Cornell University
School of Civil & Environmental Engineering

CEE 657 — Environmental Engineering Processes II

J. M. Gossett

BIOENERGETICS & STOICHIOMETRY*

I. INTRODUCTION

Classical thermodynamic concepts, along with some observations of the efficiency with which bacteria capture free energy, can be used to predict cell yields and the overall stoichiometry associated with growth. Cell yields are useful for predicting biological sludge production in microbial processes. Stoichiometries allow estimation of a number of important factors: nutrient requirements; effects of growth on alkalinity and pH; and composition of gas evolved in anaerobic processes.

We begin with the definition of an "electron equivalent" — a unit of expression which is particularly convenient in subsequent derivations.

Definition:

1 electron equivalent (eeq) of some electron donor (e.d.) ≡ amount of the substance which releases 1 mol e^- during a specified oxidation reaction.

Thus, the molar quantity which corresponds to 1 eeq is *reaction dependent.* For example, consider the oxidation of elemental iron (Fe). We may write its oxidation to ferrous form:

$$^1/_2\ Fe \Rightarrow {}^1/_2\ Fe^{+2} + e^-$$

from which we would conclude that 1 eeq Fe = $^1/_2$ mol Fe. On the other hand, if oxidation were complete to ferric form, the appropriate oxidation half-reaction would be

* These notes are largely based upon papers by Perry L. McCarty, of Stanford University. Interested students are directed to the original papers, cited at the end of this chapter — though be forewarned that there are some differences between these notes and their source material. In particular, the method of dealing with use of nitrate as N-source has been considerably altered. And, for that matter, there are nomenclature and conceptual differences among McCarty's various papers, occurring as his thoughts evolved. In large part, these notes were written to reconcile some of these differences, and to provide a consistent view of the subject.

$$^1/_3\ Fe \Rightarrow\ ^1/_3\ Fe^{+3} + e^-$$

in which 1 eeq Fe = $^1/_3$ mol Fe. Or, we might even be interested in the oxidation of ferrous (Fe^{+2}) to ferric (Fe^{+3}) form, in which 1 eeq Fe = 1 mol Fe.

In this course, we shall attempt to remove ambiguity — at least for *organic electron donors* — by universally defining electron equivalents in terms of total carbonaceous oxidation:

> 1 eeq of organic electron donor ≡ amount of the substance which releases 1 mol e^- during *total carbonaceous oxidation* (i.e., $\Rightarrow CO_2$, NH_4^+). In other words, proteinaceous nitrogen (–III oxidation state) is not oxidized, but is released as ammonia (also –III oxidation state).

Table 1 depicts oxidation half-reactions for a number of organic electron donors (e.d.), each written as a complete carbonaceous oxidation [Rxns (7) through (17)]. Thus,

1 eeq acetate = $^1/_8$ mol acetate

1 eeq glucose = $^1/_{24}$ mol $C_6H_{12}O_6$

etc.

For organic electron donors, we will adopt a definition of eeq based on complete carbonaceous oxidation — regardless of whether the actual microbial decomposition is complete. This means that 1 eeq glucose will always represent $^1/_{24}$ mol — whether glucose is completely oxidized by aerobic bacteria ($\Rightarrow CO_2$, H_2O), or merely fermented to lactate in the absence of oxygen.

It is also worth mentioning that — since our definition of eeq is based on complete carbonaceous oxidation — then

1 eeq of any organic e.d. ≡ 8 g COD.

The reason: COD is the quantity of oxygen required to completely oxidize the carbonaceous portion of an organic compound. If 1 eeq of an organic releases 1 mol e^- in complete carbonaceous oxidation, then from Rxn (3) (Table 1), it is apparent that $^1/_4$ mol of O_2 (8 g) would be consumed in accepting the 1 mol e^-.

For inorganic donors, the definition of "eeq" will necessarily remain reaction-dependent. For example, 1 eeq of ammonia equals either $^1/_8$ or $^1/_6$ mol, depending upon whether one is referring to its oxidation to nitrate [Rxn (19)] or nitrite [Rxn (20)].

For bacterial cell synthesis, we will also adopt reaction-specific definitions of "eeq" which depend upon the datum of nitrogen:

Ammonia as N-Source [Reaction (1)]

1 eeq cells = $^1/_{20}$ mol $C_5H_7O_2N$

= $^1/_{20}$ (113 g VSS) = 5.65 g VSS

= 8 g COD

Nitrate as N-Source [Reaction (2)]

1 eeq cells = $^1/_{28}$ mol $C_5H_7O_2N$

= $^1/_{28}$ (113 g VSS) = 4.04 g VSS

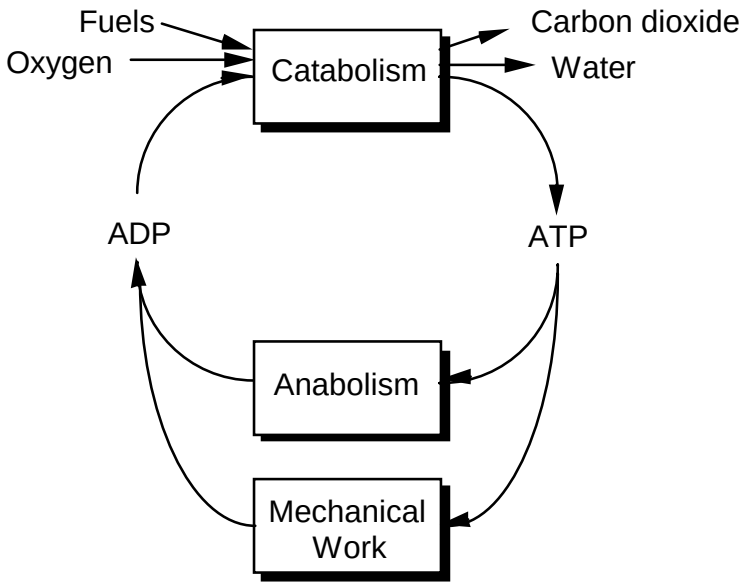
The yield of cells is closely related to the energy content of the electron donor (substrate) supplied and the electron acceptor provided.

II. HETEROTROPHIC METABOLISM

McCarty's theories are on relatively firm theoretical ground with respect to heterotrophic metabolism — particularly heterotrophic metabolism in which ammonia serves as N-source. We'll concentrate first on the application of bioenergetics to estimation of yield coefficients and growth stoichiometries for heterotrophic microorganisms. Later, we'll extend the application to autotrophic microorganisms, which involves some considerable uncertainties.

Consider what occurs in microbial metabolism: energy is made available to the microorganism via catabolism, energy which is needed for biosynthesis (anabolism) and for mechanical work (e.g., motility, transport of molecules, etc).

ENERGY FLOW IN METABOLISM



In heterotrophic, respiratory microorganisms, we may write — in a sort of shorthand — the following set of equations to represent the production and use of energy in respiration and synthesis, respectively:

Respiration

$$(ed)_{red} + (ea)_{ox} \rightarrow (ed)_{ox} + (ea)_{red}$$

Energy

Synthesis

$$(ed)_{red} + NH_4^+ \rightarrow C_5H_7O_2N$$

where

$(ed)_{red}$ = the reduced form of the electron donor — in this case, some organic substrate (e.g., glucose);

$(ea)_{ox}$ = the oxidized form of the electron acceptor (e.g, O_2);

$(ed)_{ox}$ = the oxidized form of the electron donor (e.g., CO_2);

$(ea)_{red}$ = the reduced form of the electron acceptor (e.g., H_2O);

$C_5H_7O_2N$ = empirical formula for the major elements of bacterial VSS.

In essence, the electron donor (ed) becomes oxidized and the electron acceptor (ea) is reduced. Energy is captured (e.g., as ATP) for use in biosynthesis, which in the case of heterotrophs, involves converting this same electron donor to cellular constituents ($C_5H_7O_2N$)

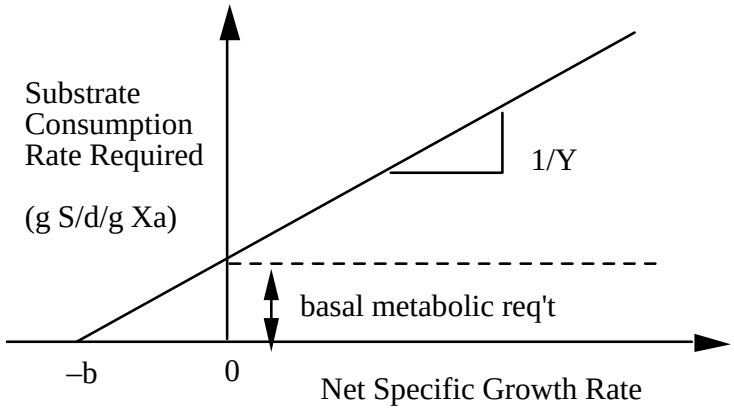
When the same electron donor is utilized for both energy and synthesis, we may consider that a fraction of all the donor consumed is used for synthesis, while the remainder fraction is used for energy:

$f_s \equiv$ fraction of electron donor used for synthesis;

$f_e \equiv$ fraction of electron donor used for energy.

$f_s + f_e = 1$

f_s and f_e depend, of course, on energetics (i.e., the energy available from the respiration reaction, in comparison to that required by the synthesis reaction). But f_s and f_e also depend upon solids retention time, θ_c. As θ_c increases (i.e., as net specific growth rate, μ, decreases), proportionately more of the electron-donor substrate is consumed to satisfy basal metabolic needs (i.e., the correction to gross growth caused by maintenance or "decay" becomes ever-more significant):



Consequently, f_s declines as θ_c increases. As we shall see later, f_e and f_s determine the overall stoichiometry of growth.

A. Energetics — Definitions

Consider the flows of electron donor (ed), electron acceptor (ea), and energy involved in the production of 1 eeq of bacterial cells.

Definitions:

1. ΔG_r

≡ free energy released per eeq of electron-donor substrate converted for energy (e.g., respiration).

Example: Consider the aerobic utilization of glucose. From the half-reactions of Table 1, we may generate the overall reaction for aerobic catabolism of glucose by subtracting Rxn (3) from Rxn (9) as follows:

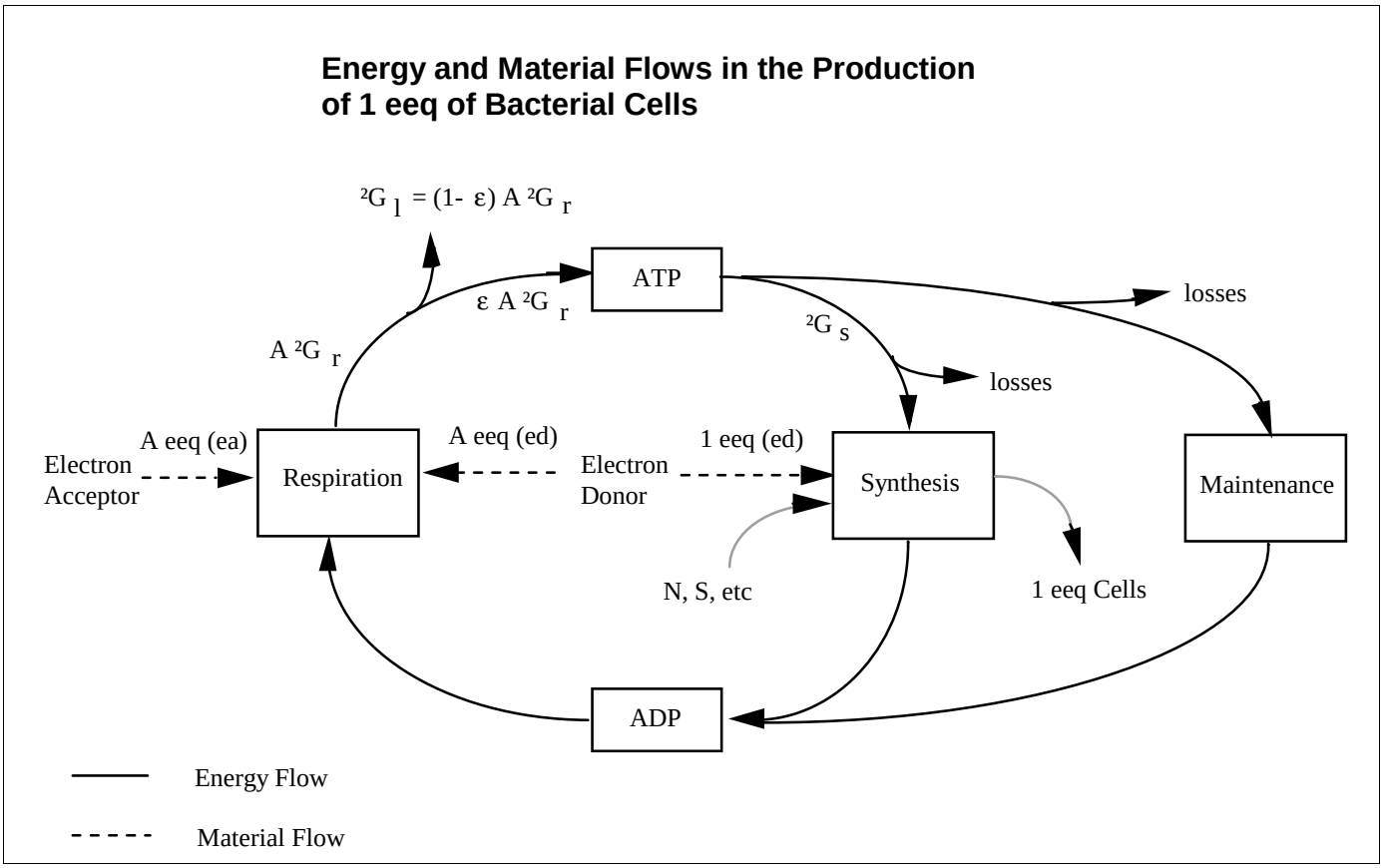
		$\Delta G^o(w)^*$ (kcal/eeq)
Rxn (9)	$^1/_{24}\ C_6H_{12}O_6 + {}^1/_4\ H_2O = {}^1/_4\ CO_2 + H^+ + e^-$	–10.0
–Rxn (3)	$^1/_4\ O_2 + H^+ + e^- = {}^1/_2\ H_2O$	–18.675
	$^1/_{24}\ C_6H_{12}O_6 + {}^1/_4\ O_2 = {}^1/_4\ CO_2 + {}^1/_4\ H_2O$	–28.675

* The terminology, $\Delta G^o(w)$, may be unfamiliar to some readers. What it represents is the standard free energy of reaction, corrected for pH 7 conditions. The standard free energy, ΔG^o, is defined for the condition where all reactants and products are at unit activity (approximately 1 M concentration, for solutes; 1 atm partial pressure, for gaseous species; and pure liquid or solid phases, for liquids and solids). But the reaction free energy (ΔG) for a biochemical reaction may differ significantly from the reaction *standard* free energy (ΔG^o), since the activities of reactants and products will seldom be unity. If we wish to calculate ΔG under *physiological conditions*, we have to correct the standard free energy for physiological concentrations according to:

$$\Delta G = \Delta G^o + RT \ln \left[\frac{\prod_j \{P_j\}^{p_j}}{\prod_i \{R_i\}^{r_i}} \right]$$

where R is the universal gas constant; T is absolute temperature (°K); the $\{P_j\}$ are the activities of the various products of reaction; the p_j are their respective stoichiometric coefficients; the $\{R_i\}$ are the activities of the reactants; and the r_i are their respective stoichiometric coefficients. Complete correction for physiological conditions involves substitution of estimated physiological activities of all reactants and products into the above equation. What $\Delta G^o(w)$ represents is a *partial* correction of ΔG for reality; for reactions in which H^+ and/or OH^- appear as reactants or products, their activities have been set equal to $\{10^{-7}\}$, reflecting neutral pH conditions, with the activities of all other reactants and products assumed to be unity. Thus, since we know that neither $\{H^+\}$ nor $\{OH^-\}$ are likely to be unity, we are at least making a partial correction to ΔG^o. Consequently, though not perfect, $\Delta G^o(w)$ values are likely to be far closer to reality than are ΔG^o values. Often, the difference between $\Delta G^o(w)$ and ΔG is not very significant. For example, the ΔG for glucose oxidation by O_2 under physiological conditions (0.2 atm O_2, 0.05 atm CO_2, 0.01 M glucose, pH 7) is estimated to be –28.773 — very close to the $\Delta G^o(w)$ value of -28.675 shown above. But in reactions where the small concentration of a reactant or product (other than H^+ or OH^-) is not "balanced" by a similarly small concentration of another reactant or product on the other side, then considerable difference may exist between $\Delta G^o(w)$ and ΔG. A good example is the ATP hydrolysis reaction, $ATP + H_2O \Rightarrow ADP + H_2PO_4^-$, in which ATP and ADP concentrations will roughly cancel each other because their ratio appears in the "RTln [——]" term, leaving $\{H_2PO_4^-\}$ as a major determinant of the correction. Thus, there is considerable difference between $\Delta G^o(w) = –7.3$ kcal/mol and the typical physiological value of $\Delta G = –12.5$ kcal/mol! Clearly some caution is in order when applying $\Delta G^o(w)$ values. We will attempt to flag those instances where additional corrections (other than for pH) are warranted.

A Mac to PC thing changed the delta's into superscript 2's



2. **ε**

≡ efficiency of energy transfer to or from the energy carrier (e.g., ATP).

McCarty assumed ε = 0.6, a value which resulted in good agreement between his bioenergetic method and experimentally observed stoichiometries.

3. **ΔG_s**

≡ carrier (ATP) energy required to synthesize 1 eeq of cells from whatever the carbon and nitrogen sources are. ΔG_s *includes* energy losses incurred in using ATP energy for synthesis. In other words, more ATP energy is spent in synthesis than the mere thermodynamically calculated value. Additional carrier energy is required because some fraction [presumably (1-ε)] will be lost due to biochemical inefficiency. Note the position of the ΔG_s label in the accompanying energy-flow scheme. ΔG_s represents the actual debit on the ATP pool caused by the synthesis of 1 eeq cells, even though only $\varepsilon\Delta G_s$ is actually "delivered" to synthesis.

4. A

≡ eeq of e.d. converted to energy per eeq of cells synthesized — ignoring decay or maintenance.

B. Energy Balance

For the moment, ignore maintenance (decay). It's OK to do so because what we seek is a means of estimating *Y*, which itself ignores decay. (Recall that in growth models, we include decay or maintenance through use of a separate *b* term for it,

$$dX_a/dt = Y(dF/dt) - bX_a$$

where *Y* represents the stoichiometric relation between *gross growth* and substrate utilization. Thus, strictly speaking, *Y* ignores decay and/or maintenance.)

ΔG_s kcal of ATP energy is required to form 1 eeq of cells. To replace this amount of ATP energy, the catabolic, ΔG_r reaction must be run *A* times. Simple thermodynamics tells us that such will potentially yield $A\Delta G_r$ kcal. However, because of inefficiencies, only $\varepsilon A\Delta G_r$ kcal will be captured in the form of ATP. (The difference, $[1-\varepsilon]A\Delta G_r$ kcal, is "lost," appearing as waste biological heat. It, along with similar losses accompanying synthesis and maintenance activities, is what keeps your body at 98.6˚F and what makes compost piles warm!).

Thus, at steady-state, a bacterium must be replacing ATP at the rate it's using ATP. Consequently,

$$\varepsilon A\Delta G_r + \Delta G_s = 0$$

where you'll note that we add the two terms because $\Delta G_r < 0$, while $\Delta G_s > 0$. Solving for *A* gives,

$$A = \frac{-\Delta G_s}{\varepsilon \Delta G_r}$$ eeq e.d. to energy per eeq cells synthesized.

We're interested in *A* because, as shall be seen, it relates directly to the yield coefficient, *Y*. We have an estimate of ε (i.e., 0.6), and we can easily calculate ΔG_r values from combinations of appropriate half-reactions (Table 1). But arriving at ΔG_s values is a bit trickier, and requires us to invoke some knowledge of biochemical pathways — along with some assumptions.

The ATP energy required for synthesis (ΔG_s) is dependent upon the energy state of the carbon source and that of the nitrogen source.

1. Ammonia as Nitrogen Source

Let us first restrict discussion to heterotrophic systems in which ammonia is the N-source. McCarty hypothesizes that synthesis can be divided into two steps:

a) Conversion of the C-source (whatever it is) into some universal intermediate, a compound occupying a central position at what amounts to a grand "intersection" of metabolic pathways. For calculation purposes, McCarty chose pyruvate as that central intermediate — a compound which indeed occupies a prominent position among the highways and byways of metabolism.

$\Delta G_p \equiv$ free energy required (or evolved) in conversion of the C-source to pyruvate (kcal/eeq pyruvate).

ΔG_p is merely the calculated free energy exchanged in conversion to pyruvate. The resulting effect on the ATP pool will either be $\varepsilon\Delta G_p$ (if $\Delta G_p < 0$) or $\Delta G_p/\varepsilon$ (if $\Delta G_p > 0$).

b) Conversion of pyruvate and NH_4^+ to 1 eeq biomass ($^1/_{20}$ $C_5H_7O_2N$);

$\Delta G_c \equiv$ ATP energy required to form $^1/_{20}$ $C_5H_7O_2N$ from pyruvate and ammonia $\cong$ 7.5 kcal.

McCarty estimated ΔG_c from studies (by others) in which cellular yields from pyruvate and ammonia were measured. Results indicated an average of 10.5 grams dry biomass solids formed per mol ATP consumed. Assuming dry biomass solids are 90% VSS, and that there are 12.5 kcal available per mol ATP$\Rightarrow$ ADP under physiological conditions, then $\Delta G_c = +7.5$ kcal ATP/eeq cells (if NH_4^+ is N-source).

Putting the two steps together then gives

$$\Delta G_s = \frac{\Delta G_p}{\varepsilon^m} + \Delta G_c$$

$m = +1 \quad (\Delta G_p > 0)$
$m = -1 \quad (\Delta G_p < 0)$

Note that no efficiency correction (ε) is required for the ΔG_c term because ΔG_c is already defined in terms of ATP energy required for synthesis. Inefficiencies have been empirically included. But not so for ΔG_p.

Substituting into our earlier equation results in the following:

Heterotrophic Growth — Ammonia as N-Source

Eq (1)

$$A = \frac{\dfrac{-\Delta G_p}{\varepsilon^m} - \Delta G_c}{\varepsilon\ \Delta G_r}$$

$m = +1 \quad (\Delta G_p > 0)$
$m = -1 \ (\Delta G_p < 0)$

in which $\varepsilon = 0.6$ and $\Delta G_c = 7.5$ kcal are usually assumed. Equation (1) is exactly as McCarty derived it.[1-4]

2. Nitrate as Nitrogen Source

Some tenuous assumptions are required when applying McCarty's bioenergetics' approach to systems using nitrate as N-source. Assumptions made in these notes differ from those made by McCarty[1-4] — specifically regarding biochemical pathways and the effects of assimilative denitrification on the cellular ATP pool. There are nomenclature differences as well. Consequently, a different equation results for estimation of *A* than is found in McCarty's papers.

To the earlier two steps in synthesis (ΔG_p and ΔG_c), we must add a third step: Conversion of nitrate to ammonia (ΔG_n). In the case of NO_3^-, our definition of 1 eeq cells is given by Rxn (2) (Table 1), from which we conclude that 1 eeq cells contains $^1/_{28}$ mol N.* We therefore need to know:

* If NO_3^- is N-source, then our total of 1 eeq e.d. is split between two uses: $^5/_7$ eeq to form $^5/_7$ eeq pyruvate; and $^2/_7$ eeq e.d. is consumed in reduction of $^1/_{28}$ mol NO_3^- to $^1/_{28}$ mol NH_4^+. This results (Rxn 1, Table 1) in $(^5/_7)(^1/_{20}) = ^1/_{28}$ $C_5H_7O_2N$, instead of the $^1/_{20}$ $C_5H_7O_2N$ achievable with ammonia as N-source. In essence, less cells are formed per eeq of e.d. to synthesis, because some of the e.d. must be used to reduce nitrate to ammonia (assimilative denitrification).

ΔG_n the ATP energy involved in the reduction of $^1/_{28}$ mol NO_3^- to $^1/_{28}$ mol NH_4^+ — a process which consumes $^2/_7$ eeq of our electron-donor substrate.

Our equation for ΔG_s (the ATP energy required for synthesis of 1 eeq of cells), now becomes:

$$\Delta G_s = {}^5\!/_7\left(\frac{\Delta G_p}{\varepsilon^m} + \Delta G_c\right) + \Delta G_n$$

Note the factor "$^5/_7$" which appears in front of the parenthetical expression (the parenthetical expression being the ΔG_s for formation of $^1/_{20}$ mol $C_5H_7O_2N$ from the e.d. and ammonia). This reflects the fact that only $^5/_7$ eeq of the donor is used to synthesize $^1/_{28}$ mol $C_5H_7O_2N$ (using the ammonia produced from reduction of nitrate by the other $^2/_7$ of the e.d.).

To estimate ΔG_n requires that we have some model in mind for assimilative nitrate reduction by heterotrophs.

Assimilative Nitrate Reduction. Many bacteria, fungi — and all plants — have the ability to utilize nitrate as N-source. The process by which they convert NO_3^- to NH_3 for synthesis is termed "assimilative nitrate reduction," and it differs markedly from the respiratory, "dissimilative nitrate reduction" which denitrifying bacteria mediate as an energy reaction.

The first step in assimilative nitrate reduction involves the reduction of NO_3^- to NO_2^- via an assimilative nitrate reductase system (NH_3-repressed); this is followed by reduction to hydroxylamine (NH_2OH) via assimilative nitrite reductase (also NH_3-repressed), with two subsequent reductions to NH_3. The 8 e^- required to effect the reduction of nitrate to ammonia are supplied by NADPH.

$$NO_3^- + 4\ NADPH + 4\ H^+ \Rightarrow NH_3 + 4\ NADP^+ + 2\ H_2O + OH^-$$

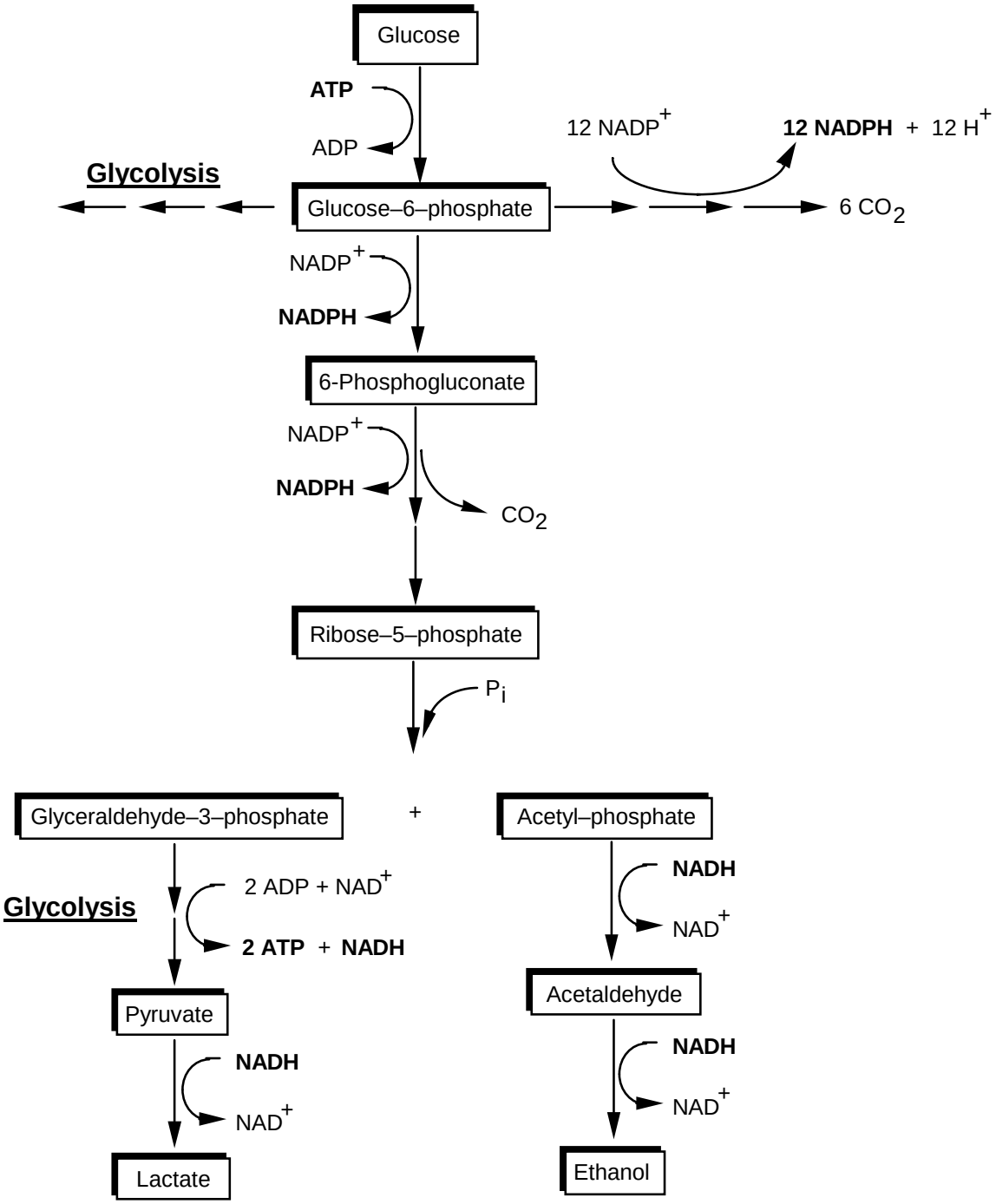
Respiratory carriers are involved, including pyridine nucleotides, flavins, quinones, and (in some cases) cytochromes. But these carriers are *not* membrane-associated like those of dissimilative nitrate reduction. Assimilative nitrate reduction does not apparently result in any ATP production, though it is exergonic.* **This is an important point.** Since we are interested in estimating the effect on the ATP pool resulting from

* Prescott, L. M., J. P. Harley, and D. A. Klein, *Microbiology*, Dubuque, IA, Wm C. Brown Publishers (1990). Page 180.

nitrate's conversion to ammonia, then we can essentially ignore it — with the exception of the steps leading to formation of the NADPH required for the reduction. Thus, the only effects on ΔG_s of using NO_3^- as N-source are: (i) the diversion of e.d. for this purpose; and (ii) the ATP consumption or production associated with NADPH generation from $NADP^+$. The subsequent reduction steps involving *use* of NADPH are assumed to have no significance with respect to the ATP pool.

Let's examine NADPH production in heterotrophs. Recall from CEE 651 that heterotrophs commonly employ the phosphogluconate pathway for production of NADPH:

PHOSPHOGLUCONATE PATHWAY



NET: **1 ATP per glucose**

2 NADH → 2 NADPH

There are two diverging routes which glucose degradation may take, depending upon cellular needs. Glucose may be completely oxidized to CO_2, generating 12 NADPH for reductive biosynthesis (with no ATP involvement); alternatively, glucose may be fermented to lactate and ethanol, in which case the net result is the formation of 1 ATP and the interconversion of 2 NADH to 2 NADPH.

We will assume the following steps regarding assimilative reduction by heterotrophs of $^1/_{28}$ mol of nitrate to $^1/_{28}$ mol ammonia:

i) $^2/_7$ eeq of our organic e.d. is first converted to $^2/_7$ eeq pyruvate, a central intermediate. This is assumed to have an effect on the ATP pool (i.e., contribution to ΔG_n) according to:

$$\Delta G_{n_{contribution}} = \frac{2}{7}\left(\frac{\Delta G_p}{\varepsilon^m}\right) \quad \text{kcal ATP/eeq cells}$$

ii) $^2/_7$ eeq of pyruvate is then converted to $^2/_7$ eeq of glucose-6-phosphate (with an estimated expenditure of 6 mol ATP per mol of glucose-6-phosphate**; at 12.5 kcal/mol ATP, this results in a total of 75 kcal ATP energy expended per mol of glucose-6-phosphate formed, or $^1/_{24}$ [75] = 3.13 kcal ATP per eeq of glucose-6-phosphate, or $^2/_7$[3.13] = 0.89 kcal ATP/eeq cells formed).

$$\Delta G_{n_{contribution}} = +\ 0.89 \quad \text{kcal ATP/eeq cells}$$

iii) Glucose-6-phosphate is completely oxidized to CO_2 via the phosphogluconate pathway, yielding 12 NADPH — with no apparent ATP either required or consumed.

iv) NADPH serves as reductant in conversion of nitrate to ammonia, with no apparent involvement of ATP.

Of the four steps, only the first two have any effect of the ATP pool. Their sum is the effective value of ΔG_n:

$$\Delta G_n = \frac{2}{7}\left(\frac{\Delta G_p}{\varepsilon^m}\right) + 0.89 \quad \text{kcal ATP/eeq cells}$$

** Prescott, L. M., J. P. Harley, and D. A. Klein, *Microbiology*, Dubuque, IA, Wm C. Brown Publishers (1990). Page 177.

We can then substitute this expression for ΔG_n into our ΔG_s equation:

$$\Delta G_s = \frac{5}{7}\left(\frac{\Delta G_p}{\varepsilon^m} + \Delta G_c\right) + \frac{2}{7}\left(\frac{\Delta G_p}{\varepsilon^m}\right) + 0.89$$

$$= \frac{\Delta G_p}{\varepsilon^m} + \frac{5}{7}\Delta G_c + 0.89$$

Substituting the expression above into our earlier equation for *A*, gives the following:

Heterotrophic Growth — Nitrate as N-Source

Eq (2)

$$A = \frac{\dfrac{-\Delta G_p}{\varepsilon^m} - \dfrac{5}{7}\Delta G_c - 0.89}{\varepsilon\ \Delta G_r}$$

$m = +1\ (\Delta G_p > 0)$
$m = -1\ (\Delta G_p < 0)$

in which $\varepsilon = 0.6$ and $\Delta G_c = 7.5$ kcal are usually assumed.

C. Estimating Yield Coefficients

Recall that the model we generally employ for microbial growth has the following form:

$$dX_a/dt = Y(dF/dt) - bX_a$$

where *Y* represents the stoichiometric relation between *gross growth* and substrate utilization. Strictly speaking, *Y* ignores decay and/or maintenance. Likewise, the ATP balance employed to estimate *A*-values (eeq e.d. to energy per eeq cells synthesized) also ignores decay and/or maintenance.

A-values can easily be used to estimate microbial yield coefficients (*Y*-values). In formation of 1 eeq cells, *A* eeq of e.d. is used for energy while 1 eeq of e.d. is used for synthesis. Consequently,

Eq (3)

$$a_e \equiv \frac{\text{eeq cells formed}}{\text{eeq e.d. used}} = \frac{1}{1 + A}$$

where a_e is really a yield coefficient based on electron equivalents, rather than usual units (g VSS per g COD or BOD_L). We can convert to conventional units by noting that 1 eeq cells = 5.65 grams of cellular VSS (4.04 grams, if NO_3^- is N-source); and 1 eeq of e.d. = 8 grams of COD. Therefore,

Ammonia as N-source

Eq (4)

$$Y \equiv \frac{\text{g } X_a \text{ formed}}{\text{g COD used}} = \frac{5.65\ a_e}{8} = \frac{5.65}{8\ (1 + A)}$$

or

Nitrate as N-source

Eq (5)

$$Y \equiv \frac{\text{g } X_a \text{ formed}}{\text{g COD used}} = \frac{4.04\ a_e}{8} = \frac{4.04}{8\ (1 + A)}$$

Example — Heterotrophic Growth

Suppose we are interested in the aerobic utilization of acetate, with ammonia as N-source:

e.d = acetate

e.a. = O_2

N = NH_4^+

Consulting Table 1:

ΔG_r = Rxn (11) – Rxn (3) = –6.609 – (18.675) = –25.284 kcal

ΔG_p = Rxn (11) – Rxn (16) = –6.609 – (–8.545) = +1.936 ($\therefore$ m = +1)

$\Delta G_n = 0$

$$A = \frac{-1.936/0.6 - 7.5}{0.6\ (-25.284)} = 0.707$$

$$\therefore\ Y = \frac{5.65}{8\ (1 + 0.707)} = 0.414\ \frac{\text{g } X_a \text{ VSS formed}}{\text{g Ac COD used}}$$

which compares remarkably well with a measured value of 0.410 g VSS/g COD [Burkhead, C.E., and R.E. McKinney, "Energy Concepts of Aerobic Microbial Metabolism," *Proc. Amer. Soc. Civil Engrs, 95* (SA2) 253 (1969)]. However, it

should be noted that measured values are notoriously imprecise — seek long enough, and you will find whatever number you like!

McCarty[2,4] has tabulated comparisons between a_e values estimated using his bioenergetics approach, and a_e values estimated from microbial yields reported in the literature (Table 2).

While there are certainly discrepancies in Table 2 between thermodynamically predicted a_e values and reported a_e values, there is generally sufficiently good agreement to encourage the use of Equations (1) and (3) in situations where experimental data are either lacking or unreliable.

TABLE 2. VALUES OF a_e FOR HETEROTROPHIC REACTIONS*

(Ammonia as N-source)

		a_e eeq of cells per eeq electron donor consumed	
Electron Donor	Electron Acceptor	Estimated from Reported Values	Estimated from Thermodynamics**
Glucose	O_2	0.79	0.72
Benzoate	O_2	0.46	0.60
Propionate	O_2	0.58	0.59
Acetate	O_2	0.58	0.59
Alanine	O_2	0.52	0.64
Methanol	CO_2	0.15	0.21
Benzoate	CO_2	0.11	0.062
Glucose	CO_2	0.27	0.28
Propionate	CO_2	0.069	0.048
Acetate	CO_2	0.06	0.047

* Excerpted from Reference [4].

** Equations (1) and (3), with $\varepsilon = 0.6$; $\Delta G_c = 7.5$ kcal/eeq

Few literature data exist for yield coefficients when NO_3^- serves as N-source. Stensel et al.[7] report a value for biological denitrification using methanol as e.d. and NO_3^- as both e.a. and N-source. Their value (0.19 g TSS/g COD) converts to an a_e value of 0.35. The thermodynamically based method presented in these notes [Equations (2) and (3)] predicts $a_e = 0.72$ — not very good agreement. Thus, the validity and usefulness of Equation (2) for estimating heterotrophic yields where nitrate is N-source remains in doubt. Additional experimental data are necessary before definitive judgment can be rendered.

D. Estimating Maximum Specific Substrate Utilization Rates

McCarty asserts that the rate of electron transfer in energy-yielding reactions (e.g., respiration) is surprisingly constant (per gram active VSS) among many types of microorganisms — including heterotrophs, autotrophs, aerobes and anaerobes.[1,5]

Define:*

$$r_e \equiv \frac{\text{mol } e^- \text{ to energy}}{\text{gram } X_a \cdot \text{ day}} \cong 1.0 \qquad @\ 25°C$$

McCarty found the actual number to vary between about 0.5 and 2.0 @ 25˚C — and, of course, it exhibited a variation with temperature which could be modeled via the Arrhenius equation,[1,5]

$$r_e \cong 2.14 \times 10^7 \exp\left(\frac{-10 \text{ kcal/mol}}{RT}\right)$$

over the range from 10˚ to 40˚C. However, given that the supposedly "constant" r_e varies between 0.5 - 2.0 among organisms at 25˚C, then temperature correction hardly seems warranted!

Knowledge of the rate at which electrons are transferred can be used to estimate maximum specific utilization rates (*k*-values).

The unit "eeq" in these notes is always unambiguously defined on the basis of *total* carbonaceous oxidation. For example, glucose will donate 24 e^- per molecule, if completely oxidized to CO_2 and H_2O. Hence, 1 eeq glucose = $^1/_{24}$ mol (no matter how many electrons are actually transferred in a particular

* Note that the nomenclature in these notes differs from that of the reference-source material.

instance). But while its potential for electron donation may be 24 e^- per molecule, there are many metabolic examples (e.g., fermentation to lactic acid) where far fewer electrons are actually transferred to a carrier such as NAD^+.

Define:

$$d \equiv \frac{\text{mol } e^- \text{ actually transferred to carrier}}{\text{mol } e^- \text{ potentially available}}$$

Thus, *d* is the fraction of potential electrons which are actually transferred to a carrier in the energy-yielding metabolism of the electron donor. For total carbonaceous oxidations, $d = 1$ [all the electrons potentially available are transferred to some intermediate carrier (NAD^+ or $NADP^+$)]. For fermentations, however, $d < 1$.

For example, in lactic acid fermentation, glucose ∅ 2 lactate via glycolysis. Only 4 e^- (i.e., 2 NADH) are involved in carrier transfers. Therefore, in this case, $d = {}^4/_{24} = {}^1/_6$. But note that there is no way — without knowing the details of the metabolic pathway — that one could have known the number of electrons externally transferred to a carrier. After all, when fermentation is complete, the number of reduced electrons associated with the two lactates equals the number associated with the original glucose. Four electrons are removed to create 2NADH; but then the 2NADH gives them back again to 2 pyruvates, forming 2 lactates.

It is typical of fermentations that there is merely a rearrangement of electrons among organic compounds, with no removal of them (unless a volatile, reduced product such as H_2 or CH_4 is evolved). Therefore, ignorance of biochemical pathways would prevent one from knowing the value of *d*. At this juncture, you might well be asking yourself, "What good is knowing *d*?"

Define:

$$k_m \equiv \frac{\text{eeq of e.d. to energy}}{\text{gram } X_a \cdot \text{day}} = \frac{r_e}{d} \qquad \text{Eq (6)}$$

While it may not yet look like it, k_m is a type of maximum specific utilization rate — albeit in unfamiliar units. We can convert k_m to more useful forms as follows:

$$k_e \equiv \frac{\text{eeq of e.d. used}}{\text{eeq cells} \cdot \text{day}} = \frac{k_m\ (5.65)^*\ (1+A)}{A} \qquad \text{Eq (7)}$$

or

* Use 4.04 if nitrate is the N-source.

$$k \equiv \frac{g\ COD\ used}{g\ X_a \cdot day} = \frac{k_m\ (1+A)\ 8}{A} \qquad \text{Eq(8)}$$

k_e is a maximum specific utilization rate based on electron equivalents, and k is a more conventional parameter, based on VSS and COD.

> [An aside: You should note that "COD used" isn't always the same thing as "COD destroyed." As earlier pointed out, fermentations often result in zero destruction of COD — merely a change in form. Glucose COD gets converted to lactate COD, for example. By "COD used," we really mean "electron donor which got processed by microorganisms, expressed in COD units." So if 20 grams glucose COD is fermented to lactate, then 20 grams COD is processed (or "used"), but zero grams of COD are actually destroyed.

You have now been provided with two types of yield coefficients (a_e and Y) and two types of maximum specific utilization rate constants (k_e and k). Careful examination of the units of each will demonstrate that we have two compatible pairs: Y is compatible with k; and a_e with k_e. We may, for example, make use of them as follows:

$$\frac{1}{\theta_c^{min}} \cong Yk - b = a_e k_e - b$$

E. Fermentations

The bioenergetics' approach can be used to estimate yield coefficients for fermentations. And if one is willing to assume an electron-transfer rate, r_e, then it is even possible to crudely estimate the maximum specific substrate-utilization rate, k., for well-studied fermentations for which d-values are known.

One well-studied fermentation is that of glucose to lactate, cited earlier. We will employ bioenergetics' techniques to compare the Y- and k-values for this fermentation to their corresponding values for aerobic oxidation of glucose.

Fermentation of Glucose to Lactate

Assume: NH_4^+ = N-source; r_e = 1.0 mol e^- transferred to energy $\cdot$ g^{-1} $X_a \cdot day^{-1}$; d = $^1/_6$; and b = 0.05 day^{-1}.

ΔG_p = Rxn (9) – Rxn (16) = –10.0 + 8.545 = –1.455 kcal/eeq

($\therefore$ m = –1)

ΔG_r = Rxn (9) – Rxn (15) = –10.0 + 7.873 = –2.127 kcal/eeq

ΔG_c = 7.5 kcal/eeq

$$\therefore \quad A = \frac{1.455\ (0.6) - 7.5}{0.6\ (-2.127)} = 5.193$$

$$Y = \frac{5.65}{8\ (1+A)} = 0.11\ \frac{g\ X_a}{g\ gluc\ COD}$$

$$k_m = \frac{r_e}{d} = \frac{1}{1/6} = 6\ \frac{eeq\ of\ gluc.\ to\ energy}{g\ X_a \cdot day}$$

$$k = k_m\left(\frac{1+A}{A}\right) 8 = 57\ \frac{g\ gluc\ COD\ used}{g\ X_a \cdot\ day}$$

$$\therefore \quad \mu_{max} = \frac{1}{\theta_c^{min}} = Yk - b = 0.11(57) - 0.05 = 6.2\ day^{-1}$$

Aerobic Oxidation of Glucose

Assume: NH_4^+ = N-source; r_e = 1.0 mol e^- transferred to energy · g^{-1} X_a· day^{-1}; d = 1; and b = 0.05 day^{-1}.

ΔG_p = Rxn (9) – Rxn (16) = –10.0 + 8.545 = –1.455 kcal/eeq

ΔG_r = Rxn (9) – Rxn (3) = –10.0 – 18.675 = –28.675 kcal/eeq ($\therefore$ m = –1)

ΔG_c = 7.5 kcal/eeq

$$\therefore \quad A = \frac{1.455\ (0.6) - 7.5}{0.6\ (-28.675)} = 0.385$$

$$Y = \frac{5.65}{8\ (1+A)} = 0.51\ \frac{g\ X_a}{g\ gluc\ COD}$$

$$k_m = 1\ \frac{eeq\ of\ gluc.\ to\ energy}{g\ X_a \cdot day}$$

$$k = k_m\left(\frac{1+A}{A}\right) 8 = 29\ \frac{g\ gluc\ COD\ used}{g\ X_a \cdot\ day}$$

$$\therefore \quad \mu_{max} = \frac{1}{\theta_c^{min}} = Yk - b = 0.51(29) - 0.05 = 14.7\ day^{-1}$$

From this exercise, we conclude several things:

(1) Glucose can potentially be processed twice as fast via fermentation (k = 57 g COD/gX_a/d) than via aerobic oxidation (k = 29 g COD/gX_a/d). This was first observed by Pasteur, working with batch cultures of facultative anaerobes. He monitored the rate of sugar utilization by yeasts under aerobic conditions; then, when he allowed the cultures to go anaerobic, the rate of substrate utilization rose dramatically. This is now referred to as the "Pasteur Effect." The explanation is simple: If you accept the premise that all organisms transfer electrons at approximately the same rate, then glucose utilization in fermentation should proceed much faster than in aerobic oxidation because far fewer electrons are being transferred (per glucose) in fermentation. In essence, fermentation alters each glucose to a far lesser degree than does total oxidation; therefore, more glucoses can be processed per organism per time.

(2) The yield coefficient for lactate fermentation of glucose (Y = 0.11 gX_a/gCOD) is only about one-fifth that for total oxidation (Y = 0.51 gX_a/gCOD). The explanation? The ΔG_r values are considerably different (–2.127 for fermentation, versus –28.675 for oxidation). Since much less energy is available per glucose in fermentation, a fermenting organism has to channel considerably more glucoses to energy than does an aerobic organism, in order to obtain enough energy to channel one glucose to biosynthesis.

(3) The maximum specific growth rate for lactate fermentation (μ_{max} = 6.2 day^{-1}) is much lower than that for aerobic glucose oxidation (μ_{max} = 14.7 day^{-1}), all other things being equal. While the *k* value is lower aerobically, the *Y* is sufficiently higher so as to more than make up for the lower *k*.

F. Stoichiometry

When the same electron donor is used for synthesis and energy production (as is usually — but not always — the case in heterotrophic metabolism), it is easy to arrive at "growth equations" which specify the stoichiometries involved. These equations are very useful for such things as predicting nutrient requirements, alkalinity changes, gas compositions, and other factors associated with microbial activities. Our stoichiometries will even include decay.

In general, we may write a growth stoichiometry as the sum of synthesis and energy reactions:

Synthesis:

$$f_s\ (ed_{red} + \text{N-source} \Rightarrow ed_{ox} + \text{cells})$$

Energy:

f_e ($ed_{red} + ea_{ox} \Rightarrow ed_{ox} + ea_{red}$)

where

f_s = fraction of e.d. to net synthesis (i.e., including all biologically formed VSS — X_a as well as the inert remains of decayed cells);

f_e = fraction of e.d. to energy;

ed_{red}, ed_{ox} = reduced and oxidized forms of the e.d., respectively;

ea_{red}, ea_{ox} = reduced and oxidized forms of the e.a., respectively.

Note that $f_s + f_e = 1$, and that f_s and f_e are functions of θ_c, as well as *A*.

Define:

R_d = half-reaction for oxidation of the e.d.;

R_a = half-reaction for formation of the e.a.;

R_c = half-reaction for oxidation of cells.

These are all defined as oxidations, as per Table 1. Half-reaction R_c is either Rxn (1) or (2), depending upon the N-source.

Our general stoichiometry may be written as follows:

Eq (9)

$$R_d - f_e R_a - f_s R_c$$

At first glance, Equation (9) may not look like an equation; but each of its components (R_d, R_a, and R_c) are chemical equations (half-reactions). Thus, Equation (9) is really a recipe for doing "equation arithmetic," with a resulting equation which is a linear combination of three half-reaction equations.

We next need to derive the functional relationship, $f_s = f(A, \theta_c)$. We can do so by simple extension of our earlier efforts at modeling MLVSS. We know that the effective yield (Y_{obs}) of biologically formed VSS (active + inert remains of decayed cells) is given by:

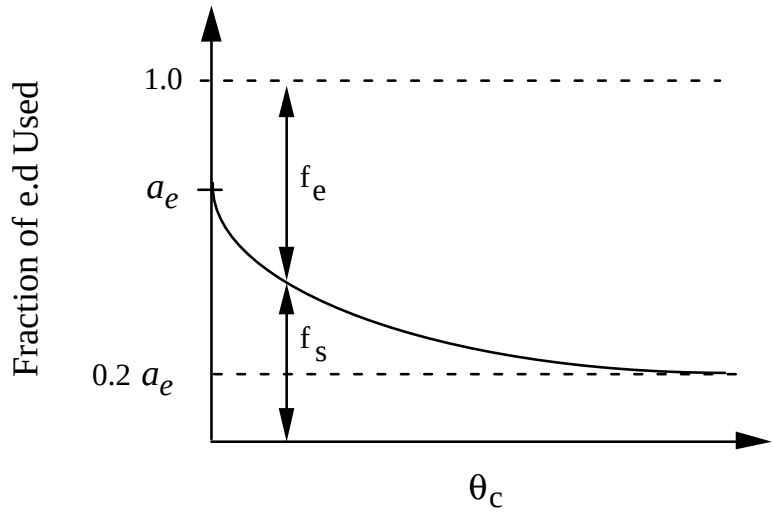
$$Y_{obs} = \frac{Y\ (1 + 0.2b\theta_c)}{1 + b\theta_c} \qquad \frac{g\ VSS}{g\ COD}$$

Similarly, f_s is the effective yield of biomass (eeq) — living and dead — per eeq of e.d. consumed. It is the "eeq analog" to Y_{obs}. The "eeq analog" to *Y* is a_e. Therefore, by analogy,

Eq (10)

$$f_s = \frac{a_e\,(1 + 0.2b\theta_c)}{1 + b\theta_c} = \left(\frac{1}{1+A}\right)\left(\frac{1 + 0.2b\theta_c}{1 + b\theta_c}\right)$$

The relationship between f_s, f_e, and θ_c is shown on the following page.



Note how the fraction of e.d. going to synthesis decreases with θ_c, from a value of a_e at θ_c = zero, to an asymptote of $0.2a_e$. (At infinite θ_c, $X_a \varnothing 0$, and all biological solids consist of the inert remains of decayed cells.) Also note that real reactors cannot function below some θ_c^{min}, so the portion of the curve at $\theta_c < \theta_c^{min}$ is merely hypothetical.

Example — Aerobic Utilization of Acetate

This is a continuation of an earlier example (page 17). Suppose we are interested in the aerobic utilization of acetate, with ammonia as N-source:

e.d = acetate

e.a. = O_2

N = NH_4^+

θ_c = 8 days; b = 0.1 day^{-1}.

From earlier example, A = 0.707 eeq to energy per eeq cells formed.

$$\therefore \quad f_s = \frac{1}{1.707}\left[\frac{1+0.2(0.1)(8)}{1+0.1(8)}\right] = 0.378$$

$$f_e = 1 - f_s = 0.622$$

R_d:

$$0.125\ CH_3COO^- + 0.375\ H_2O \varnothing 0.125\ CO_2 + 0.125\ HCO_3^- + H^+ + e^-$$

$-f_eR_a$:

$$0.155\ O_2 + 0.622\ H^+ + 0.622\ e^- \Rightarrow 0.311\ H_2O$$

$-f_sR_c$:

$$0.0756\ CO_2 + 0.0189\ HCO_3^- + 0.0189\ NH_4^+ + 0.378\ H^+ + 0.378\ e^- \Rightarrow 0.0189\ C_5H_7O_2N + 0.170\ H_2O$$

$$0.125\ CH_3COO^- + 0.155\ O_2 + 0.0189\ NH_4^+ \Rightarrow 0.0494\ CO_2 + 0.106\ HCO_3^- + 0.106\ H_2O + 0.0189\ C_5H_7O_2N$$

Which tells us that 0.125 mol acetate (= 1 eeq = 8 g COD) will result in 0.0189 mol of $C_5H_7O_2N$ (= 0.0189 mol · 113 g VSS/mol = 2.14 grams of biologically produced VSS). Thus, the observed yield, Y_{obs}= 2.14 ÷ 8 = 0.267 g VSS/gCOD. This should, of course, agree with the number arrived at via

$$Y_{obs} = \frac{Y\ (1 + 0.2b\theta_c)}{1 + b\theta_c} = \frac{0.414[1+0.2(0.1)(8)]}{1+0.1(8)} = 0.267\ \frac{g\ VSS}{g\ COD}$$

The stoichiometric equation is useful for estimating N requirements, P requirements (recognizing that P is required in a *mass* amount which is $^1/_5$ of the *mass* of N required for synthesis, or about $100(^1/_5)(^{14}/_{113})$ = 2.5% of biologically formed VSS). You can get the same information without bothering to derive the whole stoichiometry — by applying $Y_{obs}\Delta S$ to estimate VSS production, and then using knowledge of biological solids composition ($C_5H_7O_2N$) to estimate N, P requirements. But the stoichiometry provides other potentially useful information, such as alkalinity and CO_2 production/consumption. This additional information — unavailable from application of Y_{obs} alone — may be important in predicting pH effects and in designing buffer systems for biological reactors.

Example — Mixed Substrate

A complex wastewater has a BOD_L of 400 mg/L which is 75% carbohydrate, 15% grease, and 10% protein (all on a BOD basis). Based upon bioenergetic principles, estimate *Y* applicable to aerobic treatment (assuming NH_4^+ as N-source).

In such a case, the overall *Y* is merely the weighted average of the *Y*-values for each of the three components:

Carbohydrate

ΔG_r = Rxn (9) – Rxn (3) = –10.0 –18.675 = –28.675 kcal/eeq

ΔG_p = Rxn (9) – Rxn (16) = –10.0 +8.545 = –1.455 kcal/eeq

($\therefore$ m = -1)

$$A = \frac{\frac{-\Delta G_p}{\varepsilon^m} - \Delta G_c}{\varepsilon\ \Delta G_r} = \frac{+1.455(0.6) - 7.5}{0.6(-28.675)} = 0.385$$

$$a_e = \frac{1}{1 + A} = 0.722 \text{ eeq cells/eeq carbohydrate}$$

Grease (Fats and Oils)

ΔG_r = Rxn (10) – Rxn (3) = –6.6 –18.675 = –25.275 kcal/eeq

ΔG_p = Rxn (10) – Rxn (16) = –6.6 +8.545 = +1.945 kcal/eeq

($\therefore$ m = +1)

$$A = \frac{\frac{-\Delta G_p}{\varepsilon^m} - \Delta G_c}{\varepsilon\ \Delta G_r} = \frac{\frac{-1.945}{0.6} - 7.5}{0.6(-25.275)} = 0.708$$

$$a_e = \frac{1}{1 + A} = 0.585 \text{ eeq cells/eeq grease}$$

Protein

ΔG_r = Rxn (8) – Rxn (3) = –7.7 –18.675 = –26.375 kcal/eeq

ΔG_p = Rxn (8) – Rxn (16) = –7.7 +8.545 = +0.845 kcal/eeq

($\therefore$ m = +1)

$$A = \frac{\frac{-\Delta G_p}{\varepsilon^m} - \Delta G_c}{\varepsilon\ \Delta G_r} = \frac{\frac{-0.845}{0.6} - 7.5}{0.6(-26.375)} = 0.563$$

$$a_e = \frac{1}{1 + A} = 0.640 \text{ eeq cells/eeq protein}$$

Mixture

$$a_{e_{mix}} = 0.75\ a_{e_{carbohdrate}} + 0.15\ a_{e_{grease}} + 0.10\ a_{e_{protein}}$$
$$= 0.75\ (0.722) + 0.15\ (0.585) + 0.10\ (0.640)$$
$$= 0.693 \text{ eeq cells / eeq mixed substrate}$$

$\therefore\ Y_{mix} = (^{5.65}/_{8})\ a_e = 0.489 \text{ g } X_a/\text{g } BOD_L$

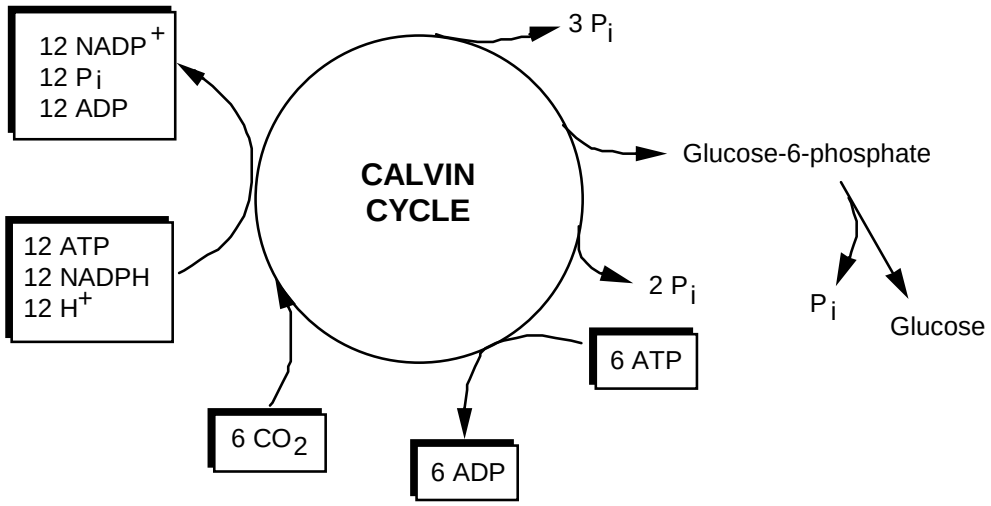
A stoichiometry for this waste's degradation can be estimated as follows: First, determine stoichiometries for each of the three components (on a 1 eeq e.d. basis each) as usual; multiply each stoichiometry by the appropriate fractional contribution which the component makes to total BOD_L; and then add the weighted stoichiometries together to provide an estimate for the mixture.

II. AUTOTROPHIC METABOLISM

Autotrophic bacteria use CO_2 as carbon source. And most of the autotrophs of concern to us are *lithotrophic* — i.e., they obtain their energy from inorganic redox reactions. Important examples include nitrifying bacteria (e.d. = NH_4^+ and NO_2^-; e.a. = O_2), sulfur bacteria (e.d. = H_2S, S^o, and $S_2O_3^=$; e.a. = O_2), hydrogen-oxidizing bacteria (e.d. = H_2; e.a. = O_2), iron-oxidizing bacteria (e.d. = Fe^{++}; e.a. = O_2), and methanogenic bacteria (all of which appear to be able to obtain energy using H_2 as e.d. and CO_2 as e.a., though organic electron donors are preferred and complete autotrophy is in doubt). In addition, some acetogens and sulfate reducing bacteria can utilize H_2 as e.d., though — like methanogens — other donors are preferred.

The reduction of CO_2 to cellular constituents ("autotrophic CO_2 fixation") occurs in a number of ways. Most autotrophs* appear to utilize the Calvin cycle, also known as the *reductive pentose cycle.* In algae and higher plants, the Calvin cycle occurs in chloroplasts. In autotrophic bacteria (e.g., nitrifying, iron-oxidizing, and sulfur-oxidizing bacteria), the enzymes of the Calvin cycle are located either in the cytoplasm or are associated with the plasma membrane.

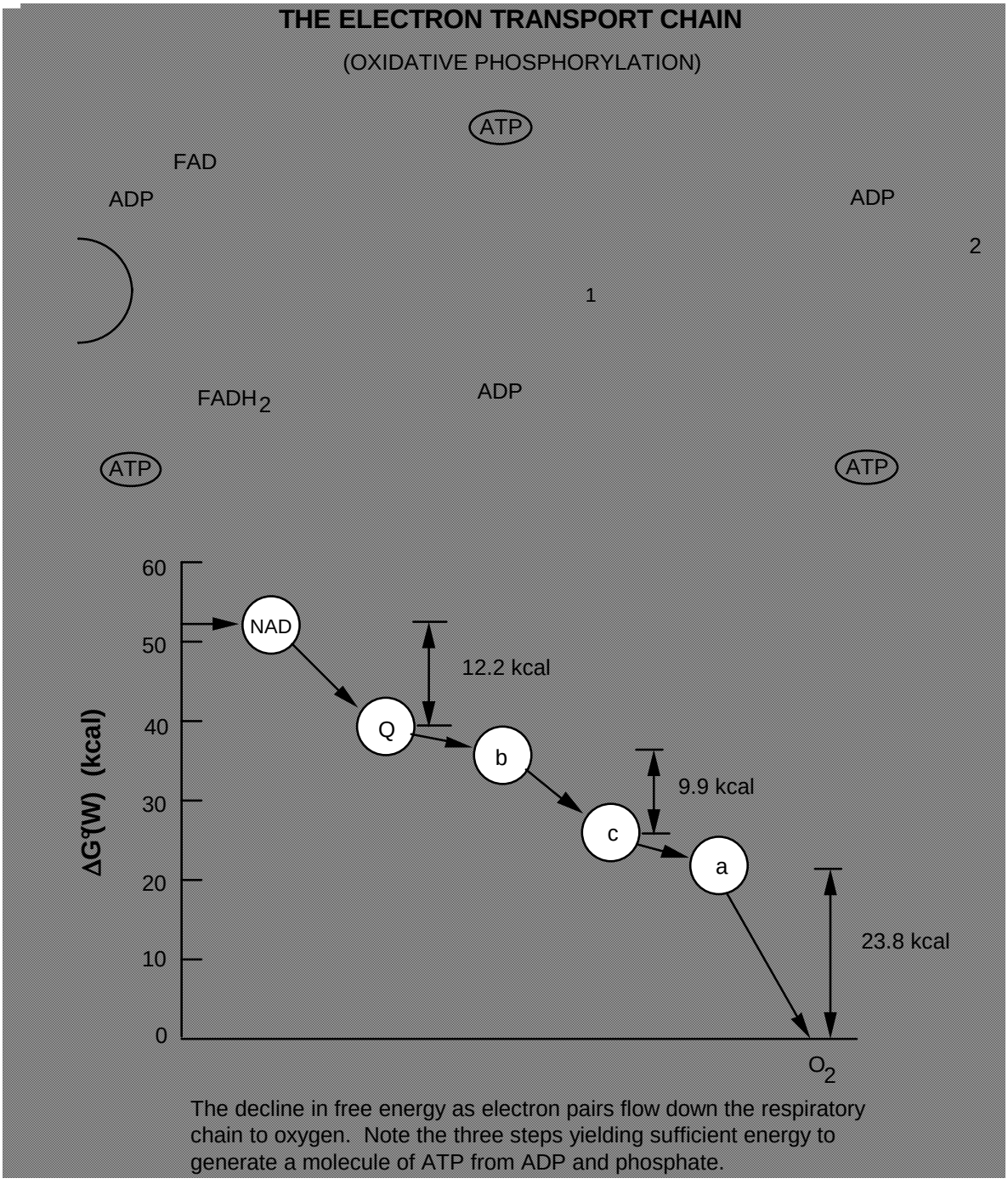
* Important exceptions include the methanogenic bacteria and acetogenic bacteria.



Overall, 18 ATP and 12 NADPH are required to reduce 6 CO_2 to one hexose. The cycle itself involves various sugar-phosphates (e.g., ribulose-5-phosphate, ribulose-1,5-diphosphate, 3-phosphoglycerate, glyceraldehyde-3-phosphate, fructose-6-phosphate, and others).

How do autotrophs derive the rather significant quantities of NADPHs required for reductive biosynthesis? Direct substrate-level reduction of $NADP^+$ by the inorganic electron donor is not possible in most instances. From Rxn (26), Table 1, it can be seen that approximately 7.38 kcal are required to reduce 1 eeq $NADP^+$ to 1 eeq NADPH. Of the inorganic donors [Rxns (18) through (25)], only H_2 and $SO_3^=$ possess sufficient oxidation potentials to form NADPH directly. As a result, most lithotrophs utilize a ***reverse-electron-transport*** system, in which ATP is expended to force electrons against the normal flow.

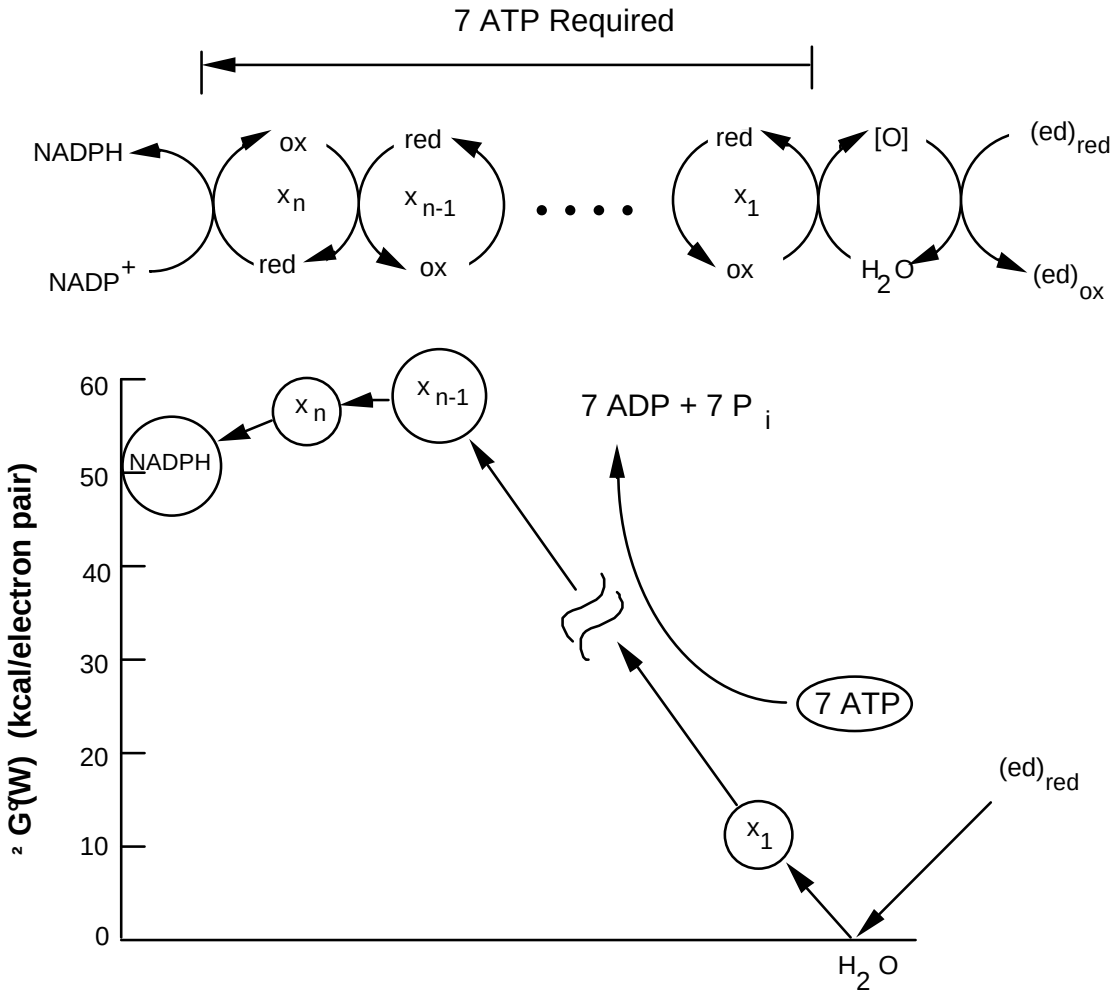
Recall that the electron-transport system of oxidative phosphorylation looks something like this:



The decline in free energy as electron pairs flow down the respiratory chain to oxygen. Note the three steps yielding sufficient energy to generate a molecule of ATP from ADP and phosphate.

Reverse Electron Transport works in a similar fashion, but with a completely different set of electron carriers — though cytochromes, flavoproteins and quinones appear prominent among them.* The big difference is that electrons from the inorganic e.d. flow "up-hill" with respect to energy level, at the expense of proton-motive force (or ATP, if you prefer to think of energy storage that way):

REVERSE ELECTRON TRANSPORT



* Brock, T.D., and M.T. Madigan, *Biology of Microorganisms*, 5th ed., Englewood Cliffs, NJ, Prentice-Hall (1988). Pages 557-559, 566-574.

McCarty assumes[**] that all autotrophs generate NADPH via a reverse-electron-transport mechanism in which the inorganic e.d. first donates electrons to a carrier-bound oxygen, [O], reducing it to the level of H_2O. This initiation step is invariably exergonic (i.e., $\Delta G < 0$), but McCarty assumes that no ATP are generated there. From H_2O, electrons are pumped against the energy gradient through expenditure of proton-motive force (or ATP), eventually being used to reduce $NADP^+$ to NADPH. The reaction whereby 1 mol H_2O donates 2 mol e^- to 1 mol $NADP^+$ requires approximately +52 kcal/pair of electrons — i.e., $2 \cdot$ [Rxn (3) – Rxn (26)]. Assuming an efficiency of 0.6 and an energy content of 12.5 kcal/mol ATP, then approximately 7 ATP would likely be expended in the production of 1 NADPH from H_2O — or 3.5 ATP per eeq. The actual number is unknown, as is its variation among autotrophic bacteria.

The nice thing about making the preceding assumptions is that calculations of A and a_e are greatly simplified — *for all autotrophs*, a uniform, constant 3.5 ATP are required for production of 1 eeq of NADPH via reverse-electron transport, and a uniform, constant 0.75 ATP/eeq are expended in CO_2 fixation (to glucose) via the Calvin cycle. At 12.5 kcal/ATP, this represents an ATP energy utilization of +53.1 kcal ATP/eeq glucose formed. If this glucose is converted to pyruvate ($\Delta G_p = -1.455$ kcal/eeq), then $\varepsilon\Delta G_p = -0.873$ kcal ATP are restored to the ATP pool. Thus, the net ATP consumption in the fixation of CO_2 to pyruvate is +53.1 – 0.873 = +52.2 kcal ATP/eeq of pyruvate formed — and this number is assumed constant among all autotrophs.

The biochemical basis is tenuous for assuming that all autotrophs expend the same number of ATPs in generation of 1 eeq NADPH from 1 eeq of e.d. A highly energetic donor would seem perfectly able to enter reverse-electron transport at a position considerably above that of H_2O, lowering the ATP requirement by shortening the electron-transport path.[*] For example, the e.d. might have a redox potential sufficient to directly reduce x_1 (or even x_{n-1}, in the case of H_2). Nevertheless, McCarty has assumed

---

[**] In fairness to McCarty, I should note that he did not explicitly make the assumptions I've attributed to him. What he explicitly assumed is that — as far as energetics are concerned — H_2O is the electron donor for pyruvate production. Thus all autotrophs have the same $\Delta G_p = +27.22$ kcal/eeq. He later assumed in his stoichiometry development that the supplied inorganic e.d. is used to reduce the O_2 (which would otherwise result from use of H_2O as an e.d.) back to H_2O. In essence, McCarty assumed that H_2O is the e.d. for synthesis — energetically — while the supplied, inorganic e.d. is the donor for synthesis —stoichiometrically. What I've tried to do is to explain the biochemical significance of his assumptions. I've also altered some of the free-energy values associated with synthesis: since we know the actual number of ATP involved in the Calvin cycle (i.e., 18 per mol glucose), why not use that information in the estimation of ΔG_s, which is, after all, defined in terms of "ATP energy" required for synthesis? I regard that approach as superior to assuming an efficiency of 60% in the fixation of 1 eeq CO_2 to 1 eeq pyruvate. In fact, calculation shows that the Calvin cycle is only about 30% efficient at converting ATP energy to hexose energy. Thus, Equations (11) and (12) differ from similar equations provided by McCarty. If errors result in estimation of Y using Eqs (11) or (12), blame me, not McCarty!

[*] Brock, T.D., and M.T. Madigan, *Biology of Microorganisms*, 5th ed., Englewood Cliffs, NJ, Prentice-Hall (1988). Page 568.

that all inorganic electron donors enter by reducing [O] to H_2O, with the resulting H_2O serving as the e.d. energetically, as is the case with plants and green algae.

The biochemical basis for assuming use of the Calvin cycle in CO_2 fixation is reasonably sound, though there are notable examples — methanogens and acetogens — which use pathways involving acetate production from CO_2. To the extent that their ATP requirement differs from that of the Calvin cycle (i.e., to the extent that their efficiencies differ), then estimates of ATP required for synthesis, ΔG_s, will be in error.

In the end — despite all of the aforementioned misgivings — one has to be impressed with the end result of McCarty's assumptions: His method works surprisingly well for predicting yield coefficients for lithotrophic autotrophs. But in the absence of firm, biochemical bases for some of the underlying assumptions, the method must be regarded as somewhat empirical.

A. Autotrophic Energy Balance

An energy balance (as per p. 9) for autotrophic growth will result in the same equation as earlier derived for heterotrophic systems:

$$A = \frac{-\ \Delta G_s}{\varepsilon\ \Delta G_r}$$ eeq e.d. to energy per eeq cells synthesized.

where A, ΔG_s, ΔG_r, and ε retain their earlier definitions (see pp. 7-10).

1. Ammonia as Nitrogen Source

As with heterotrophic growth, in autotrophic growth we conceptually divide the process of synthesis into two steps:

a) Conversion of the carbon source (CO_2) to a universal intermediate (pyruvate). Recall from a previous section that we are assuming that all autotrophs employ the same reverse-electron-transport system for NADPH production, and employ the Calvin cycle for CO_2 fixation to glucose. It was earlier estimated that +52.2 kcal ATP are needed per eeq of pyruvate formed. Since ΔG_s is defined in terms of kcal ATP energy required for synthesis, then for this step:

$$\Delta G_{s_{contribution}} = +52.2 \text{ kcal ATP/eeq cells}$$

b) Conversion of pyruvate and NH_4^+ to 1 eeq of biomass ($^1/_{20}$ mol $C_5H_7O_2N$). This is assumed to require, as with heterotrophic growth, +7.5 kcal ATP/eeq:

$$\Delta G_{s_{contribution}} = +7.5 \text{ kcal ATP/eeq cells}$$

Substituting into the expression for *A* gives the following:

Autotrophic Growth — Ammonia as N-Source

Eq (11)

$$A = \frac{-59.7}{0.6\ \Delta G_r}$$

1. Nitrate as Nitrogen Source

To the earlier two steps in synthesis, we must add a third step: conversion of nitrate to ammonia (ΔG_n), as was the case for heterotrophic growth using nitrate as N-source (p. 12):

ΔG_n + the ATP energy involved in the reduction of $^1/_{28}$ mol NO_3^- to $^1/_{28}$ mol NH_4^+ — a process which consumes $^2/_7$ eeq of our electron-donor substrate.

Our equation for ΔG_s (the ATP energy required for synthesis of 1 eeq of cells, where the definition of "1 eeq cells" = $^1/_{28}$ mol $C_5H_7O_2N$), now becomes:

$$\Delta G_s = {}^5/_7\ (59.7) + \Delta G_n$$

As with heterotrophs, we will assume that autotrophs reduce NO_3^- to NH_3 through use of NADPH, with no effect on the ATP pool — except for the ATP expended in generation of the NADPH.

We know from earlier discussion that autotrophs generally form NADPH from $NADP^+$ via reverse-electron transport, and that approximately 3.5 ATP are expended per eeq of NADPH formed (and per eeq of e.d. thus used). At 12.5 kcal/mol ATP, this represents 43.75 kcal ATP/eeq NADPH. The reduction of $^1/_{28}$ mol NO_3^- to $^1/_{28}$ mol NH_3 uses $^2/_7$ eeq of NADPH; therefore,

$$\Delta G_n = {}^2/_7\ (+43.75) = 12.5 \text{ kcal ATP/eeq cells.}$$

Substituting into our ΔG_s expression gives

$$\Delta G_s = {}^5/_7\ (59.7) + 12.5$$

= 55.1 kcal ATP/eeq cells.

The following equation then results for *A*:

Autotrophic Growth — Nitrate as N-Source

Eq (12)

$$A = \frac{-55.1}{0.6\ \Delta G_r}$$

C. Estimating Yield Coefficients

Since, stoichiometrically, the same e.d. is used for both synthesis and energy production, then the same equations hold which were presented earlier for heterotrophs:

Eq (3)

$$a_e \equiv \frac{\text{eeq cells formed}}{\text{eeq e.d. used}} = \frac{1}{1 + A}$$

Ammonia as N-source

Eq (4)

$$Y \equiv \frac{\text{g } X_a \text{ formed}}{\text{g OD used}} = \frac{5.65\ a_e}{8} = \frac{5.65}{8\ (1 + A)}$$

or

Nitrate as N-source

Eq (5)

$$Y \equiv \frac{\text{g } X_a \text{ formed}}{\text{g OD used}} = \frac{4.04\ a_e}{8} = \frac{4.04}{8\ (1 + A)}$$

Note, however, that the use of "g OD" (grams of oxygen demand) for specifying substrate consumption may be inappropriate in many lithotrophic instances. Other units of expression are often used: for example, in nitrification, "grams NH_3-N" is common for expressing e.d. consumption. With appropriate unit conversions, you should easily be able to calculate a yield coefficient from a_e in appropriate units.

Table 3 presents comparisons between autotrophic a_e values estimated using the bioenergetics approach presented here, and a_e values estimated from microbial yields reported in the literature (as cited by McCarty[2,4]). There is reasonably

good agreement between estimates from bioenergetics and estimates from measured yields. Lack of agreement for autotrophic methanogenesis from H_2/CO_2 may reflect the fact that methanogens do not fix CO_2 via the Calvin cycle; furthermore, they may not utilize reverse electron transport from H_2O (or at all), since H_2 possesses sufficient energy to directly reduce $NADP^+$. Lack of agreement for $S_2O_3^=/O_2$ may be the result of error in the measured value of microbial yield. $S_2O_3^=$ is somewhat unstable in solution, undergoing disproportionation to sulfite ($SO_3^=$) and elemental sulfur (S^o) [Bisogni, J.J, and C. T. Driscoll, "Denitrification Using Thiosulfate and Sulfide," *J. Envir. Engrg Div., Proc. ASCE, 103,* 593-604 (1977)]. Measures of $S_2O_3^=$ consumption may therefore by erroneously high, resulting in underestimation of the true yield. Thus, it's possible that the bioenergetically predicted a_e is more accurate than the "measured" value.

No reliable data were found to check the accuracy of the bioenergetic approach to autotrophic-growth situations where nitrate is used as N-source.

TABLE 3. VALUES OF a_e FOR AUTOTROPHIC REACTIONS

(Ammonia as N-source)

		a_e eeq of cells per eeq electron donor consumed	
Electron Donor	Electron Acceptor	Estimated from Reported Values*	Estimated from Thermodynamics**
S	O_2	0.22	0.19
$S_2O_3^=$	O_2	0.11	0.19
NH_4^+ (to NO_3^-)	O_2	0.096	0.095
H_2	O_2	0.24	0.22
Fe^{++}	O_2	0.038	0.064***
H_2	CO_2	0.074	0.038

* Excerpted from Reference [4].

** Equations (11) and (3), with $\varepsilon = 0.6$.

*** Corrected for pH = 2.7 conditions.

D. Estimating Maximum Specific Substrate Utilization Rates

We will assume that electron flow to carriers in the energy-producing portion of metabolism proceeds at essentially the same rate in autotrophic organisms as in heterotrophs. That is,

$$r_e \equiv \frac{\text{mol } e^- \text{ to energy}}{\text{gram } X_a \cdot \text{ day}} \cong 1.0 \qquad @\ 25^\circ C$$

For autotrophic electron donors, we define "electron equivalence" on a reaction-specific basis. For example, 1 eeq NH_4^+ equals either $^1/_8$ mol or $^1/_6$ mol, depending upon whether the reaction under consideration is a total oxidation to nitrate (Rxn 19), or merely a partial oxidation to nitrite (Rxn 20). Since the autotrophic half-reactions employed in ΔG_r calculations and stoichiometry derivations are always adjusted to reflect actual numbers of electrons transferred, then $d + 1$ in all cases. Consequently,

$$k_m \equiv \frac{\text{eeq of e.d. to energy}}{\text{gram } X_a \cdot \text{day}} = \frac{r_e}{d} = 1.0$$

Since the same e.d. is assumed to be used for both synthesis and energy production, the previously presented Equations (7), and (8) are presumed valid:

$$k_e \equiv \frac{\text{eeq of e.d. used}}{\text{eeq cells} \cdot \text{day}} = \frac{k_m\,(5.65)^*\,(1+A)}{A} \qquad \text{Eq (7)}$$

or

$$k \equiv \frac{\text{g OD used}}{\text{g } X_a \cdot \text{day}} = \frac{k_m\,(1+A)\,8}{A} \qquad \text{Eq(8)}$$

But you should realize that expressing substrate consumption in oxygen-demand (OD) units may not be appropriate in many circumstances. More suitable expressions of *k* may be easily derived from k_m through use of appropriate unit conversions.

E. Estimating Stoichiometries

* Use 4.04 if nitrate is the N-source.

If the same electron donor is used (stoichiometrically) for both synthesis and energy production, then the generalized stoichiometric equations developed for heterotrophic growth are also applicable to autotrophic systems:

Eq (9)

$$R_d - f_e R_a - f_s R_c$$

Eq (10)

$$f_s = \frac{a_e (1 + 0.2b\theta_c)}{1 + b\theta_c} = \left(\frac{1}{1+A}\right)\left(\frac{1 + 0.2b\theta_c}{1 + b\theta_c}\right)$$

IV. SUMMARY — LIMITATIONS & DISCUSSION

Yields and Stoichiometries. The bioenergetics approach is generally useful for estimating yield coefficients and growth stoichiometries — especially where ammonia is the N-source. The resulting stoichiometries allow estimation of nutrient requirements; pH & alkalinity effects of growth; and gas production & composition. The approach has not been adequately validated for situations where nitrate serves as the N-source; it is therefore recommended in such cases only when reliable *Y* data are unavailable (which is all-too-often the case!).

Maximum Specific Utilization Rates. Estimation of maximum specific utilization rates (*k*) assumes that the rate of electron transfer to energy is relatively constant (1 mol e^- per gram X_a per day). Since this rate has considerable variation among bacteria, the resulting estimates of *k* are probably less reliable than are estimates of *Y*. This may not be a severe limitation, since we usually employ θ_c values in design which are many times (perhaps 20 to 60 times) θ_c^{min}. Consequently, θ_c^{min} need not be known with great precision. But be aware of the uncertainty in bioenergetically derived *k* and θ_c^{min} values; don't make much of small differences in estimated values among organisms! A good example of this is found in nitrification. Bioenergetics predicts that *Nitrobacter* has a slightly greater θ_c^{min} than *Nitrosomonas*. Experimental evidence indicates the opposite!

Fermentations. There is no particular difficulty presented in using the bioenergetic approach to estimating *Y*-values in fermentations. However, estimates of *k* require some knowledge (or reasonable guess) as to the number of electrons involved in external transfer to carriers (such as NADH or $FADH_2$), per eeq of donor consumed for energy. Such information is needed to estimate *d*. Consequently, *k*-values for fermentations may be even more unreliable than *k*-values for total oxidations.

Complex Wastes (Mixed Substrates). Estimation of *k* and θ_c^{min} is probably ill-advised where a complex waste is simplistically regarded as being a single, hypothetical compound possessing an empirical formula (e.g., $C_{10}H_{19}O_3N$ for "municipal

wastewater"). In such cases, degradation of one of the waste components may limit the process; it is the *k* and θ_c^{min} applicable to degradation of the rate-limiting component which is most relevant. On the other hand, very little error results in *Y* estimates by such over-simplification: The *Y* estimated by considering the organic portion of municipal wastewater to be "$C_{10}H_{19}O_3N$" is very nearly the same as the average *Y* calculated from estimates of the individual *Y*-values for protein, carbohydrate, and fat, weighted according to the fraction of each component in municipal wastewater. (Recall that this approach was employed in an earlier example.) Likewise, the estimated stoichiometry is virtually unaffected by this simplistic view of a complex, mixed substrate. Only the treatment of kinetics suffers.

Limitations Arising From Ignorance of Microbiology. There is a tendency for environmental engineers to ignore the microbiological complexities of their engineered systems, resulting in a sort of "black-box" approach. An illustrative example:

> Consider the degradation of glucose in methanogenic systems. If the engineer regards the process as a single step, mediated by some mythical organism, X,
>
> $$\text{Glucose} \xrightarrow{X} \Rightarrow CO_2, CH_4$$
>
> then ΔG_r may be calculated via Rxn (9) – Rxn (6), ΔG_p via Rxn (9) – Rxn (16), leading to an estimate of *Y* and *k* which predict a $\theta_c^{min} \cong 11$ hr for this mythical organism.
>
> In contrast, the real situation is much more complicated. Some six to eight bacterial types are involved in the degradation of glucose under methanogenic conditions. Among them are fermentative bacteria which convert glucose to ethanol; others which convert ethanol to acetate and H_2; methanogenic bacteria which make methane from H_2 and CO_2; and methanogenic bacteria which cleave acetate to CH_4 and CO_2. The presence of each is critical to the overall scheme. And each has its own ΔG_r , ΔG_p, *Y*, *k*, and θ_c^{min}. For example, the bioenergetic estimate of θ_c^{min} for the ethanol producers is approximately 3 hr, while that of the acetate-cleaving methanogens is approximately 110 hr — far higher than that of our mythical "X" organism! The microbial process will fail if $\theta_c^d < \theta_c^{min}$ of the *slowest growing organism*. By treating this complex process as being mediated by a single organism, X, a designer will fail to realize process limitations resulting from a rate-limiting step in the sequential degradation chain. When it comes to kinetics, there's no substitute for a good understanding of the microbiology involved!
>
> It is worth pointing out, however, that estimated, overall stoichiometry is unaffected by this simplistic, single-organism approach. The estimated *Y* for our mythical organism, X, is very nearly equal to the sum of the *Y*-values for each organism in the sequential degradation chain. If all you care about is total

biomass yield from conversion of glucose to CH_4 and CO_2, it's OK to treat the complex process as a microbial "black box." Unless the concentrations of unconsumed, reduced metabolites (in this case, ethanol, acetate, and H_2) are significant in comparison to the consumption of the initial substrate (glucose), then total biomass production is adequately estimated via application of the overall *Y*. Likewise, the stoichiometry obtained via the "black-box" approach is an adequate estimate of the overall stoichiometry of growth when, in this case, glucose is converted to CH_4 and CO_2 — despite the actual complexity of the microbial system. It's only the kinetics which are inaccurately modeled by the simplistic, single-organism approach.

Limitations Arising From Ignorance of Biochemistry. A pathway has been assumed in the development of the bioenergetics' approach. If it is inapplicable in some instance, then so will be the estimate of *Y* and/or *k*. As an example, consider formate users: Many oxidize formate to CO_2 for energy, but use CO_2 as carbon source! In such situations, the autotrophic equation for *A* is appropriate, but might not be employed because the engineer mistakenly regards the use of formate as the realm of the heterotroph.

BIBLIOGRAPHY

1. McCarty, P.L., "Energetics and Bacterial Growth," presented at the 5th Rudolf Research Conference, Rutgers, the State University, New Brunswick, New Jersey, July 2 (1969).
2. McCarty, P.L., "Stoichiometry of Biological Reactions," presented at the International Conference, "Toward a Unified Concept of Biological Waste Treatment Design," Atlanta, Georgia, October 6 (1972).
3. McCarty, P.L., "Energetics and Bacterial Growth," in S. D. Faust and J. V. Hunter (ed.), *Organic Compounds in Aquatic Environments*, Marcel Dekker, Inc., New York, pp. 495-512 (1971).
4. McCarty, P.L., "Stoichiometry of Biological Reactions," *Progr. Water Technol., 7*, 157-172 (1975).
5. McCarty, P.L., "Energetics of Organic Matter Degradation," chapter 5 in Mitchell, R. (ed.) *Water Pollution Microbiology*, 91-118, John Wiley & Sons (1972).
6. McCarty, P.L., "Thermodynamics of Biological Synthesis and Growth," *The Proceedings of the 2nd International Water Poll. Research. Conf.*, Tokyo, 1964. Pages 169-199, Pergamon Press (1965).
7. Stensel, H.D., R.C. Loehr, and A.W. Lawrence, "Biological Kinetics of Suspended-Growth Denitrification," *Jour. Water Poll. Control Fed., 45* (2) 249-261 (1973).

TABLE 1. OXIDATION HALF REACTIONS

	HALF REACTIONS		$\Delta G^o(w)$ kcal/eeq*
	Reactions for Bacterial Cell Synthesis (R_c)		
	Ammonia as Nitrogen Source:		
1.	$^1/_{20}\ C_5H_7O_2N + {}^9/_{20}\ H_2O$	$= {}^1/_5\ CO_2 + {}^1/_{20}\ HCO_3^- + {}^1/_{20}\ NH_4^+ + H^+ + e^-$	
	Nitrate as Nitrogen Source:		
2.	$^1/_{28}\ C_5H_7O_2N + {}^{11}/_{28}\ H_2O$	$= {}^1/_{28}\ NO_3^- + {}^5/_{28}\ CO_2 + {}^{29}/_{28}\ H^+ + e^-$	
	Reactions for Electron Acceptors (R_a)		
	Oxygen:		
3.	$^1/_2\ H_2O$	$= {}^1/_4\ O_2 + H^+ + e^-$	18.675
	Nitrate:		
4.	$^1/_{10}\ N_2 + {}^3/_5\ H_2O$	$= {}^1/_5\ NO_3^- + {}^6/_5\ H^+ + e^-$	17.128
	Sulfate:		
5.	$^1/_{16}\ H_2S + {}^1/_{16}\ HS^- + {}^1/_2\ H_2O$	$= {}^1/_8\ SO_4^= + {}^{19}/_{16}\ H^+ + e^-$	–5.085
	Carbon Dioxide:		
6.	$^1/_8\ CH_4 + {}^1/_4\ H_2O$	$= {}^1/_8\ CO_2 + H^+ + e^-$	–5.763
	Reactions for Electron Donors (R_d)		
	Organic Donors		
	Domestic Wastewater:		
7.	$^1/_{50}\ C_{10}H_{19}O_3N + {}^9/_{25}\ H_2O$	$= {}^9/_{50}\ CO_2 + {}^1/_{50}\ NH_4^+ + {}^1/_{50}\ HCO_3^- + H^+ + e^-$	–7.6
	Protein:		
8.	$^1/_{66}\ C_{16}H_{24}O_5N_4 + {}^{27}/_{66}\ H_2O$	$= {}^8/_{33}\ CO_2 + {}^2/_{33}\ NH_4^+ + {}^{31}/_{33}\ H^+ + e^-$	–7.7
	Carbohydrate (Cellulose, Starch, Sugars):		
9.	$^1/_{24}\ C_6H_{12}O_6 + {}^1/_4\ H_2O$	$= {}^1/_4\ CO_2 + H^+ + e^-$	–10.0
	Grease, Fat, and Oil:		
10.	$^1/_{46}\ C_8H_{16}O + {}^{15}/_{46}\ H_2O$	$= {}^4/_{23}\ CO_2 + H^+ + e^-$	–6.6
	Acetate:		
11.	$^1/_8\ CH_3COO^- + {}^3/_8\ H_2O$	$= {}^1/_8\ CO_2 + {}^1/_8\ HCO_3^- + H^+ + e^-$	–6.609
	Propionate:		
12.	$^1/_{14}\ CH_3CH_2COO^- + {}^5/_{14}\ H_2O$	$= {}^1/_7\ CO_2 + {}^1/_{14}\ HCO_3^- + H^+ + e^-$	–6.664
	Benzoate:		
13.	$^1/_{30}\ C_6H_5COO^- + {}^{13}/_{30}\ H_2O$	$= {}^1/_5\ CO_2 + {}^1/_{30}\ HCO_3^- + H^+ + e^-$	–6.892
	Ethanol:		
14.	$^1/_{12}\ CH_3CH_2OH + {}^1/_4\ H_2O$	$= {}^1/_6\ CO_2 + H^+ + e^-$	–7.592
	Lactate:		

15.	$^1/_{12}\ CH_3CHOHCOO^- + {}^1/_3\ H_2O$	$= {}^1/_6\ CO_2 + {}^1/_{12}\ HCO_3^- + H^+ + e^-$	–7.873
	Pyruvate:		
16.	$^1/_{10}\ CH_3COCOO^- + {}^2/_5\ H_2O$	$= {}^1/_5\ CO_2 + {}^1/_{10}\ HCO_3^- + H^+ + e^-$	–8.545
	Methanol:		
17.	$^1/_6\ CH_3OH + {}^1/_6\ H_2O$	$= {}^1/_6\ CO_2 + H^+ + e^-$	–8.965
	Inorganic Donors		
18.	Fe^{++}	$= Fe^{+3} + e^-$	17.780
19.	$^1/_8\ NH_4^+ + {}^3/_8\ H_2O$	$= {}^1/_8\ NO_3^- + {}^5/_4\ H^+ + e^-$	8.245
20.	$^1/_6\ NH_4^+ + {}^1/_3\ H_2O$	$= {}^1/_6\ NO_2^- + {}^4/_3\ H^+ + e^-$	7.852
21.	$^1/_6\ S + {}^2/_3\ H_2O$	$= {}^1/_6\ SO_4^= + {}^4/_3\ H^+ + e^-$	–4.657
22.	$^1/_{16}\ H_2S + {}^1/_{16}\ HS^- + {}^1/_2\ H_2O$	$= {}^1/_8\ SO_4^= + {}^{19}/_{16}\ H^+ + e^-$	–5.085
23.	$^1/_8\ S_2O_3^= + {}^5/_8\ H_2O$	$= {}^1/_4\ SO_4^= + {}^5/_4\ H^+ + e^-$	–5.091
24.	$^1/_2\ H_2$	$= H^+ + e^-$	–9.670
25.	$^1/_2\ SO_3^= + {}^1/_2\ H_2O$	$= {}^1/_2\ SO_4^= + H^+ + e^-$	–10.595

* 25˚C; reactants and products at unit activity, except {H} = 10^{-7} (i.e, pH 7 conditions).

	HALF REACTIONS		$\Delta G^o(w)$ kcal/eeq*
	Other Reactions		
	NADPH Oxidation:		
26.	$^1/_2\ NADPH$	$= {}^1/_2\ NADP^+ + {}^1/_2\ H^+ + e^-$	–7.38
	NADH Oxidation:		
27.	$^1/_2\ NADH$	$= {}^1/_2\ NAD^+ + {}^1/_2\ H^+ + e^-$	–7.38
	Glucose-6-phosphate Oxidation:		
28.	$^1/_{24}\ C_6H_{11}O_5{\cdot}OPO_3^= + {}^7/_{24}\ H_2O$	$= {}^1/_4\ CO_2 + H^+ + {}^1/_{24}\ HPO_4^= + e^-$	–10.35**
	Chloromethane:		
29.	$^1/_6\ CH_3Cl + {}^1/_3\ H_2O$	$= {}^1/_6\ CO_2 + {}^1/_6\ Cl^- + {}^7/_6\ H^+ + e^-$	–10.89
	Dichloromethane:		
30.	$^1/_4\ CH_2Cl_2 + {}^1/_2\ H_2O$	$= {}^1/_4\ CO_2 + {}^1/_2\ Cl^- + {}^3/_2\ H^+ + e^-$	–21.23
	Chloroform:		
31.	$^1/_2\ CHCl_3 + H_2O$	$= {}^1/_2\ CO_2 + {}^3/_2\ Cl^- + {}^5/_2\ H^+ + e^-$	–53.58

* 25˚C; reactants and products at unit activity, except $\{H\} = 10^{-7}$ (i.e, pH 7 conditions).

** Estimated ΔG value, corrected for physiological concentration of $HPO_4^{=}$. $\Delta G^o(w)$ is –10.13 kcal/eeq.