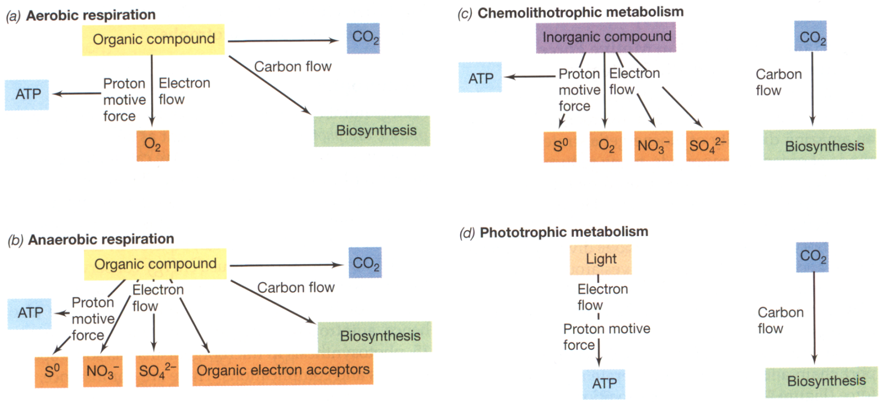
Tema 3 – Bioenergía
Bioenergetics

CI7115 – Biotecnología Ambiental

Prof. Ana Lucía Prieto Santa

Ingeniería Civil
FACULTAD DE CIENCIAS FÍSICAS Y MATEMÁTICAS
UNIVERSIDAD DE CHILE

Relaciones energéticas y flujo de carbono



Brock, 2000

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Acetate and carbon dioxide (methanogenesis of acetate)

		$\Delta G^{0\prime}$, kJ
Donor:	$\frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O = \frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^-$	−27.40
Acceptor:	$\frac{1}{8}CO_2 + H^+ + e^- = \frac{1}{8}CH_4 + \frac{1}{4}H_2O$	23.53
Net:	$\frac{1}{8}CH_3COO^- + \frac{1}{8}H_2O = \frac{1}{8}CH_4 + \frac{1}{8}HCO_3^-$	−3.87

Glucose and carbon dioxide (methanogenesis from glucose)

		$\Delta G^{0\prime}$, kJ
Donor:	$\frac{1}{24}C_6H_{12}O_6 + \frac{1}{4}H_2O = \frac{1}{4}CO_2 + H^+ + e^-$	−41.35
Acceptor:	$\frac{1}{8}CO_2 + H^+ + e^- = \frac{1}{8}CH_4 + \frac{1}{4}H_2O$	23.53
Net:	$\frac{1}{24}C_6H_{12}O_6 = \frac{1}{8}CH_4 + \frac{1}{8}CO_2$	−17.82

Hydrogen and oxygen (aerobic oxidation of hydrogen)

		$\Delta G^{0\prime}$, kJ
Donor:	$\frac{1}{2}H_2 = H^+ + e^-$	−39.87
Acceptor:	$\frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O$	−78.72
Net:	$\frac{1}{2}H_2 + \frac{1}{4}O_2 = \frac{1}{2}H_2O$	−118.59

Basic concepts in bioenergy:

- Oxidation number (state)
- Gibbs Free Energy
- Acid-base chemistry

Cálculo de número o estado de oxidación

1. The oxidation state of an element in an elementary substance is zero (ex. H_2, O_2, Fe^o)

2. The oxidation state of the ion of an element is equal to its charge

$Na^+ = +1$, $Fe^{3+} = +3$

3. The sum of oxidation numbers of all atoms in a neutral molecule is zero, Thus, H_2O is neutral because it has two H at +1 each and one O at -2.

4. In an ion, the sum of oxidation numbers of all atoms is equal to the charge on that ion. Thus, in the OH^- ion, O(-2) + H(+1) = -1.

5. In compounds, the oxidation state of O is virtually always -2, and that of H is +1.

Cálculo de número o estado de oxidación

6. In simple carbon compounds, the oxidation state of C can be calculated by adding up the H and O atoms present and using the oxidation states of these elements as given in item 5, because in a neutral compound the sum of all oxidation number must be zero. Thus, the oxidation state of carbon in methane (CH_4) is -4 (4 H at +1 each = +4); in carbon dioxide (CO_2), the oxidation state of carbon is +4 (2 O at -2 each = -4).

CH_4 ←-----------organic C-----------→ CO_2

(most reduced, -4) (most oxidized, +4)

Cálculo de número o estado de oxidación

7. In organic compounds with more than one C atom, it may not be possible to assign a specific oxidation number to each C atom, but it is still useful to calculate the oxidation state of the compound as a whole. The same conventions are used.

Thus, the oxidation state of carbon in glucose ($C_6H_{12}O_6$) is zero (12 H at +1 = 12; 6 O at -2 = -12) and the oxidation state of carbon in ethanol (C_2H_5OH) is -2 each (6 H at +1 = +6; one O at -2).

Cálculo de número o estado de oxidación

8. In all oxidation-reduction reactions there is a balance between the oxidized and reduced products. To calculate an oxidation-reduction balance, the number of molecules of each product is multiplied by its oxidation state. For instance, in calculating the oxidation-reduction balance for alcohol fermentation, there are two molecules of ethanol at -4 = -8 and two molecules of CO_2 at +4 = +8 so the net balance is zero. When constructing model reactions, it is useful to calculate redox balances to be certain that the reaction is possible (**electroneutrality** must be maintained).

Ejemplo: Fermentación del Alcohol

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH \quad + 2\ CO_2$$

(sugar) (ethanol) (carbon dioxide)

$6(\underline{0})+12(+1)+6(-2)$ $2[2\ (\underline{-2}) + 6(+1) + (-2)]$ $2[\ (\underline{+4}) + 2(-2)]$

4 carbons were reduced
to -2 = **-8 overall**

2 carbons were oxidized
to +4 = **+8 overall**

Ciclo del N y especiación

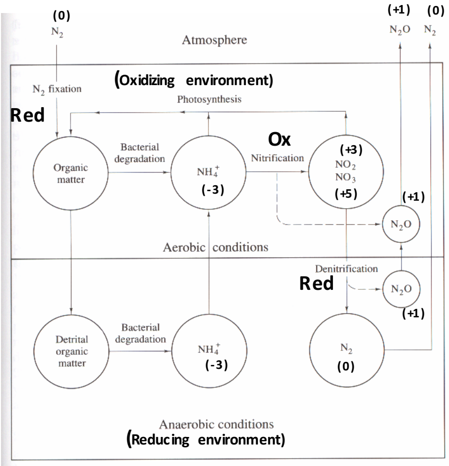

Figure 12.1 Microbial transformations of nitrogen in the global cycle. From Wollast (1981).

Ox = oxidation reaction, electron acceptor (e.g, O_2) needed
Red = reduction reaction, electron donor (e.g., H_2) needed

Schlesinger, 1991, Biogeochemistry: Analysis of Global Change.

Table 15.3. **Summary of the Principal Oxidation–Reduction Chemistry of Nitrogen**

Oxidation State	*Species*	*Remarks*
+5	NO_3^-	Nitrate ion, a strong oxidizing agent in acid solution.
+4	NO_2 N_2O_4	Nitrogen dioxide, a red-brown gas; the chief reduction product of concentrated nitric acid. Nitrogen dioxide dimerizes to form dinitrogen tetroxide, N_2O_4.
+3	NO_2^- HNO_2	Nitrite ion, in basic solution. Nitrous acid, in acid solution. Both can act either as an oxidizing agent or as a reducing agent. When acting as a reducing agent, they are oxidized to NO_3^-. When HNO_2 acts as an oxidizing agent in acid solution it is usually reduced to NO(g).
+2	NO	Nitrogen oxide (nitric oxide), a colorless gas; the chief reduction product of dilute nitric acid.
+1	N_2O	Dinitrogen oxide (nitrous oxide), also called laughing gas. Not usually formed in oxidation–reduction reactions in aqueous solution.
0	N_2	Unreactive because of the strong triple bond, $:N{\equiv}N:$.
−1	NH_2OH	Hydroxylamine, a weak base. Can serve both as an oxidizing agent and as a reducing agent.
−2	N_2H_4	Hydrazine, a colorless liquid that disproportionates to N_2 and NH_3. Hydrazine is a weak base.
−3	NH_3 NH_4^+	In basic solution. In acidic solution. Rarely used as a reducing agent in aqueous solution. When ammonia is burned in O_2 it is oxidized to either N_2 or NO depending on conditions. Oxidation of ammonium salts generally produces N_2.

Segal, Chemistry: experiment and theory. 1985

Especiación del Cloro

Table 15.4. **Summary of the Principal Oxidation–Reduction Chemistry of Chlorine**

Oxidation State	*Species*	*Remarks*
+7	ClO_4^-	Perchlorate ion, a powerful oxidizing agent.
+5	ClO_3^-	Chlorate ion, a powerful oxidizing agent.
+4	ClO_2	Chlorine dioxide, a yellow-orange gas. Not a common oxidation state of chlorine, but it is formed when ClO_3^- reacts with a number of reducing agents. Chlorine dioxide can serve as an oxidizing agent also.
+3	ClO_2^-	Chlorite ion, in basic solution. Serves as an oxidizing agent in bleaches. In acid solution $HClO_2$ disproportionates to $ClO_2(g)$ and Cl^-.
+1	ClO^- HOCl	Hypochlorite ion, in basic solution. Hypochlorous acid, in acid solution. Both serve as oxidizing agents. ClO^- disproportionates when warmed, to yield Cl^- and ClO_3^-.
0	Cl_2	Chlorine, a good oxidizing agent.
−1	Cl^-	Chloride ion, a moderately good reducing agent.

Segal, Chemistry: experiment and theory. 1985

- Note:
- Perchlorate (used in rocket fuels and now contaminating groundwater) is already highly oxidized, cannot be destroyed by oxidation; need to be reduced (electron donors, e.g., H_2 needed)
- Chloride ion is a reduced species. Thus, the reductive dechlorination process, in which chlorine is sequencially removed as chloride from a chlorinated organic compound (e.g. PCB) involves electron donors.

Energía libre de Gibb’s

1. **ΔG^0 = standard free-energy change** of the reaction at 1 atm pressure and 1 *M* concentrations
   $\Delta G^{0'}$ = free-energy change under standard conditions at pH 7
   ΔG = free-energy change under the conditions specified.

2. Calculation of ΔG^0 for a chemical reaction from the **free energy of formation, G_f^0**, of products and reactants:

$$\Delta G^0 = \sum \Delta G_f{}^0(\text{products}) - \sum \Delta G_f{}^0(\text{reactants})$$

That is, sum the ΔG_f^0 of products, sum the ΔG_f^0 of reactants, and subtract the latter from the former.

Value of ΔG:
- if negative, reaction is energy yielding and will proceed favorably (**exergonic**)
- if positive, reaction is energy consuming and will not proceed favorably (**endergonic**)

(Brock, Appendix A)

Energía libre de Gibb’s

3. For energy-yielding reactions involving H$^+$, converting from standard conditions (pH 0) to cellular conditions (pH 7):

$$\underset{(\text{@pH } 7)}{\Delta G^{0\prime}} = \underset{(\text{@pH } 0)}{\Delta G^0} + m\underset{(-39.83)}{\Delta G_f{}^0(\text{H}^+)}$$

where *m* is the net number of protons in the reaction (*m* is negative when more protons are consumed than formed) and ΔG_f^0 (H$^+$) is the free energy of formation of a proton at pH 7 (–39.83 kJ) at 25°C.

(Brock, Appendix A)

Energía libre de Gibb's

4. **Effect of concentrations on ΔG**: With soluble substrates, the concentration ratios of products formed to exogenous substrates used are generally equal to or greater than 10^{-2} at the beginning of growth and equal to or less than 10^{-2} at the end of growth. From the relation between ΔG and the equilibrium constant (see item 8), it can be calculated that ΔG for the free-energy yield in practical situations differs from the free-energy yield under standard conditions by at most 11.7 kJ, a rather small amount, and so for a first approximation, standard free-energy yields can be used in most situations.

   However, with H_2 as a product, H_2-consuming bacteria present may keep the concentration of H_2 so low that the free-energy yield is significantly affected. Thus, in the fermentation of ethanol to acetate and H_2 by syntrophic bacteria ($C_2H_5OH + H_2O \longrightarrow C_2H_3O_2^- + 2H_2 + H^+$), the $\Delta G^{0\prime}$ at 1 atm H_2 is +9.68 kJ, but at 10^{-4} atm H_2 it is –36.03 kJ. With H_2-consuming bacteria present, therefore, the ethanol fermentation becomes **exergonic**. (See also item 9.)

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(Brock, Appendix A)

Energía libre de Gibb's

5. Reduction potentials: by convention, electrode equations are written in the direction, **oxidant + *ne*$^-$ ➔ reductant** (that is, as reductions), where *n* is the number of electrons transferred. The standard potential (E_0) of the hydrogen electrode,

$$2H^+ + 2\,e^- \rightarrow H_2$$

is set by definition at 0.0 V at 1 atm pressure of H_2 gas and 1.0 *M* H^+, at 25°C. E_0' is the standard reduction potential at pH 7.

6. Relation of free energy to reduction potential:

$$\Delta G^{0\prime} = -nF\Delta E_0'$$

where *n* is the number of electrons transferred, *F* is the **Faraday constant** (96.48 kJ/V), and $\Delta E_0'$ is the E_0' of the electron-*accepting* couple minus the E_0' of the electron-*donating* couple.

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(Brock, Appendix A)

Energía libre de Gibb's

$$aA + bB \rightleftharpoons cC + dD$$

7. Equilibrium constant, *K*. For the generalized reaction

$$K = \frac{[C]^c[D]^d}{[A]^a[B]^b}$$

where A, B, C, and D represent reactants and products; *a, b, c*, and *d* represent number of molecules of each; and brackets indicate concentrations. This is true only when the chemical system is in equilibrium.

8. Relation of equilibrium constant *K* to free-energy change. At constant temperature, pressure, and pH:

$$\Delta G = \Delta G^{0\prime} + RT \ln K$$ *(for actual condition)*

where *R* is a constant (**8.29 J/mol/°K**) and *T* is the absolute temperature (in °K).

(Brock, Appendix A)

Energía libre de Gibb's

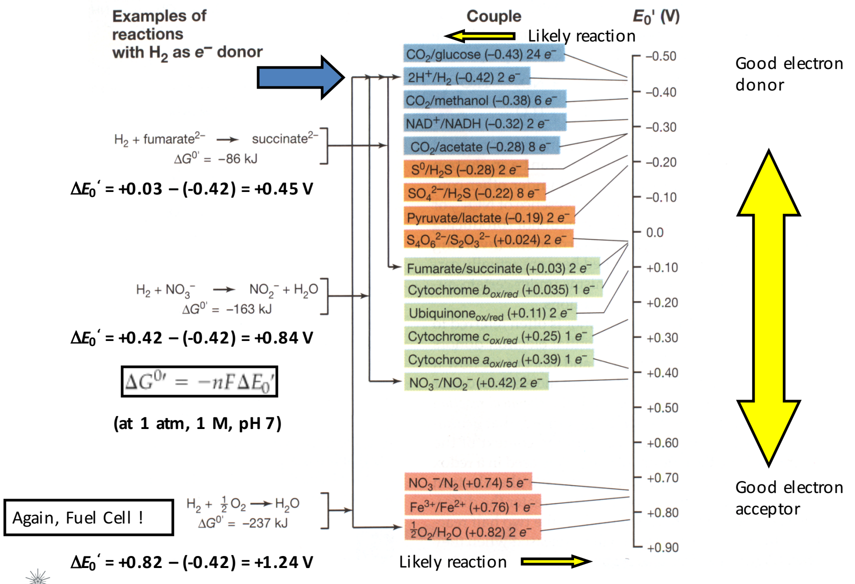
9. **Two substances can react in a redox reaction even if the standard potentials are unfavorable, provided that the concentrations are appropriate.**

   Assume that normally the reduced form of A would donate electrons to the oxidized form of B. However, if the concentration of the reduced form of A was low and the concentration of the reduced form of B was high, it would be possible for the reduced form of B to donate electrons to the oxidized form of A. Thus, the reaction would proceed in the direction opposite that predicted from standard potentials.

   A practical example of this is the utilization of H^+ as an electron acceptor to produce H_2. Normally, H_2 production in fermentative bacteria is not extensive because H^+ is a poor electron acceptor; the E_0' of the 2 H^+/H_2 pair is –0.41 V. However, if the concentration of H_2 is kept low by continually removing it (a process done by methanogenic prokaryotes, which use $H_2 + CO_2$ to produce methane, CH_4, or by many other anaerobes capable of consuming H_2 anaerobically), the potential will be more positive and then H^+ will be a suitable electron acceptor (to produce H_2).

(Brock, Appendix A)

oxidant + ne^- ➔ reductant



$H_2 + \text{fumarate}^{2-} \longrightarrow \text{succinate}^{2-}$ $\Delta G^{0'} = -86$ kJ

$\Delta E_0' = +0.03 - (-0.42) = +0.45$ V

$H_2 + NO_3^- \longrightarrow NO_2^- + H_2O$ $\Delta G^{0'} = -163$ kJ

$\Delta E_0' = +0.42 - (-0.42) = +0.84$ V

$$\boxed{\Delta G^{0\prime} = -nF\Delta E_0'}$$

(at 1 atm, 1 M, pH 7)

Again, Fuel Cell !

$H_2 + \frac{1}{2}O_2 \longrightarrow H_2O$ $\Delta G^{0'} = -237$ kJ

$\Delta E_0' = +0.82 - (-0.42) = +1.24$ V

Good electron donor

Good electron acceptor

Brock, 2000

Combustibles (Donadores de e^-)

Organic molecules are good fuels. For CO_2/organic molecule couples, E°' <0 (so they are >0 when the organic is oxidized to CO_2). When coupled to reduction half reactions for oxidants, the resulting whole reactions have positive voltages E >0.

Some inorganics are also good fuels:

best

e^- donor	Half reaction	E°' (V)
HSO_3^-	$SO_4^{2-} + 3H^+ + 2e^- = HSO_3^- + H_2O$	-0.51
H_2	$2H^+ + 2e^- = H_2$	-0.41
$S_2O_3^{2-}$	$2SO_4^{2-} + 10H^+ + 8e^- = S_2O_3^{2-} + 5H_2O$	-0.25
HS^-	$SO_4^{2-} + 9H^+ + 8e^- = HS^- + 4H_2O$	-0.22
S	$SO_4^{2-} + 8H^+ + 6e^- = S + 4H_2O$	-0.20
AsO_3^{3-}	$AsO_4^{3-} + 4H^+ + 2e^- = H_2AsO_3^- + H_2O$	+0.15
Cu^+ .	$Cu^{2+} + e^- = Cu^+$	+0.16
Se	$SeO_4^{2-} + 8H^+ + 6e^- = Se + 4H_2O$	+0.33
NH_4^+	$NO_3^- + 10H^+ + 8e^- = NH_4^+ + 3H_2O$	+0.36
Mn (II)	$MnO_2 + 4H^+ + 2e^- = Mn^{2+} + 2H_2O$	+0.47
Fe (II)	$Fe^{3+} + e^- = Fe^{2+}$	+0.77

worst

Oxidantes (receptores de e^-)

best

↓

worst

e^- acceptor	Half reaction	E°' (V)
N_2O	$N_2O + 2H^+ + 2e^- = N_2 + H_2O$	+1.31
ClO_3^-	$ClO_3^- + 6H^+ + 6e^- = Cl^- + 3H_2O$	+1.04
NO_2^-	$2NO_2^- + 8H^+ + 6e- = N_2 + 4H_2O$	+0.94
O_2	$O_2 + 4H^+ + 4e^- = 2H_2O$	+0.82
Fe^{3+}	$Fe^{3+} + e^- = Fe^{2+}$	+0.77
NO_3^-	$2NO_3^- + 12H^+ + 10e^- = N_2 + 6H_2O$	+0.74
NO_3^-	$NO_3^- + 2H^+ + 2e^- = NO_2^- + H_2O$	+0.43
NO_3^-	$NO_3^- + 10H^+ + 8\ e^- = NH_4^+ + 3H_2O$	+0.36
$C_6H_4ClCOO^-$	$C_6H_4ClCOO^- + H^+ + 2e^- = C_6H_5COO^- + Cl^-$	+0.26
$(CH_3)_2SO$	$(CH_3)_2SO + 2H^+ + 2e- = (CH_3)_2S + H_2O$	+0.23
$S_2O_3^{2-}$	$S_2O_3^{2-} + 8H^+ + 8e^- = 2HS^- + 3H_2O$	-0.21
SO_4^{2-}	$SO_4^{2-} + 9H^+ + 8e^- = HS^- + 4H_2O$	-0.22
CO_2	$CO_2 + 8H^+ + 8e- = CH_4 + 2H_2O$	-0.24
S	$S + H^+ + 2e^- = HS^-$	-0.27

Cálculos relacionados a la bioenergía

- Balancing half reactions
- Stoichiometry
- Electron and Oxygen Equivalents
- Yield
- Alkalinity/acidity

Balanceo de Reacciones Medias

Example: half reaction for reduction of CO_2 to glycine (CH_2NH_2COOH). Carbon changes oxidation state.

1) For reduction reactions: **oxidant + *ne*⁻ → reductant**

$$CO_2 \; + ? \; = \; CH_2NH_2COOH$$

In reverse, this reaction is the oxidation of glycine to CO_2.

2) Balance elements other than C, H , & O, such as nitrogen.

$$CO_2 \; + NH_4^+ + ? \; = \; CH_2NH_2COOH$$

NH_3 or NH_4^+?

Balance N with either NH_3 or NH_4^+. Balancing with NH_3 is somewhat easier to do, and it will still give the appropriate electron balance, but it will not give the correct speciation. This is because most solutions containing microbial activity have pH levels close to 7, and at these pH values, NH_4^+ is the prevalent form (pKa of ammonium is 9.2).

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3) Balance the carbon with CO_2 and HCO_3^-. Use the negative charge on HCO_3^- to cancel the positive charge on NH_4^+.

$$CO_2 \; + HCO_3^- + NH_4^+ \; + ? \; = \; CH_2NH_2COOH$$

***NOTE**. If the electron donor is both organic and a conjugate base (i.e., a species that can pick up a proton), then the negative charge on the conjugate base should be balanced by the negative charge on HCO_3^-.
e.g. for acetate:

$$CH_3COO^- + 3H_2O = CO_2 + HCO_3^- + 8H^+ + 8e^-$$

Why is this? When acetate or another conjugate base is added to water, it is added as a salt with a cation, such as Na^+ or K^+. Degradation of acetate does not destroy its associated cation, but electroneutrality must still be maintained. Therefore, the oxidation of a conjugate base, such as acetate, must be balanced in the half reaction by an equivalent concentration of HCO_3^-.

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4) Balance the oxygen with H_2O.

$$CO_2 + HCO_3^- + NH_4^+ + ? = CH_2NH_2COOH + 3H_2O$$

5) Balance the hydrogen with protons.

$$CO_2 + HCO_3^- + NH_4^+ + 6H^+ = CH_2NH_2COOH + 3H_2O$$

6) Add electrons as needed to give the same net charge on both sides of the equals sign.

$$CO_2 + HCO_3^- + NH_4^+ + 6H^+ + 6e^- = CH_2NH_2COOH + 3H_2O$$

7) Final checks:

- When adding half reactions, e- must cancel
- Correct the acid-base chemistry. Acids such as H^+ or CO_2 and bases such as NH_3 or OH^- will not appear together on the same side of the equals sign because they neutralize one another.

Ex. Acid-base reactions:
$H^+ + OH^- = H_2O$
$H^+ + NH_3 = NH_4^+$
$NH_3 + CO_2 + H_2O = NH_4^+ + HCO_3^-$
$CO_2 + OH^- = HCO_3^-$

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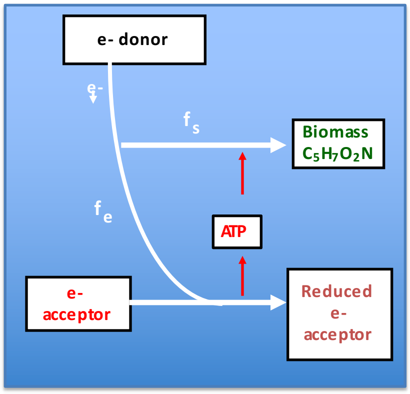

Estequiometría de reacciones biológicas

$$f_s + f_e = 1$$

$$f_s = \frac{\text{e- equivalents of \textbf{biomass} formed}}{\text{electron equivalents of electron donor consumed}}$$

$$f_e = \frac{\text{e- equivalents of \textbf{e- acceptor} consumed}}{\text{electron equivalents of electron donor consumed}}$$

$$f_e = \frac{\text{e- equivalents of \textbf{reduced e- acceptor} formed}}{\text{electron equivalents of electron donor consumed}}$$

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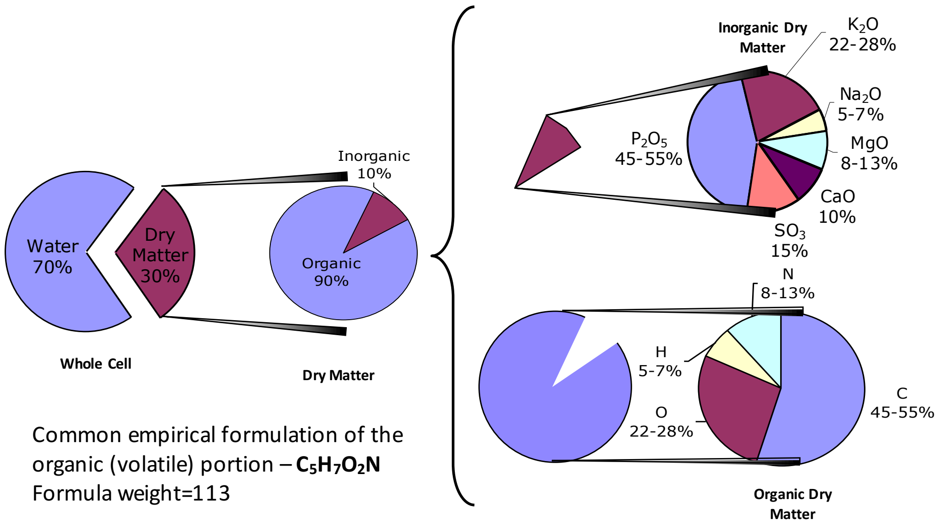
Composición química de las bacterias



Common empirical formulation of the organic (volatile) portion – $\mathbf{C_5H_7O_2N}$
Formula weight=113

Estequiometría de reacciones microbianas

Complete Reaction (R) $R = R_d + f_eR_a + f_sR_c$

Electron donor half reactions (R_d):

Oxidation reactions
(See R&M Tables 2.3, 2.2)

Organic:

$$\frac{1}{e}C_aH_bO_cN_d + \frac{2a-c}{e}H_2O = \frac{a}{e}CO_2 + \frac{d}{e}NH_3 + H^+ + e^-$$

where e = 4a+b-2c-3d

Inorganic:

$$\frac{1}{8}NH_3 + \frac{3}{8}H_2O = \frac{1}{8}NO_3^- + \frac{9}{8}H^+ + e^-$$ Ammonia oxidation

$Fe^{2+} = Fe^{3+} + e^-$ iron oxidation

Estequiometría de reacciones microbianas

Electron acceptor half reactions (R_a):

Aerobic: $\frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O$

Reduction reactions
(See R&M Tables 2.4)

Denitrification: $\frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e^- = \frac{1}{10}N_2 + \frac{3}{5}H_2O$

Sulfate reduction: $\frac{1}{8}SO_4^{2-} + \frac{5}{4}H^+ + e^- = \frac{1}{8}H_2S + \frac{1}{2}H_2O$

Methane fermentation: $\frac{1}{8}CO_2 + H^+ + e^- = \frac{1}{8}CH_4 + \frac{1}{4}H_2O$

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Estequiometría de reacciones microbianas

Cell synthesis half reactions (R_c):

Ammonia N source:

Cell growth reactions
(See R&M Tables 2.4)

$$\frac{1}{4}CO_2 + \frac{1}{20}NH_3 + H^+ + e^- = \frac{1}{20}C_5H_7O_2N + \frac{2}{5}H_2O$$

Nitrate N source:

$$\frac{5}{28}CO_2 + \frac{1}{28}NO_3^- + \frac{29}{28}H^+ + e^- = \frac{1}{28}C_5H_7O_2N + \frac{11}{28}H_2O$$

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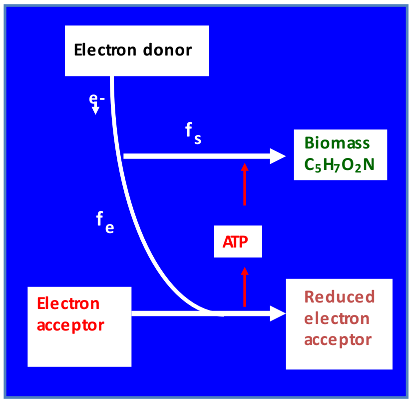
Reacciones medias para síntesis



Electron donor

e-

f_s

Biomass $C_5H_7O_2N$

f_e

ATP

Electron acceptor

Reduced electron acceptor

Cell growth using **ammonia** for N

$$\frac{1}{4}CO_2 + \boxed{\frac{1}{20}NH_3} + H^+ + e^- = \frac{1}{20}C_5H_7O_2N + \frac{2}{5}H_2O$$

Cell growth using **nitrate** for N

$$\frac{5}{28}CO_2 + \boxed{\frac{1}{28}NO_3^-} + \frac{29}{28}H^+ + e^- = \frac{1}{28}C_5H_7O_2N + \frac{11}{28}H_2O$$

Ejemplo: Utilización aeróbica de hidrocarburos

Hydrocarbon: octane
Nitrogen source: ammonia

Donor reaction: $\frac{1}{50}C_8H_{18} + \frac{8}{25}H_2O = \frac{4}{25}CO_2 + H^+ + e^-$

Acceptor reaction: $\frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O$

Synthesis: $\frac{1}{4}CO_2 + \frac{1}{20}NH_3 + H^+ + e^- = \frac{1}{20}C_5H_7O_2N + \frac{2}{5}H_2O$

Using measured value for f_s of 0.39, f_e = 1-0.39 = 0.61

R_d: $0.02\,C_8H_{18} + 0.32H_2O = 0.16CO_2 + H^+ + e^-$

f_eR_a: $0.15O_2 + 0.61H^+ + 0.61e^- = 0.31H_2O$

f_sR_c: $0.10CO_2 + 0.02NH_3 + 0.39H^+ + 0.39e^- = 0.02C_5H_7O_2N + 0.16H_2O$

$$\text{Net}: \overset{(114)}{0.02C_8H_{18}} + \overset{(32)}{0.15O_2} + \overset{(17)}{0.02NH_3} = \overset{(113)}{0.02C_5H_7O_2N} + 0{,}06CO_2 + 0.14H_2O$$

Mass balance for the degradation of 1 kg octane:

$$O_2 \text{ required} = \frac{(0.15)(32)}{(0.02)(114)} = 2.1 \text{ kg}$$

$$NH_3 \text{ required} = \frac{(0.02)(17)}{(0.02)(114)} = 0.15 \text{ kg}$$

$$\text{Biomass produced} = \frac{(0.02)(113)}{(0.02)(114)} = 1.0 \text{ kg}$$

CHECK on mass balance:

Reactants:	
Octane	1.0 kg
Ammonia	0.2 kg
O_2	2.1 kg
	3.3 kg

Products:	
CO_2	1.2 kg
Cells	1.0 kg
Water	1.1 kg
	3.3 kg

Looks good!

Medidas agregadas o Aggregate measures: e^- eq y O_2 eq

An **electron equivalent** conversion factor describes an electron donor or a collection of electron donors in terms of the moles of valence electrons (equivalents) that can be removed from it.

e- equivalents (meq) = mass (mg compound) ÷ e- eq conv. factor (mg compound/meq)

An **oxygen equivalent** conversion factor describes an electron donor or a collection of electron donors in terms of the oxygen that is theoretically needed to oxidize it. For organic electron donors, this is the COD'/WT ratio.

Oxygen equivalents (mg COD or ThOD or NOD) = **Mass** (mg compound) × **Oxygen equivalent conversion factor** (mg O_2 equivalents/ mg compound)

(e.g., COD'/wt ratio)

Ejemplo: Cuál es el factor de conversión en e- eq para el acetato?

e- donor $CH_3COOH + 2H_2O = 2CO_2 + 8H^+ + 8e^-$

MW=60

60 grams of acetic acid surrenders 8 moles of electrons or 8 e-equivalents

Electron equivalent conversion factor = 60 g / 8 eq = 7.5 g/eq = 7.5 mg/meq

The electron equivalent concentration of 100 mg/L acetic acid is 100 ÷ 7.5 = 13.3 meq/L or 13.3 mmoles of electrons per liter.

Note: If we know the concentrations of carbon, hydrogen, nitrogen and oxygen in a waste, we can compute the equivalent concentration in e-equivalents using this procedure.

Ejemplo: Cuál es el factor de conversión en e- eq para el oxígeno?

e- acceptor $O_2 + 4H^+ + 4\,e^- = 2H_2O$

32 g of O_2 accepts 4 moles of electrons, so the electron equivalent conversion factor for O_2 is 32/4 = **8 g O_2/eq**

8 g O_2 can accept 1 mol e^-
8 g OD (COD or NOD) gives up 1 mol e^-

10 mg/L O_2 accepts 10/8 = 1.25 mmoles of e^-/L
10 mg/L COD gives up 1.25 mmoles of e^-/L

Ejemplo: Cuál es la concentración O_2 eq para 100 mg/L acetato?

e- donor $CH_3COOH + 2O_2 = 2CO_2 + 2H_2O$

MW = 60 2 × 32

Computed O_2 eq conversion factor = COD’/WT = 2(32) g COD’/60 g acetate = 1.07

The O_2 eq concentration of 100 mg acetic acid /L is 100 × 1.07 = 107 mg/L.

Note: If we know the concentrations of carbon, hydrogen, nitrogen and oxygen in a waste, we can compute the oxygen equivalents using this procedure.

Ejemplo: receptores de e- expresados en O_2 eq

The half reaction for the reduction of nitrate to nitrogen is:

$$NO_3^- + 6H^+ + 5e^- = 0.5N_2 + 3H_2O$$

62 g of NO_3^- picks up 5 moles of electrons, or 12.4 g NO_3^-/mole e^-

The oxygen half reaction is:

$$O_2 + 4H^+ + 4e^- = 2H_2O$$

32 g of O_2 picks up 4 moles of electrons, or 8 g O_2/mole e^-

Therefore, 12.4 g NO_3^- has the same oxidation power as 8 g O_2

The oxygen equivalent conversion factor for nitrate is 8/12.4 = 0.645 g O_2/ g NO_3^-.

The oxidizing power of one gram NO_3^- **is equivalent to** 0.645 g O_2.

Equivalentes de O_2 para la célula

COD of cells

$C_5H_7O_2N + 5O_2 = 5CO_2 + NH_3 + 2H_2O$

$$\frac{COD}{WT} = \frac{5(32)}{113} = \mathbf{1.416}$$

NOD of cells

When oxidized, each mole of $C_5H_7O_2N$ yields 1 mole NH_3. When the NH_3 is oxidized:

$NH_3 + 2O_2 = HNO_3 + H_2O$

$$\frac{NOD}{WT} = \frac{2(32)}{113} = \mathbf{0.566}$$

ThOD of cells (COD + NOD)

$C_5H_7O_2N + 7O_2 = 5CO_2 + HNO_3 + 3H_2O$

$$\frac{ThOD}{WT} = \frac{7(32)}{113} = \mathbf{1.98}$$

Common conversion factors for electron equivalents and oxygen equivalents

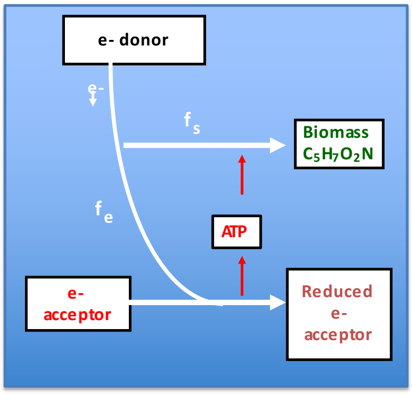
Compound	**Electron equivalent conversion factor**	**Oxygen equivalent conversion factor**
Electron donors		
methane	2.0 g CH_4/eq	4.0 g COD/g CH_4
carbohydrate	7.5 g carbohydrate/eq	1.067 g COD /g carbohydrate
protein	5.333 g protein/eq	1.50 g COD /g protein
grease (fat)	2.778 g grease/eq	2.88 g COD /g grease
sewage organics	4.02 g sewage organic/eq	1.99 g COD /g sewage organic
ammonium (to nitrate)	2.254 g NH_4^+/eq	3.55 g NOD /g NH_4^+
	1.75 g NH_4^+ as N/eq	4.57 g NOD /g NH_4^+ as N
ammonium (to nitrite)	3.0 g NH_4^+/eq	2.667 g NOD/g NH_4^+
nitrite (to nitrate)	23 g NO_2^-/eq	0.36 g NOD /g NO_2^-
	7 g NO_2^- as N/eq	1.14 g NOD/g NO_2^- as N
S (to sulfate)	5.333 g S/eq	1.50 g Sulfur OD /g S
H_2 (to H^+)	1.0 g H_2/eq	8.0 g Hydrogen OD/g H_2
Biomass ($C_5H_7O_2N$)		
N source: NH_4^+	5.65 g biomass/eq	1.42 g COD /g biomass
N source: nitrate	4.04 g biomass /eq	1.98 g ThOD/g biomass
Electron acceptors		
oxygen (to water)	8.0 g O_2/eq	1.0 g O_2/g O_2
nitrate (to nitrogen)	12.4 g NO_3^-/eq	0.645 g O_2/g NO_3^-
	2.8 g NO_3^- as N/eq	2.857 g O_2/g NO_3^- as N
sulfate (to H_2S)	12.0 g SO_4^{2-} /eq	0.667 g O_2/g SO_4^{2-}
	4.0 g SO_4^{2-} as S/eq	2.0 g O_2/g SO_4^{2-} as S
CO_2 (to methane)	5.5 g CO_2/eq	1.45 g O_2/g CO_2
	1.5 g CO_2 as C/eq	5.33 g O_2/g CO_2 as C
$Cr_2O_7^{2-}$ (to Cr^{3+}) (oxidant in COD test)	36 g $Cr_2O_7^{2-}$/eq	0.222 g O_2/g $Cr_2O_7^{2-}$
Reduced electron acceptors		
nitrogen (from nitrate)	2.8 g N_2/eq	2.857 g OD/g N_2
	2.24 L N_2/eq at STP	3.571 g OD/L N_2 at STP
H_2S (from sulfate)	4.25 g H_2S/eq	1.88 g OD/g H_2S
methane (from CO_2)	2.0 g CH_4/eq	4.0 g COD/g CH_4
	2.8 L CH_4/eq at STP	2.857 g COD/L CH_4 at STP
	3.159 L CH_4/eq at 35°C	2.532 g COD/L CH_4 at 35°C

X

= 8

g OD / eq

Conversión a equivalentes de e^- y O_2



Conversion to electron equivalents
Divide each mass by appropriate electron equivalent conversion factor

Conversion to oxygen equivalents
Multiply each mass by appropriate oxygen equivalent conversion factor

---

Conversión a equivalentes de e^- y O_2

Electron equivalent balance

e- removed from **e- donor** =
e- in biomass + e- in reduced e- acceptor.

e- equivalents in reduced **e- acceptor** =
e- equivalents of e- acceptor consumed.

$$\frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O$$

↑ ÷8 gOD/eq

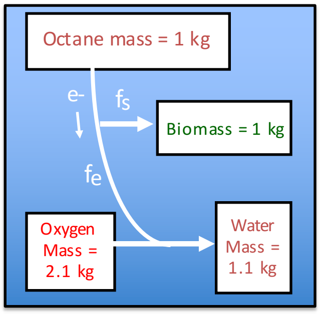
↓ x 8 gOD/eq

Oxygen equivalent balance:

O_2 eq of **e- donor** consumed = O_2 eq in biomass + O_2 eq in reduced e- acceptor.

O_2 equivalents in reduced **e- acceptor** =
O_2 equivalents of e- acceptor consumed.

Ejemplo: Cómo se balancean los equivalentes iniciales y finales para la degradación biológica de 1 kg de octano en batch?



Conversion to e- equivalents

$C_8H_{18} + 16H_2O = 8CO_2 + 50H^+ + 50e^-$

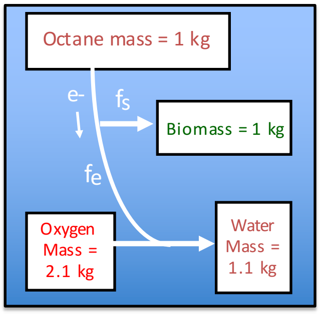
Divide each mass by appropriate electron equivalent conversion factor:

Octane: 114/50 = 2.28 g/eq
Biomass: 5.65 g VSS/eq
Oxygen: 8 g/eq

Consumed:
Octane e^- eq= 1/2.28=
0.44 kmoles e^-

Synthesis + Energy:
Biomass e^- eq=1/5.65=
0.18 kmoles e^-
O_2 e^- equiv =2.1/8=
0.26 kmoles e^-

Ejemplo: Cómo se balancean los equivalentes iniciales y finales para la degradación biológica de 1 kg de octano en batch?



[CO_2 is the missing 1 kg of product]

Conversion to oxygen equivalents

$C_8H_{18} + 25/2\ O_2 = 8CO_2 + 9H_2O$

Multiply each mass by appropriate oxygen equivalent conversion factor

Octane: (25/2 x 32)/114 = 3.5 g OD/g octane
Biomass: 1.42 g OD /g VSS
Oxygen: 1 g OD / g O_2

Consumed:
Octane COD = 1x 3.5 =
3.5 kg COD

Synthesis + Energy:
Biomass COD =1 x 1.42=
1.42 kg COD
Oxygen O_2 equiv =
2.1 kg O_2

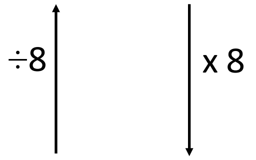
Conversión entre equivalentes de e$^-$ y O_2

Electron equivalent balance

Consumed:
Octane e$^-$ eq = 1/2.28 = **0.44 kmoles e$^-$**

Synthesis + Energy:
Biomass e$^-$ eq =1/5.65 = **0.18 kmoles e$^-$**
O_2 e$^-$ equiv = 2.1/8 = **0.26 kmoles e$^-$**

$$\frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O$$



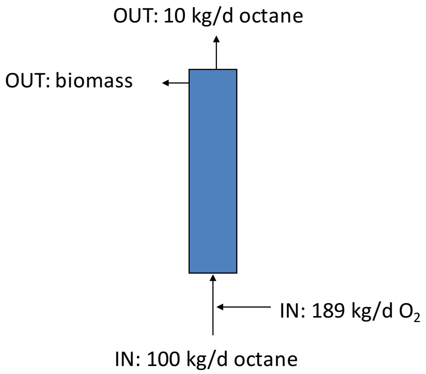
Oxygen equivalent balance:

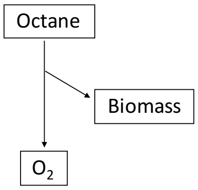
Consumed:
Octane COD = 1x 3.5 = **3.5 kg COD**

Synthesis + Energy:
Biomass COD = 1 x 1.42 = **1.42 kg COD**
Oxygen O_2 equiv = **2.1 kg O_2**

Ejemplo: degradación contínua de octano en un bioreactor

The high rate bioreactor shown below removes 90% of 100 kg/d octane and uses 189 kg/d O_2. What is the biomass production rate?





Working with equivalent factors:

$C_8H_{18} + 25/2\ O_2 = 8CO_2 + 9H_2O$
Octane: 25/2 x 32/114 = **3.5 kg COD/kg**

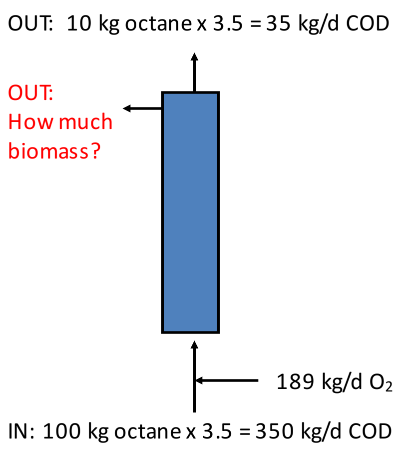
$C_5H_7O_2N + 5O_2 = 5CO_2 + NH_3 + 2H_2O$
Biomass: 5 x32/114 = **1.42 kg COD/kg**

Solución usando O_2 eq:

O_2 Conversion Factors:

Octane: 3.5 kg COD/kg

Biomass: 1.42 kg COD/kg



OUT: 10 kg octane x 3.5 = 35 kg/d COD

OUT: How much biomass?

189 kg/d O_2

IN: 100 kg octane x 3.5 = 350 kg/d COD

COD consumed = 350-35 = 315 kg/d

O_2 equivalents for energy = 189 kg/d O_2

O_2 equivalents for synthesis = 315-189
= 126 kg/d biomass as COD

Biomass produced = 126 ÷ 1.42 = 89 kg/d

f_e=189/315 = 0.60

f_s=126/315 = 0.40

Yield (Y)

$$f_s = \frac{\text{e- equivalents of biomass formed}}{\text{electron equivalents of electron donor consumed}}$$

$$\boxed{\mathbf{Y} = \frac{\text{Biomass produced}}{\text{Substrate consumed}}}$$

[g **VSS**/ g COD]

Why VSS?

- Y can be measured and is directly related to f_s.
- Y can be changed it into f_s by converting both the numerator and the denominator into electron equivalents (or into O_2 equivalents, whichever is easier).

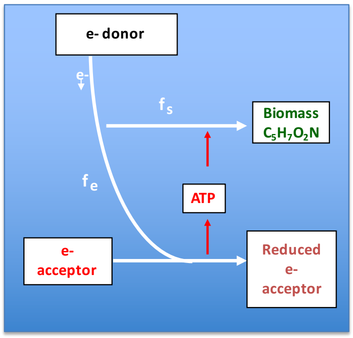
Ejemplo: Given Y = 0.49 g vss/g COD, ammonia N source. What is f_s?

Solution using **O_2 equivalents**:
f_s = 0.49 g vss/g COD x $\underline{\text{1.42 g COD/g vss}}$ = 0.70

Solution using **electron equivalents**:
f_s = 0.49 g vss/g COD x 8 g COD/eq ÷ $\underline{\text{5.65 g VS/eq}}$ = 0.70

Same result using both approaches!

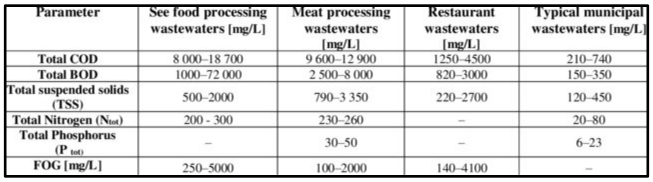
How much is f_e?



Ejemplo de estequiometría

A meat packing waste with the empirical formula: $C_3H_7O_2N$ (M.W. = 89). Determine the oxygen required per gram of waste for aerobic treatment.

Klaucans et al., 2018. (10.4028/www.scientific.net/KEM.762.61)



Parameter	See food processing wastewaters [mg/L]	Meat processing wastewaters [mg/L]	Restaurant wastewaters [mg/L]	Typical municipal wastewaters [mg/L]
Total COD	8 000–18 700	9 600–12 900	1250–4500	210–740
Total BOD	1000–72 000	2 500–8 000	820–3000	150–350
Total suspended solids (TSS)	500–2000	790–3 350	220–2700	120–450
Total Nitrogen (N_{tot})	200 - 300	230–260	–	20–80
Total Phosphorus (P_{tot})	–	30–50	–	6–23
FOG [mg/L]	250–5000	100–2000	140–4100	–

$C_3H_7O_2N + 3O_2 \rightarrow 3CO_2 + NH_3 + 2H_2O$
COD/WT = 3(32)/89 = 1.08

Will aerobic biological treatment of this wastewater require 1.08 g O_2/g waste?

If Y for aerobic growth is 0.32 g vss/g COD
f_s = 0.32 x 1.42 = 0.45 and f_e= 1-0.45 = 0.55
so the amount of oxygen required is 0.55 x 1.08 = **0.59 g O_2/g**.

Ejemplo de estequiometría: Anaerobio

Determine the nutrient requirements and methane production per gram of waste for anaerobic treatment.

Assuming an anaerobic yield of 0.07 g vss/g COD, then $f_s = 0.07 \times 1.42 = 0.1$.

First, we write the half reaction for oxidation of the electron donor (for now we'll ignore acid-base reactions): $C_3H_7O_2N + 4H_2O \rightarrow 3CO_2 + NH_3 + 12H^+ + 12e^-$

R_d is: $$\frac{1}{12}C_3H_7O_2N + \frac{1}{3}H_2O \rightarrow \frac{1}{4}CO_2 + \frac{1}{12}NH_3 + H^+ + e^-$$

R_a is: $$\frac{1}{8}CO_2 + H^+ + e^- \rightarrow \frac{1}{8}CH_4 + \frac{1}{4}H_2O$$

R_c is: $$\frac{1}{4}CO_2 + \frac{1}{20}NH_3 + H^+ + e^- \rightarrow \frac{1}{20}C_5H_7O_2N + \frac{2}{5}H_2O$$

Ejemplo de estequiometría: Anaerobio

Weighting R_a by f_e and R_c by f_s and putting them all together:

$$\frac{1}{12}C_3H_7O_2N + \frac{1}{3}H_2O \rightarrow \frac{1}{4}CO_2 + \frac{1}{12}NH_3 + H^+ + e^-$$

$$+0.9\left[\frac{1}{8}CO_2 + H^+ + e^- \rightarrow \frac{1}{8}CH_4 + \frac{1}{4}H_2O\right]$$

$$\underline{+0.1\left[\frac{1}{4}CO_2 + \frac{1}{20}NH_3 + H^+ + e^- \rightarrow \frac{1}{20}C_5H_7O_2N + \frac{2}{5}H_2O\right]}$$

$$0.083C_3H_7O_2N + 0.068H_2O \rightarrow 0.113CH_4 + 0.113CO_2 + 0.078NH_3 + 0.005C_5H_7O_2N$$

Ejemplo de estequiometría: Anaerobio

We do not need to add nitrogen.

In fact, the reaction actually produces nitrogen as ammonia, but.... if we want to know how much CO_2 is produced, we will have to correct for the fact that some of it will be neutralized by the NH_3 that is produced:

$$0.083C_3H_7O_2N + 0.068H_2O \rightarrow 0.113CH_4 + 0.113CO_2 + 0.078NH_3 + 0.005C_5H_7O_2N$$

$$0.078H_2O + 0.078NH_3 + 0.078CO_2 \rightarrow 0.078NH_4^+ + HCO_3^-$$

NET: $0.083C_3H_7O_2N + 0.146H_2O \rightarrow$
$0.113CH_4 + 0.0.035CO_2 + 0.078NH_4^+ + 0.078HCO_3^- + 0.005C_5H_7O_2N$

Ejemplo de estequiometría: Anaerobio

NET: $0.083C_3H_7O_2N + 0.146H_2O \rightarrow$
$0.113CH_4 + 0.0.035CO_2 + 0.078NH_4^+ + 0.078HCO_3^- + 0.005C_5H_7O_2N$

With the balanced acid-base reaction, we can calculate the % methane in the biogas:

$\%CH_4 = 0.113 / (0.113 + 0.035) \times 100 = 76\%$
(so there is 24% CO_2)

Methane production @ STP
(0.113)(22.4 L methane/mole) ÷ (0.083)(89) = 0.342 L/g waste

This is for STP conditions, the temperature is 0°C.

Do you know how to adjust for different temperatures?

Ejemplo de estequiometría: Anaerobio

What is the phosphorus requirement of the cells?

Suppose this wastewater has a biodegradable COD (BOD_L) of 10 g/L and no P is present in the waste? The COD removal efficiency is 100%, and P is 2% (by weight) of the vss.

P requirement =

$$\frac{10\ \text{g COD}}{\text{L}} \times \frac{\text{mol e}^-}{8\ \text{g COD}} \times \frac{(0.005)(113\ \text{g vss})}{\text{mol e}^-} \times \frac{0.02\ \text{g P}}{\text{g vss}} =$$

$$\boxed{\frac{0.014\ \text{g P}}{\text{L}}}$$

Ejemplo de estequiometría: Anaerobio

How many liters of methane will be produced per liter of waste if the treatment efficiency is 100%?

$$\frac{\text{Liters methane}}{\text{Liter waste}} = \frac{10\ \text{g COD}}{\text{L}} \times \frac{\text{mol e}^-}{8\ \text{g COD}} \times \frac{0.113\ \text{mol CH}_4}{\text{mol e}^-} \times \frac{22.4\ \text{L}}{\text{mol CH}_4} = \frac{3.15\ \text{liters}}{\text{liter of waste}}$$

NOTE: problem could just as easily be solved using the oxygen equivalent conversion factor for methane:

$$\frac{\text{Liters methane}}{\text{Liter waste}} = \frac{10\ \text{g COD}}{\text{L}} \times \frac{0.9\ \text{g COD to CH}_4}{\text{g COD}} \times \frac{\text{L CH}_4}{2.857\ \text{g COD}} = \frac{3.15\ \text{liters}}{\text{liter of waste}}$$

This is for STP conditions, the temperature is 0°C.

Do you know how to adjust for different temperatures?

Energía libre y Yield

Why do some microorganisms have a lower yield than others?

Microorganisms "prefer" to convert all their substrate into biomass ($f_s = 1.0$), but some substrate must be burned to give energy for synthesis.

When energy reactions yield very little energy per equivalent, most of the electrons must be used to produce energy for cell synthesis (f_e must be large). For these reactions, f_s and yield will be small.

Other energy reactions generate considerable energy per equivalent. Fewer equivalents can generate sufficient energy for cell synthesis (f_e can be small). For these reactions, f_s and yield will be large.

Alcalinidad

Alkalinity: The ability to neutralize strong acids

the cumulative proton concentration taken up by basic species when a solution is titrated to the CO_2 equiv pt (around pH 4.3-4.7):

Alkalinity = $-(H^+) + (HCO_3^-) + 2(CO_3^{2-}) + (NH_3) + (HS^-) + (OH^-)$

Example: Dissolve $CaCO_3$ in water

CO_3^{2-} equilibrates with HCO_3^- and $H_2CO_3^*$ according to:

$$CO_3^{2-} + H_2O \leftrightarrow HCO_3^- + OH^-$$

$$HCO_3^- + H^+ \leftrightarrow H_2CO_3^*$$

La alcalinidad cambia de forma

Biological Examples

1. Bacteria release NH_3 from protein:

Protein ➔ NH_3 (Ammonification)

2. Ammonia reacts with carbon dioxide

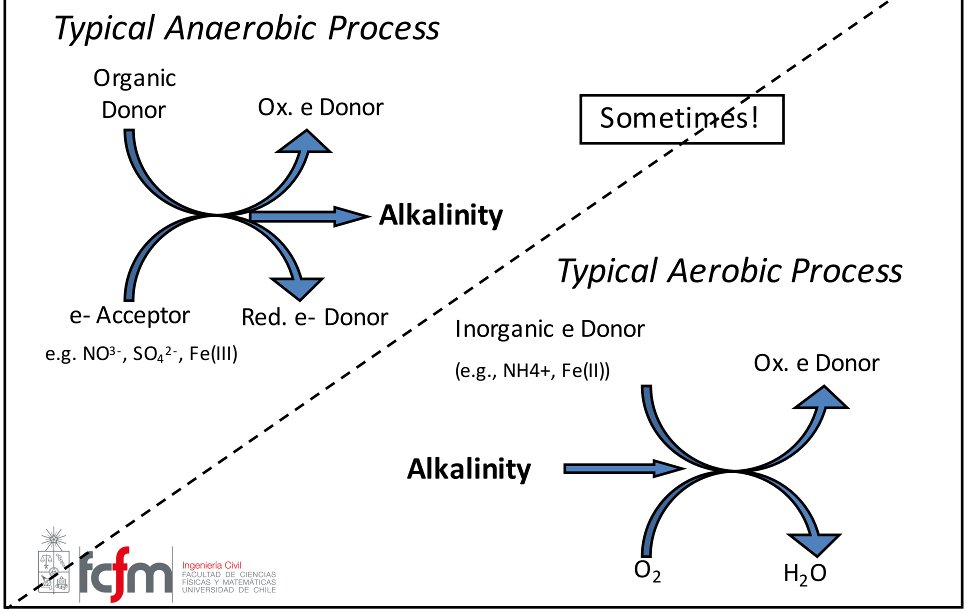
$$NH_3 + CO_2 + H_2O \rightarrow NH_4^+ + HCO_3^-$$

3. Volatile fatty acids can react with HCO_3^-:

$$CH_3COOH + HCO_3^- \rightarrow H_2CO_3 + CH_3COO^-$$

Where has the alkalinity gone?

Procesos microbianos pueden producir o consumir alcalinidad



Typical Anaerobic Process

Organic Donor

Ox. e Donor

Alkalinity

e- Acceptor

e.g. NO^{3-}, SO_4^{2-}, Fe(III)

Red. e- Donor

Sometimes!

Typical Aerobic Process

Inorganic e Donor

(e.g., NH4+, Fe(II))

Ox. e Donor

Alkalinity

O_2

H_2O

Fuentes de buffer alcalino

Alkaline buffer (salt/base)	Dissolution and Neutralization reactions	How many kg of the buffer are <u>equivalent</u> to one kmol of alkalinity?	Comments	1995 Cost($) per kmol Alkalinity
$CaCO_3$	$CaCO_3 + 2H^+ = Ca^{2+} + H_2CO_3$ $CaCO_3 + H_2O + CO_2 = Ca^{2+} + 2HCO_3^-$	100 g of $CaCO_3$ neutralizes **2** moles of strong acid, 100 g of $CaCO_3$ reacts with CO_2 to make **2** moles of HCO_3^-. We have 100 ÷ 2 = 50 kg $CaCO_3$ per kmol of alkalinity.	Poor solubility restricts alkalinity to 1400-1500 mg/L as $CaCO_3$	Not available (cheap)
Na_2CO_3	$Na_2CO_3 + 2H^+ = 2Na^+ + H_2CO_3$ $Na_2CO_3 + H_2O + CO_2 = 2Na^+ + 2HCO_3^-$	106 ÷ 2 = 53 kg	Na is inhibitory at 3,500 mg/L. Na deflocculates soil.	11.55
K_2CO_3	Like Na_2CO_3	136÷ 2 = 68 kg	K is inhibitory at > 2,500 mg/L.	Not available
$(NH_4)_2CO_3$	$(NH_4)_2CO_3 = 2NH_4^+ + CO_3^{2-}$ $CO_3^- + 2H^+ = H_2CO_3$	96 ÷ 2 = 48 kg		Not available
CaO (lime)	$CaO + 2H_2O = Ca(OH)_2$ $Ca(OH)_2 + 2H^+ = Ca^{2+} + 2H_2O$ $Ca(OH)_2 + 2CO_2 = Ca^{2+} + 2HCO_3^-$	56÷ 2 = 28 kg	Can cause severe scaling @ pH>6.8. Can create a vacuum in a closed system.	3.74
MgO	Like CaO	40 ÷ 2 = 20 kg	Low solubility reduces chance of pH overshoot.	7.71
$NaHCO_3$	$NaHCO_3 + H_2O = Na^+ + HCO_3^-$ $HCO_3^- + H^+ = H_2CO_3$	84 kg	Exactly the right form, but expensive.	36.89
NaOH	$NaOH + H^+ = Na^+ + H_2O$ $NaOH + CO_2 = Na^+ + HCO_3^-$	40 kg	Can create a vacuum in a closed system	35.20
KOH	Like NaOH	56 kg	Can create a vacuum in a closed system	Not available
NH_3	$NH_3 + H^+ = NH_4^+$ $NH_3 + CO_2 + H_2O = NH_4^+ + HCO_3^-$	17 kg	Can be toxic. Released as protein degrades.	Not available

Acidity

Acidity: the cumulative hydroxide concentration taken up by acidic species when a solution is titrated to the CO_3^{2-} equiv. pt.:

$$\text{Acidity} = (H^+) + (HCO_3^-) + 2(H_2CO_3^*) + (NH_4^+) - (OH^-)$$

Note that HCO_3^- contributes to alkalinity and acidity

The two major sources of acidity in biological systems are:

$H_2CO_3^*$ (from dissolved CO_2) and HCO_3^-

In covered reactors, CO_2 accumulates in the in gas phase, causing $H_2CO_3^*$ to accumulate and pH to drop.

HCO_3^- buffer must be added to decrease pH drop.