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# Evaluation of methods to predict bacterial yield using thermodynamics

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**Abstract**

Thermodynamic models can be used to predict bacterial yields and develop stoichiometric representation of biological reactions in the absence of empirical data. Several methods have been used by microbiologists, biotechnologists, and environmental engineers. This manuscript illustrates that these formulations are related. Yields predicted by estimation of Gibbs energy of dissipation and yields predicted by assumed efficiency of energy capture are comparable. Direct comparison of yield predictions from different methods shows the effects of assumptions inherent in the methodologies. Mathematical relationships between estimated values from the different methods help identify the best predictions from each method to bound the estimate of bacterial yield.

## Introduction

Application of biotechnology for environmental restoration and for production of medical and consumer products requires a quantitative analysis of biological processes. Mathematical models of biological systems are based on this quantitative analysis and must include a systematic mass balance approach and a biological reaction stoichiometry for quantifying substrate utilization, product formation, and biomass generation (Roels 1980, 1983; Rittmann & VanBriesen 1996). Mathematical models must further consider the coupling between biological reactions and chemical reactions that take place in a single reactor system or a single environmental compartment. Biological reactions can consume or produce acids and bases that change the system pH and affect abiotic reactions. For example, the aerobic degradation of citrate consumes acidic hydrogen and causes the system pH to rise. This changes the distribution of acid/base species and can initiate precipitation of metal hydroxides, carbonates or phosphates in the system. Biological reactions also consume electron donor and acceptor substrates, thus changing the redox conditions of the system. For example, iron-reducing bacteria utilize $Fe^{3+}$ as an electron acceptor, driving the dissolution of iron hydroxides and producing the more soluble $Fe^{2+}$.

These examples imply a one-way action of the bacteria on the chemical system; however, feedback effects are common. In the degradation of citrate, for instance, increasing pH shifts the dominant form of citrate from $HCit^{2-}$ to $Cit^{3-}$, and the bacterial growth rate slows because the organisms preferentially take up the $HCit^{2-}$ form (VanBriesen & Rittmann 2000b). Likewise, for iron-reducing bacteria, the dissolution of iron hydroxides releases base, and in poorly buffered systems, the pH rise can inhibit bacterial growth.

Modeling this type of coupled biogeochemical reaction is challenging, because the reaction stoichiometry must include a wide range of reactants and products, not just one or two rate-limiting substrates. VanBriesen & Rittmann (2000b) present a coupled biogeochemical model (CCBATCH) that utilizes equilibrium thermodynamics for aqueous reactions and dual-Monod kinetics for biodegradation, and couples the reactions through the stoichiometry of the biodegradation and the dependence of the biological rate on a specific chemical form or forms of the substrate. Banaszak et al. (2002) discuss an expanded model that includes precipitation and dissolution reactions. Other biogeochemical models also include biological reac-

tions, but without the rigorous coupling with chemical reactions (Bordon & Bedient 1986; MacQuarrie et al. 1990; Frind et al. 1990; Bedient & Rifai 1992; Bedient et al. 1992; McNabb & Narasimhan 1994; Walter et al. 1994). Utilization of a full stoichiometry (e.g., VanBriesen & Rittmann 2000b) has several important benefits. First, it can be used to link the growth of organisms to changes in the chemistry of the system due to removal of the primary substrates (VanBriesen et al. 2000; VanBriesen & Rittmann 2000b). Second, it can be used to test the consistency of values measured in bioprocess systems (Erickson et al. 1978; Ferrer & Erickson 1979; Erickson 1979a, b; Erickson & Hess 1981; Grosz & Stephanopoulos 1983) or to predict the behavior of one parameter given another, experimentally determined, parameter (Erickson et al. 1978; Erickson 1979b, 1981; Solomon et al. 1982; Oner et al. 1984; Lee et al. 1984). Finally, when product formation is the goal (as in fermentation), the stoichiometry can predict the product yield. Or, when substrate removal is the goal (as in bioremediation), the stoichiometry can predict the amount of co-substrates or nutrients needed for full removal.

In all cases that take advantage of the auto-catalytic behavior of microbes, a key parameter to define system operation is the biomass yield on substrate ($Y_{SX}$). This parameter defines the amount of biomass ($X$) produced per amount of substrate ($S$) consumed as part of synthesis. Generally, the substrate is defined as the electron donor ($D$) and the yield is then $Y_{DX}$. For heterotrophic bacteria, the electron-donor substrate is usually also the cells' carbon source. Other yields are often measured, including the biomass yield on oxygen ($Y_{OX}$) for aerobic systems and the biomass yield on electron-acceptor substrate ($Y_{AX}$) for anaerobic systems. Different yields in the same system are related and their values are fixed once a single yield is known for the system.

The stoichiometry that fixes the relationship between carbon source, electron donor, electron acceptor, and cells is based on material balances. For example, consider the synthesis of cells on citric acid ($C_6H_8O_7$):

$$a\mathrm{C_6H_8O_7} + b\mathrm{O_2} + c\mathrm{NH_4^+} \rightarrow d\mathrm{CH_2O_{0.6}N_{0.2}} + e\mathrm{H_2CO_3} + f\mathrm{H^+} + g\mathrm{H_2O}. \quad (1)$$

Six mass balances describe the stoichiometric parameters:

$$\begin{aligned} C\text{-balance}: &\quad 6a - d - e = 0 \\ H\text{-balance}: &\quad 8a + 4c - 2d - 2e - f - 2g = 0 \\ O\text{-balance}: &\quad 7a + 2b - 0.6d - 3e - g = 0 \\ N\text{-balance}: &\quad c - 0.2d = 0 \\ \text{Charge-balance}: &\quad c - f = 0 \\ \text{Degree of reductance balance}: &\quad a\gamma_{\text{citrate}} - d\gamma_x - 4e, \end{aligned} \quad (2)$$

where $\gamma_{\text{citrate}}$ and $\gamma_x$ are the degree of reductance of the carbon in citrate and cells, respectively. These six mass-balance requirements cannot fully specify the seven unknown parameters, $a$, $b$, $c$, $d$, $e$, $f$ and $g$. One additional known value is required, and that value is normally the yield of cells on substrate ($d/a$ if the yield is expressed in units of moles cells/moles substrate). Alternatively, the final value can be the respiratory quotient (RQ), which often is suggested for aerobic systems (Doran 1995; Blanch & Clark 1997). Since the RQ specifies the amount of oxygen used per mole of substrate consumed, it is equivalent to the $Y_{OX}$ value ($b/a$ in units of mole $O_2$/mole substrate). The stoichiometric representation in (1) can be developed with an observed cell yield or with a predicted true cell yield. In the first case, the net production of cells will be predicted and in the second case, the maximum production of cells in the absence of decay will be predicted. The relationship between observed (or net) yield and true (or theoretical) yield will be discussed below.

Generation of the full stoichiometric relationship (such as (1)) becomes possible with *a priori* knowledge of the cell yield. The bacterial yield can be measured in batch culture or in continuous culture; this measured value is the observed (also called experimental or net) yield, $Y_{\text{net}}$. To determine the net yield, cells are grown in a medium where all the substrates except one are in excess (thus defining a single limiting substrate). The net yield is then defined as

$$Y_{SX(\text{net})} = \frac{\Delta X}{\Delta S} = \frac{g DW \text{produced}}{g \text{Substrate used}}, \quad (3)$$

where cells and substrates are measured in concentrations ($g$ dry weight $(DW)/L$ and $g$ $S/L$). This yield is a net observed yield because growth and decay (or cell death) are occurring concurrently in the system. For comparative purposes, the yield measured this way is often converted to consistent units for cells and substrate. For example, yield can be reported in moles of carbon in cells per mole of carbon in the limiting substrate. When the limiting substrate is the electron-donor, yield is often reported in electron-equivalents

in cells per electron equivalent in the electron-donor substrate. These yield units are easily converted using the degree of reductance of cells and substrate, as discussed below.

In a batch system using Equation (3) gives a good prediction of the true yield ($Y_{SX}$) as long as rapid exponential growth is the dominant phenomenon. On the other hand, cell decay or cell death can cause Equation (3) to underestimate the theoretical yield. Many historical yields have been measured in batch systems, and some are subject to under-estimation. A lag period or formation of intermediates also complicates the meaning of $\Delta S$ and $\Delta X$ in batch systems.

Alternatively, yield can be measured in a continuous system or chemostat. A chemostat has the following general material balance on bacterial cells (Gerhardt & Drew 1994):

$$\begin{aligned}\text{cells accumulated} \;=\; &\text{cells added} - \text{cells removed} \\ &+\text{cells grown} - \text{cells died.}\end{aligned} \tag{4}$$

Mathematically, we typically represent this mass balance as:

$$\frac{dX}{dt}V = QX_0 - QX + \mu XV - bXV, \tag{5}$$

where $V$ is the volume of the reactor ($L^3$), $Q$ is the flow rate into and out of the reactor ($L^3$/time), $X_0$ is the concentration of cells in the influent ($M_{\text{cells}}/L^3$), $X$ is the concentration of cells in the effluent ($M_{\text{cells}}/L^3$), $\mu$ is the specific growth rate (1/time), $b$ is the specific death or decay rate (1/time), and $dX/dt$ is the rate of change in cell mass ($M_{\text{cells}}/L^3$-time).

When the influent has no cells, and the system is at steady state ($dX/dt = 0$), the equation simplifies to $\mu - b = \frac{Q}{V} = D$, where $D$ is the dilution rate. Thus, in a steady-state chemostat, the net growth rate can be controlled. In a chemostat system $Y_{\text{net}}$ will be close to $Y$ when $\mu \gg b$. If a decay or maintenance coefficient has been measured for the system, $Y$ can be computed from $Y_{\text{net}}$ following (Gerhardt & Drew 1994):

$$Y = Y_{\text{net}}\left(1 + \frac{b}{D}\right) \quad \text{or} \quad \frac{1}{Y} = \frac{1}{Y_{\text{net}}} - \frac{m}{D}, \tag{6}$$

where m is the maintenance coefficient ($M_s/M_x$-time) and is defined as $m = b/Y$. When decay or maintenance costs are not measured, the standard reciprocal plot $(1/Y)$ vs $(1/D)$ is often extrapolated to the maximum experimental growth rate ($\mu_{\max}$) to estimate the maximum cell yield ($Y_{\max}$), which is close to $Y$. The distinction between $Y_{\text{obs}}$ and $Y$ is critical because all experimental methods measure $Y_{\text{obs}}$ and all thermodynamic prediction methods predict $Y$.

Despite this well-established methodology for measuring bacterial yields, in some cases, such a direct measurement is not possible. For example, compounds that strongly sorb to surfaces (e.g., PAHs) can make it difficult to close the mass balance and evaluate the amount of substrate used by the bacteria for growth (Reardon et al. 2000). Compounds that are degraded through intermediates (e.g., chelating agents, BTEX, PAHs) are also problematic since we want to know the yield of cells at each step of the degradative process (VanBriesen & Rittmann 2000a). In the traditional yield measurement methodology, this would require growing organisms that are capable of only one step in degradation and/or finding a way to stop the downstream processing of the intermediates by the cells. Finally, consortia effects are difficult to separate when bioprocesses require multiple organisms for complete substrate removal. The yield for a particular organism within the consortia growing on a particular compound or intermediate is difficult to separate from the overall bulk yield of organisms, but is needed for accurate numerical modeling.

When yield measurements are difficult or impossible, estimation methods can be utilized. The search for an accurate method to predict cell yield in the absence of experimental results has a long history within the microbiology, chemical biotechnology, and environmental biotechnology fields. Different approaches have arisen from these different disciplines. Borrowing from one discipline to another has been problematic, particularly because some estimation methods are applicable only within limited cases that do not span disciplinary applications. The goal of the present work is to evaluate several prominent methods, demonstrate equivalency where it exists, and identify key limitations of each method. This evaluation will allow for the selection of the best estimate based on the specific system under consideration.

## Thermodynamics in microbial metabolism

Biological yield prediction requires a fundamental understanding of the parameters controlling bacterial systems. Unlike chemical reactions, which are con-

strained by physical and chemical laws that can inform our modeling, biological systems are best analyzed by considering the goals of the living organisms. The goal for most microbiological reactions is the creation of new cell material. Synthesis of biomass requires (1) nutrients like carbon and nitrogen to build macromolecules and synthesize enzymes, (2) electrons to send to the electron acceptor to generate Gibbs energy to drive synthesis reactions that organize elements into complex cell structures, and (3) when the carbon and nitrogen source are more oxidized than cells, electrons to reduce these elements to the level necessary for incorporation in cell structures. Many microorganisms acquire nutrients and electrons from a single substance called the primary electron-donor substrate.

Oxidation of the primary electron-donor substrate provides electrons for reduction of the primary electron-acceptor substrate to liberate Gibbs energy and to reduce nutrients to the proper oxidation state for incorporation in to cell materials (when necessary). If the primary electron-donor substrate is an organic compound, carbon (and sometimes nitrogen) from the donor becomes available for incorporation into cell mass. The portion of the electrons in the primary electron-donor substrate that are transferred to the electron-acceptor substrate to release Gibbs energy is based on balancing the *cost* in Gibbs energy of cell synthesis and the *release* of Gibbs energy from the flow of electrons from donor to acceptor (McCarty 1969; Rittmann & McCarty 2001). Likewise, the electrons needed for synthesis must be balanced with the electrons that need to be sent to the electron acceptor to liberate the required Gibbs energy. Clearly, the electron and energy balances are inter-related.

The proportioning of electrons available in the electron donor between the anabolic synthesis reaction and the catabolic electron donor oxidation reaction defines the yield of bacteria on the substrate. This is shown schematically in Figure 1 and can be represented by energy and electron balances (following McCarty 1969):

$$-f_e^0 \Delta G_{eR}^{01} = f_s^0 \Delta G_{e-\text{syn}}^{01} \tag{7}$$

$$f_e^0 + f_s^0 = 1, \tag{8}$$

in which $\Delta G_{eR}^{01}$ is the standard free energy of the catabolic redox reaction between electron donor and electron acceptor (kJ/e-eq), $\Delta G_{e-\text{syn}}^{01}$ is the standard free energy of the anabolic cell synthesis reaction (kJ/e-eq), $f_e^0$ is the fraction of electron-donor electron equivalents sent to the acceptor to drive the energy generating redox reaction, and $f_s^5$ is the fraction of electron-donor electron equivalents invested in biomass via the synthesis reaction. Thus, the energy cost for anabolic processes is balanced by the energy generated through catabolic processes. Reducing equivalents available in the electron-donor substrate are apportioned to maintain energy balance (i.e., catabolism and anabolism are coupled).

This simple division of cell behavior into anabolic synthesis and catabolic energy generation, while generally accurate, hides some of the complexity of the behavior of the organisms. Equation (7) is based on the change in Gibbs energy of the system, a state variable defined through classic thermodynamics,

$$\Delta G = \Delta H - T\Delta S, \tag{9}$$

in which the change in Gibbs energy ($G$) is related to the change in enthalpy ($H$) and the change in entropy ($S$) *for the system*. When a reaction is at equilibrium, $\Delta G = 0$, thus $\Delta H = T\Delta S$. When a reaction is not at equilibrium, the $\Delta G$ for the reaction provides insight into how far the reaction is from equilibrium and whether products or reactants will be formed as the reaction moves toward equilibrium. Thus, equilibrium thermodynamics and $\Delta G$ provide important information about many different kinds of reactions.

However, it is critical to note that equilibrium thermodynamics applies only to reversible processes since by definition irreversible processes cannot reach equilibrium. For a reversible reaction, the change in Gibbs energy accounts for changes in enthalpy and entropy between reactants and products. Entropy differences between reactants and products are reversible. Thus, reversible processes do not *generate net entropy for the universe*, but rather can only transfer entropy from one part of the universe to another (i.e., from the system to its surroundings). Irreversible processes (such as cooling, the free expansion of gases, and the growth and maintenance of living systems) on the other hand do *generate net entropy for the universe, and thus cannot be reversed and cannot reach thermodynamic equilibrium*. The direct application of equilibrium thermodynamics to irreversible processes including cell growth is not advisable. However, thermodynamics can offer insight into these systems and, properly modified, Gibbs energy calculations can again provide important information.

Net entropy production within living cells results from maintenance and growth processes that are in-

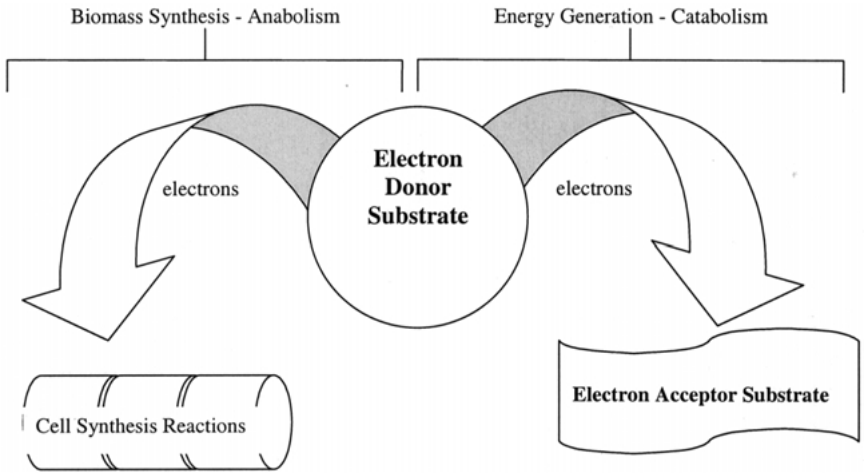


*Figure 1.* Energy and electron flow in catabolism and anabolism.

herently irreversible. Cells rid themselves (but not the universe) of this "new" entropy by yielding products of higher entropy than substrates ("feeding on negative entropy" as described by Schrodinger (1944) and reviewed recently by von Stockar & Liu (1999)), or by dissipating heat. Thus, bacterial growth represented in Figure 1 and based on equilibrium thermodynamics and energy balance, is incomplete. While representing an accurate schematic of the division of *electrons* between the two pathways requiring reducing equivalents, it is an oversimplification of the energy relationships. The Gibbs energy generated by the electron donor – electron acceptor couple is available to the organisms, but only a fraction of this Gibbs energy can be directly utilized, while a fraction must be "spent" in order to form higher entropy products or to dissipate heat. The net entropy generation in the universe must be offset by a reduction in Gibbs energy for the system.

Thus, a more complete energy balance for the system might be:

$$-f_e^0 \Delta G_{eR}^{01} = f_s^0 \Delta G_{e-\text{syn}}^{01} + \left(\frac{D_s^{01}}{r_{Ax}}\right)\left(\frac{1}{\gamma_x}\right), \quad (10)$$

where $D_s^{01}/r_{Ax}$ is the Gibbs energy which must be dissipated to produce 1 C-mole of biomass in the system (kJ/c-mole) and $\gamma_x$ is the degree of reductance of the carbon in the biomass formed (C-mol/e-eq). Thus, net entropy production due to the irreversible processes of cell growth increases the cost of cell synthesis (the right side of the energy balance is increased). The dissipation energy, first described by Roels (1983) as $D/\Phi_x$ and later incorporated into yield predictions by Heijnen & van Dijken (1992) as $D_s^{01}/r_{Ax}$, describes the well-observed phenomena that microorganisms dissipate heat during growth with no net product formation.

An alternative formulation of the energy balance can be derived by representing the loss of Gibbs energy as an inefficiency of energy capture by the organisms. Then, the energy balance can be written in terms of a proposed efficiency term ($K$):

$$-f_e^0 K \Delta G_{eR}^{01} = f_s^0 \Delta G_{e-\text{syn}}^{01}, \quad (11)$$

where the efficiency is defined as the fraction of Gibbs energy available from the electron donor/electron acceptor pair ($f_e^0 \Delta G_{eR}^{01}$) that is conserved in the formed biomass ($f_s^0 \Delta G_{e-\text{syn}}^{01}$). Thus, the Gibbs energy lost due to generation of entropy in the irreversible growth process is represented as a decrease in energy available for synthesis (the left side of the energy balance is decreased). This efficiency term, introduced by McCarty (1969) as $K$ and by Roels (1983, 1987) as $\eta_{th}$, has been rederived for bacterial energetics using non-equilibrium thermodynamics by Westerhoff et al. (1983, 1987). It can be computed from experimental yield values or estimated and is related to the dissipation energy. The relationship between the terms will be evaluated in detail below.

Once the efficiency or the dissipation energy is known, the cell yield can be computed from the thermodynamic relationships between the substrates in the overall macroscopic equation of growth (see Equation (1) above). Methods to couple efficiency and dissipa-

tion to yield predictions are discussed in detail in the following sections.

## Cell yield prediction

### *Predicting the maximum theoretical yield of cells on substrate*

The maximum bacterial yield in a system has a theoretical upper limit constrained by the Second Law of thermodynamics: entropy must increase. One maximum theoretical yield on substrate (in C-mole cells/C-mole substrate) has been defined as

$$Y_{DX}^{th} = \frac{\gamma_D}{\gamma_X}, \tag{12}$$

or the ratio of the degree of reductance of the carbon in the electron-donor substrate ($\gamma_D$) to the degree of reductance of the carbon in the cells ($\gamma_X$) (Heijnen & Roels 1981). Thus, the maximum yield of cells that is possible is determined by the electrons available in the donor and the electrons needed to synthesize cells. While Equation (12) is an over simplification (as will be shown later), it is a good first estimation for the maximum yield possible from a given substrate and can be used to constrain yield predictions. Referring to Figure 1, this maximum yield would occur if the cells sent *all* the electrons from the electron-donor substrate to synthesis ($f_s^0 = 1$ and $Y_{DX}^{th} = 1^{\text{electron}-\text{equivalent cells}}/_{\text{electron}-\text{equivalentsubstrate}}$).

### *The degree of reductance correlation approach of Roels*

Initial attempts to predict cell yields relied on simple relationships. Linton & Stephenson (1978) first proposed a simple, two-part discontinuous function for predicting the yield of heterotrophic organisms based on the heat of combustion for the growth substrate. The relationship was first applied to yield and efficiency predictions by Roels (1983). Evaluating a large body of yield data originally published by Heijnen & Roels (1981), Roels (1983) concluded that, when the degree of reductance of carbon in the electron-donor substrate ($\gamma_D$) is greater than approximately 4.67 (e.g., ethanol with $\gamma_D = 6$), the yield of cells on substrate appears to be nearly constant in carbon units (0.5–0.6 C-mole cells/C-mole substrate), while the thermodynamic efficiency ($\eta_{th}$) decreases with increasing degree of reductance in the substrate. However, when $\gamma_D$ is less than 4.67 (e.g., citrate with $\gamma_D = 3$), $\eta_{th}$ is nearly constant (average of 0.58), and the yield decreases with decreasing degree of reductance. Thus, for aerobic growth with ammonia as nitrogen source, Roels (1983) concluded:

$$\begin{aligned} &\text{if } \gamma_D \leq 4.67, \text{ then } Y_{DX} = 0.13\gamma_D \text{ and } \eta_{th} = 0.58 \\ &\text{if } \gamma_D > 4.67, \text{ then } Y_{DX} = 0.60 \text{ and } \eta_{th} = \frac{2.7}{\gamma_D} \end{aligned} \tag{13}$$

While Equation (13) is clearly an empirical approximation for a limited subset of organisms, it provides a first estimate for the yield for aerobic heterotrophic growth.

### *The dissipation correlation approach of Heijnen and van Dijken*

The yield prediction method proposed by Heijnen & van Dijken (1992) is based on a statistical relationship among carbon chain length ($C$), electron-donor substrate degree of reductance ($\gamma_D$), and the Gibbs energy of dissipation for the biological reaction that involves oxidation of the primary electron-donor substrate, reduction of the primary electron-acceptor substrate, and production of biomass as the only product. The Gibbs energy of dissipation, coupled with the Gibbs energy values of the catabolic and anabolic reactions, is used to predict the cell yield. The method is summarized here, and the reader is referred to the works of Heijnen and co-workers for further details (Heijnen 1991, 1994, 1999; Heijnen et al. 1992; Heijnen & van Dijken 1992, 1993; Tijhuis et al. 1993).

*Estimating dissipation.* The Gibbs energy of dissipation can be determined experimentally or it can be predicted by a correlation developed by Heijnen & van Dijken (1992):

$$\begin{aligned} D_s^{01}/r_{AX} = {} & 200 + 18 \times (6 - C)^{1.8} \\ & + \text{Exp}[\{3.8 - \gamma_D)^2\}^{0.16} \times (3.6 \\ & + 0.4C)], \end{aligned} \tag{14}$$

where $D_s^{01}/r_{Ax}$ represents the Gibbs energy of dissipation in kJ/C-mole, $C$ is the number of carbon atoms in a mole of the substrate, and $\gamma_D$ is the degree of reductance of the carbon in the electron-donor substrate. This correlation was developed based on analysis of experimental yields from multiple data sets representing aerobic and anaerobic growth on a variety of substrates (Herbert et al. 1956; Linton & Stephenson 1978; van Verseveld 1979; Heijnen & Roels 1981;

Stouthamer 1988; Pronk et al. 1990; Rutgers 1990; Verduyn et al. 1991). While some of these data represented $Y_{\text{net}}$ rather than $Y$, Heijnen & van Dijken (1992) did not consider the effects of specific details of experimental methods when they developed the correlation. Since $Y_{\text{net}} \leq Y$ it's possible that Equation (14) over-predicts $D_s^{01}/r_{AX}$.

*Estimating yield.* Following estimation of the Gibbs energy of dissipation, the cell yield is computed from Equation (15), derived from four elementary reactions and mass balances:

$$Y_{DX} = \\ = \frac{\gamma_D}{\gamma_X} \frac{\Delta G_{eD}^{01} - \Delta G_{eA}^{01}}{(\Delta G_{eD}^{01} - \Delta G_{eA}^{01}) + \left[\left(\frac{D_s^{01}}{r_{AX}}\right)\left(\frac{1}{\gamma_X}\right) + (\Delta G_{eX}^{01} - \Delta G_{eD}^{01})\right]}, \tag{15}$$

where $Y_{DX}$ is the yield of bacterial cells on the electron donor substrate (in C-mole cells/C-mole substrate), $\gamma_D$ is the degree of reductance of the electron-donor substrate, $\gamma_X$ is the degree of reductance of the carbon in the bacterial cells, $\Delta G_{eD}^{01}$ is the Gibbs energy of the electron-donor oxidation half reaction at the standard state per mole of electrons (kJ/e-mole), $\Delta G_{eA}^{01}$ is the Gibbs energy of the acceptor oxidation half reaction at the standard state per mole of electrons (kJ/e-mole), and $\Delta G_{eX}^{01}$ is the Gibbs energy of the cell material oxidation half reaction at the standard state per mole of electrons (kJ/e-mole). Prediction of yield in electron units (e-mole cells/e-mole substrate) requires multiplication of Equation (15) by $\gamma_X/\gamma_D$.

Heijnen et al. (1992) also suggest that a simplification is possible, based on the assumption that $\Delta G_{eX}^{01} \approx \Delta G_{eD}^{01}$ or the Gibbs energy of combustion of biomass is approximately the same as the Gibbs energy of combustion of most carbon-source, electron-donor substrates. This energetic regularity, noted previously by Minkevich & Eroshin (1973), leads to the simplification:

$$Y_{DX} = \frac{\gamma_D}{\gamma_X} \frac{\Delta G_{eD}^{01} - \Delta G_{eA}^{01}}{(\Delta G_{eD}^{01} - \Delta G_{eA}^{01}) + \left[\left(\frac{D_s^{01}}{r_{Ax}}\right)\left(\frac{1}{\gamma_X}\right)\right]}. \tag{16}$$

This simplification relaxes the troublesome requirement for $\Delta G_{eX}^{01}$, a value computed for the half reaction for cell combustion to inorganic forms of carbon and nitrogen and requiring knowledge of the Gibbs energy of formation for biomass from elements ($\Delta G_{fX}^{01}$). $\Delta G_{fX}^{01}$ is not well known, having been estimated by Roels (1980) as −67 kJ/C-mole for biomass having an average composition ($CH_{1.8}O_{0.5}N_{0.2}$) and by Grosz & Stephanopoulos (1983) as −45 kJ/C-mole for *E. coli*. Heijnen et al. (1992) use the Roels (1980) value in calculations when the simplification (Equation (16)) is not used.

Justification for the simplification is provided in that it does not significantly increase the overall error across the data set (see Figure 3, panels A and B in Heijnen et al. 1992), because the value of ($\Delta G_{eX}^{01} - \Delta G_{eD}^{01}$) is only about 5–10% of the denominator value in Equation (15). Of course, this simplification can lead to significant differences in predicted yields for *certain* organic electron donors. Evaluation of this method and the effect of its simplification is presented below.

After developing Equation (15), Heijnen et al. (1992) proposed a new "black-box" thermodynamic efficiency definition: $\eta_{th} = \frac{Y_{DX}}{Y_{DX}^{th}}$, or the ratio of the actual and thermodynamic maximal yield of biomass on electron donor, where $Y_{DX}^{th}$ determined by setting the dissipation to zero in Equation (15).

$$Y_{DX}^{th} = \frac{\gamma_D}{\gamma_X} \frac{\Delta G_{eD}^{01} - \Delta G_{eA}^{01}}{(\Delta G_{eX}^{01} - \Delta G_{eA}^{01})}. \tag{17}$$

The assumption of zero dissipation is equivalent to an assumption that the reactions are reversible and thus produce no *net entropy increase* in the universe that would require heat dissipation. Setting the dissipation Gibbs energy to zero in the simplified version (Equation (16)) is equivalent to assuming the regularity value is a good estimate of $\Delta G_{eD}^{01}$ and the predicted maximum theoretical yield would be equivalent to the approximation suggested by Heijnen & Roels (1981), Equation (12).

### *The efficiency approach of McCarty*

An alternative thermodynamic yield prediction method was proposed by McCarty and coworkers (McCarty 1965, 1969; Lawrence & McCarty 1970, 1971, 1972a, b; Christensen & McCarty 1975; McCarty 1975; Rittmann & McCarty 2001). This method is similar in some ways to the method using the Gibbs energy dissipation, but relies on estimates of thermodynamic efficiency of bacterial growth instead of dissipation. The McCarty method is suitable for compounds containing more than six carbons and having more complex structures. It can also be used for inorganic electron donors, electron acceptors other than

oxygen, and fermentation reactions (Rittmann & McCarty 2001). The McCarty method was expanded by VanBriesen & Rittmann (2000a) to consider reactions that generate biodegradation intermediates rather than full mineralization products (e.g., $CO_2$, $NH_4^+$). This method is general enough that it can be applied to predict cell yields and stoichiometries for single reaction steps in a pathway, as long as the steps are simple oxidations. However, for organics that are degraded through activation reactions, further modifications are necessary to consider the effect on the cell yield of the diversion of electrons to oxygenase reactions (Woo & Rittmann 2000; Yuan & VanBriesen 2002; VanBriesen 2001).

The McCarty method is based on the concept that electron and energy balances control the behavior of biological systems. This method is widely used by environmental engineers (Noguera et al. 1988; Metcalf & Eddy 1991; Corseuil & Weber 1994; Alvarez et al. 1994; Hooker et al. 1994; Edwards & Grbic-Galic 1994; Beller et al. 1996; Zitomer 1998; Hayes et al. 1998; Burland & Edwards 1999; Arcangeli & Arvin 1999; Woo & Rittmann 2000). In addition to extensive application, the method has also been re-derived using non-equilibrium thermodynamics by Westerhoff et al. (1983, 1987). As with the previous method, I review the fundamentals of the McCarty method in this section.

For cell growth to occur, an electron-donor substrate is oxidized, and electrons are shuttled to the electron acceptor to generate energy or to the carbon and nitrogen sources to reduce these elements to the oxidation state necessary for incorporation into cells (as shown in Figure 1 previously). As discussed above, the energy and electron balances are represented as:

$$-f_e^0 K \Delta G_{eR}^{01} = f_s^0 \Delta G_{e-\text{syn}}^{01} \tag{18}$$

$$f_e^0 + f_s^0 = 1, \tag{19}$$

in which $\Delta G_{eR}^{01}$ is the standard free energy of the redox reaction between electron donor and electron acceptor (kJ/e-eq), $\Delta G_{e-\text{syn}}^{01}$ is the standard free energy of the cell synthesis reaction (kJ/e-eq), $f_e^0$ is the fraction of electron-donor electron equivalents sent to the acceptor to drive the energy generating redox reaction, $f_s^0$ is the fraction of electron-donor electron equivalents invested in biomass via the synthesis reaction, and $K$ is the efficiency of energy capture in the energy-generation reaction. All Gibbs energies are in the standard state of $T = 25\ ^\circ\text{C}$, $P = 1$ atm, 1 M reactants and products, except $[H^+] = 10^{-7} \cdot \Delta G_{eR}^{01}$ is computed as the difference between the free energy of the donor and acceptor half reactions, $\Delta G_{eA}^{01} - \Delta G_{eD}^{01}$ where each reaction is written in standard reduction format. $\Delta G_{eA}^{01}$ and $\Delta G_{eD}^{01}$ values are tabulated in many standard texts (Stumm & Morgan 1996; Rittmann & McCarty 2001). If the half reaction value is not tabulated, the calculation is straightforward using values for the standard free energy of formation ($\Delta G_f^{01}$) for all the species in the half reaction. If the standard free energy of formation of a compound is not known, it can be estimated using group contribution theory (Mavrovouniotis 1990, 1991).

*Synthesis.* $\Delta G_{e-\text{syn}}^{01}$ is computed based on a simplified two step synthesis involving conversion of the substrate to a common intermediate (pyruvate for carbon) and conversion of this intermediate to cells.

$$\Delta G_{e-\text{syn}}^{01} = \frac{(\Delta G_{e-\text{pyr}}^{01} - \Delta G_{eCS}^{01})}{\kappa^m} + \frac{\Delta G_{e-\text{cells}}^{01}}{\kappa}, \tag{20}$$

where $\Delta G_{e-\text{pyr}}^{01}$ is the Gibbs energy of the standard reduction half reaction for pyruvate (a common cellular intermediate), $\Delta G_{eCS}^{01}$ is the Gibbs energy of the standard reduction half reaction for the carbon source (equivalent to $\Delta G_{eD}^{01}$ for many heterotrophic organisms), $\Delta G_{e-\text{cells}}^{01}$ is the cost to synthesize biomass from pyruvate (assumed to be constant), and $\kappa$ is again an assumed inefficiency of energy capture. $\kappa$ is often taken as identical in value to $K$, but this equivalence is unproven. $m$ is an integer used to account for the fact that the conversion of the carbon source to pyruvate can be energy-consuming or energy-generating. When $(\Delta G_{e-\text{pyr}}^{01} - \Delta G_{eCS}^{01})$ is positive, energy is required for the conversion, and MORE energy is needed due to inefficiencies ($m = +1$). When $(\Delta G_{e-\text{pyr}}^{01} - \Delta G_{eCS}^{01})$ ($m = +1$) is negative, energy is generated during the conversion, but LESS energy is available than computed, again due to inefficiencies ($m = -1$).

Eliminating the first term in (20) is equivalent to as assumption of regularity of the energy of the electron donor (Minkevich & Eroshin 1973), since pyruvate is a substrate that follows the observed regularity ($\Delta G_{e-\text{pyr}}^{01} = 35.6\text{kJ/e-eq} \approx \Delta G_{e-\text{cells}}^{01} = 34.7\,\text{kJ/e-eq}$), and the regularity assumption would predict that the carbon source compound ($\Delta G_{eCS}^{01}$) would also be close to this value for cells. However, as in the previous method, this simplification fails when the carbon source does not obey the observed

regularity. The key piece of Equation (20) is clearly $\Delta G^{01}_{e-\text{cells}}$, a value that generally dominates $\Delta G^{01}_{e-\text{syn}}$, except when the substrate is significantly nonregular (e.g., autotrophic growth).

$\Delta G^{01}_{e-\text{cells}}$ is computed using terms associated with biomass formation. Originally $\Delta G^{01}_{e-\text{cells}}/\kappa$ was reported as 7.5 kcal/e-eq by McCarty (1971). More recently, Rittmann & McCarty (2001) suggest a value for $\Delta G^{01}_{e-\text{cells}} = 3.33$ kJ/gcells, a value that is consistent with the original 7.5 value and assumes $\kappa = 0.6$ and cells are represented by $C_5H_7O_2N$, with molecular weight of 113 g/mole and degree of reductance of 4. VanBriesen & Rittmann (2000a) provide full details for the calculation of $\Delta G^{01}_{e-\text{cells}}$ from different assumed formulae for biomass and discuss the meaning and values of the biomass constants inherent in its development.

Equation (20) is generally successful in predicting synthesis costs for a wide range of organisms. Theoretically, if a verifiable, constant value for $\Delta G^{01}_{eX}$ were available, a simplification could be made based on the second part of Equation (20) being the difference between the Gibbs energy of the pyruvate half reaction and the cell half reaction ($\Delta G^{01}_{e-\text{cells}} = \Delta G^{01}_{eX} - \Delta G^{01}_{e-\text{pyr}}$):

$$\Delta G^{01}_{e-\text{syn}} = \frac{(\Delta G^{01}_{e-\text{pyr}} - \Delta G^{01}_{eCS})}{\kappa^m} + \frac{\Delta G^{01}_{eX} - \Delta G^{01}_{e-\text{pyr}}}{\kappa}. \quad (21)$$

For heterotrophs with $\Delta G^{01}_{eD} = \Delta G^{01}_{eCS}$ *and* $\Delta G^{01}_{eD} < \Delta G^{01}_{e-\text{pyr}}$ so that $m = 1$, Equation (21) can be simplified to:

$$\Delta G^{01}_{e-\text{syn}} = \frac{\Delta G^{01}_{eX} - \Delta G^{01}_{eD}}{\kappa}. \quad (22)$$

These simplifications are not generally made in practice of this methodology, because the method shows high sensitivity to the value of $\Delta G^{01}_{eX}$, and this value is not known well nor known to be a constant for a wide range of organisms. The calculation is shown here to allow easier comparison between the methods.

*Energy use and efficiency.* The energy balance (Equation (18)) says that the energy made available from transfer of electrons from the donor to the acceptor ($-f^0_e K \Delta G^{01}_{eR}$) is invested to synthesize $f^0_s$ electron equivalents of biomass ($f^0_s \Delta G^{01}_{e-\text{syn}}$). While not specifically stated by McCarty, implicit in this analysis is that the Gibbs energy not captured from the redox reaction and used for synthesis is dissipated as heat to export the net entropy generated within the cell due to the irreversible nature of growth and maintenance reactions. The efficiency of energy capture in the energy-generation reaction, $K$, was determined empirically by McCarty (1969) to range from 0.2–0.8. Such a wide range for a "constant" in a mathematical methodology is not ideal, naturally; however, McCarty also noted several limiting regularities. In general, $K$ is between 0.2–0.3 for aerobic heterotrophs, between 0.3–0.6 for autotrophic growth, and from 0.4–0.7 for anaerobic heterotrophic growth (McCarty 1969, 1971). However, a $K$ value of 0.6 is now widely and generally used in environmental biotechnology (Rittmann & McCarty 2001) with a frame of reference that includes typical species found in environmental applications: $CO_2$, $HCO_3^-$, $H_2O$(aq), $N_{2(g)}$, $O_{2(g)}$, $H^+$ at pH = 7. As noted by Heijnen & van Dijken (1993) efficiency values are dependent upon the thermodynamic frame of reference (the implications of this limitation will be discussed below). Analysis of experimentally determined yields (VanBriesen, unpublished) suggests the discontinuous relationship between efficiency and degree of reductance of the carbon source is applicable to the determination of $K$ for a frame of reference consisting of $H_2CO_3$, $H_2O$(aq), $N_{2(g)}$, $O_{2(g)}$, and $H^+$ at pH = 7. For aerobic heterotrophs, the thermodynamic efficiency can be predicted as:

$$K = 0.1\gamma_D \text{ for } \gamma_D \le \gamma_X \quad K = 0.3 \text{ for } \gamma_D > \gamma_X. \quad (23)$$

Despite these preliminary results, the McCarty efficiency approach is generally utilized with a constant empirically determined efficiency value suitable for the frame of reference originally selected by McCarty.

*Estimating yield.* Equation (19) says that the number of electrons sent to synthesis and energy generation cannot exceed the number of electrons available from the electron-donor substrate. Coupling these two equations numerically allows solution for the unknown $f^0_s$ and $f^0_e$, which are then utilized to recombine the half reactions for anabolism and catabolism. To simplify the mathematics, $A$ is typically defined as the ratio of $f^0_e$ to $f^0_s$:

$$A = \frac{f^0_e}{f^0_s} = \frac{-\Delta G^{01}_{e-\text{syn}}}{K \Delta G^{01}_{eR}}, \quad (24)$$

and represents the electron equivalents to the acceptor normalized by the electron equivalents to the biomass.

Equation (24) coupled with Equation (19) leads to the definitions of $f_e^0$ and $f_s^0$:

$$f_s^0 = \frac{1}{1+A} \quad \text{and} \quad f_e^0 = \frac{A}{1+A}. \tag{25}$$

In electron units, the yield is equivalent to $f_s^0$. For conversion to carbon units:

$$\left(Y \frac{C - \text{mole cells}}{C - \text{mole substrate}}\right) = f_s^0 \frac{\gamma_D}{\gamma_X}. \tag{26}$$

Combining Equations (18), (19), and (26), we find

$$Y_{DX} = \frac{\gamma_D}{\gamma_X}\left[\frac{K\Delta G_{eR}^{01}}{K\Delta G_{eR}^{01} - \Delta G_{e-\text{syn}}^{01}}\right]. \tag{27}$$

While it is tempting to see the simple formulation in Equation (26) and assume that $f_s^0$ is an efficiency term, we must recall that it is calculated using the electron and energy balances (Equations (18) and (19) above) and as such already *includes, but it not identical to* the efficiency term $K$. As $f_s^0$ approaches 1, more of the available electrons are being sent to synthesis and fewer to the electron acceptor substrate; however, $f_s^0$ reaches a maximum value *below 1*. One can see that at $f_s^0 = 1$, $f_e^0 = 0$, and the energy balance becomes undefined. Thus, the true value expressing the energy conversion efficiency value in the McCarty thermodynamic method is $K$, and its value is defined between 0 and 1, while $f_s^0$ is a yield term in electron units. With $K = 1$ in Equation (24), the maximum yield is predicted from Equation (25) as:

$$f_s^{0-th} = \frac{1}{1 + \frac{-\Delta G_{e-\text{syn}}^{01}}{\Delta G_{eR}^{01}}}$$

$$f_s^{0-th} = \frac{\Delta G_{eR}^{01}}{\Delta G_{eR}^{01} - \Delta G_{e-\text{syn}}^{01}}. \tag{28}$$

The maximum yield in carbon units is then

$$Y_{DX}^{th} = \frac{\gamma_D}{\gamma_X} f_s^{0-th}$$

$$Y_{DX}^{th} = \frac{\gamma_D}{\gamma_X} \frac{\Delta G_{eR}^{01}}{\Delta G_{eR}^{01} - \Delta G_{e-\text{syn}}^{01}}. \tag{29}$$

Substituting equation (22) with $\kappa = 1$ for $\Delta G_{e-\text{syn}}^{01}$ and $\Delta G_{eR}^{01} = \Delta G_{eA}^{01} - \Delta G_{eD}^{01}$ would lead to the same thermodynamic maximum yield as described by Heijnen & van Dijken (1992), Equation (17) above, based on a dissipation of zero.

*Acceptability of methods*

In addition to $\eta_{th}$ and $K$, there have been many other bacterial efficiency terms proposed in the literature for use in yield prediction. Heijnen & van Dijken (1993) review these terms. As suggested by Roels (1993) and easily demonstrated mathematically, all these proposed efficiency values are related, making their use equivalent to the method of McCarty. All show some relationship to the efficiency value $K$, to the Gibbs energy dissipation in the system, and to the overall yield of cells on substrate. *Their utility is determined not by their obvious relationship with known yields and bacterial reactions, but their ability to predict yields for novel substrates and bacterial species in the absence of data*.

Heijnen & van Dijken (1992) suggest that all bacterial efficiency methodologies have intrinsic problems based on the requirement for additional values. However, the Gibbs energy of dissipation is itself a required value, determined either experimentally or estimated for simple carbon compounds, following Equation (14). Heijnen & van Dijken (1992, 1993) stress that the Gibbs energy of dissipation can be calculated from the observed yield, and this calculation does not require additional information or assumptions; however, this calculation is based on the macroscopic biological equation and requires an implicit assumption of the biomass formulation and the energy associated with biomass (see for example Appendix F in Heijnen & van Dijken (1992)). These authors contend that use of efficiencies is inherently limited by selection of frame of reference and go so far as to note that the energy value of biomass (−67 kJ/C-mole) is strongly dependent upon this frame of reference (Heijnen & van Dijken 1993). However, they fail to note that the Gibbs energy of dissipation calculated from an observed yield will likewise depend on this biomass value and, thus, on the selection of the frame of reference. Similarly, prediction of the yield using Equation (15) and the dissipation energy estimated by Equation (14) depends on an energy value for cells that is inherently dependent on the frame of reference. Utilization of the simplification provided in Equation (16) eliminates this dependency on frame of reference, but is itself based on an added assumption of the regularity of energy in substrates. Thus, the Gibbs energy of dissipation used to predict a bacterial yield is based

on a frame of reference selection, as is the efficiency approach.

This analysis of thermodynamic yield methodologies would not be complete without consideration of the requirements for a "black-box" model proposed by Heijnen & van Dijken (1992). Because Heijnen & van Dijken (1992) demonstrated that the use of Gibbs energy of dissipation is consistent with "black box" requirements, the consistency of the McCarty method is evaluated here. A "black-box" model requires that the method not depend upon detailed knowledge of the biochemical reactions involved in anabolism and catabolism. Rather, the method must rely only upon information dealing with the carbon source, electron donor and acceptor, nitrogen source, and biomass composition. The McCarty method relies on the identity of the electron donor, electron acceptor, carbon and nitrogen sources, and the chemical composition of the cells only; as such it is a true "black-box" method. Detailed knowledge of the biochemical reactions involved in anabolism and catabolism are ***not*** required for accurate predictions of mineralization reactions as implied by Heijnen (1994). Further, Heijnen & van Dijken (1992) suggest that thermodynamic methods for yield prediction must be generally applicable to all microbial growth systems and must conform to the Second Law of Thermodynamics. Utilizing half reactions for donor and acceptor reactions allows the McCarty method to be applied to all microbial growth systems. The use of a generalized cell synthesis half reaction from inorganic forms of nitrogen and carbonate allows consideration of cells with different chemical composition if needed (as described in Van-Briesen & Rittmann, 2000a). Since the maximum predicted yield occurs for $K = 1$, the second law of thermodynamics is not violated in the method.

Finally, Heijnen & van Dijken (1992) suggest suitable methods must not suffer from intrinsic problems that they define predominately as being dependent upon the choice of reference state. The McCarty method (as all previous yield prediction methodologies) depends upon the reference state. As discussed above, while the Gibbs energy of dissipation is independent of the reference state, its use in predicting yield terms is not. Thus, this requirement that methods avoid dependence on a reference state, if strictly applied, would preclude predictions of yield values from thermodynamics by the McCarty method and it would equally exclude the method of Heijnen & van Dijken (1992).

*Equivalence of methods*

Before proceeding to an evaluation of the relative predictive power of the two methods, I digress briefly to identify the equivalence of the methods. Table 1 provides a summary of the yield prediction methodology equations.

Equating the yield prediction Equations ((15) and (27)) gives:

$$\begin{aligned} Y_{DX} &= \frac{\gamma_D}{\gamma_X}\frac{-\Delta G_{eR}^{01}}{(-\Delta G_{eR}^{01}) + \left[\left(\frac{D_s^{01}}{r_{Ax}}\right)\left(\frac{1}{\gamma_X}\right) + (\Delta G_{eX}^{01} - \Delta G_{eD}^{01})\right]} \\ &= \frac{\gamma_D}{\gamma_X}\left[\frac{K\Delta G_{eR}^{01}}{K\Delta G_{eR}^{01} - \Delta G_{e-\mathrm{syn}}^{01}}\right]. \end{aligned} \tag{30}$$

Simplifying and solving for $K$ provides a relationship between $K$ and the dissipation energy,

$$K = \frac{\Delta G_{e-\mathrm{syn}}^{01}}{\left[\left(\frac{D_s^{01}}{r_{Ax}}\right)\left(\frac{1}{\gamma_X}\right) + (\Delta G_{eX}^{01} - \Delta G_{eD}^{01})\right]}. \tag{31}$$

This analysis uncovers the near-equivalence of $K$ and $\eta_{th}$ proposed by Roels (1993):

$$\eta_{th} = \frac{-\Delta g_x^r}{\left[\frac{D_s^{01}}{r_{Ax} - g_x^r}\right]}, \tag{32}$$

where $\Delta g_x^r$ is the Gibbs energy content of the biomass in kJ/c-mole (equivalent to $-\gamma_X \Delta G_{e-\mathrm{syn}}^{01}$ here). The difference between $K$ and $\eta th$ appears only in the requirement for an additional efficiency term ($\kappa$) in Equation (5), precluding the substitution of $\Delta G_{e-\mathrm{syn}}^{01}$ for $(\Delta G_{eX}^{01} - \Delta G_{eD}^{01})$ in Equation (31).

## Results

In this section, two analyses will be presented. First, yield values for a selection of substrates will be computed using each of the methods and the predictions compared. The substrates are common organic electron donors for which Gibbs energy of formation values are known. However, accurate experimental yield values are not necessarily known for these substances. This analysis will allow comparison of the methods to one another. Second, a small data set of yields measured under ideal conditions and verified through carbon balance will also be predicted with both methods to allow comparison of the predictions to observed yields.

*Table 1.* Equations for yield prediction methodologies

| Method | Calculations | Equation numbers |
|---|---|---|
| Roels degree of reductance correlation approach | if $\gamma_D \leq 4.67$, then $Y_{DX} = 0.13\gamma_D$ and $\eta_{th} = 0.58$<br>if $\gamma_D > 4.67$, then $Y_{DX} = 0.60$ and $\eta_{th} = 2.7/\gamma_D$ | (13) |
| Heijnen & van Dijken dissipation correlation approach | $D_s^{01}/r_{Ax} = 200 + 18x(6-C)^{1.8} + \mathrm{Exp}[\{(3.8-\gamma_D)^2\}^{0.16}x(3.6+0.4C)]$<br>$Y_{DX} = \frac{\gamma_D}{\gamma_X}\frac{-\Delta G_{eR}^{01}}{(-\Delta G_{eR}^{01})+\left[\left(\frac{D_s^{01}}{r_{Ax}}\right)\left(\frac{1}{\gamma_X}\right)+(\Delta G_{eX}^{01}-\Delta G_{eD}^{01})\right]}$ | (14) and (15) |
| McCarty efficiency approach | $\Delta G_{e-\mathrm{syn}} = \frac{(\Delta G_{e-\mathrm{pyr}}^{01}-\Delta G_{eCS}^{01})}{\kappa^m} + \frac{\Delta G_{e-\mathrm{cells}}^{01}}{\kappa}$<br>$Y_{DX} = \frac{\gamma_D}{\gamma_X}\left[\frac{K\Delta G_{eR}^{01}}{K\Delta G_{eR}^{01}-\Delta G_{e-\mathrm{syn}}^{01}}\right]$ | (20) and (27) |

*Intra-method consistency*

As indicated in discussions above, thermodynamic yield prediction methods (whether based on correlation or efficiency estimates) require multi-step calculations and the utilization of specific literature values for substrates. Table 2 provides all the required information for yield prediction for each of the methods for a selection of organic electron-donor carbon-source compounds. Both methods are capable of prediction of yields for organisms growing on a variety of different electron donors, electron acceptors, and carbon sources. However, predicting yields for autotrophic and fermentative growth require additional assumptions for the methods. Thus, the focus here is on heterotrophic growth.

Table 3 provides calculations necessary to complete the yield predictions. $\Delta G_{eD}^{01}$ (column 2) was computed using $\Delta G_{eDf}^{01}$ (Table 2, column 5) and the Gibbs energy of formation for $H_2CO_3$ ($-324$ kJ/mole), $NH_4^+$ ($-79.7$ kJ/mole), $H_2O$ ($-237.4$ kJ/mole) and $H^+$ at pH 7 ($-40.0$ kJ/mole). Values for these constants are from Thauer et al. (1977). The Gibbs dissipation (column 3) is computed following Equation (14). The synthesis cost $\Delta G_{e-\mathrm{syn}}^{01}$ (Table 3, column 4) is computed following Equation (20) with $\kappa = 0.3$ and $\Delta G_{e-\mathrm{cells}}^{01} = 10.41$ kJ/e-eq for cells with an assumed formula of $CH_2O_{0.6}N_{0.2}$. Not shown is the energy-generating couple $\Delta G_{eR}^{01}$, computed as $(\Delta G_{eA}^{01} - \Delta G_{eD}^{01})$ where $\Delta G_{eD}^{01}$ is given (Table 3, column 2) and $\Delta G_{eA}^{01} = -78.72$ kJ/e-eq for oxygen as electron acceptor, $\Delta G_{eA}^{01} = -72.20$ kJ/e-eq for nitrate as electron acceptor, $\Delta G_{eA}^{01} = 20.85$ kJ/e-eq for sulfate as electron acceptor, $\Delta G_{eA}^{01} = -74.27$ kJ/e-eq for ferric iron as electron acceptor, and $\Delta G_{eA}^{01} = 25.53$ kJ/e-eq for $CO_2$ as electron acceptor.

With the data in Table 2 and the calculated values in Table 3, the yield based on the dissipation correlation approach (following Heijnen & van Dijken 1992) is then predicted using Equation (15) (the simplification, Equation (16) can also be used; these results are not shown). The yield based on the efficiency approach (following Rittmann & McCarty 2001) is predicted from the data in Table 2 and the $\Delta G_{eR}^{01}$ and $\Delta G_{e-\mathrm{syn}}^{01}$ using Equations (24)–(26). Despite the relationship between $K$ and the degree of reductance of the carbon source electron-donor substrate described above in Equation (23), the McCarty method is generally employed with a constant $K$. Therefore for these analyses, I assume the thermodynamic efficiency is constant with $K = \kappa = 0.3$. For these and subsequent calculations, predicted yields are reported in C-mole cells/C-mole substrate. Since the assumption is made that the electron-donor is the carbon source for these heterotrophic systems, we

*Table 2.* Data needed to predict yield for substrates using the dissipation correlation approach or the efficiency approach

| | Formula | Number of carbons (C) | Degree of reductance ($\gamma_D$) | $\Delta G^{01}_{eDf}$ (kJ/mole) |
|---|---|---|---|---|
| Acetate | $C_2H_4O_2$ | 2 | 4 | −369.41 |
| Acetoin | $C_4H_8O_2$ | 4 | 5 | −280 |
| Butanediol | $C_4H_{10}O_2$ | 4 | 5.5 | −33 |
| Butyrate | $C_4H_8O_2$ | 4 | 5 | −351.63 |
| Citrate | $C_6H_8O_7$ | 6 | 3 | −1168.3 |
| Dihydroxyacetone | $C_3H_6O_3$ | 3 | 4 | −445.18 |
| Ethane | $C_2H_6$ | 2 | 7 | −32 |
| Ethanol | $C_2H_6O$ | 2 | 6 | −181.75 |
| Ethylene glycol | $C_2H_6O_2$ | 2 | 5 | −323.21 |
| Fumarate | $C_4H_4O_4$ | 4 | 3 | −604.21 |
| Galactose | $C_6H_6O_6$ | 6 | 4 | −915.38 |
| Gluconate | $C_6H_{12}O_7$ | 6 | 3.67 | −1154.0 |
| Glucose | $C_6H_{12}O_6$ | 6 | 4 | −923.53 |
| Glycerate | $C_3H_6O_3$ | 3 | 4 | −658.1 |
| Glycerol | $C_3H_8O_3$ | 3 | 4.667 | −477.06 |
| Glycine | $C_2H_5O_2N$ | 2 | 3 | −370.79 |
| Glyoxylate | $C_2H_2O_3$ | 2 | 2 | −468.6 |
| Lactate | $C_3H_6O_3$ | 3 | 4 | −517.81 |
| Lactose | $C_{12}H_{22}O_{11}$ | 12 | 4 | −1515.24 |
| Malate | $C_4H_6O_5$ | 4 | 3 | −845.08 |
| Malonate | $C_3H_4O_4$ | 3 | 2.667 | −700 |
| Mannitol | $C_6H_{14}O_6$ | 6 | 4.33 | −942.61 |
| Propane | $C_3H_8$ | 3 | 6.67 | −24 |
| Propanediol | $C_3H_8O_2$ | 3 | 5.33 | −327 |
| Propanol | $C_3H_8O$ | 3 | 6 | −175.81 |
| Propionate | $C_3H_6O_2$ | 3 | 4.67 | −361.08 |
| Pyruvate | $C_3H_4O_3$ | 3 | 3.33 | −474.63 |
| Sorbitol | $C_6H_{14}O_6$ | 6 | 4.33 | −492.2 |
| Succinate | $C_4H_6O_4$ | 4 | 3.5 | −690.23 |
| Tartrate | $C_4H_6O_6$ | 4 | 2.5 | −1010 |

Values for $\Delta G^{01}_{eDf}$ for the substrates are from Thauer et al. (1977), Stull et al. (1969) and Rutgers (1990).

could just as accurately predict and report yield values in electron-equivalents in cells/electron-equivalent in substrate. For the reader interested in repeating the calculations using each method, Appendix A shows the step-by-step methodology for a sample compound, citrate.

Table 4 shows the predicted yields by each method and the percent difference between the predictions for the set of 30 compounds *with oxygen as the electron acceptor*. Figure 2 shows a comparison of the predicted yields by the two methods for all electron acceptors evaluated ($O_2$, $NO_3^-$, $SO_4^{2-}$, $Fe^{3+}$, $CO_2$). The line shown is the best-fit going through the origin. The methods show good correlation ($R^2 = 0.9$), with the average difference less than 15% for aerobic heterotrophs and between 15–25% for other electron-acceptor substrates. This is slightly more than the typical error reported for the methods (13% reported by Heijnen & van Dijken (1992) for the dissipation correlation approach; 12–15% for the efficiency approach (see below and VanBriesen 2001).

### *Method accuracy*

In order to evaluate the accuracy of the thermodynamic yield prediction methods, a set of experimentally determined yields is needed. As discussed above, determination of experimental or observed yields is well established, but system controls and measurement of maintenance costs are rarely sufficient to

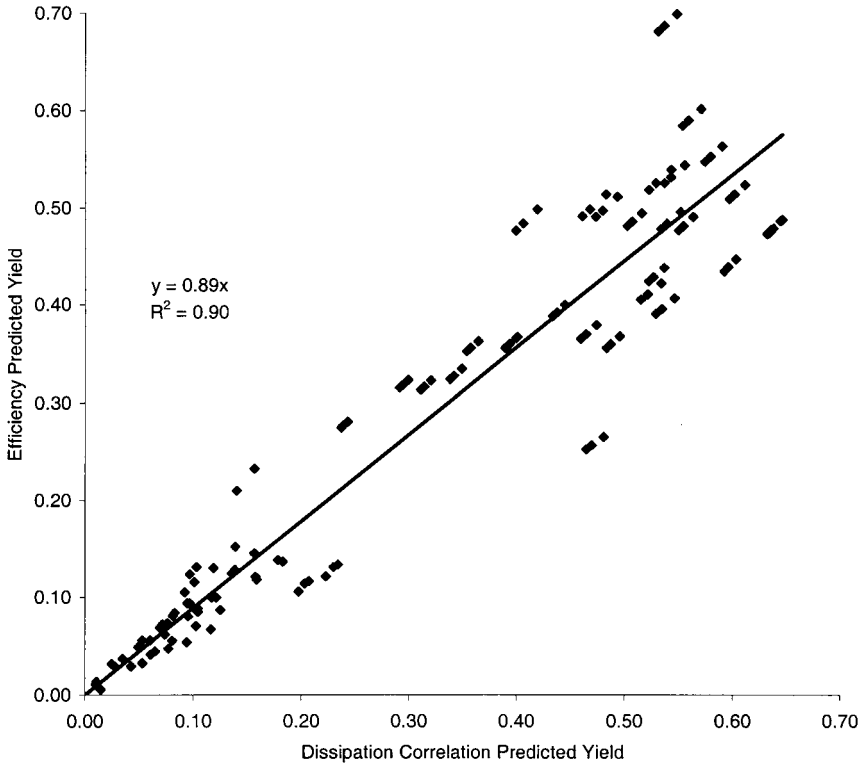


*Figure 2.* Comparison of yield predictions for compounds given in Table 2 using the dissipation correlation approach and the efficiency approach. Electron acceptors evaluated include $O_2$, $NO_3^-$, $SO_4^{2-}$, $Fe^{3+}$, and $CO_2$.

allow extrapolation from observed to true yields. For the comparisons in this work, I selected aerobic yields reported by Rutgers et al. (1989). Organisms were grown in chemostats at close to their maximum specific growth rate ($\mu_{max}$) under carbon-limited conditions with no product formation (as verified by carbon balance), using constant pH and temperature. The reciprocal yield and the reciprocal dilution rate produce a linear relationship that was extrapolated to determine the yield and standard deviation for the maximum growth rate. While not as accurate as experimental determination of maintenance costs followed by numerical adjustment of observed yield to theoretical yield following Equation (6) this method is likely to produce a yield *close* to the theoretical yield.

It is worth noting that reported yield values for many other substrates are available in the literature; however, for comparison with theoretical yield predictions, it is *critical* that only experimental values under controlled conditions that minimize aspects of bacterial growth that reduce yields. Thus, the limited data set selected were conducted under conditions designed to *minimize* (1) energy spilling or catabolic-anabolic uncoupling (Chen & Liu 1999), (2) formation of intermediates and soluble microbial products (Noguera et al. 1994; VanBriesen & Rittmann 2000a), (3) formation of storage polymers (van Loosdrecht et al. 2000), and (4) diversion of energy and electrons for maintenance (Pirt 1965). Further, because unit conversions for cell yields measured in dry weight or weight of protein require assumptions about the composition of the bacterial cells, yields that are reported in c-mole/c-mole will be most directly comparable to predictions from the thermodynamic methods.

Table 5 presents data and computed values for yield predictions for both methods. Table 6 provides the observed yields from Rutgers et al. (1989) and the predicted yields using the two methods and the percent error between the prediction and the observation. Figure 3 shows the predicted theoretical yields plotted against the reported maximum yields with errors as reported in Rutgers et al. (1989). The solid line is for the observed yields, and dotted lines bracket 15% above and below the observed yields.

For the dissipation correlation approach, yield values predicted are based on Equation (15) using an

*Table 3.* Computed values needed to predict yield for substrates using the dissipation correlation approach or the efficiency approach

| | $\Delta G_{eD}^{01}$ (kJ/e-eq) | $D_s^{01}/r_{AX}$ (kJ) | $\Delta G_{e-\text{syn}}^{01}$ (kJ/e-eq) |
|---|---|---|---|
| Acetate | 31.06 | 432.12 | 49.83 |
| Acetoin | 32.04 | 510.43 | 46.57 |
| Butanediol | 43.99 | 737.23 | 32.18 |
| Butyrate | 28.45 | 510.43 | 58.53 |
| Citrate | 37.94 | 466.80 | 34.00 |
| Dihydroxyacetone | 39.74 | 347.64 | 33.46 |
| Ethane | 25.06 | 1010.62 | 69.83 |
| Ethanol | 29.89 | 706.20 | 53.73 |
| Ethylene glycol | 37.49 | 524.36 | 34.13 |
| Fumarate | 39.30 | 389.36 | 33.59 |
| Galactose | 39.10 | 236.05 | 33.65 |
| Gluconate | 38.97 | 222.72 | 33.69 |
| Glucose | 38.76 | 236.05 | 33.75 |
| Glycerate | 22.40 | 347.64 | 78.70 |
| Glycerol | 37.84 | 428.06 | 34.03 |
| Glycine | 34.45 | 478.41 | 38.53 |
| Glyoxylate | 56.71 | 620.73 | 28.37 |
| Lactate | 34.09 | 347.64 | 39.73 |
| Lactose | 40.72 | 432.12 | 33.16 |
| Malate | 39.02 | 389.86 | 33.67 |
| Malonate | 38.05 | 477.89 | 33.97 |
| Mannitol | 38.12 | 333.87 | 33.94 |
| Propane | 25.51 | 1163.93 | 68.33 |
| Propanediol | 32.65 | 574.67 | 44.53 |
| Propanol | 28.66 | 811.86 | 57.83 |
| Propionate | 29.16 | 428.62 | 56.17 |
| Pyruvate | 37.22 | 373.41 | 34.21 |
| Sorbitol | 38.12 | 333.87 | 33.94 |
| Succinate | 33.25 | 297.06 | 42.53 |
| Tartrate | 46.09 | 548.52 | 31.55 |

$\Delta G_{eD}^{01}$ using $\Delta G_{eDf}^{01}$ (Table 1, column 4) and the Gibbs energies for $H_2CO_3$ (−324 kJ/mole), $NH_4^+$ (−79.7 kJ/mole), $H_2O$ (−237.4 kJ/mole) and $H^+$ at pH 7 (−40.0 kJ/mole). Values for these constants are from Thauer et al. (1977).
$D_s^{01}/r_{AX}$ following Equation (14); $\Delta G_{e-\text{syn}}^{01}$ following Equation (20) with $\kappa = 0.3$ and $\Delta G_{e-\text{cells}}^{01} = 10.41$ kJ/e-eq for cells with an assumed formula of $CH_2O_{0.6}N_{0.2}$; $\Delta G_{e-\text{pyr}}^{01} = 35.6$ kJ/e-eq.

*Table 4.* Comparison of yield predictions using the dissipation correlation approach or the efficiency approach

| Substrate | Yield by dissipation correlation approach | Yield by efficiency approach | % difference in yield predictions |
|---|---|---|---|
| Acetate | 0.474 | 0.379 | 22.36 |
| Acetoin | 0.552 | 0.496 | 10.67 |
| Butanediol | 0.548 | 0.699 | −24.11 |
| Butyrate | 0.534 | 0.422 | 23.36 |
| Citrate | 0.364 | 0.362 | 0.58 |
| Dihydroxyacetone | 0.563 | 0.491 | 13.80 |
| Ethane | 0.483 | 0.514 | −6.21 |
| Ethanol | 0.543 | 0.539 | 0.72 |
| Ethylene glycol | 0.571 | 0.602 | −5.24 |
| Fumarate | 0.401 | 0.367 | 8.98 |
| Galactose | 0.646 | 0.488 | 27.87 |
| Gluconate | 0.603 | 0.447 | 29.68 |
| Glucose | 0.644 | 0.486 | 27.87 |
| Glycerate | 0.481 | 0.265 | 57.88 |
| Glycerol | 0.590 | 0.563 | 4.69 |
| Glycine | 0.349 | 0.335 | 4.31 |
| Glyoxylate | 0.243 | 0.280 | −14.26 |
| Lactate | 0.536 | 0.438 | 20.18 |
| Lactose* | 0.516 | 0.495 | 4.25 |
| Malate | 0.400 | 0.366 | 8.98 |
| Malonate | 0.321 | 0.322 | −0.56 |
| Mannitol | 0.611 | 0.524 | 15.44 |
| Propane | 0.419 | 0.499 | −17.24 |
| Propanediol | 0.556 | 0.544 | 2.13 |
| Propanol | 0.494 | 0.511 | −3.49 |
| Propionate | 0.546 | 0.406 | 29.35 |
| Pyruvate | 0.445 | 0.400 | 10.80 |
| Sorbitol | 0.611 | 0.524 | 15.44 |
| Succinate | 0.496 | 0.368 | 29.64 |
| Tartrate | 0.299 | 0.323 | −7.56 |
| **Average absolute difference** | | | **14.9** |

* Lactose has $C > 6$; however, for estimation with the dissipation correlation method, it was assumed that a smaller fragment of the full compound ($C = 2$) was used for synthesis. Dissipation correlation approach uses equations (14) and (15) with $\Delta G_{eX}^{01} = 38.8$ kJ/e-eq (based on $\Delta G_{fX}^{01} = -67$ kJ/C-mole), $\gamma_X = 4.2$, and $\Delta G_{eA}^{01} = -78.72$ kJ/e-eq for oxygen. Efficiency approach based on $K = \kappa = 0.3$ and Equations (18), (19), and (26). Percent difference computed as $100\% x \frac{(Y_{\text{diss}} - Y_{\text{eff}})}{((Y_{\text{diss}} + Y_{\text{eff}})/2)}$.

assumed $\Delta G_{fX}^{01} = -67$ kJ/C-mole (Roels 1980). Calculations were also made with the $\Delta G_{fX}^{01} = -45$ kJ/C-mole (Grosz & Stephanopoulos 1983) and with the simplification (Equation (16)) based on assuming regularity (results not shown). Differences between the predictions based on the full method (using Equation (15)) and the simplification based on Equation (16) range from 2% to 6%. Not unexpectedly, the largest discrepancies appear for substrates that have $\Delta G_{eD}^{01}$ values significantly different from the regularity value (e.g., glyoxylate). It is also worth noting that the differences between results using Equation (15) with the different values of $\Delta G_{fX}^{01}$ were also small (1–3%), indicating the method is not very sens-

*Table 5.* Data and computed values for yield predictions using the dissipation correlation approach or the efficiency approach

| Substrate | Formula | Number of carbons (C) | Degree of reductance | $\Delta G^{01}_{eDf}$ (kJ/mole) | $\Delta G^{01}_{eD}$ (kJ/e-eq) | $D^{01}_s/r_{AX}$ (kJ) | $\Delta G^{01}_{e-\text{syn}}$ (kJ/e-eq) |
|---|---|---|---|---|---|---|---|
| Acetate | $C_2H_4O_2$ | 2 | 4 | −369.41 | 31.06 | 432.12 | 49.83 |
| Citrate | $C_6H_8O_7$ | 6 | 3 | −1168.34 | 37.94 | 466.80 | 34.00 |
| Ethanol | $C_2H_6O$ | 2 | 6 | −181.75 | 29.89 | 706.21 | 53.73 |
| Fructose | $C_6H_{12}O_6$ | 6 | 4 | −915.38 | 39.10 | 236.05 | 33.65 |
| Glycerol | $C_3H_8O_3$ | 3 | 4.67 | −477.06 | 37.84 | 428.06 | 34.03 |
| Glyoxylate | $C_2H_2O_3$ | 2 | 2 | −468.60 | 56.71 | 620.73 | 28.37 |
| Malonate | $C_3H_4O_4$ | 3 | 2.67 | −700.0 | 38.05 | 477.19 | 33.97 |
| Succinate | $C_4H_6O_4$ | 4 | 3.5 | −690.23 | 33.25 | 297.06 | 42.53 |
| Tartrate | $C_4H_6O_6$ | 4 | 2.5 | −1010.00 | 46.09 | 548.52 | 31.55 |

*Table 6.* Yield prediction with the dissipation correlation approach or the efficiency approach

| Substrate | Observed yield (C-mole cells/C-mole substrate) | Yield by dissipation correlation approach | Percent error for dissipation correlation approach | Yield by efficiency approach | Percent error for efficiency approach |
|---|---|---|---|---|---|
| Acetate | 0.406 ± 0.006 | 0.474 | −16.8 | 0.379 | 6.7 |
| Citrate | 0.390 ± 0.011 | 0.364 | 6.6 | 0.362 | 7.1 |
| Ethanol | 0.558 ± 0.013 | 0.543 | 2.7 | 0.539 | 3.4 |
| Fructose | 0.505 ± 0.049 | 0.646 | −27.9 | 0.488 | 3.4 |
| Glycerol | 0.569 ± 0.019 | 0.590 | −3.7 | 0.564 | 1.1 |
| Glyoxylate | 0.220 ± 0.016 | 0.243 | −10.5 | 0.280 | −27.5 |
| Malonate | 0.238 ± 0.041 | 0.321 | −34.9 | 0.323 | −35.6 |
| Succinate | 0.385 ± 0.014 | 0.496 | −28.7 | 0.368 | 4.5 |
| Tartrate | 0.280 ± 0.001 | 0.299 | −6.9 | 0.323 | −15.4 |
| **Average absolute error** | | | **15.4** | | **11.6** |

Observed yield values from Rutgers et al. (1989). Dissipation correlation approach uses Equations (14) and (15) with $\Delta G^{01}_{eX} = 38.8$ kJ/e-eq (based on $\Delta G^{01}_{fX} = -67$ kJ/C-mole). Efficiency approach based on $K = 0.3$ and Equations (18), (19), and (26). Percent error $100x\left(\frac{Y_{\text{obs}}-Y_{\text{pred}}}{Y_{\text{obs}}}\right)$.

itive to this empirical value, while it is slightly more sensitive to $\Delta G^{01}_{eCS}$.

Comparing the dissipation correlation approach with the reported yield values shows absolute errors ranging from 3–35%, with the highest error for malonate. The average absolute value of the error using this method for this dataset was 15%. This is higher than the average error reported by Heijnen & van Dijken (1992) of 12%. This is not unexpected, since this dataset includes substrates that were not part of the original dataset used to develop the correlation (Equation (14)), while the 12% error reported by Heijnen & van Dijken (1992) was for prediction of the same substrates as were in the dataset used to generate the correlation.

The yield predicted by the efficiency approach is predicted from the data in Table 5 and the $\Delta G^{01}_{eR}$ and $\Delta G^{01}_{e-\text{syn}}$ following Equations (24)–(26), using an assumed constant thermodynamic efficiency of $\kappa = K = 0.3$. Absolute values of errors range from 1–35%, with an overall average error of 12%. Interestingly, as for the dissipation correlation approach,

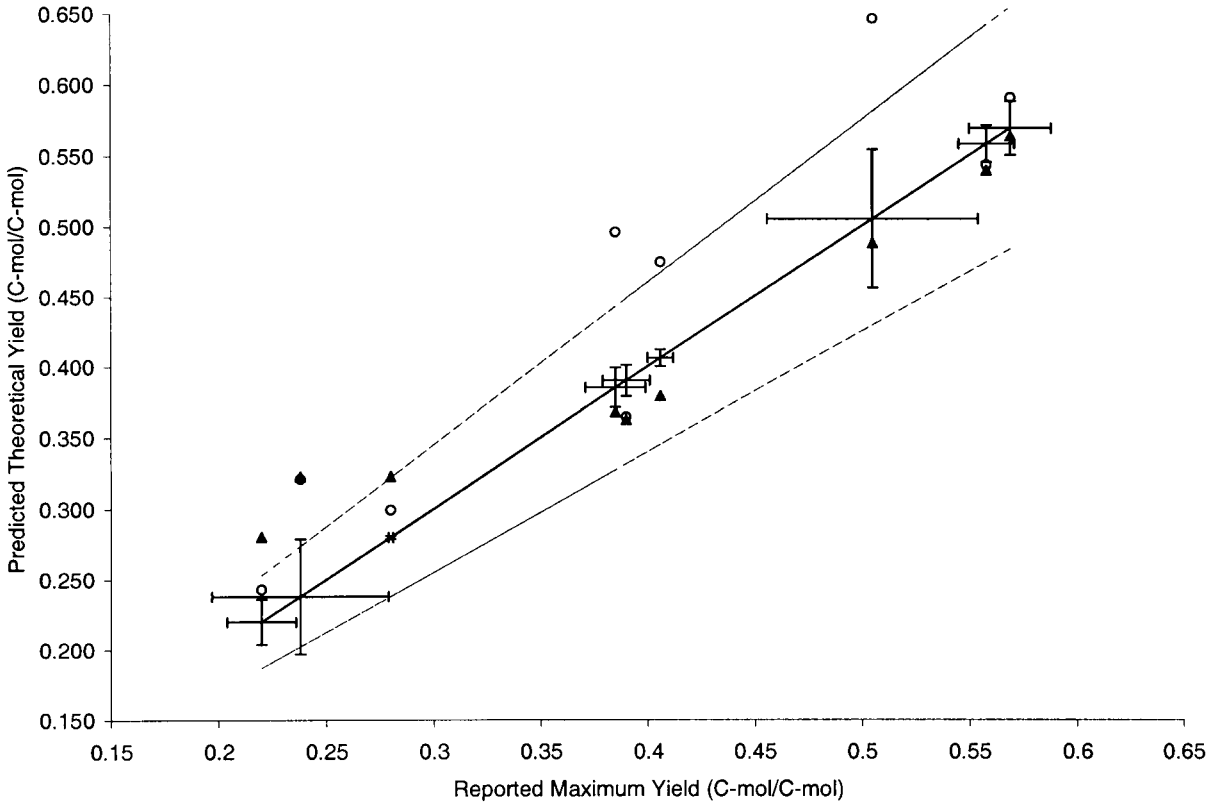


*Figure 3.* Comparison between predicted theoretical and reported maximum yield values for substrates in Tables 5 and 6. Circles are predictions based on dissipation correlation approach. Triangles are predictions based on efficiency approach. Solid line is observed yields; reported errors are shown as bars (Rutgers et al. 1989). Dotted lines are 15% above and below reported yields.

malonate is again the substrate with the highest prediction error (35% error for each method).

## Conclusions

Thermodynamic models are used to predict bacterial yields and develop stoichiometric representation of biological reactions in the absence of empirical data. Several competing methods have been used by microbiologists, biotechnologists, and environmental engineers. Comparison of these different methodologies shows them to be mathematically equivalent and to predict yield values within 15% of one another. This is close to the predicted error for the individual methods (15% for the dissipation correlation approach and 12% for the efficiency approach). Caution is warranted in utilization of predicted yields due to the high degree of variability in the behavior of different organisms growing on the same substrate and due to the effect of environmental conditions on bacterial yield. However, when yield prediction is necessary, the methods evaluated in this paper produce acceptable and consistent results.

## Appendix A: Example of prediction of yield of citrate

Citrate is biodegraded following Equation (1), repeated here for convenience:

$$aC_6H_8O_7 + bO_2 + cNH_4^+ \rightarrow dCH_2O_{0.6}N_{0.2} + eH_2CO_3 + fH^+ + gH_2O.$$

The yield of cells on citrate can be predicted following the dissipation method or the efficiency method. These methods are demonstrated in this Appendix.

### *Dissipation method*

First, the dissipation is computed following Equation (14), repeated here:

$$D_s^{01}/r_{Ax} = 200 + 18 \times (6 - C)^{1.8} + \text{Exp}[\{(3.8 - \gamma_D)^2\}^{0.16} x (3.6 + 0.4C)].$$

For citrate, $C = 6$ and $\gamma_D = 3$, thus

$$D_s^{01}/r_{Ax} = 200 + 18x(6-6)^{1.8} + \text{Exp}[\{(3.8-3)^2\}^{0.16} \times \times (3.6 + 0.4 \times 6)] = 466.8 \text{ kJ/C-mole}.$$

Next, Equation (15) is used to predict the yield:

$$Y_{DX} = \frac{\gamma_D}{\gamma_X} \frac{\Delta G_{eD}^{01} - \Delta G_{eA}^{01}}{(\Delta G_{eD}^{01} - \Delta G_{eA}^{01}) + \left[\frac{D_s^{01}}{r_{Ax}} x \frac{1}{\gamma_X} + \Delta G_{eX}^{01} - \Delta G_{eD}^{01})\right]},$$

where $\Delta G_{eD}^{01} = 37.94$ kJ/eeq, $\Delta G_{eA}^{01} = -78.72$ kJ/eeq, $\Delta G_{eX}^{01} = 38.8$ kJ/eeq (derived from $\Delta G_{fX}^{01} = -67$ kJ/Cmole (Roels 1980)), $\gamma_D = 3$, $\gamma_X = 4.2$, and $D_s^{01}/r_{Ax}$ was calculated above as 466.8 kJ/C-mole.

$$\begin{aligned} Y_{DX} &= \frac{3}{4.2} \frac{37.94 - (-78.72)}{(37.94 - (-78.72)) + \left[466.8 x \frac{1}{4.2} + (38.8 - 37.94)\right]} \\ &= 0.364 \text{ CmolCells/CmolCitrate} \\ &= 0.510 \text{ electron-equivalentsCells/electron-equivalentsCitrate} \end{aligned}$$

*Efficiency method*

This method begins with computing the cost to generate cells from the substrate following Equation (20),

$$\Delta G_{e-\text{syn}}^{01} = \frac{(\Delta G_{e-\text{pyr}}^{01} - \Delta G_{eCS}^{01})}{\kappa^m} + \frac{\Delta G_{e-\text{cells}}^{01}}{\kappa},$$

$\kappa = 0.3$, $\Delta G_{e-\text{cells}}^{01} = 10.41$ kJ/e-eq, $\Delta G_{e-\text{pyr}}^{01} = 35.6$ kJ/e-eq, and $\Delta G_{eCS}^{01} = \Delta G_{eD}^{01} = 37.94$ kJ/eeq. Since $(\Delta G_{e-\text{pyr}}^{01} - \Delta G_{eCS}^{01}) < 0$, $m = -1$. Thus,

$$\Delta G_{e-\text{syn}}^{01} = \frac{(35.6 - 37.94)}{0.3^{-1}} + \frac{10.41}{0.3} = 34.0 \text{ kJ/eeq}.$$

Next, $A$ is calculated following Equation (24):

$$A = \frac{f_e^0}{f_s^0} = \frac{-\Delta G_{e-\text{syn}}^{01}}{K \Delta G_{eR}^{01}},$$

where $\Delta G_{eD}^{01} = 37.94$ kJ/eeq, $\Delta G_{eA}^{01} = -78.72$ kJ/eeq, $\Delta G_{eR}^{01} = -78.72 - 37.94 = -116.66$ kJ/eeq, $\Delta G_{e-\text{syn}}^{01}$ was computed above and $K = 0.3$.

$$A = \frac{f_e^0}{f_s^0} = \frac{-34.0}{0.3(-116.66)} = 0.9715.$$

Next, $f_s^0$ is computed from $A$ following Equation (25):

$$f_s^0 = \frac{1}{1 + A} = \frac{1}{1 + 0.97148} = 0.507$$

and

$$f_e^0 = 0.493.$$

And finally, the yield is computed from Equation (26)

$$\begin{aligned} Y = f_s^0 &= 0.507 \text{ electron-equivalentCells/electron-equivalentCitrate} \end{aligned}$$

$$\begin{aligned} Y = f_s^0 \frac{\gamma_D}{\gamma_X} &= 0.507 \frac{3}{4.2} \\ &= 0.362 \text{ CmolCells/CmolCitrate}. \end{aligned}$$

Thus for citrate, the two methods show less than a 1% difference in predicted yield. Based on the observed yield of 0.390 Cmole/Cmole for citrate, the methods show a 6–7% error.

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