden[1]* and Qusheng Jin[2]

Department of Geoscience, University of Wisconsin, Madison, Wisconsin 53706,[1] and Department of Geological Sciences, University of Oregon, Eugene, Oregon 97403[2]

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A literature compilation demonstrated a linear relationship between microbial growth yield and the free energy of aerobic and anaerobic (respiratory and/or fermentative) metabolism of glucose, ethanol, formate, acetate, lactate, propionate, butyrate, and H_2. This relationship provides a means to estimate growth yields for modeling microbial redox metabolism in soil and sedimentary environments.

The growth yield coefficient, commonly expressed in units of grams (dry weight) of cells per mole of energy source utilized, is a key parameter in microbial growth models. Growth yield is related to the free energy of catabolic reactions compared to the energy required for cell synthesis (8, 9). A variety of quantitative relationships between catabolic free energy and cell growth yield have been proposed and debated (see reference 20 for details). Most of these relationships have focused on how the energy content (degree of reductance) of organic substrates influences growth yield coupled to aerobic respiration (2) or on the comparison of aerobic versus fermentative bacterial growth (9). A relationship between catabolic free energy and growth yield across the full range of electron-accepting pathways typically observed in natural systems is not yet available (10). Such a relationship would be useful for parameterization of numerical models of microbial activity in aquifers, aquatic sediments, and hydromorphic soils, where anaerobic metabolic pathways are important in driving carbon and energy flow.

This paper compiles experimental data on growth yields for the metabolism of glucose, ethanol, short-chain fatty acids (e.g., formate, acetate, lactate, propionate, and butyrate), and H_2 coupled to aerobic and anaerobic (respiratory and fermentative) metabolic pathways (see Table S1 and S2 in the supplemental material). These energy substrates are those typically involved in the degradation of polymeric organic carbon in sedimentary environments (6, 16), as well as those that may be used to promote reductive degradation or immobilization of organic and inorganic contaminants in subsurface environments (5). The suite of electron-accepting pathways represents the major redox pathways that are active in soils and sediments, including aerobic respiration, denitrification, dissimilatory nitrate reduction to ammonium, amorphous Mn(IV) and Fe(III) oxide reduction, elemental sulfur reduction, sulfate reduction, and methanogenesis (4). The results of the survey were analyzed via regression of growth yield versus estimated catabolic free energy and in terms of a standard energetics-based approach to estimation of cell growth yield (12).

Our analysis does not consider the impact of growth rate and maintenance energy on yield coefficients (11). Such effects have been documented for aerobic heterotrophic and fermentative growth (17) but not for most of the other metabolic pathways considered here. In general, yield values vary by a factor of 2 as a function of growth rate (17), and this phenomenon may account for some scatter in yield values as a function of estimated catabolic free energy (Fig. 1A). In addition, variations in growth rates under natural conditions in soils and sediment could lead to alterations in yield that would not be accounted for by our analysis of laboratory growth data. Thus, our results provide a starting point rather than the final word on the prediction of *in situ* growth yield based on free energy calculations.

Data compilation. A total of 123 experimental yield determinations comprising 38 metabolic pathways were acquired from the primary literature, review articles, and book chapters (see Tables S1 and S2 in the supplemental material). The literature search was conducted by accumulating all available growth yield data for a given energy substrate, specifying each metabolic pathway one by one. The search was exhaustive; hence, missing pathways likely represent gaps in the published record. It should be noted that many papers examined provided information on metabolic rates or qualitative descriptions of growth but not quantitative data on growth yield or data (e.g., cell counts) that could be converted to yield values. The procedures used to convert (when necessary) growth yield data to the common unit of grams of cells per mole of electron donor utilized are explained in Table S2 in the supplemental material. A cell biomass of 4×10^{-13} to 6×10^{-13} g per cell (E. Roden, unpublished data) was assumed to convert cell densities of Mn(IV)- and Fe(III)-reducing microorganisms to dry weights. A cell biomass formula of $C_5H_7O_2N$ (molecular weight, 113 g/mol) (12) was assumed when needed to convert measurements of moles of cellular carbon to cell dry weight.

Free energy calculations. The publications from which the growth yield values were obtained usually did not provide detailed information on the concentrations of reactants and products required to compute free energy change values (i.e.,

* Corresponding author. Mailing address: Department of Geology and Geophysics, 1215 W. Dayton St., Madison, WI 53706. Phone: (608) 890-0724. Fax: (608) 262-0693. E-mail: eroden@geology.wisc.edu.

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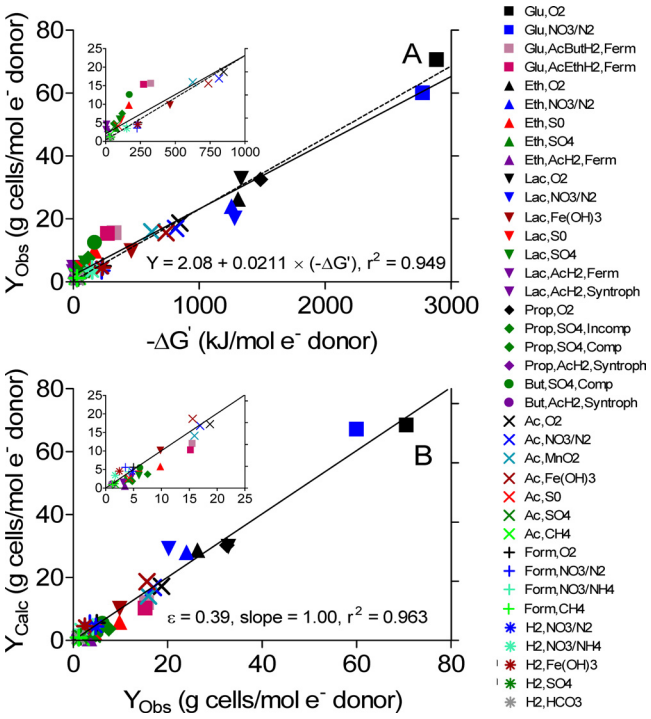

FIG. 1. (A) Experimental yield values versus estimated free energy release for various catabolic pathways involved in the metabolism of glucose and its anaerobic metabolic intermediates (see Table S1 in the supplemental material). Each data point represents either a single value or the average of 2 to 7 values (see Table S2 in the supplemental material). (B) Yield values calculated using the energetics-based approach described in reference 12 plotted versus experimental values. The solid lines in both panels represent the results of linear least-squares regression analyses; the dashed lines in panel A show a linear regression analysis of theoretical (calculated) yield values versus catabolic free energy release. The legend indicates the catabolic pathway in the format of "electron donor, electron acceptor," except in the case of methane production from a carbon substrate, which is indicated by "CH4" [abbreviations: Glu, glucose; Eth, ethanol; Lac, lactate; Prop, propionate; But, butyrate; Ac, acetate; Form, formate; H2, dissolved hydrogen; O2, oxygen; NO3, nitrate; N2, dissolved nitrogen; NH4, ammonium; MnO2, amorphous M(IV) oxide; Fe(OH)3, amorphous Fe(III) oxide; S0, elemental sulfur; SO4, sulfate; CH4, methane; HCO3, bicarbonate]; NO3/N2 and NO3/NH4 denote reduction of nitrate to N_2 and ammonium, respectively; Ferm and Syntroph refer to growth via fermentation without and with syntrophic methanogenesis, respectively; Comp and Incomp refer to complete or incomplete oxidation of propionate or butyrate coupled to sulfate reduction, respectively.

ΔG) for the various redox reactions (see Table S1 in the supplemental material). Hence, a standard set of reactant and product concentrations typical of most experimental culture systems (as discerned from available data) was adopted to estimate the free energies of reaction (see Table S3 in the supplemental material). Formation energies from references 18 and 19 were used for the calculations. In general, a 2- to 10-fold change in reactant or product concentrations had little effect (<10%) on estimated ΔG values, as is typical of such calculations (19). Testing showed that the concentration of dissolved H_2 as an end product of fermentation (ethanol, lactate, or glucose) or an intermediate during syntrophic growth (lactate, propionate, or butyrate fermentation coupled to methanogenesis) was the only case that had a dramatic effect on estimated ΔG. For experiments in which H_2 was the electron donor for growth, an aqueous concentration equivalent to that expected for a solution in equilibrium with equal volumes of 100% H_2 (0.624 mM) was assumed. For experiments where H_2 was an end product of fermentation, an aqueous concentration corresponding to a system containing a total volumetric H_2 concentration of 1 mmol liter^{-1}, partitioned between equal volumes of aqueous (20 μM) and gas phase, was assumed. For experiments where H_2 was an intermediate in syntrophic metabolism, an aqueous H_2 concentration of 1×10^{-8} M (ca. 1 Pa) was assumed based on values typically observed in such experiments (15). Amorphous Fe(III) oxide [$Fe(OH)_3$(s)] reduction was assumed to produce a mixture of magnetite [Fe_3O_4(s)] and dissolved Fe^{2+} in proportions typical of experimental observations (1, 7, 14).

Growth yield versus free energy. There was a linear correlation between growth yield and estimated catabolic reaction free energy (Fig. 1A). This result is implied in the energetics-based interpretation of microbial growth yield (12), as evidenced in an early analysis of aerobic and fermentative bacterial growth yields (9). This synthesis shows that the correlation between free energy release and growth yield holds across a much wider range of catabolic reaction pathways and helps to fill an important gap (10) in our understanding of growth yields coupled to anaerobic heterotrophic pathways.

The linear correlation in Fig. 1A can be explained in terms of the theoretical relationship between catabolic free energy and cell growth yield (9, 12). To illustrate this point, theoretical yield estimates were computed as described in reference 12. Pyruvate was assumed to be the cellular intermediate involved in the conversion of a carbon source in cellular carbon, and ammonium was assumed to be the nitrogen source. The energy required to synthesize one mole of cells (18.8 kJ/mol $C_5H_7O_2N$) is based on an assumed value of 10.5 g of cell dry weight per mole of ATP consumed and a free energy of −52.3 kJ/mol for ATP hydrolysis under physiological conditions (9). With all substrates except H_2, the electron donor was also taken as the carbon source; with H_2, acetate was assumed to be the carbon source, since organic growth components are typically added to culture medium with H_2 as the electron donor. The only adjustable parameter in the yield calculations was the conversion efficiency (ε), which accounts for heat loss during electron transfer. An ε value of 0.39 produced a 1:1 correlation between observed and calculated yield values (Fig. 1B). This value is within the range of 0.4 to 0.8 originally estimated for aerobic and fermentative carbon metabolism (9) and remarkably close to the range of 0.37 to 0.39 obtained from a reanalysis of aerobic growth yield data (10). As expected, regression of the computed yield values versus catabolic free energy release produced a relationship very close to that for the observed yield data (dashed line in Fig. 1A).

Utility. Accurate yield coefficients are important for both kinetic (see, e.g., reference 13) and equilibrium (see, e.g., reference 3) modeling of competing catabolic pathways in sediments. The correlations shown in Fig. 1 provide complementary means to estimate growth yield based on free energy calculations. Despite the considerable scatter among observed and calculated yield values (particularly for lower-energy-yielding pathways; see insets in Fig. 1), the fundamental connection

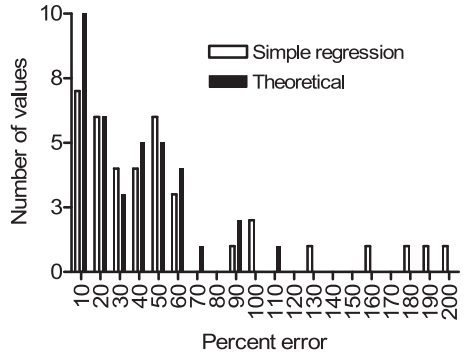

FIG. 2. Histogram showing the distribution of percent errors between observed and calculated yield values from the simple linear regression in Fig. 1A and the theoretical approach. Percent error was calculated as $|Y_{Obs} - Y_{Calc}|/Y_{Obs} \times 100$, where | | denotes absolute value and Y denotes yield. Values listed on the y axis correspond to the number of observations having a percent error less than or equal to the corresponding value on the x axis but larger than the preceding x axis value, e.g., 10 indicates percent error values of ≤10%, 20 indicates percent error values between 10 and 20%, etc.

between free energy release and growth yield is evident. Thus, yield values for pathways where no experimental data are available may be calculated, assuming that reasonable estimates of catabolic free energy can be obtained. To constrain the likely accuracy of such estimates, a histogram of percent error between observed and calculated yield values for the 38 pathways was constructed (Fig. 2). The results suggest that it should be possible to estimate yield values within a factor of 2 (percent error, ≤100) for most pathways using either the simple linear relationship shown in Fig. 1A or the theoretical approach. The three largest percent error values are the simple linear regression estimates of yield for H_2 metabolism [via dissimilatory nitrate reduction to ammonium, amorphous Fe(III) oxide reduction, and methanogenesis], which suggests that theoretical calculations may provide better yield estimates in this case. In general, the theoretical approach may be preferable, as it directly links the catabolism of a specific energy source to growth with a specified carbon source. However, the relationship in Fig. 1A provides a straightforward way to estimate yield without the need for assumptions regarding the carbon (and nitrogen) sources used for cell growth.

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