Biodegradation **12:** 265–281, 2001.

Thermodynamic yield predictions for biodegradation through oxygenase activation reactions

Jeanne M. VanBriesen
Department of Civil and Environmental Engineering, Carnegie Mellon University, Pittsburgh, PA 15213-3890, USA (e-mail: jeanne@cmu.edu)

Key words: biodegradation, microbial thermodynamics, modeling, oxygenation, yield

Abstract

Activation reactions involve modification of recalcitrant substrates to forms that are more readily degradable. These reactions require specialized enzymes and cosubstrates, including molecular oxygen and reduced electron carriers. In these reactions, microorganisms invest electrons and cannot capture energy or carbon for synthesis. The subsequent degradation of the intermediates formed in activation reactions releases electrons, energy, and carbon that the organisms use for growth. The overall yield is reduced due to the required activation investments. A mathematical method to predict cell yields of oxygenase activation reactions is developed using electron and energy balances. Predicted yields are compared with experimental yields for methane, organic chelating agents, and aromatic hydrocarbons.

Introduction

Recalcitrant organic compounds often contain structural components that challenge microbial enzyme systems. Polycyclic aromatic hydrocarbons (PAHs), nitroaromatics (e.g., TNT), halogenated compounds (e.g., PCBs), and strong organic chelating agents (e.g., EDTA) contain complex structures that require dedicated enzyme systems for bacterial degradation. In many cases these specialized enzymes catalyze reactions that the organisms use to 'activate' the organic substrate for subsequent oxidation. One major type of activation reaction is the reduction of molecular oxygen for insertion into the substrate compound. Unlike reduction of molecular oxygen as an electron acceptor for energy generation via respiration, oxygenase-catalyzed reactions reduce oxygen without capturing energy. The electrons needed for this reduction may come from the oxidation of the substrate, downstream intermediates, co-metabolic substrates, or biomass. Like the energy associated with the reduction, these electrons are also lost; they are no longer available for energy generation or cell synthesis reactions. The investment of reducing equivalents in activating the organic compound and the spilling of the energy associated with the reduction of molecular oxygen profoundly change the stoichiometric relationships, reducing the overall yield of organisms in the system.

In this work, I present a modification to the thermodynamic yield prediction methodology developed by McCarty (e.g., McCarty 1975; Rittmann & McCarty 2001) that allows prediction of overall yields for biological degradation pathways involving oxygenase-catalyzed steps. The modification is easy to implement, does not require detailed understanding of the degradation pathway, and results in significant improvement to predicted yield values. Analyses of theoretical predictions and experimental yield values for several compounds requiring oxygenase reactions for their transformation (organic chelating agents and aromatic hydrocarbons) are presented as demonstration of the method.

Cell yield and its prediction

Cell yield (Y) is a fundamental biological parameter that allows prediction of the full stoichiometry of biological growth/substrate utilization reactions and thus is necessary for modeling coupled biological and chemical reactions. The yield is combined with mass balances using either the method of regularities (Minkevich & Eroshin 1973; Erickson et al. 1978a,b; Erickson 1979; Minkevich 1985) or the method of half reactions (McCarty 1965, 1969, 1971, 1972a,b, 1975; Heijnen et al. 1992; Heijnen & vanDijken 1992; Tijhuis et al. 1993; Heijnen 1994; VanBriesen & Rittmann 2000) to predict the overall stoichiometry of the biological reactions.

Often the net yield for the oxidation of an organic electron-donor substrate can be measured experimentally. However, for compounds that participate in competitive abiotic reactions (e.g., sorption or volatilization), or complex multi-step reactions, experimental yields are difficult to determine. Yields for multi-organism, multi-substrate systems may also need to be estimated without extensive experimental work (Heijnen & vanDijken 1992). In these cases, a systematic method for *predicting* yields in biological degradation reactions is required.

Several methods have shown promise and continue to be used to provide the best 'estimate' of this crucial parameter. Roels (1983, 1987) introduced the concept of thermodynamic efficiency and the related concept of Gibbs Energy Dissipation for the overall growth and substrate utilization reaction. He suggests a rule-of-thumb value of thermodynamic efficiency of 0.60 will produce a useful first estimate of the expected growth yield. Heijnen & vanDijken (1992) provide an expansion of this concept based on a correlation between the Gibbs Energy dissipation and the number of carbon atoms and the degreee of reductance of the electron donor substrate. This method provides a first estimate of the yield more specifically related to the structure of the substrate. However, the correlation developed is based on a limited set of pure culture laboratory experiments of growth on simple carbon compounds. As such, its utility for more complex, anthropogenic compounds whose structures require specialized enzyme systems for initiation of mineralization reactions, is unclear.

In the late 1960's a thermodynamic yield prediction methodology was introduced in the environmental engineering literature by McCarty (1965, 1969, 1971, 1972a,b, 1975). This methodology is fundamentally equivalent to the method of Heijnen (VanBriesen 2001), although, like Roels (1983, 1987), McCarty suggests use of an efficiency term and provides a rule of thumb value for different types of organisms. This methodology has been employed extensively in the environmental biotechnology field (e.g., Noguera et al. 1988; 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) and is presented most recently by Rittmann & McCarty (2001). The major limitation to this method is that it is generally applied by assuming simple degradation pathways to complete mineralization. In natural systems, with anthropogenic compounds this assumption comes into question. Recently, VanBriesen & Rittmann (2000) modified the McCarty yield prediction method to allow predictions in systems where complex intermediates form and are then utilized during the microbial degradation process. Subsequently, Woo & Rittmann (2000) built on this methodology to evaluate degradation of phenanthrene, a polycyclic aromatic hydrocarbon that is degraded through a pathway involving multiple oxygenase-catalyzed reactions. In this work, I further expand the thermodynamic prediction methodology introduced by McCarty (1965, 1969, 1971, 1972a,b, 1975) to develop a generalized method to predict yields for complex organic compounds that are degraded via oxygenation reactions.

The McCarty method of yield prediction is based on the assumption that electron and energy balances control the behavior of biological systems (i.e., catabolism and anabolism are coupled). For cell growth to occur, an electron-donor substrate is oxidized and electrons are shuttled to the electron acceptor to release Gibbs free energy or to the carbon and nitrogen sources to reduce these elements to the oxidation state necessary for incorporation into cells. Mathematically, the energy and electron balances are represented as:

$$-f_e^0 \kappa \Delta G_{eR}^{01} = f_s^0 \Delta G_{e\text{-}syn}^{01} \tag{1}$$

$$f_e^0 + f_s^0 = 1 \tag{2}$$

in which ΔG_{eR}^{01} is the standard free energy of the redox reaction between electron donor and electron acceptor, $\Delta G_{e\text{-}syn}^{01}$ is the standard free energy of the cell synthesis reaction, κ is the efficiency of energy capture in the energy-generation reaction (generally assumed to be 0.6 (Rittmann & McCarty 2001)), f_e^0 is the frac-

tion of electron-donor electron equivalents sent to the acceptor to drive the energy generating redox reaction and f_s^0 is the fraction of electron-donor electron equivalents invested in biomass via the synthesis reaction. All Gibbs energies are in the standard state of T = 25 °C, P = 1 atm, 1 M reactants and products (thus the specification of the superscript 0), except $[H^+] = 10^{-7}$ (thus the specification of the superscript 1). All Gibbs energies are in units of kJ/e-eq and the subscript e is introduced here to specify these units in terms of electron equivalents. The original McCarty method was also presented in terms of units of electron equivalents but did not employ a specific notation for these units. The introduction of this more specific notation is needed to distinguish the energy associated with a half reaction written per electron equivalent with the energy associated with a half reaction written per *mole* of substrate and also to introduce notational consistency with other yield prediction methods (e.g., Heijnen & vanDijken 1992). ΔG_{eR}^{01} is computed as the difference between the free energy of the donor and acceptor half reactions when both reactions are written as *reductions* ($\Delta G_{eA}^{01} - G_{eD}^{01}$). $\Delta G_{e\text{-}syn}^{01}$ is estimated from experimental data (discussed below).

Equation (1) says that the energy made available from transfer of electrons from the donor to the acceptor ($-f_e^0 \kappa \Delta G_{eR}^{01}$) is invested to synthesize f_s^0 electron equivalents of biomass ($f_s^0 \Delta G_{e\text{-}syn}^{01}$). Equation (2) 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_s^0 and f_e^0. The yield is then predicted from:

$$\gamma \left(\frac{\text{moles cell carbon}}{\text{moles substrate carbon}} \right) = f_s^0 \frac{\gamma_s}{\gamma_c} \qquad (3)$$

where γ_c is the degree of reductance of the cells and γ_s is the degree of reductance of the substrate.

Once the f_s^0 is known, the overall stoichiometry can also be generated using the half reactions for the electron donor (R_D), the electron acceptor (R_A) and cell synthesis (R_C), following Rittmann & McCarty (2001):

$$\text{Overall Reaction} = -R_D + f_e^0 R_A + f_s^0 R_C \qquad (4)$$

Biological investment reactions using oxygenase enzymes

Many organic compounds resemble natural metabolites and are degraded by standard metabolic enzymes. However, anthropogenic compounds can have structural moities that preclude degradation without key initiation reactions. These 'activation' reactions alter the structure and thus reactivity of compounds towards standard enzyme systems. Organisms perform activation reactions by either reducing molecular oxygen and inserting it into the compound using mono- or di-oxygenase enzymes or reducing the compound via electrons supplied by intracellular carriers (i.e., $NADH_2^+$). In this work, I consider activation by oxygenase enzymes.

Oxygenase-catalyzed reactions involve the addition of oxygen atoms (from molecular oxygen) into an organic structure. Reducing the oxygen to its −2 oxidation state requires an investment of electrons, which come from oxidation within the structure of the compound or from reduced electron carriers. When electron carriers supply the reducing equivalents, these may be recouped in later oxidative degradation of the substrate, its intermediates, or other electron donors (through cometabolism). Here I do not consider cometabolism so the required activation electrons are derived from the main pathway after the oxygenase reactions.

In the original McCarty method, available electrons from the electron donor are assumed to be captured for transfer to either the electron acceptor or for cell synthesis; however, in the case of oxygenase reactions, this assumption is invalid. Some electrons are utilized for the reduction of molecular oxygen necessary to insert it into the substrate. In addition to requiring electrons, the reduction of oxygen releases Gibbs energy. The reduction of oxygen to water is very energetically favorable ($\Delta G_{e\text{-}ox}^{01} = -78.14$ kJ/eeq or $\Delta G_{ox}^{01} = -625.12$ kJ/mole O_2), and oxygen is reduced as it is inserted into the substrate molecules. In the standard thermodynamic method, this energy is implicitly assumed to be available to the organisms, since the method deals only with the Gibbs energy of formation of reactants and products, and oxygen is a reactant while water is a product. Despite the highly exergonic nature of the reaction, this Gibbs energy is not conserved by the formation of ATP (Mason and Cammack 1992) and instead is dissipated by the organism as enthalpy.

Also, since the Gibbs energy release is not coupled to positive electron transfer, and the cells require electrons for synthesis reactions, the energy released is unavailable for synthesis until subsequent steps make electrons available. Similarly, oxygenase catalyzed reactions often involve rearrangement of the structure of the primary substrate to accommodate the oxygen, making it more easily degraded in subsequent steps, but often do not involve release of carbon. If the primary electron-donor substrate is also the cells' carbon-source substrate (as if often the case with degradation of anthropogenic compounds by specialized bacteria), synthesis with the energy released due to the oxygen insertion would again not be possible until subsequent steps release carbon. One possible solution to this problem would be for cells to utilize a substrate-level phosphorylation, where Gibbs energy from a reaction is coupled directly (not through NADH) to production of ATP. However, there is no evidence to suggest that substrate-level phosphorylation is coupled to oxygenase-catalyzed reactions. In the methane monooxygenation system, methane and methanol are utilized by bacteria with nearly the same yield indicating that the energy associated with the insertion of oxygen into the compound is not available to the organisms (Linton & Stephenson 1978; Wolf & Higgins 1979; Heijnen & Roels 1981; Brock & Madigan 1991). For that reason, I suggest the Gibbs energy change associated with the reduction of oxygen to water in the system must be removed from the released energy calculated for the energy balance (Equation (1)). When the Gibbs energy of the electron donor half reaction is calculated, it must be reduced by the energy change that came from the reduction of oxygen since this energy is unavailable to the cells. Likewise, the electrons used to reduce molecular oxygen are not available and an adjustment to the electron balance (Equation (2)) is also necessary. Once these adjustments are made, f_s^0 can again be estimated and the yield predicted using Equation (3).

Thus, modeling systems involving the utilization of oxygenase enzymes requires key changes to the structure of the original McCarty thermodynamic method.

Energy balance modifications

When the electron-donor substrate is mineralized using an oxygenase enzyme activation, the half reaction for the donor must explicitly include the oxygen cosubstrate. However, the Gibbs free energy associated with this electron donor half reaction will be reduced by the energy directly related to the reduction of the oxygen:

$$(\Delta G_D^{01})^{ox} = \Delta G_D^{01} + \Delta G_{e\text{-}ox}^{01} x N_O \qquad (5)$$

where $(\Delta G_D^{01})^{ox}$ is the Gibbs energy of the electron donor half-reaction, modified for consideration of the energy "invested" in the oxygenase reaction (note units are kJ/mole substrate), ΔG_{e-ox}^{01} is the Gibbs energy of the oxygen reduciton reaction (-78.14 kJ/electron equivalent), and N_O is the number of electrons used to reduce oxygen for activation. For one oxygenase reaction, $N_O = 4$. This newly calculated Gibbs energy for the electron donor reaction, $(\Delta G_D^{01})^{ox}$ in kJ/mole of substrate is divided by the number of electrons transferred in the half reaction that includes molecular oxygen as a cosubstrate (denoted N_T here) to return the units to kJ/electron equivalent transferred in the reaction.

$$(\Delta G_{eD}^{01})^{ox} = \frac{(\Delta G_D^{01})^{ox}}{N_T} \qquad (6)$$

The calculation of the Gibbs energy associated with the electron donor/acceptor couple then continues normally $\Delta G_{eR}^{01} = (\Delta G_{eA}^{01} - (G_{eD}^{01})^{ox})$.

Alternatively, the calculations in (5) and (6) can be directly incorporated into the overall energy balance (Equation (1)), leading to

$$-f_e^0 \kappa \left(\Delta G_{eA}^{01} - \frac{\Delta G_D^{01}}{N_T} - (\Delta G_{e\text{-}ox}^{01})^{ox} \frac{N_O}{N_T} \right) = f_s^0 \Delta G_{e\text{-}syn}^{01} \qquad (7)$$

where ΔG_D^{01} is the molar Gibbs free energy of the electron-donor reduction half-reaction that includes the molecular oxygen cosubstrate.

Electron balance modifications

The electron balance (Equation (2) above) is modified to consider the diversion of electron equivalents away from synthesis and energy generation:

$$f_e^o + f_s^o + f_{ox} = 1 \qquad (8)$$

where f_{ox} is the fraction of electron-donor electron equivalents shunted to activation and not available for synthesis or energy generation. $f_{ox} = N_O / EQs$ where EQs is the total number of electron equivalents available in the electron-donor substrate. Figure 1 shows a schematic of electron and energy flow in

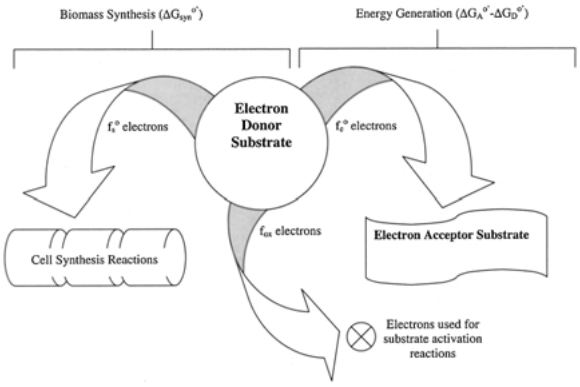

Figure 1. Representation of electron and energy flow in bacterial systems using oxygenase enzymes.

bacterial growth using oxygenase activation reactions. Three pathways for electron transfer are shown, to cell synthesis, to energy generation and to activation reactions.

Carbon source modifications

The original presentation by McCarty (1965, 1969, 1971, 1972a,b, 1975) suggested $\Delta G^{01}_{e\text{-}syn}$ should be computed based on a simplified two step synthesis involving conversion of the carbon-source substrate to a common intermediate (pyruvate) and conversion of this intermediate to cells.

$$\Delta G^{01}_{e\text{-}syn} = \frac{\Delta G^{01}_{epyr} - \Delta G^{01}_{eCS}}{K^m} + \Delta G^{01}_{e\text{-}cells} \qquad (9)$$

The estimate of the Gibbs energy associated with building cells from the intermediate ($\Delta G^{01}_{e\text{-}cells}$) was based on the degree of reductance of the cells (γ_s) and the carbon fraction of the cells (σ_c), generally believed to be relatively constant for microorganisms. Several constants are also used (namely, Y_{ATP}, $\Delta G_{ATP\,hydrolysis}$) that are now known to be variable. Average values are used for these 'constants', and the predicted Gibbs energy for synthesis of biomass ($\Delta G^{01}_{e\text{-}cells}$ for the assumed formulae of $CH_2O_{0.6}N_{0.2}$ with $\gamma_c = 4.2$ and a carbon fraction of $\sigma_c = 0.454$ g carbon/g cells) is 34.7 kJ/e-eq (note, the value reported by McCarty and widely used for this parameter is 7.5 kcal/e-eq or 31.35 kJ/e-eq and is based on an assumed formulae of $C_5H_7O_2N$).

For chemoheterotrophic organisms, the original McCarty method generally involves assuming the electron donor and the carbon source are the same organic compound, thus $\Delta G^{01}_{eCS} = \Delta G^{01}_{eD}$. For oxygenase-catalyzed activation reactions, this assumption is not valid. The primary electron donor substrate is transformed to an oxygen-adduct substrate that can then be metabolized. This transformation step usually does not release carbon. Further, the assumption that the cells can directly utilize a complex anthropogenic compound as carbon source can lead to significant error in yield prediction.

Several possible modifications to the original McCarty calculations are possible. In the simplest modification, the downstream oxygen adduct intermediate can be assumed to be the carbon source, its' Gibbs energy computed, and the synthesis costs from this intermediate calculated using Equation (9). However, in complex multi-step degradation pathways the first intermediate may be subsequently converted to other intermediates, with or without the release of carbon. Woo and Rittmann (2000), considering the stoichiometry of biodegradation of naphthalene, assumed pyruvate (released in many of the conversion steps) was actually the carbon source for the cells, thereby, using a constant value for $\Delta G^{01}_{e\text{-}syn}$ for each step in the complex pathway. The limitation to this modification is that it requires significant pathway information. One of the goals of any yield prediction methodology is to generate a prediction based solely on the original compound without knowledge of its biochemical pathways.

An alternative to use of a downstream intermediate as putative carbon source is to assume that a complex pathway releases simple carbon compounds that are likely to move directly into metabolic pathways, as does pyruvate. This allows the simplification of Equation (9) to $\Delta G^{01}_{e\text{-}syn} = \Delta G^{01}_{e\text{-}cells}$. While clearly this assumption will not hold for many cases (e.g., autotrophic growth), it can be a useful assumption when pathway information is not available but the complex structure of the organic electron-donor substrate suggests it is not a potential carbon source in its unmodified form. Support for judicious use of this assumption comes from several sources.

For simple compounds, the Gibbs energy of the carbon source and pyruvate will be numerically close, as originally reported by Minkevich & Eroshin (1973). Heijnen et al. (1992) also conclude that since the Gibbs energy of cell synthesis is close in value to the Gibbs energy of many simple carbon sources, the difference between the Gibbs energy of the carbon source and the Gibbs energy of cells can be ignored with less than 5% increase in error of yield prediction. Further, the cell synthesis energy estimate ($\Delta G^{01}_{e\text{-}cells}$ = 34.7

kJ/eeq) shows a good agreement with the Gibbs energy of the cell synthesis half reaction of 33.8 kJ/e-eq, predicted from the $\Delta G_f^{01} = -67$ kJ/C-mole observed by Roels (1983, 1987).

Analysis across a wide range of *simple* carbon-source substrates indicates use of a constant $\Delta G_{e\text{-}syn}^{01} = \Delta G_{e\text{-}cells}^{01} = 34.7$ kJ/e-eq for aerobic heterotrophic systems leads to less than 10% variability in the predicted yield values (VanBriesen 2001). Therefore, *when the downstream intermediate used for cell synthesis in a heterotrophic organism is not known*, the right side of the energy balance Equation (1) can be estimated using $\Delta G_{e\text{-}syn}^{01} = \Delta G_{e\text{-}cells}^{01} = 34.7$ kJ/e-eq. The fact that this simplification works well in heterotrophic systems when the physiological carbon source is unknown but likely to conform to a regularity Gibbs value, does not negate the use of the more complete original McCarty formulation (Equation (9)) when the original substrate is the carbon source, when the downstream intermediate that acts as carbon source is known, or when the carbon source is independent of the electron-donor substrate (i.e., autotrophic growth).

Predicting the new yield

Following adjustments to the electron and energy balances and selection of a representative carbon source compound, the energy and electron balances are solved as coupled equations to produce values of f_s^0 and f_e^0 as in the original McCarty method. Equation (3) can then be used to predict a cell yield. Equation (4) must be modified to predict the overall stoichiometry (following VanBriesen & Rittmann, 2000):

$$\text{Overall Reaction} = -T\,R_D + f_e^0 R_A + f_s^0 R_C \quad (10)$$

Equation (10) will produce a reaction in terms of T moles of electron-donor substrate whereas it is often desired to write the overall reaction in terms of one mole of the electron-donor substrate. A rearrangement to

$$\text{Overall Reaction} = -N_T R_D + \frac{f_e^0}{T} N_T R_A + \frac{f_s^0}{T} N_T R_C$$

produces a stoichiometry for one mole of electron-donor substrate.

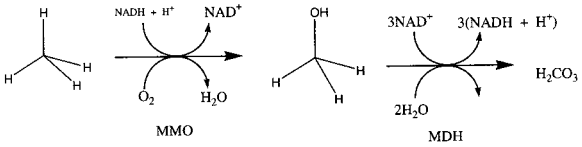

Figure 2. Methane degradation pathway. MMO = methane monooxygenase. MDH = methanol dehydrogenase.

Demonstrations of the modified yield prediction method

Methane transformation

To illustrate the modified method for thermodynamic yield prediction, I consider the simplest compound to be transformed by an oxygenase enzyme, methane. The methane mono-oxygenase pathway is shown in Figure 2. The reduction of molecular oxygen requires four electrons, two that come from the oxidation of methane ($\gamma = 8$) to methanol ($\gamma = 6$) and two that are supplied by a reduced electron carrier, NADH+H$^+$. The half reaction for methane mineralization, written as a reduction, is:

$$\begin{aligned} &H_2CO_3 + 8H^+ + 8e^- \rightarrow CH_4 + 3H_2O \\ &\Delta G_D^{01} = \Delta_{CS}^{01} = 181.0\ \text{kJ/mole} \\ &\Delta G_{eD}^{01} = \Delta G_{eCS}^{01} = 22.63\ \text{kJ/electron-equivalent} \end{aligned} \quad (11)$$

The Gibbs energy for the donor half reaction shown in (11) and all others discussed in this paper were computed based on the Gibbs energy of formation of all the compounds in the reaction (all values used are provided in Appendix A). Reaction (11) indicates that the mineralization of one mole of methane releases 8 electrons for use in cell synthesis and energy generation.

Table 1 provides relevant thermodynamic parameters for the original McCarty yield estimation method in the second column. The original McCarty method is based on use of the primary electron-donor substrate as the carbon-source substrate, thus, ($\Delta G_{eD}^{01} = \Delta G_{eCS}^{01}$ (methane) = 22.62 kJ/eeq); the method also assumes an efficiency, κ, of 0.6. And, of course, this method does not employ the adjustment for energy and electron investment in the oxygenase reaction, as described above. The predicted yield is 0.98 moles cells/mole methane, an error of 79% from the observed yield of 0.55 (Linton & Stephenson 1978; Heijnen & Roels 1981). The observed yield (Y_{obs}) for methane has been verified repeatedly and was meas-

Table 1. Key parameters and calculated values for methane mineralization reactions

Parameter	Original McCarty method	New oxygenase method
ΔG_D^{01} (kJ/mole)	181.0	495.8
γ_S (degree of reductance of substrate)	8	8
γ_C (degree of reductance of cells)	4.2	4.2
EQs (electrons in 1 mole substrate)	8	8
N_O (electrons invested in oxygenase reaction)	0	4
N_T (electrons transferred)	8	4
$\Delta G_{e\text{-}ox}^{01}$ (kJ/eeq) = ΔG_{eA}^{01}	−78.14	−78.14
$\Delta G_{e\text{-}ox}^{01}$ (kJ/eeq)	22.62	–
$(\Delta G_{eD}^{01})^{ox}$ (kJ/eeq)	–	45.81
ΔG_{eR}^{01} (kJ/eeq)	−100.76	−123.95
ΔG_{eCS}^{01} (kJ/eeq)	22.62	22.62
$\Delta G_{e\text{-}cells}^{01}$ (kJ/eeq)	34.75	34.75
$\Delta G_{e\text{-}syn}^{01}$ (kJ/eeq) following Equation (9)	56.63	56.63
κ	0.6	0.6
f_{ox}	0	0.5
$T = N_T/EQs = f_s^0 + f_e^0$	1	0.5
f_s^0	0.52	0.28
f_e^0	0.48	0.22
Predicted yield (Cmole/Cmole); following Equation (3)	0.98	0.54
Experimental yield (Cmole/Cmole)	0.55	0.55
(Linton & Stephenson 1978; Heijnen & Roels 1981)		
Error $\left(\frac{\lvert\text{ExpYield} - \text{PredYield}\rvert}{\text{ExpYield}} * 100\%\right)$	+78.8%	−1.7%

ured under experimental conditions of rapid growth with excess substrate, designed to reduce the influence of maintenance cost on the observed yield. The yield predicted by the thermodynamic method is the Theoretical Yield, so comparison with observed yields can only be made when experimental conditions make these values nearly equivalent, or maintenance costs are estimated and used to adjust the observed yield.

As a first step to improving yield predictions using the thermodynamic method for methane, I rewrite the electron-donor half reaction in a more representative manner including the oxygen cosubstrate. Considering that four electrons from the oxidation of the methane are utilized to reduce the cosubstrate oxygen atoms (N_O = 4), the full mineralization via the monooxygenation pathway is more accurately written as:

$$\begin{aligned} &H_2CO_3 + 4H^+ + 4e^- \rightarrow CH_4 + H_2O + O_2 \\ &\Delta G_D^{01} = 495.8\,\text{kJ/mole} \end{aligned} \tag{12}$$

This more representative reaction shows a significantly higher Gibbs energy available; however, as discussed above it is unlikely that the cells can capture this energy so we reduce the overall Gibbs energy predicted by the energy associated with the reduction of the molecular oxygen. Then,

$$(\Delta G_D^{01})^{ox} = 495.8 + (-78.1 \times 4) = 183.26\,\text{kJ/mole}. \tag{13}$$

Adjusting this value in terms of the electrons released in the half-reaction, the adjusted Gibbs energy associated with the standard reduction electron donor half reaction is $(\Delta G_{eD}^{01})^{ox}$ = 45.8 kJ/eeq. We notice immediately that the Gibbs energy *per mole* associated with the mineralization of the donor substrate has not changed significantly. This is necessary and expected since Equation (12) is the sum of Equation (11) and the half reaction for oxygen reduction to water so substracting the Gibbs energy associated with this half reaction should bring us back, numerically, to the same value. However, the Gibbs energy *per electron equivalent* has changed substantially since only half the electrons are available for catabolic or anabolic processes.

Again, key thermodynamic parameters are provided in Table 1 for the calculations following the new methodology (column 3). A total of 8 electrons were available from methane and 4 are released in this reaction while 4 are invested. Thus, $N_O = 4$ and $N_T = 4$; $f_{ox} = 0.5$ and $T = 0.5$; and $f_s^0 + f_e^0 = T = 0.5$. The overall yield using this modified methodology is predicted then as 0.54 moles/mole for the mineralization of methane through the monooxygenation pathway, a 2% error from the experimental value.

In comparison, using the correlation-based prediction method of Heijnen (Heijnen et al. 1992; Heijnen & vanDijken 1992), the Gibbs energy of dissipation is estimated using an empirical relationship based on the carbon chain length (C = 1 for methane) and the degree of reductance of the carbon source (γ_s = 8 for methane). The predicted yield using this method is 0.53–0.56 moles cells/moles methane (a 2–3% error); the range is based on whether a simplification based on the assumption of a regular value for the cell synthesis cost is made. Thus, while the original thermodynamic half-reaction method of McCarty was unsuccessful in predicting the yield for methane, the modified method produces a prediction with error similar to other prediction methods.

In this example, neither the original McCarty method nor the modification used an assumed carbon source of a downstream intermediate (methanol) or a simple carbon source. Since methanol and methane are 1 carbon compounds, we do not expect them to conform to energy regularities noted by Minkevich & Froshin (1973) nor do we expect that downstream intermediates or cell synthesis carbon sources to be *less complex*. In fact use of the regularity assumption for the carbon source calculation in the case of methane biotransformation leads to yield predictions that are less accurate (1.21 C-mole/C-mole, a 120% error, for the original McCarty method, and 0.65 C-mole/C-mole, a 18% error, for the modified oxygenase method).

Chelate degradation

The aerobic degradation of nitrilotriacetate (NTA) is a second example of an oxygenase-catalyzed reaction. It is degraded by *Chelatobacter heintzii* following a monooxygenation-initiated reaction (Tiedje et al. 1973; Tiedje & Mason 1974; Firestone & Tiedje 1978; Egli 1990; Uetz et al. 1992; Egli 1994). The first step in the pathway for NTA degradation is shown in Figure 3. Following the initial cleavage to iminodiacetate

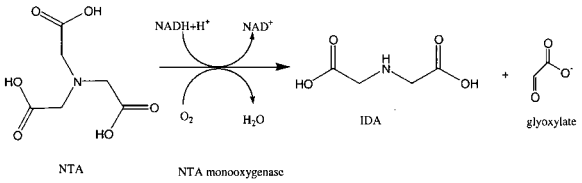

Figure 3. Initial Step in NTA degradation. NTA = nitrilotriacetate. IDA = iminodiacetate.

(IDA) and glyoxylate, an IDA dehydrogenase enzyme cleaves IDA to glyoxylate and glycine. The experimental yield for *C. heintzii* is reported as 0.24 grams of cell dry weight (CDW) per gram of NTA (Bally et al. 1994) with a maintenance coefficient of 0.02 g NTA/g DW-hour and a maximum growth rate ($\mu_{\max}$) of 0.17–0.18/hour (Bally et al. 1994). The maintenance coefficient and yield are used to compute the cell decay constant as follows:

$$\begin{aligned} b\left(\frac{1}{hr}\right) &= \text{Yield}\left(\frac{0.24gCDW}{gNTA}\right) \\ &\quad \times \text{MaintCoef}\left(\frac{0.02gNTA}{gCDW - hr}\right) \\ &= 0.0048\left(\frac{1}{hr}\right) \end{aligned} \tag{14}$$

The cell decay constant is used to compute the growth rate (μ) from the maximum growth rate ($\mu_{\max}$) as follows:

$$\begin{aligned} \mu &= \mu_{\max}\left(\frac{0.17\text{–}0.18}{hr}\right) - b\left(\frac{0.0048}{hr}\right) \\ &= 0.1652\text{–}0.1752\left(\frac{1}{hr}\right) \end{aligned} \tag{15}$$

The theoretical yield (in the absence of decay) is computed from the observed yield and the ratio of the maximum growth rate to the actual growth rate:

$$\begin{aligned} Y_{TRUE} &= Y_{OBS}\left(\frac{0.24gCDW}{gNTA}\right) \\ &\quad \times \frac{\mu_{\max}\left(\frac{0.17\text{–}0.18}{hr}\right)}{\mu\left(\frac{0.1652\text{–}0.1752}{hr}\right)} \\ &= 0.2465\text{–}0.24697\frac{gCDW}{gNTA} \end{aligned} \tag{16}$$

Assuming the organic cell formulation, $CH_2O_{0.6}N_{0.2}$, represents 90–95% of the dry weight (Stouthamer 1973; Andrews 1989), a molar true yield can be computed

$$0.2465\text{–}0.24697\frac{gCDW}{gNTA}\frac{188gNTA}{\phi NTA}\frac{0.9\text{–}0.95gVSS}{gCDW}$$

$$\frac{\phi cells}{26.4gVSS} = 1.58\text{–}1.67\frac{\phi cells}{\phi NTA}. \qquad (17)$$

Given the six carbons in each molecule of NTA, and the assumed cell formulae of $CH_2O_{0.6}N_{0.2}$ with one mole of carbon per mole of cells, the yield then converts to 0.263–0.278 C-mole cells/C-mole NTA (average value is 0.271 c-mole/c-mole).

As with the methane example, initially, I complete two calculations to compare predicted yields with this observed yield. First, the original mineralization method as outlined by McCarty (1972b, 1975). Second, the energy and electron balance modification presented in this work. For the NTA degradation system, the full mineralization without consideration of the oxygenase enzyme system is represented as:

$$\begin{aligned} &6H_2CO_3 + NH_4^+ + 14H^+ + 18e^- \rightarrow C_6H_6O_6N^{3-} \\ &\quad + 12H_2O \\ &\Delta G_D^{01} = \Delta G_{CS}^{01} = -580.32\,\text{kJ/mole} \\ &\Delta G_{eD}^{01} = \Delta G_{eCS}^{01} = -32.24\,\text{kJ/mole} \end{aligned} \qquad (18)$$

Considering that four electrons from the oxidation of the NTA are utilized to reduce the cosubstrate oxygen atoms, the full mineralization via the monooxygenation pathway is more accurately written as:

$$\begin{aligned} &6H_2CO_3 + NH_4^+ + 10H^+ + 14e^- \rightarrow C_6H_6O_6N^{3-} \\ &\quad + 10H_2O + O_2 \end{aligned} \qquad (19)$$

Thus, from the total 18 electrons in NTA, 4 are invested (N_O) and 14 (N_T) are available for synthesis or energy generation; $f_{ox} = 4/18$ and $T = 14/18$. The relevant thermodynamic parameters are given in Table 2, along with the yield predictions.

The error compared with the experimental yield decreases from 80% when energy and electron corrections are ignored to 44% when the adjustments are made. By comparison, using Heijnen's correlation a yield of 0.366 mole cell carbon/mole substrate carbon is predicted, a 35% overprediction. The Heijnen method can also be modified by assumption that the carbon source from NTA degradation is actually the 2C glyoxylate. When this is done, the predicted yield is 0.14–0.22 c-mole/c-mole, a 19–48% underprediction. Similarly, the McCarty method (original and the modification described in this paper) can be used with an assumed downstream intermediate as the carbon source. Calculations with IDA as carbon source indicate yields of 0.45 c-mole/c-mole (66% error) for the original McCarty method and 0.36 c-mole/c-mole (32% error) with the modified method.

One reason for the consistent overprediction of the original and the modified McCarty methods is the selection of $K = 0.6$ for the efficiency. This value has been routinely used in mixed culture yield predictions by McCarty and others (e.g., Noguera et al. 1988; McFarland & Sims 1991; Woo & Rittmann 2000)). However, McCarty's original analysis estimated this value as 0.5–0.6 for autotrophic growth, 0.4–0.8 for anaerobic heterotrophic growth, and 0.2–0.4 for aerobic heterotrophic growth (McCarty 1965). While a value of 0.6 is typically used (Rittmann & McCarty 2001), recent analysis of experimental yield data published subsequent to McCarty's work confirms the range of 0.2–0.4 for aerobic heterotrophs in pure culture and indicates a value of 0.3 is most predictive for this class of organisms (VanBriesen, 2001). Use of K = 0.3 results in yield predictions for NTA of 0.36 c-mole/c-mole (32% error) for the original McCarty method and 0.30 c-mole/c-mole (11% error) with the modified method.

Ethylenediaminetetraacetic acid (EDTA) is a hexadentate anthropogenic chelating agent that is more recalcitrant to biodegradation than NTA. Early reports on EDTA indicated that the molecule was not biodegradable (Potos 1965; Bunch & Ettinger 1967; Alexander 1973; Gardiner 1976; Gerike & Fischer 1979). Subsequent work indicated EDTA is degradable in soils (Tiedje 1975; Tiedje 1977; Bolton et al. 1993), in sediments (Tiedje 1975; Tiedje 1977), in aerated lagoons (Belly et al. 1975), and in some wastewaters (Means et al. 1980; Lauff et al. 1990; Nortemann 1992; Palumbo et al. 1994). Organisms capable of growth with EDTA as carbon and energy source have been isolated (Lauff et al. 1990; Nortemann et al. 1991; Nortemann 1992; Henneken et al. 1994; Palumbo et al. 1994; Henneken et al. 1995; Miyazaki et al. 1997; Kluner et al. 1998; Thomas et al. 1998; Nortemann 1999) and all use a mono-oxygenation initiated pathway.

Figure 4 shows the initial steps of the degradation pathway (Kluner et al. 1998) The conversion of EDTA to ethylenediaminetriacetate (ED3A) is a monooxygenation reaction resulting in cleavage of a glyoxylate moity from the parent structure (#1). The subsequent conversion of ED3A to N,N' ethylenediaminediactate

Table 2. Key parameters and calculated values for NTA mineralization reactions

Parameter	Original McCarty method	New oxygenase method
ΔG_D^{01} (kJ/mole)	700.32	1015.12
γ_S (degree of reductance of substrate)	3	3
γ_C (degree of reductance of cells)	4.2	4.2
EQs (electrons in 1 mole substrate)	18	18
N_O (electrons invested in oxygenase reaction)	0	4
N_T (electrons transferred)	18	14
$\Delta G_{e\text{-}ox}^{01}$ (kJ/eeq) = ΔG_{eA}^{01}	−78.14	−78.14
ΔG_{eD}^{01} (kJ/eeq)	38.91	–
$(\Delta G_{eD}^{01})^{ox}$ (kJ/eeq)	–	50.18
ΔG_{eR}^{01} (kJ/eeq)	117.04	128.32
ΔG_{eCS}^{01} (kJ/eeq)	38.91	38.91
$\Delta G_{e\text{-}cells}^{01}$ (kJ/eeq)	34.75	34.75
$\Delta G_{e\text{-}syn}^{01}$ (kJ/eeq) following Equation (9)	32.86	32.86
κ	0.6	0.6
f_{ox}	0	0.22
$T = N_T/EQs = f_s^0 + f_e^0$	1	0.78
f_s^0	0.68	0.55
f_e^0	0.32	0.23
Predicted yield (Cmole/Cmole); following Equation (3)	0.487	0.39
Experimental yield (Cmole/Cmole) (Bally et al. 1994)	0.271	0.271
Error $\left(\frac{\lvert\text{ExpYield} - \text{PredYield}\rvert}{\text{ExpYield}} * 100\%\right)$	+80%	+44%

(N,N'-EDDA) also involves cleavage of a glyoxyate and uses the same oxygenase enzyme (#2). The alternative conversion of ED3A to iminoacetaldehydeaceate (IAA) and iminodiacetate (IDA) is also a monooxygenation (alt #2). While the conversion of EDDA has not been documented, recent work has indicated IAA is converted to IDA (probably via a dehydrogenase although this is unconfirmed) and the two molecules of IDA are converted using an oxygenase enzyme (Liu et al. 2001) (#3 and #4). Thus, full EDTA mineralization probably requires a total of 4 oxygenations. Overall the mineralization of EDTA can be represented as:

$$\begin{aligned} &10H_2CO_3 + 2NH_4^+ + 34e^- + 28H^+ \\ &\quad \rightarrow C_{10}H_{12}O_8N_2^{4-} + 22H_2O \\ &\Delta G_D^{01} = \Delta G_{CS}^{01} = -1088.6\,\text{kJ/mole} \\ &\Delta G_{eD}^{01} = \Delta G_{eCS}^{01} = -32.02\,\text{kJ/mole} \end{aligned} \tag{20}$$

Thus, 34 electron equivalents are released per mole mineralized. Taking into account the electrons used for the four oxygenase reactions ($N_O = 16$; $N_T = 18$), the electron donor half reaction is rewritten as:

$$\begin{aligned} &10H_2CO_3 + 2NH_4^+ + 18e^- + 12H^+ \\ &\quad \rightarrow C_{10}H_{12}O_8N_2^{4-} + 14H_2O + 4O_2 \end{aligned} \tag{21}$$

BNC1, the EDTA degrading organism isolated by Nortemann (1992), has a reported yield of 0.271 g cell dry weight/g EDTA (Henneken et al. 1998). The maximum cell growth rate is reported to vary between 0.03–0.07 hr^{-1}, and the decay coefficient is reported as 15.5 mg EDTA/gCDW-hr (Henneken et al. 1994). Following Equations (15)–(17), the true yield is estimated as 2.26–2.64 moles cells (as $CH_2O_{0.6}N_{0.2}$)/moles EDTA, or 0.226–0.264 moles cell carbon/moles substrate carbon (average value = 0.245 c-mole/c-mole).

Table 3 provides the relevant thermodynamic parameters and yield predictions. The modification improves the error on the yield prediction from 120% to 21%. While EDTA has more than six carbons, we can compare this result with the yield predicted by the Heijnen correlation method if we assume the downstream glyoxylate is the carbon source (a 2C compound). This methodology predicts a yield of 0.14–0.22, depending upon the assumptions selected

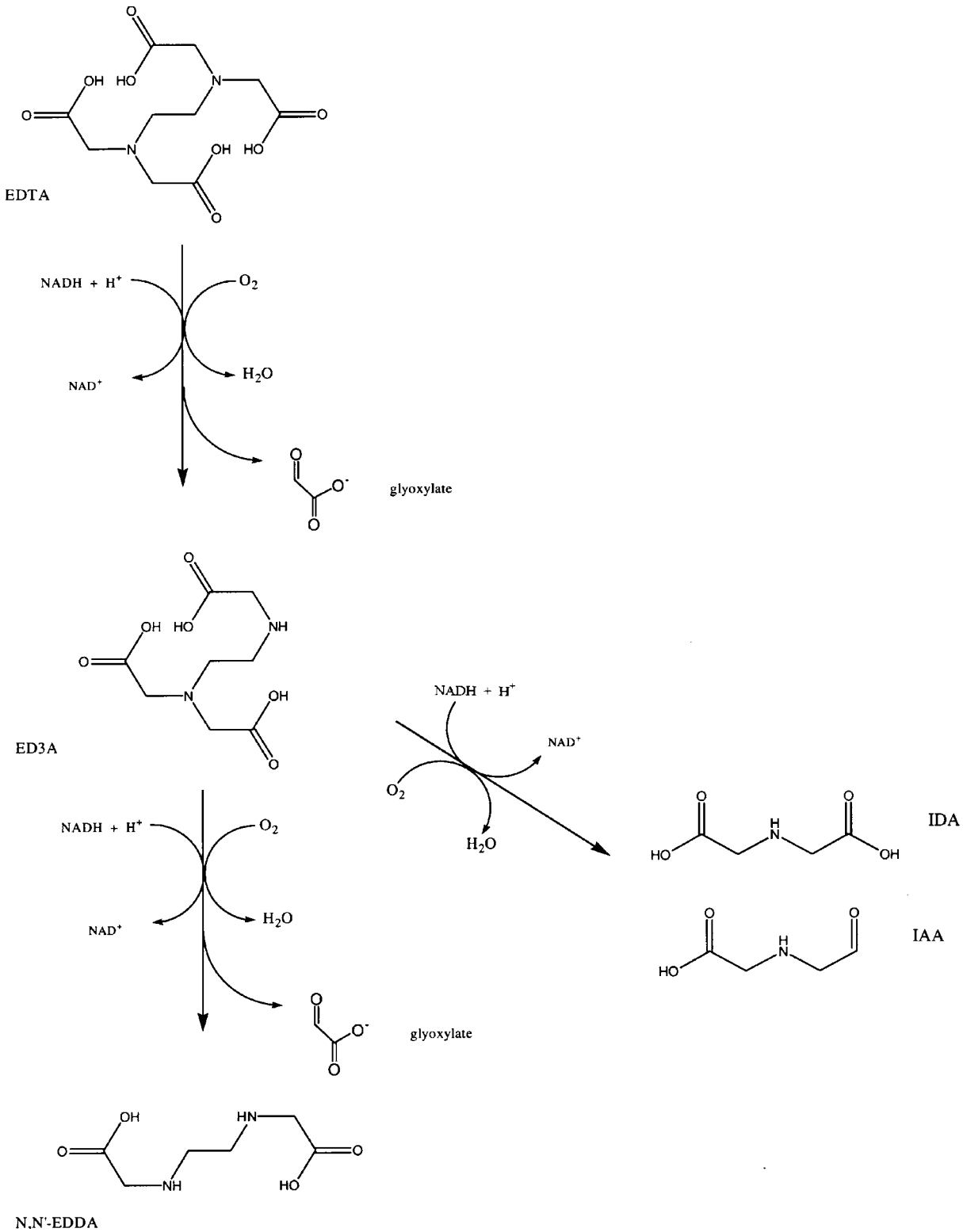

Figure 4. Initial Steps in EDTA degradation. EDTA = ethylenediaminetetraacetate. ED3A = ethylenediaminetriacetate; NNEDDA = N,N′ ethylenediaminediacetate; IDA = iminodiacetate; IAA = iminoacetaldehydeacetate.

Table 3. Key parameter and calculated values for EDTA mineralization reactions

Parameter	Original McCarty method	New oxygenase method
ΔG_D^{01} (kJ/mole)	1248.6	2347.8
γ_S (degree of reductance of substrate)	3.4	3.4
γ_C (degree of reductance of cells)	4.2	4.2
EQs (electrons in 1 mole substrate)	34	34
N_O (electrons invested in oxygenase reaction)	0	16
N_T (electrons transferred)	34	18
$\Delta G_{e\text{-}ox}^{01}$ (kJ/eeq) = ΔG_{eA}^{01}	−78.14	−78.14
ΔG_{eD}^{01} (kJ/eeq)	36.72	–
$(\Delta G_{eD}^{01})^{ox}$ (kJ/eeq)	–	52.38
ΔG_{eR}^{01} (kJ/eeq)	−114.84	−130.52
ΔG_{eCS}^{01} (kJ/eeq)	36.72	36.72
$\Delta G_{e\text{-}cells}^{01}$ (kJ/eeq)	34.75	34.75
$\Delta G_{e\text{-}syn}^{01}$ (kJ/eeq) following Equation (9)	34.18	34.18
κ	0.6	0.6
f_{ox}	0	0.47
$T = N_T/EQs = f_s^0 + f_e^0$	1	0.53
f_s^0	0.67	0.37
f_e^0	0.33	0.16
Predicted yield (Cmole/Cmole); following Equation (3)	0.54	0.298
Experimental yield (Cmole/Cmole) (Henneken et al. 1998)	0.245	0.245
Error $\left(\frac{\lvert\text{ExpYield} - \text{PredYield}\rvert}{\text{ExpYield}} * 100\%\right)$	+120%	+22%

Table 4. Stoichiometry for electron donor half reactions for aromatic mineralization reactions

	Benzene	
Original	$C_6H_6 + 19H_2O \rightarrow 30H^+ + 30e^- + 6H_2CO_3$	ΔG_{eD}^{01} = 26.66 kJ/eeq
New method	$C_6H_6 + 19H_2O + 2O_2 \rightarrow 22H^+ + 22e^- + 6H_2CO_3$	$(\Delta G_{eD}^{01})^{ox}$ = 64.98 kJ/eeq
	Toluene	
Original	$C_7H_8 + 21H_2O \rightarrow 36H^+ + 36e^- + 7H_2CO_3$	ΔG_{eD}^{01} = 26.09 kJ/eeq
New method	$C_7H_8 + 17H_2O + 2O_2 \rightarrow 28H^+ + 28e^- + 7H_2CO_3$	$(\Delta G_{eD}^{01})^{ox}$ = 56.03 kJ/eeq
	Phenol	
Original	$C_6H_5OH + 17H_2O \rightarrow 28H^+ + 28e^- + 6H_2CO_3$	ΔG_{eD}^{01} = 27.81 kJ/eeq
New method	$C_6H_5OH + 13H_2O + 2O_2 \rightarrow 20H^+ + 20e^- + 6H_2CO_3$	$(\Delta G_{eD}^{01})^{ox}$ = 70.41 kJ/eeq

in the method, a 11–75% error. The original and modified McCarty methods can also use this presumed carbon source of glyoxylate or can be based on a constant cell synthesis cost assumption. For the original method, these alternatives result in yield predictions of 0.54–0.58 (still greater than 100% error). For the new oxygenase method, the predicted yield is 0.30–0.32 (22–30% error).

As in the case of NTA, use of $K = 0.3$ results in improved yield predictions for EDTA. The original McCarty method with $K = 0.3$ predicts an EDTA yield of 0.4–0.43 C-mole/C-mole, representing a 63–75% overprediction error; while using the modification described in this paper with $K = 0.3$ predicts an EDTA yield of 0.2–0.24 C-mole/C-mole, a 2–19% underprediction.

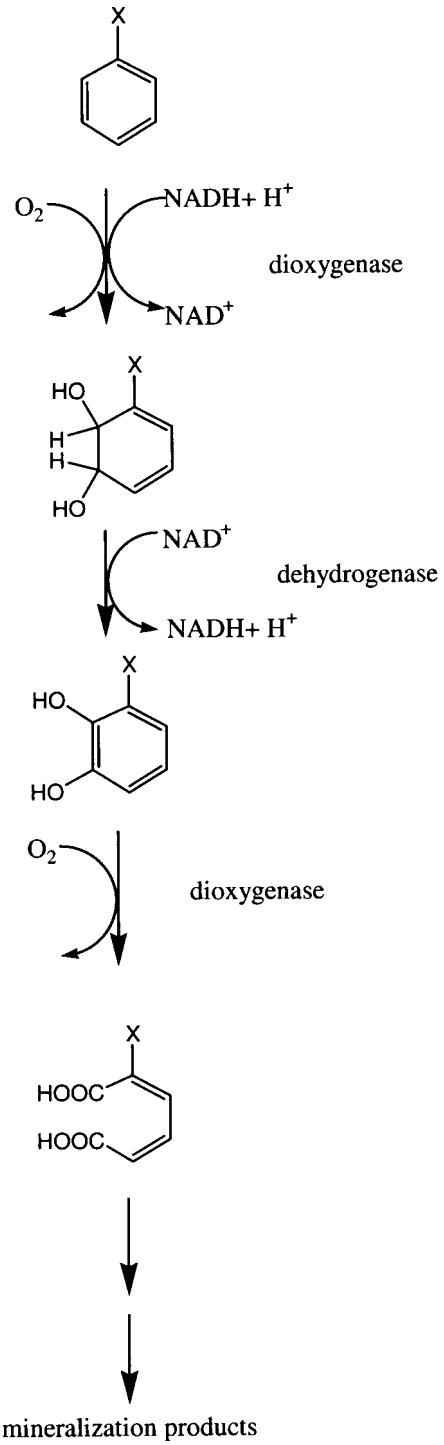

Figure 5. Initial Steps in Aromatic degradation. X is either CH_3 for toluene or Cl for monochlorbenzene. For phenol, with one OH already on the ring, the initial reaction is a mono-oxygenation but the subsequent dehydrogenase and dioxygenations are the same.

Table 5. Calculated values for aromatic mineralization reactions

Prameter	Benzene	Toluene	Phenol
Predicted yield (original McCarty method)	0.66	0.68	0.63
Predicted yield (Oxygenase method)	0.51	0.54	0.47
Experimental yield (Reardon et al. 2000)	0.427	0.525	0.358
Error with original method	+55%	+29%	+76%
Error with Oxygenase method	+18.5%	+3%	+31%

Aromatic hydrocarbon degradation

Aromatic hydrocarbons are often degraded through oxygenase enzymes that cleave the benzene ring. Figure 5 shows the general pathway for mineralization of substituted aromatic compounds. Table 4 shows the stoichiometry of the electron donor half reactions based on the standard and the modified thermodynamic methods.

Three aromatic compounds are shown as examples in Table 4 – benzene, toluene, and phenol. These compounds were selected because they were the subject of a recent kinetic study that included adequate detail in yield experiments and a literature survey of previous reported yield values (Reardon et al. 2000). Table 5 contains predicted yields following the simple mineralization method and the new oxygenase method introduced in this paper. The oxygenase method significantly reduces the error in yield predictions (from 29–76% to 3–31%). Improved predictions are again seen with use of $K = 0.3$ along with the assumption that the carbon source for synthesis is a simple carbon compound resulting from the cleavage of the benzene ring; predicted yields are then within 10% of observed values for all three aromatic hydrocarbons.

Conclusions

The McCarty thermodynamic method for prediction of cell yields is based on the assumption that the oxidation of the primary electron donor releases all available electrons to the synthesis or energy generation pathways (Rittmann & McCarty 2001). However, biodegradation of anthropogenic compounds may require investment of electrons and involvement of oxygen as a cosubstrate to activate the organic compound. This investment diverts electrons from synthesis and energy

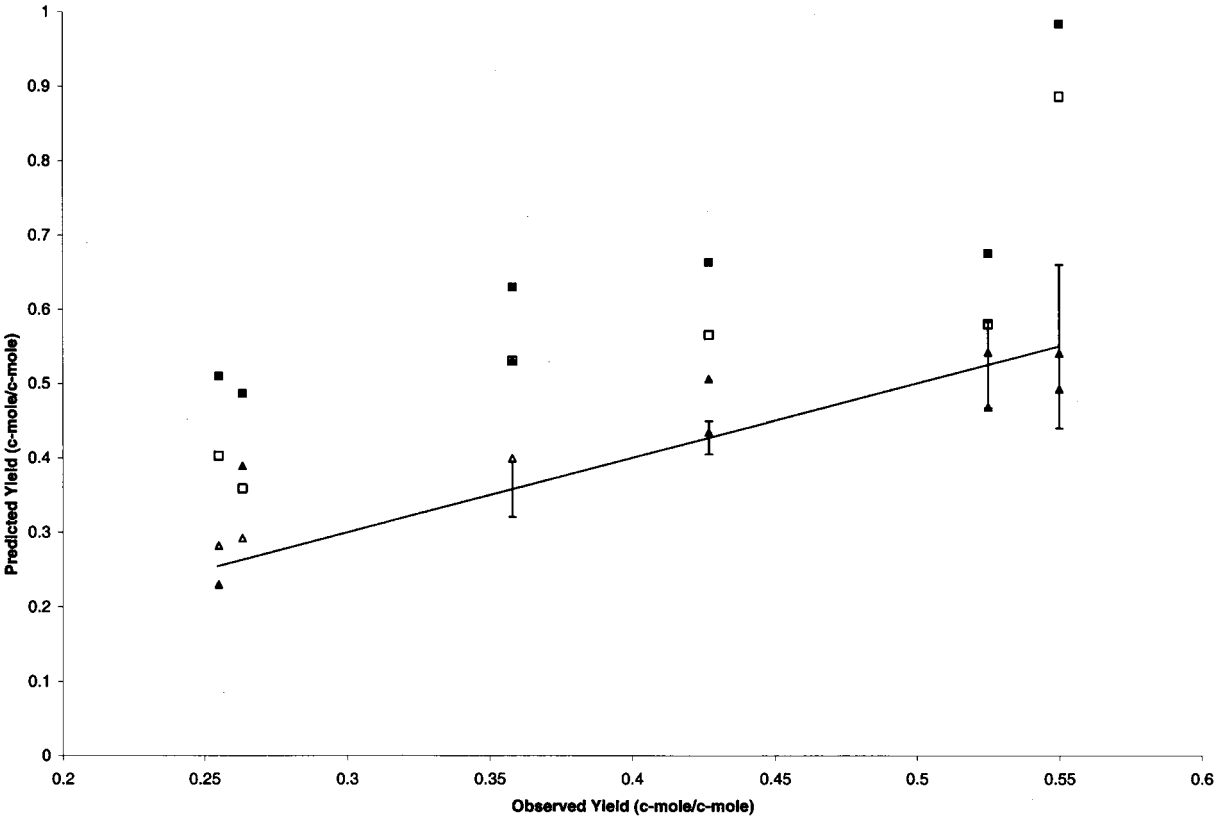

Figure 6. Comparison of experimental yields and yields predicted by the standard McCarty thermodynamic method (squares; solid is $K = 0.6$ and open is $K = 0.3$), and the newly developed Oxygenase-adjusted method (triangles; solids is $K = 0.6$ and open is $K = 0.3$). Solid line with represents the Observed Values with maximum and minimum reported values shown by the brackets when data are available.

generation, thus reducing the overall cell yield. Modification of energy and electron balances is required to consider the utilization of substrate electrons for reduction of oxygen via oxygenase enzymes.

Figure 6 shows results for all the substrates considered in this paper. Observed yields are plotted against predicted yields based on the standard thermodynamic method and predicted yields based on the method introduced here (results for $K = 0.6$ and $K = 0.3$ are shown; carbon source simplifications are not shown). The solid line defines the observed yields with error bars shown based on the variability in the observations when these data were available. Squares are the prediction based on the original method (solid $K = 0.6$ and open $K = 0.3$), in all cases exceed the observed values, and fall outside the range of reported values. The modification described here (shown as triangles; solid $K = 0.6$ and open $K = 0.3$) provides significant improvement for estimation of cell yields for bacterial growth on complex, anthropogenic compounds degraded through oxygenase-catalyzed reactions.

Acknowledgement

The author acknowledges the excellent insights provided by the anonymous reviewers.

Appendix A. Gibbs Energy of Formation for Relevant Compounds

Compound	Gibbs energy of formation (kJ/mole)	Reference
Methane	−50.82	(Brock and Madigan 1991)
NTA	−954.79	Estimated following (Mavrovouniotis 1990, 1991). Details provided in (VanBriesen 1998).
EDTA	−1208.34	Estimated following (Mavrovouniotis 1990, 1991). Details provided in (VanBriesen 1998).
Benzene	128.84	(Stull, Westrum & Sinke 1969)
Toluene	116.32	(Stull, Westrum & Sinke 1969)
Phenol	−49.79	(Stull, Westrum & Sinke 1969)
Glyoxylate	−461.07	(Long 1961)
IDA	−655.2	Estimated following (Mavrovouniotis 1990, 1991). Details provided in (VanBriesen 1998).
H_2O	−237.57	(Brock and Madigan 1991)
H_2CO_3	−624.05	(Brock and Madigan 1991)
NH_4^+	−79.61	(Brock and Madigan 1991)
H^+ (at pH 7)	−39.83	(Brock and Madigan 1991)

References

Alexander M (1973) Nonbiodegradable and other recalcitrant molecules. Biotechnol. Bioeng. 15: 611–647

Alvarez PJJ, Anid PJ & Vogel, TM (1994) Kinetics of toluene degradation by denitrifying aquifer microorganism. J. Environ. Eng. 120: 1327–1337

Andrews G (1989) Estimating cell and product yield. Biotechnol. Bioeng. 33: 256–265

Arcangeli J-P & Arvin E (1999) Modeling the growth of a methanotrophic biofilm: estimation of parameters and variability. Biodegradation 10: 177–191

Bally M, Wilberg F, Kuhni M, Egli T (1994) Growth and regulation of enzyme synthesis in the nitrilotriacetic acid (NTA)-degrading bacterium *Chelatobacter heintzii* ATCC 29600. Microbiol. 140: 1927–1936

Beller HR, Spormann AM, Sharma PK, Cole JR & Reinhard M (1996) Isolation and characterization of a novel toluene-degrading, sulfate-reducing bacterium. Appl. Environ. Microbiol. 62: 1188–1196

Belly RT, Lauff JJ & Goodhue CC (1975) Degradation of ethylenediaminetetraacetic acid by microbial populations from an aerated lagoon. Appl. Microbiol. 29: 787–794

Bolton H Jr, Li SW, Workman DJ & Girvin DC (1993) Biodegradation of synthetic chelates in subsurface sediments from the southeast coastal plain. J. Environ. Quality 22: 125–132

Brock TD & Madigan MT (1991) Biology of microorganisms. 6th ed. Englewood Cliffs, NJ: Prentice Hall

Bunch RL & Ettinger MB (1967) Biodegradability of potential organic substitutes for phosphates. Proceedings of Ind. Waste Conf 22 Purdue Engng Extn Ser No 129: 393–396

Burland S & Edwards EA (1999) Anaerobic benzene biodegradation linked to nitrate reduction. Appl. Environ. Microbiol. 65: 529–533

Corseuil HX & Weber WJ (1994) Potential biomass limitations on rates of degradation of monoaromatic hydrocarbons by indigenous microbes in subsurface soils. Water Res. 28: 1415–1423

Edwards FA & Grbic-Galic D (1994) Anaerobic degradation of toluene and oxylene by a methanogenic consortium. Appl. Environ. Microbiol. 60: 313–322

Egli T (1990) Biodegradation of synthetic chelating agents with special reference to nitrilotriacetic acid (NTA). Experientia 404–406

Egli T (1994) Biochemistry and physiology of the degradation of nitrilotriacetic acid and other metal complexing agents. In: C. Ratledge (Ed) Biochemistry of microbial degradation. (pp 179–195). New York: Kluwer Academic Publishers

Erickson LF (1979) Energetic efficiency of biomass and product formation. Biotechnol. Bioeng. 21: 725–743

Erickson LE, Minkevich IG & Eroshin VK (1978a) Application of mass and energy balance regularities in fermentation. Biotechnol. Bioeng. 20: 1595–15621

Erickson LE, Selga SE & Viesturs UF (1978b) Application of mass and energy balance regularities to product formation. Biotechnol. Bioeng. 20: 1623–1638

Firestone MK & Tiedje JM (1978) Pathway of degradation of nitrilotriacetate by a pseudomonas species. Appl. Environ. Microbiol. 35: 955–961

Gardiner J (1976) Complexation of trace metals by ethylenediaminetetraacetic acid (EDTA) in natural waters. Water Res. 10: 507–514

Gerike P & Fischer WK (1979) A correlation study of biodegradability determinations with various chemicals in various tests. Ecotoxicol. Environ. Safety 3: 159–173

Hayes AM, Flora JRV & Khan J (1998) Research note: electrolytic stimulation of denitrification in sand columns. Water Res. 32: 2830–2834

Heijnen JJ (1994) Thermodynamics of microbial growth and its implications for process design. Trends in Biotechnology 12: 483–492

Heijnen JJ & Roels JA (1981) A macroscopic model describing yield and maintenance relationships in aerobic fermentation processes. Biotechnol. Bioeng. 23: 739–763

Heijnen JJ & vanDijken JP (1992) In search of a thermodynamic description of biomass yields for the chemotrophic growth of microorganisms. Biotechnol. Bioeng. 39: 833–858

Heijnen JJ, vanLoosdrecht MCM & Tijhuis L (1992) A black box mathematical model to calculate auto- and heterotrophic biomass yields based on Gibbs energy dissipation. Biotechnol. Bioeng. 40: 1139–1154

Henneken L, Nortemann B & Hempel DC (1998) Biological degradation of EDTA: reaction kinetics and technical approach. J. Chem. Technol. Biotechnol. 73: 144–152

Henneken LB, Kluner T, Nortemann B & Hempel DC (1994) Abbau von EDTA mit freien und immobilisierten Bakterien. Wasser Abwasser 135: 354–358

Henneken LB, Nortemann B & Hempel DC (1995) Influence of physiological conditions on EDTA degradation. Appl. Microbiol. Biotechnol. 44: 190–197

Hooker BS, Skeen RS & Petersen JR (1994) Biological destruction of CCI4: II. Kinetic modeling. Biotechnol. Bioeng. 44: 211–218

Kluner T, Hempel DC & Nortemann B (1998) Metabolism of EDTA and its metal chelates by whole cells and cell-free extracts of strain BNC1. Appl. Microbiol. Biotechnol. 49: 194–201

Lauff JJ, Steele DB, Coogan LA & Breitfeller JM (1990) Degradation of the ferric chelate of EDTA by a pure culture of an *Agrobacterium* sp. Appl. Environ. Microbiol. 56: 3346–3353

Linton JD & Stephenson RJ (1978) A preliminary study on growth yields in relation to the carbon and energy content of various organic growth substrates. FEMS Microbiol. Lett. 3: 95–98

Liu Y, Louie TM, Payne J, Bohuslavek J, Bolton H Jr & and Xun L (2001) Identification, purification, and characterization of Iminodi acetate oxidase from the EDTA degrading bacterium BNCl. Appl. Environ. Microbiol. 67: 696–701.

Long C (1961) Biochemist's handbook. London, England: E&F.N. Spon Ltd.

Mason JR & Cammack R (1992) The electron transport proteins of hydroxylating bacterial dioxygenases. Ann. Rev. Microbiol. 46: 277–305

Mavrovouniotis ML (1990) Group contributions for estimating standard Gibbs energies of formation of biochemical compounds in aqueous solution. Biotechnol. Bioeng. 36: 1070–1108

Mavrovouniotis ML (1991) Estimation of standard Gibbs energy changes of biotransformations. J. Biological Chem. 266: 14,440–14,445

McCarty PL (1965) Thermodynamics of biological synthesis and growth. In: J Baers (Ed) Advances in Water Pollution Research: Proceedings of the 2nd International Conference on Water Pollution Research. (pp 169–199) Vol. 2. Oxford, England: Pergamon Press, Inc

McCarty PL (1969) Energetics and bacterial growth. The Fifth Rudolf Research Conference

McCarty PL (1971) Energetics and bacterial growth. In: SD Editors & JV Hunter (Eds) Organic Compounds in Aquatic Environments. Faust New York: Marcel Dekker, Inc

McCarty PL (1972a) Energetics of organic matter degradation. In: Ralph Mitchell (Ed), Water Pollution Microbiology. New York: Wiley-Interscience

McCarty PL (1972b) Stoichiometry of biological reactions. Paper presented at the International Conference toward a Unified Concept of Biological Waste Treatment Design

McCarty PL (1975) Stoichiometry of biological reactions. Progress in Water Technology 7: 157–172

McFarland MJ & Sims RC (1991) Thermodynamic framework for evaluating PAH degradation in the subsurface. Ground Water 29: 885–896

Means JL, Kucak T & Crerar DA (1980) Relative degradation rates of NTA, EDTA, and DTPA and environmental implications. Environ. Poll. (Ser. B) 1: 45–60

Minkevich IG (1985) Estimation of available efficiency of microbial growth on methanol and ethanol. Biotechnol. Bioeng. 27: 792–799

Minkevich IG & Eroshin VK (1973) Productivity and heat generation of fermentation under oxygen limitation. Folia Microbiologica 18: 376–385

Miyazaki H, Suzuki S & Imada K (1997) Isolation and characterization of a bacterium that decomposes (ethylenediaminetetraacetato)ferrate (III) complex. Environ. Sci. 10: 257–262

Noguera DR, Brusseau GA, Rittmann BE & Stahl DA (1988) A unified model describing the role of hydrogen in the growth of *desulfovibrio vulgaris* under different environmental conditions. Biotechnol. Bioeng. 59: 732–746

Nortemann B (1992) Total degradation of EDTA by mixed cultures and a bacterial isolate. Appl. Environ. Microbiol. 58: 671–676

Nortemann B (1999) Mini-review: biodegradation of EDTA. Appl. Microbiol. Biotechnol. 51: 751–759

Nortemann B, Imberg B & Hempel DC (1991) Biodegradation of ethylenediaminetetraacetic acid (EDTA). In: H. Verachtert & W. Verstraete (Eds), Proceedings of the International Symposium of Environmental Biotechnology, Antwerp, Belgium: Koninklijke Vlaamse Ingenieursverenigung.

Palumbo AV, Lee SY & Boerman P (1994) The effect of media composition on EDTA degradation by *Agrobacterium* sp. Appl. Biochem. Biotechnol. 45/46: 811–822

Potos C (1965) Effects of EDTA on wastewater treatment. Water Pollution Control Federation 37(9): 1247–1255

Reardon KF, Mosteller DC & Rogers JDB (2000) Biodegradation kinetics of Benzene, Toluene, and Phenol as single and mixed substrates for *pseudomonas putida F1*. Biotechnol. Bioeng. 69: 385–400

Rittmann BE & McCarty PL (2001) Environmental Biotechnology: Principles and Applications. New York: McGraw-Hill Book Company

Roels JA (1983) Energetics and kinetics in biotechnology. Amsterdam: Elsevier.

Roels JA (1987) Thermodynamics of growth. In: J Kiistiansen & B Bu'Lock (Eds) Basic Biotechnology. New York: Academic Press.

Stouthamer AH (1973) A theoretical study on the amount of ATP required for synthesis of microbial cell material. Antonie Van Leeuwenhoek 29: 545–565

Stull DR, Westrum EF Jr & Sinke GC (1969) Chemical thermodynamics of organic compounds. New York: John Wiley and Sons, Inc.

Thomas RAP, Lawlor K, Bailey M & Macaski LE (1998) Biodegradation of metal-EDTA complexes by an enriched microbial population. Appl. Environ. Microbiol. 64: 1319–1322

Tiedje JM (1975) Microbial degradation of ethylenediaminetetraacetate in soils and sediments. Appl. Microbiol. 30: 327–329

Tiedje JM, Firestone MK, Mason BB & Warren CB (1973) Pathway of catabolism of nitrilotriacetate (NTA) by *Pseudomonas*. Abstracts of the Annual Meeting of Microbiologists: 171

Tiedje JM & Mason BB (1974) Biodegradation of nitrilotriacetate (NTA) in soils. Proceedings of Soil Science Society of America 38: 278–283

Tiedje JM (1977) Influence of environmental parameters on EDTA biodegradation in soils and sediments. J. Environ. Qual. 6: 21–25

Tijhuis LM, vanLoosdrecht CM & Heijnen JJ (1993) A thermodynamically based correlation for maintenance Gibbs energy requirements in aerobic and anaerobic chemotrophic growth. Biotechnol. Bioeng. 42: 509–519

Uetz T, Schneider R, Snozzi M & Egli T (1992) Purification and characterization of a two-component monooxygenase that hy-

droxylates nitrilotriacetate from '*Chelatobacter*' strain ATCC 29600. J. Bacteriol. 174: 1179–1188

VanBriesen JM (1998) Modeling coupled biogeochemical processes in mixed waste systems. PhD Dissertation, Northwestern University.

VanBriesen JM (2001) Evaluation of yield predictions using thermodynamics. in preparation.

VanBriesen, JM & Rittmann BE (2000) Mathematical description of microbiological reactions involving intermediates. Biotechnol. Bioeng. 67: 35–52.

Wolf RS & Higgins IJ (1979) Microbial biochemistry of methane – a study in contrasts. In: JR Quayle (Ed) International Review of Biochemistry: Microbial Biochemistry, Vol 21, (pp 257–353) Baltimore MD: University Park Press.

Woo SH & Rittmann BE (2000) Microbial energetics and stoichiometry for biodegradation of aromatic compounds involving oxygenation reactions. Biodegradation 11(4): 213–227.

Zitomer DH (1998) Stoichiometry of combined aerobic and methanogenic COD transformation. Water Res. 32: 669–676