1 Stoichiometry

Based on: “Thermodynamics for Microbial Electrocatalysis” by René Rozendal and Korneel Rabaey.

1.1 Introduction

A key aspect of process development and performance assessment is the relationship between *input* (type of substrate/product, concentration) and *output* (removal). This requires an understanding of the thermodynamics of the system, the calculations for which are all too often considered as cumbersome and prone to errors. Here, we present a method that simplifies thermodynamic calculations. This method is based on the *Growth Reference System* (GRS) as designed by J.J. Heijnen in 1999.

1.2 Growth Reference System

The most important feature of the GRS is the fact that, compared to the conventional thermodynamic system, it chooses a different reference for all thermodynamic data. Whereas the conventional thermodynamic system is defined in such a way that the Gibbs energy of formation is zero for the elements in their most stable state (e.g., $H_2(g)$, $O_2(g)$, $N_2(g)$, C(s), etc.) at standard conditions (i.e., 1 bar, 298.15 K, 1 mol/L, pH 0), the GRS is defined in such a way that the Gibbs energy is zero for the end products typically occurring in microbial systems ($HCO_3^-(aq)$, $SO_4^{2-}(aq)$, $NO_3^-(aq)$, H_2O, $H^+(aq)$, etc.) at biochemical standard conditions (i.e., 1 atm, 298.15 K, 1 mol/L, pH 7). In addition, the GRS defines three simple numbers for each chemical compound, i.e., (i) degree of reduction (γ), (ii) Gibbs energy per electron, and (iii) enthalpy per electron. These numbers further simplify calculations.

1.2.1 Degree of reduction

For each component participating in an electrochemical reaction, the GRS defines a degree of reduction, which can be calculated using Table 1. The interesting feature of this definition is that the degree of reduction is zero for the following typical end products in microbial systems: bicarbonate (HCO_3^-), sulfate (SO_4^{2-}), nitrate (NO_3^-), water (H_2O), and protons (H^+). These compounds are also referred to as the reference compounds. For instance, the degree of reduction of bicarbonate is calculated as 1 (H) + 4 (C) + 3×-2 (O) + 1 (negative charge) = 0. All other compounds have a degree of reduction different from zero. For instance, acetate ($C_2H_3O_2^-$) has a positive degree of reduction of 2×4 (C) + 3×1 (H) + 2×-2 (O) +1 (negative charge) = 8, while oxygen gas, a typical electron acceptor in microbial systems, has a negative degree of reduction of 2×-2 (O) = -4. The value of the degree of reduction is equal to the electron content of a specific compound, i.e., the number of electrons an electron donor can donate or an electron acceptor can accept per mole of compound in order to be completely oxidized or reduced to reference compounds. It is thus the oxidation state of these atoms in the reference compounds.

One additional advantage of the definition of the degree of reduction of compounds in the GRS is that it simplifies the calculation of reaction stoichiometry, especially also in the context of BESs. The reason for this is that the degree of reduction is equivalent to the electron content of a specific compound. Moreover, in most half reactions there are only few compounds that have a degree of reduction other than zero. Therefore, an electron balance can be quickly made. Using this electron balance, the reaction stoichiometry can be easily solved using simple sequential steps. A calculation example for both a half reaction and a complete reaction stoichiometry can be found in Example 1.

Example 1: Calculation examples for determining reaction stoichiometry using the GRS

In the anode half reaction for acetate oxidation to bicarbonate and electrons, acetate is the only compound that has a degree of reduction other than zero (i.e., 8 e^-).

$C_2H_3O_2^- + 4H_2O \leftrightarrows 2HCO_3^- + 9H^+ + 8e^-$

All other compounds involved in this reaction, i.e., bicarbonate, water, and protons, all have a zero degree of reduction and do not influence the balance of the degree of reduction. Therefore, by using the balance of degree of reduction, one can instantly derive that the amount of electrons generated in the anode reaction is eight:

$C_2H_3O_2^- \rightarrow 8\ e^-$

Then, by sequentially solving carbon balance using bicarbonate;

$C_2H_3O_2^- \rightarrow 2\ HCO_3^- + 8\ e^-$

the oxygen balance using water;

$C_2H_3O_2^- + 4\ H_2O \rightarrow 2\ HCO_3^- + 8\ e^-$

and the hydrogen balance using protons:

$C_2H_3O_2^- + 4\ H_2O \rightarrow 2\ HCO_3^- + 9\ H^+ + 8\ e^-$

one can quickly derive the complete stoichiometry of a half reaction. As a final check, one can then verify that the charge is equal on both sides of the equation.

Equally simple as for half reactions, also the complete reaction stoichiometries can be determined. For example, for the reaction stoichiometry of a reactor operated on acetate at biochemical standard conditions, the only compounds that have a degree of reduction other than zero are acetate (i.e., 8 e^-) and oxygen (i.e., -4 e^-). Therefore, by using the balance of degree of reduction, one can instantly derive that acetate and oxygen react in a 1:2 ratio:

$C_2H_3O_2^- + 2\ O_2 \rightarrow$

Then, by sequentially solving carbon balance using bicarbonate;

$C_2H_3O_2^- + 2\ O_2 \rightarrow 2\ HCO_3^-$

the oxygen balance using water;

$C_2H_3O_2^- + 2\ O_2 \rightarrow 2\ HCO_3^-$ (no water involved)

and the hydrogen balance using protons:

$C_2H_3O_2^- + 2\ O_2 \rightarrow 2\ HCO_3^- + H^+$

one can quickly derive the stoichiometry of the complete reaction. Note that determining the electron donor to oxygen ratio is also a rapid method for determining the COD value of a certain compounds. E.g. for the above case: 1 mol of acetate requires 2 moles of oxygen. Multiplied by the molecular weight of oxygen gas this gives 2×32=64 g COD.

Exercise 1:

Balance the nitrification half-reactions and the total reaction by following the rules above.

Exercise 2:

Calculate the COD of acetate, ethanol and glucose.

1.2.2 Gibbs energy per electron

The GRS also defines a Gibbs energy value *per electron* for each specific compound. This new value is easily calculated from Gibbs energy of formation data in conventional thermodynamic tables in two simple steps. First, a reference half reaction is written out, which is the production reaction of a specific compound from reference compounds (e.g., HCO_3^-(aq), SO_4^{2-}(aq), NO_3^-(aq), H_2O, H^+(aq), etc.) and electrons. E.g. for acetate:

$$2\ HCO_3^- + 9\ H^+ + 8\ e^- \rightarrow C_2H_3O_2^- + 4\ H_2O \qquad (1)$$

Secondly, using the conventional thermodynamic tables, the Gibbs energy change of this specific reference reaction is calculated at biochemical standard conditions and subsequently divided by the amount of electrons (i.e., the degree of reduction γ) involved in the reaction:

$$\Delta G_e^{0'} = \frac{\Delta G_{ref}^{0'}}{\gamma} \qquad (2)$$

In which $\Delta G_e^{0'}$ is the Gibbs energy value *per electron* present in a specific compound at biochemical standard conditions and $\Delta G_{ref}^{0'}$ is the Gibbs energy change of the reference reaction of this specific compound at biochemical standard conditions. E.g., calculating the $\Delta G_e^{0'}$ for acetate:

$$\Delta G_{ref}^{0'} = -2 \times \Delta G_{f\_HCO_3^-}^{0'} - 9 \times \Delta G_{f\_H^+}^{0'} + \Delta G_{f\_C_2H_3O_2^-}^{0'} + 4 \times \Delta G_{f\_H_2O}^{0'} \quad (3a)$$

$$\Delta G_{ref}^{0'} = -2 \times -586.85 - 9 \times -39.87 + -369.41 + 4 \times -237.18 = 214.4 \text{ kJ/mol} \quad (3b)$$

$$\Delta G_e^{0'} = \frac{214.4}{8} = 26.80 \text{ kJ/e - mol} \quad (3c)$$

Table 2 lists γ and $\Delta G_e^{0'}$ values for chemical compounds relevant in the context of existing bioelectrochemical conversions. A very important note of caution is thermodynamic values listed in Table 2 should never be used in combination with conventional thermodynamic data due to the different reference used (i.e., standard conditions vs. biochemical standard conditions; see above), as this unavoidably results in miscalculation.

1.3 Simplifying thermodynamical calculations

At first glance, the GRS might appear to be a highly elaborate recalculation of already existing data. However, when using the system, it quickly becomes clear that it can drastically simplify thermodynamic calculations in microbial systems. Electrode potential and cell voltages are calculated from the Gibbs energy change of a reaction (Text Box), which can be calculated from the tabulated data in the GRS according to:

$$\Delta G_r^{0'} = \sum \left(\nu_X \times \gamma_X \times \Delta G_{e,X} \right) \quad (4)$$

with ν_X is the stoichiometric reaction coefficient of compound X involved in the reaction (positive for products and negative for reactants). The most important advantage of using the GRS is that most of the compounds involved in the bioelectrochemical reaction are reference compounds, such as HCO_3^-(aq), SO_4^{2-}(aq), NO_3^-(aq), H_2O, and H^+(aq), which can be eliminated from the calculations, as the value of γ and $\Delta G_e^{0'}$ for these compounds is zero per definition. For instance, the reaction equation of an MFC operated on acetate only contains two compounds that are not reference compound (i.e., acetate and oxygen). Therefore, the theoretical cell voltage of this reaction at biochemical standard conditions can be easily calculated according to (according to Text Box; thermodynamic data from Table 2):

$$C_2H_3O_2^- + 2\ O_2 \rightarrow 2\ HCO_3^- + H^+ \quad (5)$$

$$\Delta G_r^{0'} = -1 \times \gamma_{C_2H_3O_2^-} \times \Delta G_{e\_C_2H_3O_2^-}^{0'} - 2 \times \gamma_{O_2} \times \Delta G_{e\_O_2}^{0'} + 2 \times \gamma_{HCO_3^-} \times \Delta G_{e\_HCO_3^-}^{0'} + 1 \times \gamma_{H^+} \times \Delta G_{e\_H^+}^{0'} \quad (6a)$$

$$\Delta G_r^{0'} = -1 \times 8 \times 26.801 - 2 \times -4 \times -78.719 + 0 + 0 = -844.16 \text{ kJ/mol} \quad (6b)$$

$$E^{0'} = -\frac{\Delta G_r^{0'}}{nF} = -\frac{-844.16}{8 \times 96.4853} = 1.09 \text{ V} \quad (6c)$$

This advantage is even more evident when calculating the equilibrium potentials of half

reactions, as many half reactions only have one compound that is not a reference compound. According to the IUPAC convention, equilibrium potentials for half reactions involving a certain electron acceptor/electron donor couple are calculated from the reduction reaction, i.e., the electron consuming reaction with the electron acceptor in the left side of the equation and the electron donor in the right side of the equation (e.g., Eq. (1) for acetate). Similar to above, the equilibrium potential of the half reaction for acetate oxidation to bicarbonate is then calculated according to (based on Eq. (1) according to Eq. (4) and Text Box; thermodynamic data from Table 2):

$$2\ HCO_3^- + 9\ H^+ + 8\ e^- \rightarrow C_2H_3O_2^- + 4\ H_2O$$

$$\begin{aligned}\Delta G_r^{0'} = &-2\times\gamma_{HCO_3^-}\times\Delta G^{0'}_{e\_HCO_3^-} - 9\times\gamma_{H^+}\times\Delta G^{0'}_{e\_H^+} \\ &+1\times\gamma_{C_2H_3O_2^-}\times\Delta G^{0'}_{e\_C_2H_3O_2^-} + 4\times\gamma_{H_2O}\times\Delta G^{0'}_{e\_H_2O}\end{aligned} \quad (7a)$$

$$\Delta G_r^{0'} = 0 - 0 + 1\times 8\times 26.801 + 0 = 214.4\ \text{kJ/mol} \quad (7b)$$

$$E^{0'} = -\frac{\Delta G_r^{0'}}{nF} = -\frac{214.41}{8\times 96.485} = -0.278\ \text{V} \quad (7c)$$

Or, if the half reaction only contains one compound that is not a reference compound, the equilibrium potential of the half reaction can just simply be calculated from the Gibbs energy value *per electron* according to:

$$E^{0'} = -\frac{\Delta G_e^{0'}}{F} \quad (8)$$

Again, in the example of the half reaction for acetate oxidation to bicarbonate:

$$2\ HCO_3^- + 9\ H^+ + 8\ e^- \rightarrow C_2H_3O_2^- + 4\ H_2O$$

$$E^{0'} = -\frac{\Delta G^{0'}_{e\_C_2H_3O_2^-}}{F} = -\frac{26.801}{96.485} = -0.278\ \text{V} \quad (9)$$

Or for the half reaction for oxygen reduction to water:

$$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O \quad (10)$$

$$E^{0'} = -\frac{\Delta G^{0'}_{e\_O_2}}{F} = -\frac{-78.719}{96.485} = 0.815\ \text{V} \quad (11)$$

Table 3, 4 and 5 list the Gibbs energy change and the equilibrium potential of specific half reactions at biochemical standard conditions. Table 3 lists the thermodynamic data for selected half reactions involving bicarbonate as the electron acceptor; Table 4 lists the thermodynamic data for selected half reactions involving an organic electron acceptor; and Table 5 lists the thermodynamic data for selected half reactions involving an inorganic electron acceptor (other than bicarbonate).

Equilibrium potentials of half reactions can conveniently be used for calculating the theoretical cell voltage by subtracting the equilibrium potential of the anode reaction from that of the cathode reaction. E.g., for an MFC operated on acetate:

$$E_{cell}^{0'} = E_{cathode}^{0'} - E_{anode}^{0'} = 0.815 - 0.278 = 1.09 \text{ V} \quad (12)$$

Evidently, this will give the same result as Eq. 6.

1.4 Concentration effects

The thermodynamic data of the GRS and thus the numbers in Table 3, 4, and 5 are reported at biochemical standard conditions, which are defined as 1 atm, 298.15 K, 1 mol/L, and pH 7. Evidently, a real system is not always operating at biochemical standard conditions. Therefore, the Gibbs energy change and equilibrium potential typically need to be corrected for the effect of partial pressure, temperature, concentration, and pH. For the half reaction:

$$\nu_A A + \nu_B B + \nu_H H^+ + n\, e^- \rightarrow \nu_C C + \nu_D D \quad (13)$$

in which compound A, B, and protons are converted into compound C and D, consuming n electrons and with ν_i being the reaction coefficient of the specific reactants, protons, and products, the Gibbs energy change of the half reaction can be corrected for the effect of the pressure, temperature, concentration, and pH using the following equation:

$$\Delta G_r = \Delta G_r^{0'} + RT\, ln(\Pi) - RT\, ln\left(\left[\frac{10^{-pH}}{10^{-7}}\right]^{\nu_H}\right) \quad (14)$$

The last term in Eq. 14 is a consequence of the chosen reference of pH 7 in the GRS. Π is defined as:

$$\Pi = \frac{a_C^{\nu_C} a_D^{\nu_D}}{a_A^{\nu_A} a_B^{\nu_B}} \approx \frac{[C]^{\nu_C}[D]^{\nu_D}}{[A]^{\nu_A}[B]^{\nu_B}} \quad (15)$$

With a_i the activity of a specific compound and $[i]$ the concentration (in mol/l) or partial pressure (in atm) of a specific compound i. In dilute systems, which is typically the case in microbial systems, calculations can be conveniently simplified by estimating the activities as concentrations.

Accordingly, the equilibrium potential of the half reaction can be corrected for the effect of the pressure, temperature, concentration, and pH using the following equation (i.e., Eq. 14 divided by -*nF*):

$$E_r = E_r^{0'} - \frac{RT}{nF} ln(\Pi) + \frac{RT}{nF} ln\left(\left[\frac{10^{-pH}}{10^{-7}}\right]^{\nu_H}\right) \quad (16)$$

Due to the exponential nature of the pH scale, the pH value can have a very strong effect on the equilibrium potential, which is demonstrated in Figure 1 for half reactions that involve as many protons involved in the reaction as electrons (i.e., ν_H=n).

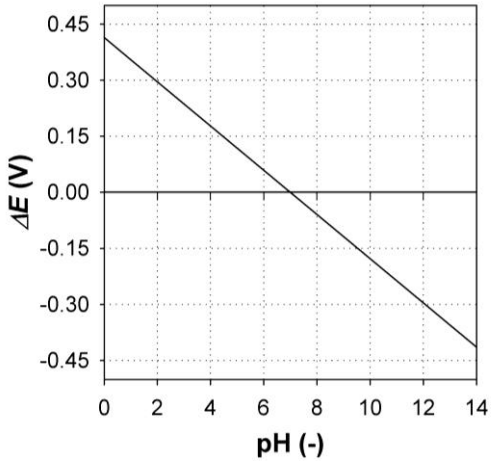

Figure 1. Effect of pH on the equilibrium potential of half reactions that involve as many protons involved in the reaction as electrons (i.e., ν_H=n) at 298.15 K.

As can be seen in Figure 1, the equilibrium potential of such reactions decreases with increasing pH and shifts by about 0.059 V per pH unit. Or, more generally, the equilibrium potential of half reaction $\nu_A A+\nu_B B+\nu_H H^+ + n\,e^- \rightarrow \nu_C C+\nu_D D$ (i.e., involving protons as reactants), *decreases* by about $\left[\frac{\nu_H RT}{nF}\right] ln(10)$ V per pH unit *increase* and *increases* by about $\left[\frac{\nu_H RT}{nF}\right] ln(10)$ V per pH unit *decrease*. At a temperature of 298.15 K, this means that the equilibrium potential *decreases* by about $\left[\frac{\nu_H}{n}\right]$ times 0.059 V per pH unit *increase* and *increases* by about $\left[\frac{\nu_H}{n}\right]$ times 0.059 V per pH unit *decrease*.

Similarly, also concentrations/partial pressure can have a significant effect on the equilibrium potential (Figure 2). In general, the equilibrium potential of half reaction $\nu_A A+\nu_B B+\nu_H H^+ + n\,e^- \rightarrow \nu_C C+\nu_D D$, increases by about $\left[\frac{\nu_i RT}{nF}\right] ln(10)$ V per 10 fold increase of reactants A or B and decreases by about $\left[\frac{\nu_i RT}{nF}\right] ln(10)$ V per 10 fold increase of products C or D. At a temperature of 298.15 K, this means that the equilibrium potential increases by about $\left[\frac{\nu_i}{n}\right]$ times 0.059 V per 10 fold increase of reactants A or B and decreases by about $\left[\frac{\nu_i}{n}\right]$ times 0.059 V per 10 fold increase of products C or D.

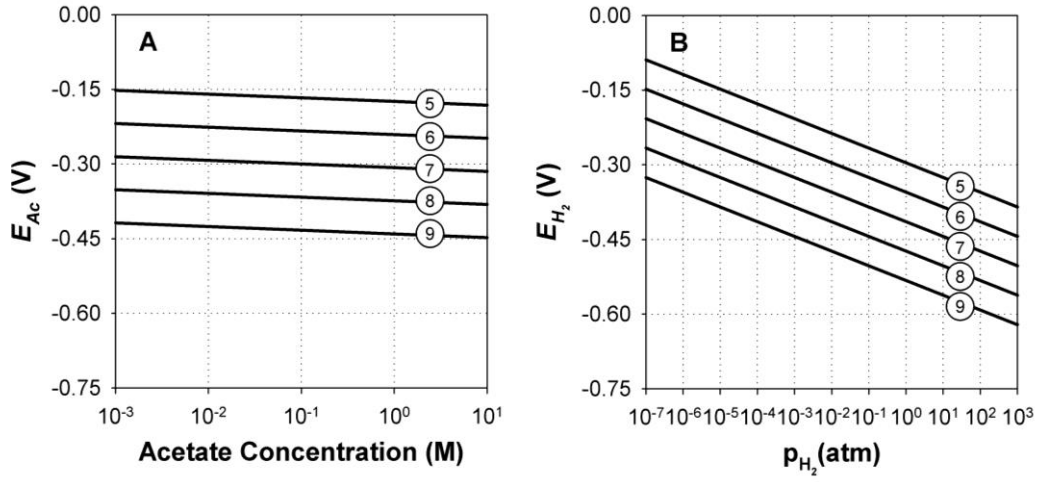

Figure 2. Effect of concentration/partial pressure on the equilibrium potential of specific half reactions: (A) effect of acetate concentration on the equilibrium potential of the half reaction 2 HCO_3^- + 9 H^+ + 8 e^- ⇆ Acetate + 4 H_2O in the pH range 5 to 9 (298.15 K; 0.01 M HCO_3^-), (B) effect of hydrogen partial pressure on the equilibrium potential of the half reaction 2 H^+ + 2 e^- ⇆ H_2 in the pH range 5 to 9 (298.15 K).

The extent of this concentration effect on the equilibrium potential highly depends on the type of reactant/products involved in the reaction. Figure 2A shows that at constant pH the effect of concentration on the equilibrium potential of acetate oxidation is relatively low in the typical concentration range occurring in microbial systems (10^{-3}-10^{1} M). The reason for this is that most electron donors have a high degree of reduction (i.e., n is high, so $\left[\frac{\nu_i}{n}\right]$ is low). E.g., for acetate $\left[\frac{\nu_i}{n}\right]$ equals 1/8 and hence the equilibrium potential only decreases by about 0.0074 V per 10 fold increase of the acetate concentration (at a temperature of 298.15 K).

For hydrogen, however, a more significant shift in equilibrium potential can be observed. One of reasons for this is that it only has a degree of reduction of 2 (i.e., n is low, so $\left[\frac{\nu_i}{n}\right]$ is high) and hence the shift in equilibrium potential is about 0.03 V per 10 fold change in partial pressure (at a temperature of 298.15 K). In addition, hydrogen is known for its wide partial pressure range in microbial systems (10^{-7}-10^{3} atm), which, as can be seen in Figure 2, can cause a large shift in equilibrium potential. The latter is also of particular importance when discussing whether hydrogen is a possible intermediate in microbially catalysed cathode reactions. As can be seen from Figure 2, hydrogen can already start evolving from about -0.33 V at pH 7 if the hydrogen partial pressure is kept below 10^{-3} atm, which is indeed a typical hydrogen partial pressure that can be observed in syntrophic methanogenic populations. Hence, a potential above -0.41 V or a

hydrogen concentration below the detection limit of a standard gas chromatograph, is not necessarily proof that hydrogen is not an intermediate.

An important note of caution with the above calculations is that they do not take acid-base speciation into account. The thermodynamic effects of acid-base speciation are generally very small, but can be of importance in some biological systems where thermodynamic gains are small, such as in syntrophic methanogenic populations or the alcohol production through the reduction of volatile fatty acids with hydrogen as the electron donor. Under those conditions, acid-base speciation should be included in thermodynamic calculations as described by Dolfing and coworkers. For instance, Dolfing and co-workers calculated that if the Gibbs energy data of the most dominantly species (i.e., the weak acid/base vs. the conjugated base/acid) present under the actual working conditions is used for calculations, the maximum error of neglecting acid-base speciation is about 1.72 kJ/mol. This value is indeed 2 to 3 orders of magnitude smaller than the actual Gibbs energies of the specific half reactions tabulated in Table 3, 4, and 5.

Exercise 3: The reaction $HCOOH \rightarrow H_2 + CO_2$ is thermodynamically unfavourable. Prove this by calculating the ΔG_r. However, it is possible to microbially produce hydrogen from formate if hydrogen is consumed by another species, i.e. the methanogens: $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$. Calculate the new ΔG_r assuming that the methanogens keep the concentration of H_2 [H_2] at 10^{-5} atm. The reaction occurs at pH 7 and temperature 25°C.

1.5 Stoichiometry in conventional wastewater treatment

In this section, the stoichiometry of the most common reactions in conventional wastewater treatment is explained. The basic reactions involve the removal of organic material (mainly C) and nutrients (N, P, S).

1.5.1 Organic matter removal

Organic matter in domestic wastewater can on average chemically be represented by the molecule $C_{10}H_{19}O_3N$. In order to simplify, we use glucose as the model compound of organic waste.

- Removal under aerobic conditions:

Glucose is used as the electron donor, oxygen as the electron acceptor. Using the data in Table 3, 4 and 5, we can balance the half reactions and calculate the overall Gibbs free energy as follows:

	$\Delta G^{0'}_r$ (kJ/mol)
Glucose + 12 H_2O ⇆ 6 HCO_3^- + 30 H^+ + 24 e^-	-954
6 * (O_2 + 4 H^+ + 4 e^- ⇆ H_2O	-315)
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Glucose + 6 O2 + 6 H2O → 6 HCO_3^- + 6 H^+	-2844

This reaction has a highly negative Gibbs free energy, implicating that this reaction is thermodynamically feasible for bacteria to perform.

- Removal under anoxic/anaerobic conditions

Whenever O_2 is present, bacteria will always consume O_2 as the electron acceptor. However, this requires aeration of the system, which will imply a cost. When O_2 is not present, bacteria will look for alternative electron acceptors, such as NO_3^-, SO_4^{2-} or HCO_3^-. Reactions involving nitrate or sulfate reduction are discussed further, as they are essential steps in the removal of nutrients. When HCO_3^- is used as electron acceptor, this reaction yields methane, a possible energy source. However, it is difficult to perform this reaction at atmospheric temperatures.

	$\Delta G^{0'}_r$ (kJ/mol)
Glucose + 12 H_2O ⇆ 6 HCO_3^- + 30 H^+ + 24 e^-	-954
3 * (HCO_3^- + 9 H^+ + 8 e^- ⇆ CH_4 + 4 H_2O	183)
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Glucose → 3 HCO_3^- + 3 H^+ + 3 CH_4	-405

The Gibbs free energy of this reaction is less negative than the reaction with oxygen, implicating a less spontaneous process. Whenever oxygen is present, bacteria will use it as electron acceptor, since it is thermodynamically more favorable.

1.5.2 Removal of nutrients

- **Nitrogen removal**

In most conventional biological wastewater treatment systems, N is removed by the combination of aerobic nitrification and anoxic denitrification.

Nitrification

The nitrification reaction uses ammonium as the electron donor and oxygen as the electron

acceptor. Note that this process is performed by 2 distinct types of bacteria: ammonia oxidizing species (AOB) oxidize ammonia to nitrite (NO_2^-), and nitrite oxidizing bacteria (NOB) oxidize nitrite to nitrate (NO_3^-).

	$\Delta G^{0'}_r$ (kJ/mol)
$NH_4^+ + 3\ H_2O \leftrightarrows NO_3^- + 10\ H^+ + 8\ e^-$	-281
$2 * (O_2 + 4\ H^+ + 4\ e^- \leftrightarrows 2\ H_2O$	-315)
---	---
$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + 2\ H^+ + H_2O$	-911

Denitrification

Through denitrification, nitrite and nitrate are removed from the system as the gaseous N_2. For this process, an electron donor is required. Preferably, this e- donor is present in the wastewater as residual COD. In some cases however, it needs to be added externally.

	$\Delta G^{0'}_r$ (kJ/mol)
$5 * (Glucose + 12\ H_2O \leftrightarrows 6\ HCO_3^- + 30\ H^+ + 24\ e^-$	-954)
$24 * (NO_3^- + 6\ H^+ + 5\ e^- \leftrightarrows 0.5\ N_2 + 3\ H_2O$	-361)
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$5\ Glucose + 24\ NO_3^- \rightarrow 12\ N_2 + 6\ H^+ + 12\ H_2O + 30\ HCO_3^-$	-13434

Exercise 4: Calculate how much glucose is needed per gram of nitrate-N removed. Also calculate how much methanol would be needed as carbon source (instead of glucose; methanol is converted to HCO_3^-). Calculate the cost of the addition of both carbon sources (methanol € 0.35/kg; glucose € 0.3/kg)?

- **Sulfur removal**

Sulfate is the most abundant inorganic S- species in wastewater. It can be reduced to sulfide by a specific group of sulfate reducing bacteria (SRB). This is a very common reaction in sewer systems, which is to be avoided, since it causes severe corrosion of the sewer pipes. It uses COD, usually present in untreated sewage, as the electron donor.

	$\Delta G^{0'}_r$ (kJ/mol)
Glucose + 12 H_2O ⇆ 6 HCO_3^- + 30 H^+ + 24 e^-	-954
3 * (SO_4^{2-} + 9 H^+ + 8 e^- ⇆ HS^- + 4 H_2O	167)
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Glucose + 3 SO_4^{2-} → 3 HS- + 6 HCO_3 + 3 H^+	-453

1.6 Biomass production

The balanced reactions described above only include catabolic processes, these are metabolic processes performed by microorganisms to gain energy. However, microbes will always consume part of the electron donor for biomass synthesis. Table 1 lists the fraction of the electron donor used for biomass formation (mol biomass/mol donor consumed). These values are given on a mole basis, not on a mass (or gram) basis.

Table 1: Mole fractions of the electron donor used for biomass synthesis in the different reactions

Electron donor	Electron acceptor	Fraction for synthesis
Glucose	O_2	0.7
Glucose	HCO_3^-	0.05
Methanol	NO_3^-	0.5
NH_4^+	O_2	0.1

Note that the anaerobic organic removal process leads to less biomass production than the aerobic organic removal process; less biomass is thus produced in the anaerobic process. As biomass (sludge) is a waste product of wastewater treatment with a concomitant treatment cost, this is a big advantage of the anaerobic process.

Tables:

Table 1. Degree of reduction of atoms and charge as defined in the Growth Reference System (after Heijnen[1])

Atom/Charge	γ
C	4
H	1
O	-2
N	5
S	6
Halogen (F, Cl, Br, I) [a]	-1
Positive Charge	-1
Negative Charge	+1

[a] Added by the authors.

Table 2. Degree of reduction (γ) and Gibbs energy per electron $\Delta G_e^{0'}$ of specific compounds as defined in the Growth Reference System (biochemical standard conditions: 1 atm, 298.15 K, 1 mol/L, pH 7). Data from Heijnen[1] unless otherwise indicated.

Chemical Compound	Chemical Formula	γ (electrons)	$\Delta G_e^{0'}$ (kJ/e-mol)
Reference compounds			
Bicarbonate	HCO_3^-	0	0
Sulfate	SO_4^{2-}	0	0
Nitrate	NO_3^-	0	0
Water	H_2O	0	0
Proton	H^+	0	0
Halide ions	(F^-, Cl^-, Br^-, I^-)	0	0 [a]
Fe^{3+}	Fe^{3+}	0	0
Alkanes			
Methane	CH_4	8	22.925
Ethane	C_2H_6	14	25.404
Propane	C_3H_8	20	25.948
Carboxylic acids			
Formate	CHO_2^-	2	39.186
Acetate	$C_2H_3O_2^-$	8	26.801
Propionate	$C_3H_5O_2^-$	14	26.939
Butyrate	$C_4H_7O_2^-$	20	27.000
Oxalate	$C_2HO_4^-$	2	52.522
Citrate	$C_6H_7O_7^-$	18	32.282
Fumarate	$C_4H_3O_4^-$	12	33.662
Succinate	$C_4H_5O_4^-$	14	28.405
Lactate	$C_3H_5O_3^-$	12	31.488
Pyruvate	$C_3H_3O_3^-$	10	34.129
Gluconate	$C_6H_{11}O_7^-$	22	39.106
β-hydroxybutyrate	$C_4H_7O_3^-$	18	30.220 [a]
Carbohydrates			
Glucose	$C_6H_{12}O_6$	24	39.744
Fructose	$C_6H_{12}O_6$	24	39.820
Galactose	$C_6H_{12}O_6$	24	39.481
Sucrose	$C_{12}H_{22}O_{11}$	48	40.690
Glycogen (per glucose unit)	$C_6H_{10}O_5$	24	40.482
Alcohols			
Methanol	CH_4OH	6	36.032
Ethanol	C_2H_5OH	12	30.353
n-Propanol	C_3H_7OH	18	29.144
n-Butanol	C_4H_9OH	24	28.466 [a]

Ethylene glycol	$C_2H_6O_2$	10	37.292
Propanediol	$C_3H_8O_2$	16	33.177
Butanediol	$C_4H_{10}O_2$	22	31.374
Glycerol	$C_3H_8O_3$	14	37.625
Ketones/Aldehydes			
Formaldehyde	CH_2O	4	45.326
Acetone	C_3H_6O	16	28.718
Acetoin	$C_4H_8O_2$	20	32.625
Amino acids			
Aspartate	$C_4H_6NO_4^-$	20	5.313 [a]
Cysteine	$C_3H_7NO_2S$	26	8.926 [a]
Cystine	$C_6H_{12}N_2O_4S_2$	50	7.941 [a]
Glutamate	$C_5H_8NO_4^-$	26	10.056 [a]
Phenyl alanine	$C_9H_{11}NO_2$	48	17.862 [a]
Tryptophane	$C_{11}H_{12}N_2O_2$	62	12.582 [a]
Halogenated compounds			
Ethylene	C_2H_4	12	32.520 [b]
Chloroethylene	C_2H_3Cl	10	45.964 [b]
1,1-Dichloroethylene	$C_2H_2Cl_2$	8	65.426 [b]
cis-1,2-Dichloroethylene	$C_2H_2Cl_2$	8	64.873 [b]
trans-1,2-Dichloroethylene	$C_2H_2Cl_2$	8	65.405 [b]
Trichloroethylene	C_2HCl_3	6	101.296 [b]
Tetrachloroethylene	C_2Cl_4	4	175.286 [b]
Inorganic compounds			
Hydrogen	H_2	2	39.870
Carbon monoxide	CO	2	47.477
Sulfite	SO_3^{2-}	2	50.296
Sulfur	S^0	6	19.146
Thiosulfate	$S_2O_3^{2-}$	8	23.584
Polysulfide	S_5^{2-}	32	20.003 [c]
Bisulfide	HS^-	8	20.850
Nitrite	NO_2^-	2	-41.650
Nitric oxide	NO	3	-38.989 [d]
Nitrous oxide	N_2O	8	-57.540
Ammonium	NH_4^+	8	-35.109
Nitrogen	N_2	10	-72.194
Oxygen	O_2	-4	-78.719
Hydrogen peroxide	H_2O_2	-2	-130.23 [a]
Chlorate	ClO_3^-	-6	-59.424 [e]
Perchlorate	ClO_4^-	-8	-84.220 [e]
Fe^{2+}	Fe^{2+}	1	-74.270

Table 3. Gibbs energy change $\Delta G_r^{0'}$ and equilibrium potential $E_r^{0'}$ of specific half reactions with bicarbonate as the electron acceptor. Calculated using the Growth Reference System (biochemical standard conditions: 1 atm, 298.15 K, 1 mol/L, pH 7). Data from Heijnen[1] unless otherwise indicated.

Half reaction (e-acceptor + x e^- ⇆ e-donor)	$\Delta G_r^{0'}$ (kJ/mol)	$E_r^{0'}$ (V)
Alkanes		
HCO_3^- + 9 H^+ + 8 e^- ⇆ Methane + 4 H_2O	183	-0.238
2 HCO_3^- + 16 H^+ + 14 e^- ⇆ Ethane + 6 H_2O	356	-0.263
3 HCO_3^- + 23 H^+ + 20 e^- ⇆ Propane + 9 H_2O	519	-0.269
Carboxylic acids		
HCO_3^- + 2 H^+ + 2 e^- ⇆ Formate + H_2O	78	-0.406
2 HCO_3^- + 9 H^+ + 8 e^- ⇆ Acetate + 4 H_2O	214	-0.278
3 HCO_3^- + 16 H^+ + 14 e^- ⇆ Propionate + 7 H_2O	377	-0.279
4 HCO_3^- + 23 H^+ + 20 e^- ⇆ Butyrate + 10 H_2O	540	-0.280
2 HCO_3^- + 3 H^+ + 2 e^- ⇆ Oxalate + 2 H_2O	105	-0.544
6 HCO_3^- + 23 H^+ + 18 e^- ⇆ Citrate + 11 H_2O	581	-0.335
4 HCO_3^- + 15 H^+ + 12 e^- ⇆ Fumarate + 8 H_2O	404	-0.349
4 HCO_3^- + 17 H^+ + 14 e^- ⇆ Succinate + 8 H_2O	398	-0.294
3 HCO_3^- + 14 H^+ + 12 e^- ⇆ Lactate + 6 H_2O	378	-0.326
3 HCO_3^- + 12 H^+ + 10 e^- ⇆ Pyruvate + 6 H_2O	341	-0.354
6 HCO_3^- + 27 H^+ + 22 e^- ⇆ Gluconate + 11 H_2O	860	-0.405
4 HCO_3^- + 21 H^+ + 18 e^- ⇆ β-hydroxybutyrate + 9 H_2O	544	-0.313
Carbohydrates		
6 HCO_3^- + 30 H^+ + 24 e^- ⇆ Glucose + 12 H_2O	954	-0.412
6 HCO_3^- + 30 H^+ + 24 e^- ⇆ Fructose + 12 H_2O	956	-0.413
6 HCO_3^- + 30 H^+ + 24 e^- ⇆ Galactose + 12 H_2O	948	-0.409
12 HCO_3^- + 60 H^+ + 48 e^- ⇆ Sucrose + 25 H_2O	1953	-0.422
6 HCO_3^- + 30 H^+ + 24 e^- ⇆ Glycogen + 13 H_2O	972	-0.420
Alcohols		
HCO_3^- + 7 H^+ + 6 e^- ⇆ Methanol + 2 H_2O	216	-0.373
2 HCO_3^- + 14 H^+ + 12 e^- ⇆ Ethanol + 5 H_2O	364	-0.315
3 HCO_3^- + 21 H^+ + 18 e^- ⇆ n-Propanol + 8 H_2O	525	-0.302
4 HCO_3^- + 28 H^+ + 24 e^- ⇆ n-Butanol + 11 H_2O	683	-0.295
2 HCO_3^- + 12 H^+ + 10 e^- ⇆ Ethylene glycol + 4 H_2O	373	-0.387
3 HCO_3^- + 19 H^+ + 16 e^- ⇆ Propanediol + 7 H_2O	531	-0.344
4 HCO_3^- + 26 H^+ + 22 e^- ⇆ Butanediol + 10 H_2O	690	-0.325
3 HCO_3^- + 17 H^+ + 14 e^- ⇆ Glycerol + 6 H_2O	527	-0.390
Ketones/Aldehydes		
HCO_3^- + 5 H^+ + 4 e^- ⇆ Formaldehyde + 2 H_2O	181	-0.470

$3\ HCO_3^- + 19\ H^+ + 16\ e^- \leftrightarrows$ Acetone + $8\ H_2O$	459	-0.298
$4\ HCO_3^- + 24\ H^+ + 20\ e^- \leftrightarrows$ Acetoin + $10\ H_2O$	653	-0.338
Amino acids		
$4\ HCO_3^- + NH_4^+ + 14\ H^+ + 12\ e^- \leftrightarrows$ Aspartate + $8\ H_2O$	387	-0.334
$3\ HCO_3^- + NH_4^+ + SO_4^{2-} + 22\ H^+ + 18\ e^- \leftrightarrows$ Cysteine + $11\ H_2O$	513	-0.295
$6\ HCO_3^- + 2\ NH_4^+ + 2\ SO_4^{2-} + 42\ H^+ + 34\ e^- \leftrightarrows$ Cystine + $22\ H_2O$	959	-0.292
$5\ HCO_3^- + NH_4^+ + 21\ H^+ + 18\ e^- \leftrightarrows$ Glutamate + $11\ H_2O$	542	-0.312
$9\ HCO_3^- + NH_4^+ + 48\ H^+ + 40\ e^- \leftrightarrows$ Phenyl alanine + $25\ H_2O$	1138	-0.295
$11\ HCO_3^- + 2\ NH_4^+ + 55\ H^+ + 46\ e^- \leftrightarrows$ Tryptophane + $31\ H_2O$	1342	-0.302

Table 4. Gibbs energy change $\Delta G_r^{0'}$ and equilibrium potential $E_r^{0'}$ of specific half reactions with an organic electron acceptor. Calculated using the Growth Reference System (biochemical standard conditions: 1 atm, 298.15 K, 1 mol/L, pH 7). Data from Heijnen[1] unless otherwise indicated.

Half reaction (e-acceptor + x e^- ⇆ e-donor)	$\Delta G_r^{0'}$ (kJ/mol)	$E_r^{0'}$ (V)
Reduction of fatty acids to alcohols		
Formate + 5 H^+ + 4 e^- ⇆ Methanol + H_2O	138	-0.357
Acetate + 5 H^+ + 4 e^- ⇆ Ethanol + H_2O	150	-0.388
Propionate + 5 H^+ + 4 e^- ⇆ Propanol + H_2O	147	-0.382
Butyrate + 5 H^+ + 4 e^- ⇆ Butanol + H_2O	143	-0.371
Reduction of alcohol groups		
Methanol + 2 H^+ + 2 e^- ⇆ Methane + H_2O	-33	0.170
Ethanol + 2 H^+ + 2 e^- ⇆ Ethane + H_2O	-9	0.044
Propanol + 2 H^+ + 2 e^- ⇆ Propane + H_2O	-6	0.029
Glycerol + 2 H^+ + 2 e^- ⇆ Propanediol + H_2O	4	-0.021
Double bond saturation		
Fumarate + 2 H^+ + 2 e^- ⇆ Succinate	-6	0.033
Dehalogenation		
Tetrachloroethylene + 4 H^+ + 8 e^- ⇆ Ethylene + 4 Cl^-	-311	0.403
Tetrachloroethylene + H^+ + 2 e^- ⇆ Trichloroethylene + Cl^-	-93	0.484
Trichloroethylene + H^+ + 2 e^- ⇆ 1,1-Dichloroethylene + Cl^-	-84	0.437
Trichloroethylene + H^+ + 2 e^- ⇆ cis-1,2-Dichloroethylene + Cl^-	-89	0.460
Trichloroethylene + H^+ + 2 e^- ⇆ trans-1,2-Dichloroethylene + Cl^-	-85	0.438
1,1-Dichloroethylene + H^+ + 2 e^- ⇆ Chloroethylene + Cl^-	-64	0.330
cis-1,2-Dichloroethylene + H^+ + 2 e^- ⇆ Chloroethylene + Cl^-	-59	0.307
trans-1,2-Dichloroethylene + H^+ + 2 e^- ⇆ Chloroethylene + Cl^-	-64	0.330
Chloroethylene + H^+ + 2 e^- ⇆ Ethylene + Cl^-	-69	0.360

Table 5. Gibbs energy change $\Delta G_r^{0'}$ and equilibrium potential $E_r^{0'}$ of specific half reactions with an inorganic electron acceptor. Calculated using the Growth Reference System (biochemical standard conditions: 1 atm, 298.15 K, 1 mol/L, pH 7). Data from Heijnen[1] unless otherwise indicated.

Half reaction (e-acceptor + x e^- ⇆ e-donor)	$\Delta G_r^{0'}$ (kJ/mol)	$E_r^{0'}$ (V)
Nitrogen species		
$NO_3^- + 6\ H^+ + 5\ e^- \leftrightarrows 0.5\ N_2 + 3\ H_2O$	-361	0.748
$NO_2^- + 4\ H^+ + 3\ e^- \leftrightarrows 0.5\ N_2 + 2\ H_2O$	-278	0.959
$NO_3^- + 2\ H^+ + 2\ e^- \leftrightarrows NO_2^- + H_2O$	-83	0.432
$NO_2^- + 2\ H^+ + 1\ e^- \leftrightarrows NO + H_2O$	-34	0.349
$NO + H^+ + e^- \leftrightarrows 0.5\ N_2O + 0.5\ H_2O$	-113	1.173
$0.5\ N_2O + H^+ + e^- \leftrightarrows 0.5\ N_2 + 0.5\ H_2O$	-131	1.356
$NO_3^- + 10\ H^+ + 8\ e^- \leftrightarrows NH_4^+ + 3\ H_2O$	-281	0.364
$NO_2^- + 8\ H^+ + 6\ e^- \leftrightarrows NH_4^+ + 2\ H_2O$	-198	0.341
Sulfur species		
$SO_4^{2-} + 9\ H^+ + 8\ e^- \leftrightarrows HS^- + 4\ H_2O$	167	-0.216
$SO_3^{2-} + 7\ H^+ + 6\ e^- \leftrightarrows HS^- + 3\ H_2O$	66	-0.114
$S_2O_3^{2-} + 4\ H^+ + 4\ e^- \leftrightarrows HS^- + 1.5\ H_2O$	72	-0.188
$S^0 + H^+ + 2\ e^- \leftrightarrows HS^-$	52	-0.269
$SO_4^{2-} + 8\ H^+ + 6\ e^- \leftrightarrows S^0 + 4\ H_2O$	115	-0.198
$SO_4^{2-} + 2\ H^+ + 2\ e^- \leftrightarrows SO_3^{2-} + H_2O$	101	-0.521
$S_5^{2-} + 5\ H^+ + 8\ e^- \leftrightarrows 5\ HS^-$	194	-0.251
$5\ S^0 + 2\ e^- \leftrightarrows S_5^{2-}$	66	-0.341
Other		
$2\ H^+ + 2\ e^- \leftrightarrows H_2$	80	-0.413
$O_2 + 4\ H^+ + 4\ e^- \leftrightarrows 2\ H_2O$	-315	0.816
$O_2 + 2\ H^+ + 2\ e^- \leftrightarrows H_2O_2$	-54	0.282
$H_2O_2 + 2\ H^+ + 2\ e^- \leftrightarrows 2\ H_2O$	-260	1.350
$ClO_3^- + 6\ H^+ + 6\ e^- \leftrightarrows Cl^- + 3\ H_2O$	-357	0.616 [a]
$ClO_4^- + 8\ H^+ + 8\ e^- \leftrightarrows Cl^- + 4\ H_2O$	-674	0.873 [a]
$Fe^{3+} + e^- \leftrightarrows Fe^{2+}$	-74	0.770
$Fe(CN)_6^{3-} + e\text{-} \leftrightarrows Fe(CN)_6^{4-}$	35	0.361 [b]

[a] Value obtained from [2]
[b] Value obtained from [3]

Gibbs energy (ΔG) is a thermodynamic property that represents the maximum amount of useful work that can be produced from a specific chemical reaction or the minimum amount of work that needs to be delivered to make a specific chemical reaction happen. If Gibbs energy change of a reaction is negative, work can be produced from the system and the reaction is referred to as exergonic. If the Gibbs energy change of a reaction is positive, work needs to be delivered to the system and the reaction is referred to as endergonic. The Gibbs energy change of a (half) reactions can be easily converted into cell voltage and potentials using the following equation:

$$E = -\frac{\Delta G}{nF}$$

In which E is the electrode potential resulting from a certain (half) reaction, ΔG is Gibbs energy change of that specific (half) reaction, n the amount of electrons involved in the specific (half) reaction, and F Faraday's number (96485.3 C/mol).

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