Περιβαλλοντική Βιοτεχνολογία- Environmental Biotechnology

Ενότητα 2: Stoichiometry and Bacterial Energetics

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Τμήμα Χημικών Μηχανικών

Introduction

Design of systems for biological treatment

↓

Mass balances

↙ ↘

For a given quantity of waste $\Rightarrow$ chemicals & end-products

- Energy (e.g. O_2)
- Nutrient (e.g. N , P)
- Environmental needs

  ($Ca(OH)_2$ or H_2SO_4 for pH control)

- Excess microorganisms (costly disposal problem)
- CH_4 (source of energy)

Introduction

Stoichiometry ⇩ Relationships among reactants & products

Energetics ⇩ Balancing for elements, electrons & charge

The microbial reactions complicate the stoichiometry.

- Microbial reactions often involve oxidation and reduction of more than one species.
- The microorganisms have two roles
  - catalysts for the reaction
  - products of the reaction
- The microorganisms carry out most chemical reactions in order to capture some of the energy released for **cell synthesis** and for **maintaining** cellular activity

An example stoichiometric equation

Porges et al (1956) for a casein-containing wastewater

carbon source | electron donor

$$C_8H_{12}O_3N_2 + 3O_2 \rightarrow C_5H_7O_2N + NH_3 + 3\ CO_2 + H_2O$$

casein | **microorganisms**

M. B.	**184**	**96**	**113**	**17**	**132**	**18**
(kg/d)	**1000**	**520**	**610**			

Could we predict the stoichiometry of such a reaction?

- Empirical formula for cells
- How the electron-donor substrate is partitioned between energy generation and synthesis
- The proportion of the electron-donor substrate that is used to synthesize new biomass to the energy gained from catabolism and the energy needed for anabolism.

Empirical formula for microbial cells

$$\mathbf{C_5H_7O_2N} \rightarrow \text{empirical formula}$$

Depends on :

- The characteristics of the microorganisms involved
- The substrates being used for energy
- The availability of other nutrients required for microbial growth

  (e.g. in a nitrogen-deficient environment more fatty material or carbohydrates are produced)

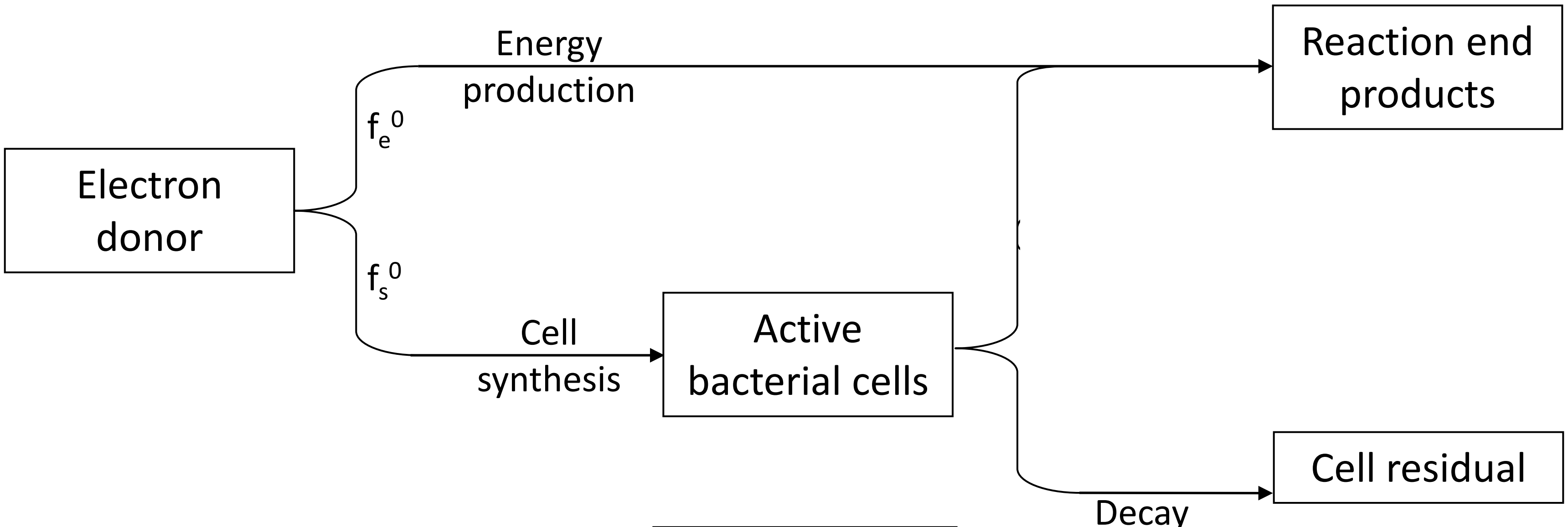
$$C_nH_aO_bN_c + (2n+0.5a-1.5c-b)/2\ O_2 \rightarrow n\ CO_2 + c\ NH_3 + (a-3c)/2\ H_2O$$

$$COD' / weight = \frac{(2n + 0.5a - 1.5c - b)\ 16}{12n + a + 16b + 14c}$$

$$n = \%C/12T,\ \ a = \%H/T,\ \ b = \%O/16T \text{ and } c = \%N/14T$$

$$T = \%C/12 + \%H + \%O/16 + \%N/14$$

Substrate partitioning and cellular yield



$$\mathbf{f_e^0 + f_s^0 = 1}$$

True yield :

Y = f_s^0 (M_c g cells/mol cells) / [(n_e e^- eq/mol cells)(8 g COD/e^- eq donor)]

- f_s^0 = g cell produced per g COD'
- Y = g cell produced per g COD
- M_c = empirical formula weight of cells
- n_e = number of electron eq. in a mole of cells

Substrate partitioning and cellular yield

The growth rate of microbial cells :

$$\frac{dX_a}{dt} = Y\left(-\frac{dS}{dt}\right) - bX_a$$

The net yield :

$$Y_n = \frac{dX_a/dt}{-dS/dt} = Y - b\frac{X_a}{-dS/dt}$$

$f_e + f_s = 1$

$f_e > f_e^0$

$f_s < f_s^0$

$Y_n \downarrow$ because

$X_a \Uparrow$ **or** $(-dS/dt) \Downarrow$

Maintentance energy:

$$Y_n = 0, \quad \frac{-dS/dt}{X_a} = \frac{b}{Y} = m$$

Energy reactions

Microorganisms obtain their energy for growth and maintenance from **oxidation – reduction reactions**.

Oxidation-reduction reactions always involve an electron **donor** and an electron **acceptor**.

Aerobic conditions: electron donor → organic matter or e.g. NH_3

electron acceptor → oxygen

Anaerobic conditions: electron donor → organic matter

electron acceptor → NO_3^-, SO_4^{2-}, CO_2

Fermentation: electron donor → organic matter

electron acceptor → organic matter

Energy reactions

Example: **Glucose is the electron donor**

Free Energy (kJ/mol glucose)

Aerobic oxidation

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$ -2,880

Denitrification

$$5C_6H_{12}O_6 + 24NO_3^- + 24H^+ \rightarrow 30CO_2 + 42H_2O + 12N_2$$ -2,720

Sulfate reduction

$$2C_6H_{12}O_6 + 6SO_4^{2-} + 9H^+ \rightarrow 12CO_2 + 12H_2O + 3H_2S + 3HS^-$$ -492

Methanogenesis

$$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4$$ -428

Ethanol Fermentation

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 3CH_3CH_2OH$$ -244

Energy reactions

Energy reactions using half-reactions.

The oxidation half-reaction for glucose:

$$\frac{1}{24}C_6H_{12}O_6 + \frac{1}{24}H_2O \rightarrow \frac{1}{4}CO_2 + H^+ + e^-$$

The reduction half-reaction for nitrate:

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

The overall balanced reaction:

$$\frac{1}{24}C_6H_{12}O_6 + \frac{1}{5}NO_3^- + \frac{1}{5}H^+ \rightarrow \frac{1}{4}CO_2 + \frac{7}{20}H_2O + \frac{1}{10}N_2$$

$$\mathbf{5C_6H_{12}O_6 + 24NO_3^- + 24H^+ \rightarrow 30\,CO_2 + 42H_2O + 12N_2}$$

Energy reactions
Steps of half-reactions

Organic Compounds

Step 1: Write the oxidized form of the element of interest on the left and the reduced form on the right.

$$\mathrm{CO_2} \rightarrow CH_3CHNH_2COOH$$

Step 2: Add other species that are formed or consumed in the reaction. In oxidation-reduction reactions, water is almost always a reactant or a product; here it will be included as a reactant in order to balance the oxygen present in the organic compound. As a reduction half-reaction, electrons must also appear on the left side of the equation. Since the nitrogen is present in the reduced form as an amino group in alanine, N must appear on the left side of the equation in the reduced form, either as NH_3 or NH_4^+. In this case, we arbitrarily select NH_3 for illustration.

$$\mathrm{CO_2} + H_2O + NH_3 + e^- \rightarrow CH_3CHNH_2COOH$$

Energy reactions
Steps of half-reactions

Step 3: Balance the reaction for the element that is reduced and for all elements except oxygen and hydrogen. In this case, carbon and nitrogen must be balanced.

$$3\mathrm{CO}_2 + H_2O + NH_3 + e^- \rightarrow CH_3CHNH_2COOH$$

Step 4: Balance the oxygen through addition or subtraction of water. Elemental oxygen is not to be used here, as oxygen must not have its oxidation state changed.

$$3\mathrm{CO}_2 + NH_3 + e^- \rightarrow CH_3CHNH_2COOH + 4H_2O$$

Step 5: Balance hydrogen by introducing H^+.

$$3\mathrm{CO}_2 + NH_3 + 12H^+ + e^- \rightarrow CH_3CHNH_2COOH + 4H_2O$$

Energy reactions
Steps of half-reactions

Step 6: Balance the charge on the reaction by adding sufficient e- to the left side of the equation.

$$3CO_2 + NH_3 + 12H^+ + 12e^- \rightarrow CH_3CHNH_2COOH + 4H_2O$$

The coefficient on electrons should equal the number of electron equivalents in the reduced compound, or 12e$^-$ eq in alanine, which it does.

Step 7: Convert the equation to the electron-equivalent form by dividing by the coefficient on e$^-$.

$$\frac{1}{4}CO_2 + \frac{1}{12}NH_3 + H^+ + e^- \rightarrow \frac{1}{12}CH_3CHNH_2COOH + \frac{1}{3}H_2O$$

Energy reactions
Steps of half-reactions

Inorganic Compounds

Step 1: $CrO_4^{2-} \rightarrow Cr^{3+}$

Step 2: $CrO_4^{2-} + H^+ + e^- \rightarrow Cr^{3+} + H_2O$

Step 3: $CrO_4^{2-} + H^+ + e^- \rightarrow Cr^{3+} + H_2O$ $(\text{Cr balanced in Step 2; thus no change here})$

Step 4: $CrO_4^{2-} + H^+ + e^- \rightarrow Cr^{3+} + 4H_2O$

Step 5: $CrO_4^{2-} + 8H^+ + e^- \rightarrow Cr^{3+} + 4H_2O$

Step 6: $CrO_4^{2-} + 8H^+ + 3e^- \rightarrow Cr^{3+} + 4H_2O$

Step 7: $\frac{1}{3}CrO_4^{2-} + \frac{8}{3}H^+ + e^- \rightarrow \frac{1}{3}Cr^{3+} + \frac{4}{3}H_2O$

Energy reactions
Steps of half-reactions

The conditions in which a reaction takes place determine the form of the products (e.g. NH_4^+ instead of NH_3 at pH neutral).

Anaerobic methanogenic fermentation of alanine:

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

$$\frac{1}{12}CH_3CHNH_2COOH + \frac{5}{12}H_2O \rightarrow \frac{1}{6}CO_2 + \frac{1}{12}NH_4^+ + \frac{1}{12}HCO_3^- + H^+ + e^-$$

$$Sum: \frac{1}{12}CH_3CHNH_2COOH + \frac{1}{6}H_2O \rightarrow \frac{1}{8}CH_4 + \frac{1}{24}CO_2 + \frac{1}{12}NH_4^+ + \frac{1}{12}HCO_3^-$$

Energy reactions
Steps of half-reactions

For each half-reaction that involves organic nitrogen, **HCO_3^-** enters into the reaction.

$$\frac{1}{5}CO_2 + \frac{1}{10}HCO_3^- + H^+ + e^- \rightarrow \frac{1}{10}CH_3COCOO^- + \frac{2}{5}H_2O$$

Different half-reaction forms sometimes used are illustrated:

$$\frac{1}{4}CO_2 + \frac{1}{12}NH_3 + H^+ + e^- \rightarrow \frac{1}{12}CH_3CHNH_2COOH + \frac{1}{3}H_2O$$

$$\frac{1}{6}CO_2 + \frac{1}{12}NH_4^+ + \frac{1}{12}HCO_3^- + H^+ + e^- \rightarrow \frac{1}{12}CH_3CHNH_2COOH + \frac{5}{12}H_2O$$

$$\frac{1}{6}CO_2 + \frac{1}{12}NH_4^+ + \frac{1}{12}HCO_3^- + \frac{11}{12}H^+ + e^- \rightarrow \frac{1}{12}CH_3CHNH_2COO^- + \frac{5}{12}H_2O$$

$$\frac{1}{6}CO_2 + \frac{1}{12}NH_4^+ + \frac{1}{12}HCO_3^- + \frac{1}{2}H_2 \rightarrow \frac{1}{12}CH_3CHNH_2COOH + \frac{5}{12}H_2O$$

$$\frac{1}{12}NH_4^+ + \frac{1}{4}HCO_3^- + \frac{13}{12}H^+ + e^- \rightarrow \frac{1}{12}CH_3CHNH_2COO^- + \frac{7}{12}H_2O$$

Overall reactions for biological growth

Bacterial growth involves **two** basic reactions :

- One for energy production and
- One for cellular synthesis

The **electron donor** provides electrons to the **electron acceptor** for energy production.

Combination of half-reaction for synthesis (R_c) and the acceptor half-reaction (R_a).

Nitrogen source (N) : $\mathbf{NH_4^+, NO_2^-, NO_3^-, N_2}$

Cell mass: $\mathbf{C_5H_7O_2N}$

Electron acceptor : $\mathbf{O_2, NO_3^-, Fe_3^+, SO_4^{2-}, CO_2}$

Overall reactions for biological growth

Overall energy reaction : $R_e = R_a - R_d$

Overall synthesis reaction : $R_s = R_c - R_d$

Where R_d the donor half-reaction ("-" because the donor is oxidized).

Overall reaction : $R = f_e R_e + f_s R_s = f_e (R_a - R_d) + f_s (R_c - R_d)$

But also applies : $f_s + f_e = 1$ $\quad R_d (f_s + f_e) = R_d$

$$R = f_e R_a + f_s R_c - R_d$$

(the net consumption of reactants and production of products when the microorganisms consume one electron equivalent of electron donor)

Overall reactions for biological growth

Synthesis reaction

$$R_c : \frac{1}{5} CO_2 + \frac{1}{20} NH_4^+ + \frac{1}{20} HCO_3^- + H^+ + e^- \rightarrow \frac{1}{20} C_5H_7O_2N + \frac{9}{20} H_2O$$

$$-R_d : \frac{1}{30} C_6H_5COO^- + \frac{13}{30} H_2O \rightarrow \frac{1}{5} CO_2 + \frac{1}{30} HCO_3^- + H^+ + e^-$$

$$R_s : \frac{1}{30} C_6H_5COO^- + \frac{1}{20} NH_4^+ + \frac{1}{60} HCO_3^- \rightarrow \frac{1}{20} C_5H_7O_2N + \frac{1}{60} H_2O$$

Overall reaction $R = f_e R_e + f_s R_s$

$$f_e R_e : 0.02 C_6H_5COO^- + 0.12 NO_3^- + 0.12 H^+ \rightarrow 0.12 CO_2 + 0.06 N_2 + 0.02 HCO_3^- + 0.1 H_2O$$

$$f_s R_s : 0.0133 C_6H_5COO^- + 0.02 NH_4^+ + 0.0067 HCO_3^- \rightarrow 0.02 C_5H_7O_2N + 0.0067 H_2O$$

$$R : 0.0333 C_6H_5COO^- + 0.12 NO_3^- + 0.02 NH_4^+ + 0.12 H^+ \rightarrow$$

$$0.02 C_5H_7O_2N + 0.06 N_2 + 0.12 CO_2 + 0.0133 HCO_3^- + 0.1067 H_2O$$

Overall reactions for biological growth

Example:

Electron donor : **benzoate** ($C_6H_5COO^-$)

Electron acceptor : $\mathbf{NO_3^-}$

Nitrogen source : $\mathbf{NO_3^-}$

$f_s = 0.55$, $f_e = 0.45$

Overall reaction

$$f_eR_a : 0.09NO_3^- + 0.54H^+ + 0.45e^- \rightarrow 0.045N_2 + 0.27H_2O$$

$$f_sR_c : 0.0196NO_3^- + 0.0982CO_2 + 0.5696H^+ + 0.55e^- \rightarrow 0.0196C_5H_7O_2N + 0.2161H_2O$$

$$-R_d : 0.0333C_6H_5COO^- + 0.4333H_2O \rightarrow 0.2CO_2 + 0.0333HCO_3^- + H^+ + e^-$$

$$R : 0.0333C_6H_5COO^- + 0.1096NO_3^- + 0.1096H^+ \rightarrow$$
$$0.0196C_5H_7O_2N + 0.045N_2 + 0.1018CO_2 + 0.0333HCO_3^- + 0.0528H_2O$$

We see that 0.09 mole nitrate is converted to nitrogen gas, and 0.0196 mole is converted into the organic nitrogen of the cells.

Bacterial growth of chemolithotrophic microorganisms

<u>Example (Nitrification stoichiometry)</u>:

Electron donor: NH_4^+
Electron acceptor : O_2
Nitrogen source : NH_4^+
Carbon source : CO_2
$f_s = 0.10$ $f_e = 0.90$
Waste amount (Q) : 1000 m^3/d
Concentration NH_4^+-N : 22 mg/l

For each 0.13(14)=1.82 g ammonium-nitrogen, 0.225(32)=7.2 g oxygen is consumed. Also, 0.005(113)=0.565 g cells and 0.125(14)=1.75 g NO_3^--N are produced. The amount of ammonium-nitrogen treated= (22 mg/l)(1000 m^3)(103 liters/m^3)(kg/10^6 mg)=22 kg.
Thus,
Oxygen consumption=22 kg(7.2 g/1.82 g)=87 kg
Cell dry weight produced=22 kg(0.565 g/1.82 g)=6.83kg
Effluent NO_3^--N conc.=22 mg/l(1.75 g/1.82 g)=21 mg/l

$$f_eR_a : 0.225O_2 + 0.9H^+ + 0.9e^- \rightarrow 0.45H_2O$$

$$f_sR_c : 0.02CO_2 + 0.005NH_4^+ + 0.005HCO_3^- + 0.1H^+ + 0.1e^- \rightarrow 0.005C_5H_7O_2N + 0.045H_2O$$

$$-R_d : 0.125NH_4^+ + 0.375H_2O \rightarrow 0.125NO_3^- + 1.25H^+ + e^-$$

$$R : 0.13NH_4^+ + 0.225O_2 + 0.02CO_2 + 0.005HCO_3^- \rightarrow 0.005C_5H_7O_2N + 0.125NO_3^- + 0.25H^+ + 0.12H_2O$$

Bacterial growth of methanogenic microorganisms

Example (methanogenesis stoichiometry):

Electron donor : $C_8H_{17}O_3N$
Electron acceptor : CO_2
Nitrogen source : NH_4^+
Carbon source : $C_8H_{17}O_3N$
$f_s = 0.08$ $f_e = 0.92$
Waste amount (Q) : 150 m^3/d
Concentration $C_8H_{17}O_3N$: 23,000 mg/l
Process efficient : 95%
Conditions: T = 35°C, P = 1 atm

The formula weight of $C_8H_{17}0_3N$ is 175, and the equivalent weight is 0.025(175), or 4.375 g.
Methane fermentation of one equivalent of organic matter produces 0.115 mol methane and 0.044 mol carbon dioxide. Thus,
Methane produced=[(273 + 35)/273][0.0224 m^3gas/mol][3,280,000g/d][0.115 mol/4.375 g]=2,180 m^3/d
Percent methane=100[0.115/(0.115 +0.044)]=72 percent

$$R_d : \frac{1}{40}NH_4^+ + \frac{1}{40}HCO_3^- + \frac{7}{40}CO_2 + H^+ + e^- \rightarrow \frac{1}{40}C_8H_{17}O_3N + \frac{7}{20}H_2O$$

$$f_eR_a : 0.115CO_2 + 0.92H^+ + 0.92e^- \rightarrow 0.115CH_4 + 0.23H_2O$$

$$f_sR_c : 0.016CO_2 + 0.004NH_4^+ + 0.004HCO_3^- + 0.08H^+ + 0.08e^- \rightarrow 0.004C_5H_7O_2N + 0.036H_2O$$

$$-R_d : 0.025C_8H_{17}O_3N + 0.35H_2O \rightarrow 0.025NH_4^+ + 0.025HCO_3^- + 0.175CO_2 + H^+ + e^-$$

$$R : 0.025C_8H_{17}O_3N + 0.084H_2O \rightarrow 0.004C_5H_7O_2N + 0.115CH_4 + 0.044CO_2 + 0.021NH_4^+ + 0.021HCO_3^-$$

Simple fermentation reactions

Electron donor: organic compound

Electron acceptor: organic compound

$$C_6H_{12}O_6 \rightarrow 2\,CO_2 + 2\,CH_3CH_2OH$$

$$R_a : \frac{1}{6}CO_2 + H^+ + e^- \rightarrow \frac{1}{12}CH_3CH_2OH + \frac{1}{4}H_2O$$

$$-R_d : \frac{1}{24}C_6H_{12}O_6 + \frac{1}{4}H_2O \rightarrow \frac{1}{4}CO_2 + H^+ + e^-$$

$$\mathrm{R_e} : \frac{1}{24}C_6H_{12}O_6 \rightarrow \frac{1}{12}CH_3CH_2OH + \frac{1}{12}CO_2$$

$$0.78R_a : 0.13CO_2 + 0.78H^+ + 0.78e^- \rightarrow 0.065CH_3CH_2OH + 0.195H_2O$$

$$0.22R_c : 0.044CO_2 + 0.011NH_4^+ + 0.011HCO_3^- + 0.22H^+ + 0.22e^- \rightarrow 0.011C_5H_7O_2N + 0.099H_2O$$

$$-R_d : 0.0417C_6H_{12}O_6 + 0.25H_2O \rightarrow 0.25CO_2 + H^+ + e^-$$

$$R : 0.0417C_6H_{12}O_6 + 0.011NH_4^+ + 0.011HCO_3^- \rightarrow 0.011C_5H_7O_2N + 0.065CH_3CH_2OH + 0.076CO_2 + 0.044H_2O$$

f_s = 0.22

f_e = 0.78

Mixed fermentation reactions

Electron donor: one or more organic compounds (e.g. municipal wastewaters)

Electron acceptor: one or more organic compounds (e.g. *E.coli*)

$$R_a = \sum_{i=1}^{n} e_{ai} R_{ai}$$

where

$$e_{ai} = \frac{equiv_{ai}}{\sum_{j=1}^{n} equiv_{aj}} \quad and \quad \sum_{i=1}^{n} e_{ai} = 1$$

$$R_d = \sum_{i=1}^{n} e_{di} R_{di}$$

where

$$e_{di} = \frac{equiv_{di}}{\sum_{j=1}^{n} equiv_{dj}} \quad and \quad \sum_{i=1}^{n} e_{di} = 1$$

Here, $\mathbf{e_{ai}}$ is the fraction of the *n* reduced end products that is represented by product *ai*.

$\mathbf{Equiv_{ai}}$ represents the equivalents of *ai* produced.

The sum of the fractions of all reduced end products equals 1.

Mixed fermentation reactions

CITRATE FERMENTATION TO TWO REDUCED PRODUCT

Bacteroides sp. converts 1 mol citrate into 1 mol formate, 2 mol acetate, and 1 mol bicarbonate. Write the overall balanced energy reaction (R_e) for this fermentation.

The reduced end products are formate and acetate. Bicarbonate, like carbon dioxide, is an oxidized end product and not considered in constructing the electron balance. The first step is to determine the number of equivalents ($equiv_{ai}$) formed for each reduced product.

$e_{formate}$ =2/(2+ 16) or 0.111, and $e_{acetate}$ = 16/(2+16) or 0.889.

The sum of $e_{formate}$ plus $e_{acetate}$ equals 1.0.

$$\frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^- = \frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O$$

$$\frac{1}{2}HCO_3^- + H^+ + e^- = \frac{1}{2}HCOO^- + \frac{1}{2}H_2O$$

Mixed fermentation reactions

$$0.111R_{formate}: 0.0555HCO_3^- + 0.111H^+ + 0.111e^- \rightarrow 0.0555HCOO^- + 0.0555H_2O$$

$$0.889R_{acetate}: 0.111CO_2 + 0.111HCO_3^- + 0.889H^+ + 0.889e^- \rightarrow 0.111CH_3COO^- + 0.333H_2O$$

$$R_a: 0.111CO_2 + 0.166HCO_3^- + H^+ + e^- \rightarrow 0.0555HCOO^- + 0.111CH_3COO^- + 0.388H_2O$$

The overall energy reaction (R_e) is then found using R_a - R_d, where R_d represents the half-reaction for citrate. Combining these produces the following for R_e:

$$0.0555(COO^-)CH_2COH(COO^-)CH_2COO^- + 0.056H_2O \rightarrow 0.0555HCOO^- + 0.111CH_3COO^- + 0.056CO_2$$

If this equation is normalized by dividing through by 0.0555, the moles of citrate in one equivalent, the following mole-normalized equation is obtained:

$$(COO^-)CH_2COH(COO^-)CH_2COO^- + H_2O \rightarrow HCOO^- + 2CH_3COO^- + CO_2$$

Energetics and bacterial growth

Microorganisms carry out oxidation-reduction reactions in order to obtain energy for **growth** and **cell maintenance**. The amount of energy released per electron equivalent of an electron donor oxidized varies considerably from reaction to reaction. It is not surprising then that the amount of growth that results from an equivalent of donor oxidized varies considerably as well.

Cell maintenance has energy requirements for activities such as cell movement and repair of cellular proteins (that decay because of normal resource recycling or through interactions with toxic compounds).

When cells grow rapidly in the presence of non limiting concentrations of all factors required for growth, cells make the maximum investment of energy for synthesis. However, when an essential factor, such as the electron-donor substrate, is limited in concentration, then a larger portion of the energy obtained from substrate oxidation must be used for cell maintenance.

$$Y_n = \frac{dX_a / dt}{-dS / dt} = Y - b\frac{X_a}{-dS / dt}$$

Energetics and bacterial growth

The electron equivalents are easily related to measurements of widespread utility in enviromental engineering practice, such as COD :

1 electron equivalent = 8 g O_2

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

COMPUTING COD'

A wastewater contains 12.6 g/l of ethanol. Estimate the e^- eq/l and the COD' (g/l) for this wastewater.

There are 12 e^- eq/mol ethanol. Since 1 mol of ethanol weighs 46 g, the equivalent weight is 46/12 or 3.83 g/e^- eq. The ethanol concentration in the wastewater is thus 12.6/3.83 or 3.29 e^- eq/l.

The COD' thus becomes (8 g OD/e^- eq)(3.29 e – eq/l) = 26.3 g/l.

$$\frac{1}{6}CO_2 + H^+ + e^- = \frac{1}{12}CH_3CH_2OH + \frac{1}{4}H_2O$$

Free energy of the energy reaction

Balanced half-reaction for 2-chlorobenzoate formation:

$$\frac{1}{28}HCO^{-}_{3(aq)} + \frac{3}{14}CO_{2(g)} + \frac{1}{28}Cl^{-}_{(aq)} + \frac{29}{28}H^{+}_{(aq.10^{-7})} + e^{-} \rightarrow \frac{1}{28}C_6H_4ClCOO^{-} + \frac{13}{28}H_2O_{(l)}$$

The free energies of formation for each species are (in kJ/e$^-$ eq):

$$\frac{1}{28}(\text{-}586.85),\ \frac{3}{14}(\text{-}394.36),\ \frac{1}{28}(\text{-}31.35),\ \frac{29}{28}(\text{-}39.87),\ 0,\ \frac{1}{28}(\text{-}237.9),\ \frac{13}{28}(\text{-}237.18)$$

The half-reaction free energy is calculated as the sum of the product free energies minus the sum of the reactant free energies, or **$\Delta G^{O\prime}$ = 29.26 kJ/e$^-$eq.**

Free energy of the energy reaction

One can adjust the reaction free energy for nonstandard concentrations of reactants and products:

$$\upsilon_1 \mathrm{A}_1 + \upsilon_2 \mathrm{A}_2 + \ldots \rightarrow \upsilon_m \mathrm{A}_m + \upsilon_{\mathrm{m+1}} \mathrm{A}_{\mathrm{m+1}} + \ldots + \upsilon_n \mathrm{A}_n$$

which can be written in an even more general form: $0 = \sum_{i=1}^{n} \upsilon_{ir} A_i$

The value of υ_{ir} is negative if constituent A_i appears on the left side of the above equation and positive if it appears on the right side. The nonstandard free energy change for this reaction can be determined from:

$$\Delta G_r = \Delta G_r^0 + RT \sum_{i=1}^{n} \upsilon_{ir} \ln a_i$$

Here, υ_{ir} represents the stoichiometric coefficient for constituent A_i in reaction r, and a_i represents the activity of constituent A_i.

T is absolute temperature (K).

Free energy of the energy reaction

$$\frac{1}{12}CH_3CH_2OH + \frac{1}{4}O_2 = \frac{1}{6}CO_2 + \frac{1}{4}H_2O$$

For this energy reaction, ΔG_r^0 equals $\Delta G_r^{0'}$ because H^+ is not a component of the equation; n = 4; and υ_{ik} equals -1/12, -1/4, 1/6, and 1/4 for ethanol, oxygen, carbon dioxide, and water, respectively.

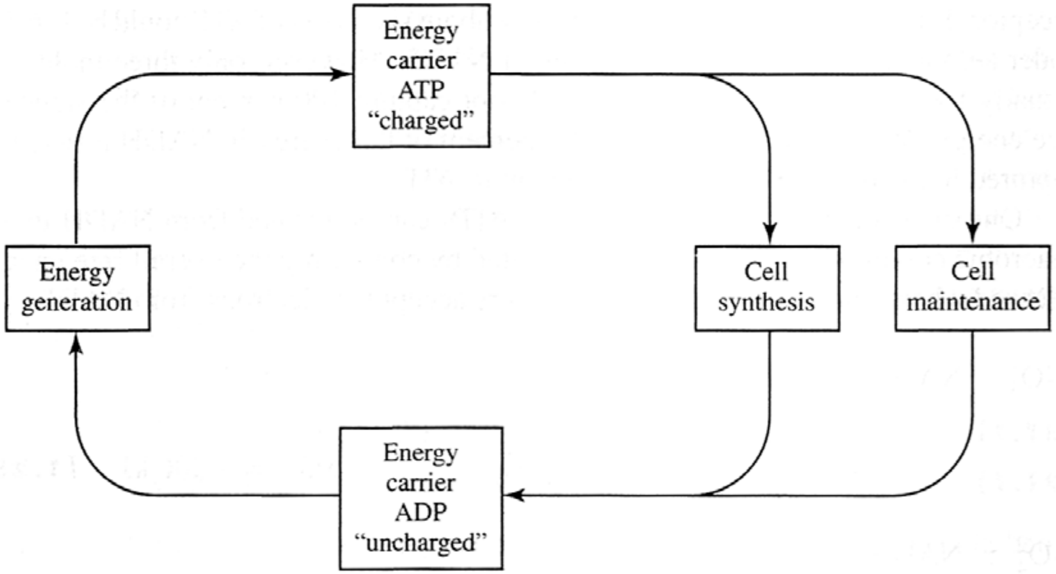
$$\Delta G_r = \Delta G_r^0 + RT \ln \frac{[CO_2]^{1/6}[H_2O]^{1/4}}{[CH_3CH_2OH]^{1/12}[O_2]^{1/4}}$$

We assume that the concentration of ethanol in an aqueous solution is 0.002 M, the oxygen partial pressure is that in the normal atmosphere at sea level (0.21 atm), the carbon dioxide concentration is that in normal air (0.0003 atm), and the temperature is 20°C. We also assume that the activities of the three constituents are equal to their molar concentration or partial pressure. (These values could be corrected if one knew the respective activity coefficients for each constituent. In this case the activity coefficient is likely to be close to 1.0.) With aqueous solutions in which water is the major solvent, the activity of H_20 is sufficiently close to 1. Then,

$$\Delta G_r = -109{,}900 + 8.314(273+20)\ln \frac{[0.0003]^{1/6}[1]^{1/4}}{[0.002]^{1/12}[0.21]^{1/4}}$$

$$= -111{,}000 J/e^- eq \text{ or } -111 kJ/e^- eq$$

Yield coefficient and reaction energetics



Transfer of energy from energy generation to cell synthesis or maintenance via an energy carrier, represented by ATP.

The energy carriers are "spent" to drive cell synthesis or cell maintenance. As with all reactions, a certain amount of thermodynamic free energy is lost with each transfer.

Yield coefficient and reaction energetics

We must determine the **energy change** resulting from the conversion of the carbon source to **pyruvate** (as a representative intermediate) (ΔG_p).

$$\mathbf{\Delta G_p = 35.09 - \Delta G_c^{0'}}$$

Where $\mathbf{\Delta G_c^{0'}}$ is the half-reaction free energy of carbon source, which for heterotrophic bacteria is the electron donor.

e.g. For pyruvate: $\Delta G_c^{0'}$ = 27.4 kJ/e^- eq.

In photosynthesis we can determined the energy involved $\Delta G_c^{0'}$ = -78.72 kJ/e^- eq (for the half-reaction H_2O-O_2), thus $\mathbf{\Delta G_p = 35.09 - (-78.72) = 113.8}$ kJ/e^- eq.

When pyruvate is converted to cellular carbon, the energy required is : $\mathbf{\Delta G_{pc}}$ **= 3.33 kJ/g cells or 18.8 kJ/e^- eq**

Yield coefficient and reaction energetics

Energy is always lost in the electron transfers. The energy requirement for cell synthesis becomes:

$$\Delta G_s = \Delta G_p/\varepsilon^n + \Delta G_{pc}/\varepsilon$$

where **ε : term of energy-transfer efficiency (0.55 – 0.70), typical value = 0.6**

For some electron donors, such as glucose **n = -1 (energy is obtained by its conversion to pyruvate or ΔG_p <0).**
In other cases, such as with acetate **n = 1 (energy is required in its conversion to pyruvate or ΔG_p >0).**

Energy required in **A equivalent electron donor** for the synthesis of 1 equivalent cells.

Energy balance:

$$A\, \varepsilon\, \Delta G_r + \Delta G_S = 0 \quad \rightarrow \quad A = (\Delta G_p/\varepsilon^n + \Delta G_{pc}/\varepsilon) / \varepsilon\, \Delta G_r$$

where ΔG_r is the free energy released per equivalent of donor oxidized for energy generation.

Yield coefficient and reaction energetics

Since part of the donor consumed is used for energy (**A equivalents in this case**) and the other part for synthesis (**1.0 equivalents in this case**), the total donor used is **1 +A**.

$$f_s^0 = \frac{1}{1+A} \qquad f_e^0 = 1 - f_s^0 = \frac{A}{1+A}$$

Yield coefficient and reaction energetics

EFFECTS OF ε ON HETEROTROPHIC YIELD

Compare estimates for f_s^0 and Y for aerobic oxidation of acetate, assuming ε=0.4, 0.6, and 0.7, that pH=7, and that all other reactants and products are at unit activity. Ammonium is available for synthesis.

$$\Delta G_p = 35.09 - 27.40 = 7.69\, kJ / e^{-} eq.$$

Since this is an aerobic reaction $\Delta G_a^{0'} = -78.72\, kJ / e^{-} eq.$

$$\Delta G_r = \Delta G_a^{0'} - \Delta G_d^{0'} = -78.72 - 27.40 = -106.12\, kJ / e^{-} eq.$$

Since ΔG_p is positive, n = +1. Also, since ammonium is available for cell synthesis, ΔG_{pc} equals 18.8 kJ/e^- eq. Hence,

$$A = \frac{\dfrac{7.69}{\varepsilon^{+1}} + \dfrac{18.8}{\varepsilon}}{-106.12\varepsilon}$$

References

The images where their origin is not mentioned are derived from the book:

Environmental Biotechnology : Principles and Applications,

Bruce E. Rittmann and Perry L. McCarty,

McGraw-Hill Series in Water Resources and Environmental Engineering

Τέλος Ενότητας

Χρηματοδότηση

- Το παρόν εκπαιδευτικό υλικό έχει αναπτυχθεί στο πλαίσιο του εκπαιδευτικού έργου του διδάσκοντα.
- Το έργο «**Ανοικτά Ακαδημαϊκά Μαθήματα στο Πανεπιστήμιο Αθηνών**» έχει χρηματοδοτήσει μόνο την αναδιαμόρφωση του εκπαιδευτικού υλικού.
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Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης

Σημειώματα

Σημείωμα Ιστορικού Εκδόσεων Έργου

Το παρόν έργο αποτελεί την έκδοση 1.0.0.

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