

Excerpted from: Rockne, K. J. *Alternative Electron Acceptors for Polycyclic Aromatic Hydrocarbon Biodegradation* (1997). Ph.D. Dissertation, University of Washington, Seattle, WA.

Appendix B

Energetics equations for determination of electron acceptor to PAH stoichiometry.

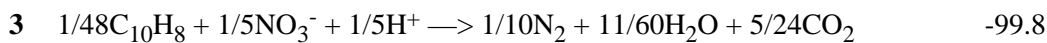
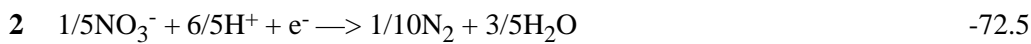
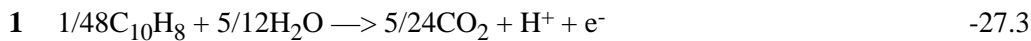
Based on the analysis by McCarty (1975).

The development of stoichiometric ratios for electron acceptor reduction coupled to PAH oxidation is presented here. There are two analyses for the amount of electron donor coupled to cell growth based on McCarty (1975): one based on thermodynamic considerations and one based on rate relationships taking into account endogenous energy needs by bacteria.

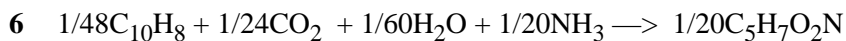
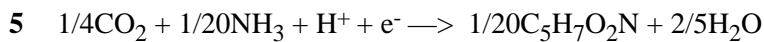
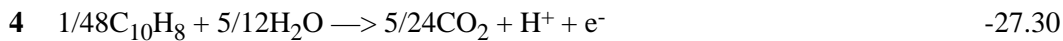
Thermodynamic estimation of A, the amount of electron donor used for cell synthesis:

Energy reaction

ΔG_r for mineralization of Naphthalene: kJ/e-mol



Synthesis reactions

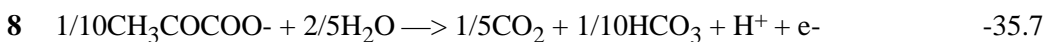
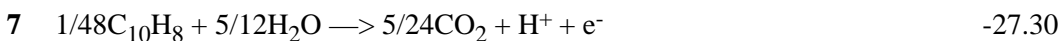


(continued)

Synthesis reactions- intermediate state- pyruvate

$$\Delta G_s = \Delta G_{\text{NAP} \rightarrow \text{pyr}} + \Delta G_{\text{pyr} \rightarrow \text{cells}} \quad (\text{step 2} = 31.4 \text{ kJ/e-mol})$$

so, for $\Delta G_{\text{NAP} \rightarrow \text{pyr}}$:



$$\Delta G_{\text{NAP} \rightarrow \text{pyr}} \text{ total} \quad 8.42$$

and production of cellmass from pyruvate has a Gibbs free energy of 31.4 kJ/e-mol

so:

$$9 \quad \Delta G_s = \Delta G_{\text{NAP} \rightarrow \text{pyr}} + \Delta G_{\text{pyr} \rightarrow \text{cells}} = 8.42/0.6 + 31.4 = 45.4$$

$$10 \quad A = \Delta G_s / (k \Delta G_r) = -\{45.4 / (0.6 \times -99.8)\} = 0.76$$

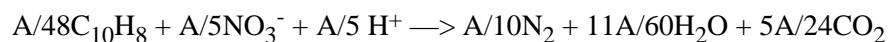
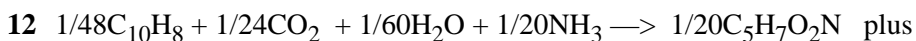
These reactions can be simplified by combining the Gibbs free energy tabulated in Appendix A and combining equations 3, 9, and 10:

$$11 \quad A = ((\Delta G_r \text{ PAH ox} + 35.72)/k + 31.4) / (k * (\Delta G_r \text{ PAH ox} + \Delta G_r \text{ EA red}))$$

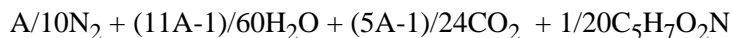
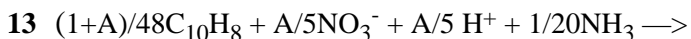
These values are tabulated in Table B-2.

With a value for A, the overall equation = Synthesis equation + A*Energy equation

For naphthalene under denitrification conditions:



overall



so the ratio of electron acceptor reduced to electron donor oxidized is:

14 $(A/5)/(1+A)/48 \rightarrow$ rearranging gives:

15 $(A/(1+A)) * EA/ED$ ratio from the energy equations in Appendix A and Table 2.1

for nitrate/naphthalene:

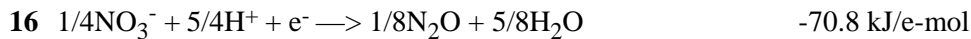
$$0.76/1.76*0.2(1/48) = 0.432(EA/ED) = 0.432*9.6 = 4.14$$

for sulfide/naphthalene:

$$6.48/7.48*0.125/2/(1/48) = 0.866(EA/ED) = 0.866*12 = 10.4$$

So for EA's with large A values, the stoichiometric ratio is close to that from the energy equation.

Alternatively, with denitrification to nitrous oxide:



From equ. 11:

$$\mathbf{17} \quad A = (((-27.3) + 35.72)/0.6 + 31.4)/(0.6*(-27.3 + -70.8))$$

$$A = 0.772$$

and from equation 15:

$$EA/ED = 0.772/1.772 * \text{ratio} = 0.437*0.25/(1/48) = 5.25$$

within 20% of the value for denitrification to nitrogen gas

The stoichiometric parameters for the other PAHs under denitrifying and sulfidogenic conditions are tabulated in Tables B-1 and B-2.

Estimation of stoichiometric ratio of EA utilization to ED consumption including endogenous growth:

An attempt was made to account for endogenous respiration during the long time periods of the batch incubations presented in Chapter 4.

McCarty (1975) presented the following equation relating the fraction of electron donor used for cell synthesis (f_s) to the cell decay rate (b) and solids retention time (θ_c) in a reactor:

$$18 \quad f_s = a_e \{ 1 - f_d b \theta_c / (1 + b \theta_c) \}$$

where: a_e - cell yield coefficient representing the fraction of ED used for cell synthesis
 θ_c - mean cell residence time
 f_d - biodegradable fraction of cells

The average time for the PAH biodegradation batch experiments was approximately 30 and 140 days for the nitrate- and sulfate-reducing cultures, respectively. A typical value for the cell decay rate is 0.05 per day (Tchobanoglous and Burton 1991). Taking these values, equation 18 simplifies to:

$$19 \quad f_s = a_e(1 - 0.62f_d) \quad \text{For the nitrate cultures}$$

or $f_s = a_e(1 - 0.88f_d) \quad \text{For the sulfate cultures}$

Values for f_d are reported to be approximately 0.8. In addition, A and a_e are related by the following equation from McCarty (1972):

$$20 \quad a_e = (1 + A)^{-1}$$

Combining with equation 19 and taking a value of 0.8 for f_d :

$$21 \quad f_s = 0.5(1 + A)^{-1} \quad \text{For the nitrate reducers}$$

or $f_s = 0.3(1 + A)^{-1} \quad \text{For sulfate reducers}$

For naphthalene oxidation coupled to denitrification, equation 21 gives:

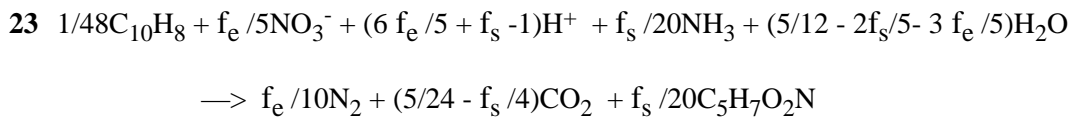
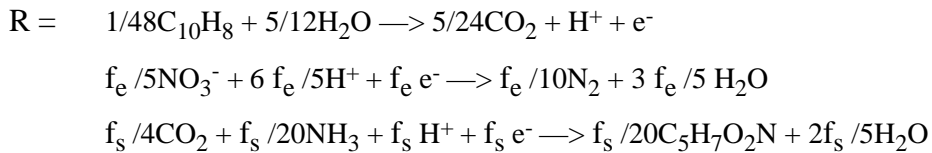
$$f_s = 0.5/1.76 = 0.28$$

Using equation 19, we see that the EA/ED ratio would increase due to increased amount of EA reduction coupled to endogenous respiration. So now, the overall equation is rewritten:

$$22 \quad R = R_d - f_e R_a - f_s R_c$$

where: R_d - ED oxidation half reaction
 R_a - EA reduction half reaction
 f_e - fraction of ED coupled to energy
 R_c - cell synthesis half reaction

Because $f_e + f_s = 1$, every parameter is known and the overall equation can be computed. Naphthalene oxidation coupled to denitrification becomes:



So the calculated EA/ED ratio is:

$$24 \quad EA/EA = f_e /5/(1/48) \quad \text{or} \quad f_e *EA/ED \text{ from energy equations.}$$

These values are tabulated in Table B-1.

Table B-1. Stoichiometric parameters calculated for naphthalene, biphenyl, phenanthrene, and dibenzofuran.

Stoichiometric parameter	PAH			
	Naphthalene	Biphenyl	Phenanthrene	Dibenzofuran
NO₃⁻ -> N₂				
A ¹	0.76	0.78	0.77	0.64
Complete mineralization stoichiometry ²	9.6	11.6	13.2	10.8
With production of cell mass ³	4.1	5.1	5.7	4.2
f _e ⁴	0.72	0.72	0.72	0.70
With endogenous respiration ⁵	6.9	8.3	9.5	7.5
SO₄⁼ -> H₂S				
A ¹	6.5	7.0	6.8	4.4
Complete mineralization stoichiometry ²	12	14.5	16.5	13.5
With production of cell mass ³	10.4	12.7	14.4	11
f _e ⁴	0.960	0.963	0.962	0.944
With endogenous respiration ⁵	11.5	14	15.9	12.8

¹- Calculated from equation 11 and Gibbs free energy values tabulated in Table B-2

²- From energy equations for mineralization of PAH to carbon dioxide. Tabulated in Table 2.1 and in Appendix A.

³-Using the energetics equations 11 and 15.

⁴-From equation 21 and: f_e + f_s = 1

⁵-Including endogenous growth. Using equations 11, 21, and 24