C & EE 255B
Prof. M. K. Stenstrom
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BALANCING REDOX EQUATIONS

Balancing redox (oxidation-reduction) equations is a simple and very useful technique of performing balances from empirical equations describing microbial stoichiometry. Each basic equation (synthesis or growth, respiration, and decay) can all be balanced and added together to describe a process. Moreover, the equations can be balanced for each type of metabolism (aerobic or oxic, anoxic, and anaerobic). This handout describes the techniques for balancing the equations and then shows some common examples. Be careful when using empirical redox techniques; the reactions can be balanced, but other information must be used to determine if the reaction actually occurs.

RULES

Each redox equation contains two parts -- the oxidation and reduction parts. Each is balanced separately.

1. The first rule is to balance the major atoms with known end products. The end products of the redox equations must be stated or determined from other sources. The redox equations give you no information about the actual end products. Common end products for carbon are CO_2 or cells. Other end products can occur as well. Major atoms are defined as all atoms except oxygen and hydrogen.

2. The next step is to balance the oxygen atoms by adding water (H_2O) molecules.

3. Next balance the hydrogen with hydrogen ions (H^+).

4. Finally balance the charge with electrons (e^-).

After these four steps one obtains a balanced half-reaction -- either the oxidation reaction or the reduction reaction. Oxidation reactions will produce electrons (electrons appear on the right-hand side of the equation). Reduction reactions will consume electrons (electrons appear on the left-hand side of the equation).

SOME EXAMPLES

Consider the oxidation of glucose, $C_6H_{12}O_6$:

Step 1. Balance the major atoms. In this case we will use CO_2 as the end product for carbon.

$$C_6H_{12}O_6 \text{ -------->} \quad 6\ CO_2$$

Step 2. Balance the oxygen with water:

$$C_6H_{12}O_6 + 6\ H_2O \text{ -------->} \quad 6\ CO_2$$

Step 3. Balance the hydrogen with H^+:

$$C_6H_{12}O_6 + 6\ H_2O \text{ -------->} \quad 6\ CO_2 + 24\ H^+$$

Step 4. Balance the charge with electrons:

$$C_6H_{12}O_6 + 6\ H_2O \text{ -------->} \quad 6\ CO_2 + 24\ H^+ + 24\ e^- \qquad (1)$$

Note that the charge and all atoms are balanced. This is the balanced half-reaction. It is an oxidation reaction. Note that electrons are produced or are removed from the substrate (glucose). The oxidation state of carbon is zero on the left hand side and + 2 on the right hand side of the equation. You can determine the oxidation state by adding up the other valences to see what valence is needed so that the whole molecule sums to zero (or -1, etc for an ion). Therefore, for glucose, we have twelve H at +1 each, six O at -2 each. This sums to +12 for the H and -12 for O, which means the six carbons are at zero. For CO_2, we get -4 for the O so the C must be +4. CO_2 is the highest oxidation state for carbon, meaning it is the most oxidized form. The lowest oxidation state for C is in methane, CH_4, and carbon is -4.

For this reaction to occur it must be balanced by a second half-reaction. It contains a reactant that is called the hydrogen acceptor or electron acceptor. Metabolisms are distinguished by which electron acceptors are used. Aerobic metabolism uses oxygen, anoxic metabolism uses sulfate or nitrate or nitrite, and anaerobic metabolism uses CO_2 or an organic molecule. Environmental engineers need to differential between anoxic and anaerobic, since the desirable metabolism makes the difference between a successful and unsuccessful process. Many microbiologists do not make this distinction. Anaerobic reactions are sometimes called fermentations when an organic compound is the electron acceptor.

For aerobic reactions we can write the balanced half-reaction as follows:

Step 1. Balance the major atoms. No major atoms!

$$O_2 \text{ ---------->}$$

Step 2. Balance the oxygen with waters:

$$O_2 \text{ ---------->} \ 2\ H_2O$$

Step 3. Balance the hydrogen:

$$O_2 + 4\ H^+ \text{ ---------->} \ 2\ H_2O$$

Step 4. Balance the charge with electrons:

$$O_2 + 4\ H^+ + 4\ e^- \text{---------->} \ 2\ H_2O \qquad (2)$$

Equations (1) and (2) can now be added to balance the electrons to zero by multiplying equation (2) by 6 and adding them together as follows:

$$C_6H_{12}O_6 + 6\ H_2O \text{ -------->} \quad 6\ CO_2 + 24\ H^+ + 24\ e^-$$
$$6\ O_2 + 24\ H^+ + 24\ e^- \text{---------->} \ 12\ H_2O$$

Summation

$$C_6H_{12}O_6 + 6\ O_2 \text{ -------->} \quad 6\ CO_2 + 6\ H_2O \qquad (3)$$

This equation represents the maximum amount of oxygen needed to oxidize glucose, and is sometimes called the respiration or Theoretical Oxygen Demand (ThOD) equation. From this equation one can calculate the ThOD as 6 moles of O_2 per mole of glucose, or 1.07 g O_2/g glucose. Be careful to calculate the ratios correctly, as follows:

$$\text{ThOD} = 6*32 /(6*12 + 12 + 6*16) = 1.07 \qquad (4)$$

Equation (1) can be written with different metabolisms (electron acceptors) using the following half equations instead of oxygen:

Anoxic:

$$NO_3^- + 6\,H^+ + 5\,e^- \text{------->} 0.5\,N_2 + 3\,H_2O \qquad (5)$$

$$NO_2^- + 4\,H^+ + 3\,e^- \text{-------->} 0.5\,N_2 + 2\,H_2O \qquad (6)$$

$$SO_4^= + 10\,H^+ + 8\,e^- \text{------->} H_2S + 4\,H_2O \qquad (7)$$

Anaerobic

$$CO_2 + 8\,H^+ + 8\,e^- \text{------->} CH_4 + 2\,H_2O \qquad (8)$$

At this point in the notes and lecture, you should be able to write balanced redox equations for any reactants, given their end products for the major atoms.

EMPIRICAL MICROBIAL EQUATIONS

So far in this handout we have described redox equations and how to balance them without regard to how they may be used. The empirical equations are quite useful to describe the stoichiometry of biological processes. Recall that there are three basic paths for growth of microorganisms on a substrate, as follows:

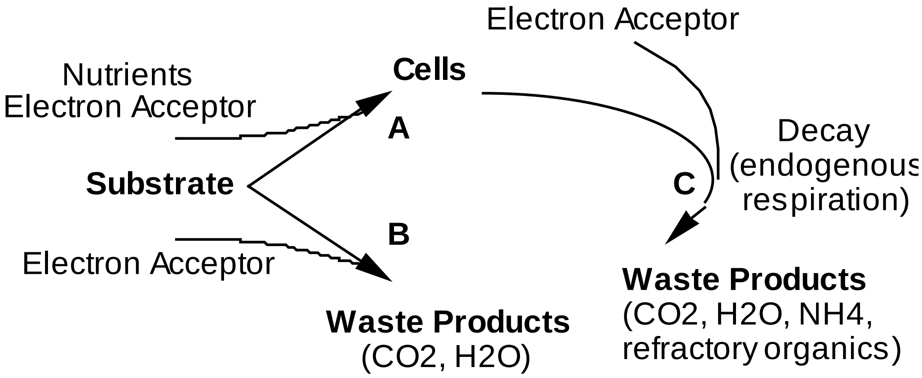

Figure 1. Stoichiometric relationships in microbial growth.

Equation A above is the synthesis or growth equation. Equation B is the respiration equation, while equation C is the decay equation. Equation B and C always use the highest oxidation state of the end products as their normal condition. An exception relates to ammonia. In a fully nitrifying system, the end-product form of nitrogen is nitrate. In non-nitrifying systems, ammonia is the end product.

SYNTHESIS

We can write the synthesis equation if we know an empirical formula for cells. In the 50s and later, activated sludge process cells were analyzed with a carbon-hydrogen-nitrogen analyzer and cell empirical formulas were found to be close to $C_5H_7NO_2$ for a wide range of activated sludge processes. Later work confirmed the earlier results, although for some systems with very long sludge ages (SRT or MCRT), the empirical formula maybe different due to the accumulation of biologically inert, organic products. If we want to include phosphorus in the empirical cell formula, $C_5H_7NO_2P_{0.1}$ is a good approximation. We can see for nitrogen composes about 12% of the biomass while phosphorus composes only 2.7% of the biomass. These fractions can be used to estimate the nutrient requirements for a nutrient-poor wastewater, such as an industrial wastewater, or the mass of nutrients removed through biomass wasting.

We begin to balance of our equations in the same was as before by balancing the major atoms first. Let us consider sucrose as a substrate.

$$C_{12}H_{22}O_{11} \text{ -------------> } C_5H_7NO_2$$

Balance the major atoms, C and N. Use ammonia as the nitrogen source

$$5\ C_{12}H_{22}O_{11} + 12\ NH_4^+ \text{ -------------> } 12\ C_5H_7NO_2$$

Balance the oxygen with water.

$$5\ C_{12}H_{22}O_{11} + 12\ NH_4^+ \text{ -------------> } 12\ C_5H_7NO_2 + 31\ H_2O$$

Balance the hydrogen with hydrogen ions

$$5\ C_{12}H_{22}O_{11} + 12\ NH_4^+ \text{ -------------> } 12\ C_5H_7NO_2 + 31\ H_2O + 12\ H^+ \qquad (9)$$

Note that there are no electrons needed to balance the equation. This results because there is no net oxidation or reduction. Equation (9) is the synthesis equation and describes the conversion of the substrate to cells. There will usually be some reduction or oxidation in the synthesis equation. Equation (9) shows 100% conversion of substrate to cells, which is not possible, since some of it must be oxidized to produce energy. For cells to obtain energy from a reaction, there must be an oxidation. We now must combine the synthesis equation with the respiration equation to obtain a net equation.

RESPIRATION

Equation (10) is the respiration equation and we want to add it to equation (9) in the correct amount.

$$C_{12}H_{22}O_{11} + 12\ O_2 \text{ -------------> } 12\ CO_2 + 11\ H_2O \qquad (10)$$

To combine the synthesis and net equations we use a yield term. Yields are measured experimentally and reported in units of mass cells per mass substrate. Mass yields for heterotrophic organisms are always significantly less than 1. Suppose we have a yield of 0.6 mass cells/mass substrate. We must first convert the yield to a molar yield, using the molecular weights of sucrose (342) and cells (113) as follows:

$$Y \text{ (molar)} = 0.6 * 342/113 = 1.816.$$

We must now combine equations (9) and (10) to produce an equation that has one mole of substrate on the left and 1.816 moles of cells on the right. There are many ways of obtaining the correct amounts of synthesis and respiration, but the easiest approach is to multiply equation (9) by a coefficient to produce 1.816 moles of cells. Next multiply equation (10) by a second coefficient to produce 1.0 moles of substrate when equations (9) and (10) are summed. Multiply equation (9) by 1.816/12 or 0.151.

$$0.757\ C_{12}H_{22}O_{11} + 1.816\ NH_4^+ \text{ -------------> } 1.816\ C_5H_7NO_2 + 4.69\ H_2O + 1.816\ H^+$$

Now multiply equation (10) by (1.0 - 0.757)

$$0.243\ C_{12}H_{22}O_{11} + 2.92\ O_2 \text{ -------------> } 2.92\ CO_2 + 2.68\ H_2O$$

Adding together provides the net equation, as follows:

$$C_{12}H_{22}O_{11} + 1.816\ NH_4^+ + 2.92\ O_2 \text{ --------> } 1.816\ C_5H_7NO_2 + 2.92\ CO_2 + 7.37\ H_2O + 1.816\ H^+ \qquad (11)$$

The net equation can be used to define the mass of end-products and the stoichiometry of a process.

In biological systems, we may want to use a theoretical yield (Y), or an observed yield (Y_{obs}). The theoretical yield represents the split between paths A and B in Figure 1. It is determined from a series of experiments performed at different SRTs. The cell production is measured and a maximum cell production is calculated (extrapolated actually) to a minimum or washout cell retention time. This is the maximum cell production possible from a given microbial culture. The yield is calculated as the cell production per unit mass of substrate metabolized or consumed. An observed yield is what is actually measured and is specific for the cell retention time and reactor conditions at the time of the experiment. The observed yield includes the reduction in cell mass that occurs because of cell decay (path C in Figure 1). Decay is greater for long cell retention times, which means that Y_{obs} will be lower for systems operating at longer SRTs.

The net equation, equation (11), can be written using either the observed or theoretical yield. If the theoretical yield is used, then the cell decay equation must be added to obtain the net equation for a particular SRT. It is more tedious to write the net equation using the theoretical yield and then add the decay equation; however, if done correctly, one obtains the same result using the theoretical yield or observed yield.

DECAY

The final equation we need is the cell decay equation or endogenous respiration equation. We write it as follows:

$$C_5H_7NO_2 + 5\ O_2 + H^+ \text{ --------> } 5\ CO_2 + NH_4^+ + 2\ H_2O \qquad (12)$$

The above equation is written with two half reactions as before, balanced and assuming the end product for nitrogen is ammonia. This assumes that there is no nitrification. If nitrification occurs, then we must write the nitrogen end products as nitrate. We can start over with equation (12) by writing nitrate instead of ammonia and perform the entire sequence to balance the equation, or we can add the equation describing oxidation of ammonia, as follows:

$$NH_4^+ + 2\ O_2 \text{ -------> } H_2O + NO_3^- + 2\ H^+ \qquad (13)$$

The ammonia on the left cancels the ammonia on the right and the sum is:

$$C_5H_7NO_2 + 7\ O_2 \text{ --------> } 5\ CO_2 + NO_3^- + 3\ H_2O + H^+ \qquad (14)$$

Note the additional oxygen required, 5 moles O2/mole cell versus 7 moles O2/mole cell.

Once we know how to write the redox reactions, we can use them to predict the amounts of reactants and products that will be needed or obtained from a particular reaction.

APPLICATIONS

OXYGEN DEMAND

There are many potential applications for the redox equations. The first is defining oxygen demand. Recall that there are several types of oxygen demand, Biochemical Oxygen Demand$_n$ or BOD$_u$ (where n is a unit of time in days, most commonly 5 days). Chemical Oxygen Demand (COD) and Total Oxygen Demand (COD) are both determined in laboratory experiments, while the Theoretical Oxygen Demand (ThOD) is calculated from redox-equations. Equation (4) defines the ThOD of glucose. It can be defined in units of moles oxygen per mole of glucose (6 moles in this case), or in units of mass, 1.07 g oxygen/g glucose (6*32/180). If a mass of the compound is dissolved or suspended in water, the oxygen demand can also be expressed as a concentration. To use the empirical equations to define BOD, COD and TOD we must relate theory to an experiment. There are instruments that attempt to oxidize a sample to its highest oxidation state, and can only approximate the ThOD, since the reactions cannot be 100% complete. For some compounds such as glucose, the approximation is very close so that the TOD ≈ ThOD. For compounds containing nitrogen, the TOD can be significantly less than the ThOD because of the production of NO_x as opposed to nitrate. This is a function of the instrument used to measure TOD.

COD is defined by an experimental procedure (see *Standard Methods*). COD is becoming less popular since it uses dichromate, mercury, and sulfuric acid, which are very hazardous. The COD test attempts to measure the total oxygen demand of a compound but only approximates it due to incomplete reactions. Some ring compounds are only partially oxidized, and nitrogen is always converted to **ammonia, not nitrate.**

In a BOD laboratory test, a sample of the wastewater is contacted with a known amount of oxygen and allowed to react with microorganisms. The amount of oxygen consumed is measured over time. The BOD of a sample depends upon the biodegradability of a compound and the "seed" or the organisms that are used as the inoculum. Generally one uses a seed that has been acclimated to the wastewater. The BOD of a compound can be very close to its ThOD, or zero. The end product for nitrogen can be either ammonia or nitrate in the BOD test, depending upon the seed. If a seed from a nitrifying culture is used, some portion or nearly all of the ammonia can be oxidized to nitrate. The oxygen demand due to nitrification is called the nitrogenous oxygen demand or second-stage demand. It is called second-stage because it is often exerted in the later part of the BOD test. The BOD of a sample is rarely greater than the COD; this can occur if a second-stage BOD is present, or if a compound for some reason is biodegradable but not oxidizable by dichromate (very rare!). A useful concept is the ultimate BOD, or BOD$_u$. This is the BOD that would be exerted in infinite time. The BOD$_u$ of biodegradable compounds is equal to the COD or TOD, depending on the presence of nitrogen and nitrification. The utility of BOD$_u$ is that it represents the maximum oxygen demand possible in a biological treatment system, which is usually our design goal.

The strength of the BOD test is also its greatest weakness. The test attempts to measure what actually happens in a biochemical reaction. This can be highly variable since the microorganisms are subject to many variables. For example, a biodegradable waste which would ordinarily have a high BOD can be mixed with a toxic material and the BOD will be measured as zero. Trying to quantify the conditions can be important to understanding the impact of a wastewater discharge into the environment.

When using empirical equations to approximate oxygen demand, we use the following conventions:

ThOD	Write the end products, including nitrogen, in their highest oxidation state (e.g., CO_2 and NO_3^-).
TOD	Use ThOD as an *approximation* for TOD. Measured TOD is always less than the ThOD because of incomplete oxidation in the test device and because some of the nitrate will be reduced to various nitrous oxides (NO_x) during combustion.

COD Write the end products except for nitrogen in their highest oxidation state. Use ammonia as the nitrogen end product.

BOD_n Approximate the BOD_5 or BOD_u by the COD and some knowledge about the biodegradability of the compounds. For most municipal wastewaters, the BOD_5 is 70 to 80% of the BOD_u. A relation of $BOD_5/0.65 = COD$ is often used.

PROCESS STOICHIOMETRY

Once the net equation (equation 11) is obtained, the mass of reactants and end products can be estimated. Equation (11) can be used to estimate oxygen requirements, sludge (cells) production, CO_2 production, alkalinity production or destruction, and nutrient requirements.

$$C_{12}H_{22}O_{11} + 1.816\ NH_4^+ + 2.92\ O_2 \text{ --------------> } 1.816\ C_5H_7NO_2 + 2.92\ CO_2$$

$$7.37\ H_2O + 1.816\ H^+ \qquad (15)$$

Equation (15) shows that 1.816 moles of ammonia and 2.92 moles of oxygen are required to metabolize one mole of substrate. 2.92 moles of CO_2 are produced and 3.632 moles of alkalinity are consumed. The decay equation can be used in a similar fashion.

For cases where the influent substrate can be approximated by an empirical formula, the number of moles is easily calculated and the maximum value of the reactants and products can be calculated. For example, it is easy to determine the maximum possible methane production of a known substrate. When converting to moles, do not restrict yourself to gram modes. One can work in kg moles, pound moles, ton moles, etc.

DENITRIFICATION

The most common way for a biological process to remove nitrogen is to first nitrify it to nitrate, then denitrify it to nitrogen gas. See the nitrogen cycle. For denitrification, equation (5) is used, which requires that an electron donor be provided. The electron donor can be any biodegradable carbon compound, but easily degradable, low molecular weight compounds are preferred, such as acetate or methanol. This preference results because it is easier to operate a reactor if nitrate is rate limiting, not the electron donor compound. This also allows denitrification reactors to have short hydraulic retention times.

The required carbon can be calculated by balancing the nitrate electron acceptor (equation 5) with the appropriate carbon source. Methanol is balanced as follows:

$$6\ NO_3^- + 5\ CH_3OH + 6H^+ \rightarrow 3\ N_2 + 5\ CO_2 + 13\ H_2O \qquad (16)$$

Nitrite can also be used as an electron acceptor, as shown for acetic acid:

$$NO_2^- + 0.375\ CH_3COO^- + 1.375\ H^+ \rightarrow 0.5\ N_2 + 0.75\ CO_2 + 1.25\ H_2O \qquad (17)$$

If the carbon source must be added, equation (16) or (17) can be used to calculate a ratio, and the ratio of 1.90 (g methanol/g N) is well known. It is also necessary to reduce the DO to zero to obtain denitrification which means that some additional carbon is usually required.

Denitrification can reduce the oxygen required by a biological process, if the influent substrate (BOD) can be used as the electron donor. This is achieved by recycling nitrified effluent or mixed-liquor to the influent zones of the treatment plant, bringing easily degradable carbon from the influent together with nitrate.

The reduction of oxygen demand can be calculated using the stoichiometric concepts demonstrated in these notes. The amount of carbon needed to reduce the nitrate to nitrogen does not need to be treated (the amount of carbon oxidized by nitrate reduction is subtracted from the carbon that would have been oxidized by oxygen reduction).

For example, suppose 250 mg/L of substrate that can be approximated sucrose is treated in a process described by equation (11). First write the oxidation of sucrose using nitrate as the electron acceptor, as follows:

$C_{12}H_{22}O_{11} + 9.6\ NO^-_3 + 9.6\ H^+ \text{---->}\ 12\ CO_2 + 4.8\ N_2 + 15.8\ H_2O$ (18)

If 21 mg/L of NO_3-N is to be nitrified, convert it to millimoles (21/14= 1.5), and then calculate 1.5/9.6 millimoles of sucrose or 53.4 mg/L sucrose. The ratio is 53.4/21 or 2.5 mg sucrose/mg N (compares to 1.9 for methanol). The "return" of the oxygen (oxygen used to convert ammonia to nitrate is then reused to convert substrate to CO_2) is often called the denitrification credit, and is significant in the design and operation of denitrifying treatment plants.

The oxygen required to nitrify and denitrify can also be estimated by balancing the oxidation of ammonia to nitrogen gas, as follows:

$¾\ O_2 + NH_4^+ \rightarrow ½\ N_2 + H^+ + 1.5\ H_2O$ (19)

The mass ratio is 1.71 mgO2/mg-N, which is much less than 4.57 required for nitrification. The ratio of 4.57 can be calculated from equation 13 (2*32/14) Note that the reaction in equation (19) does not occur biologically, and is a "short cut" across the nitrogen cycle. It is useful for calculating the oxygen requirements instead of using equations 13 and 16. The difference between 4.57 and 1.71 (2.86) is recovered when the carbon source, such as the influent substrate, is oxidized by nitrate reduction. Of course, a reduction in oxygen demand occurs only if a carbon source that is used would otherwise have been oxidized. If you have to add a carbon source, there is no oxygen savings, but an increase, since the carbon must also be used to remove the dissolved oxygen in the denitrifying reactor.

Anammox is a newly discovered microbial way for converting ammonia to nitrogen gas, and part of the process is anaerobic. One-half of the ammonia is first oxidized to nitrite aerobically, as follows:

$NH_4^+ + 1.5\ O_2\ \text{------->}\ NO_2^- + H_2O + 2\ H^+$ (20)

Next the nitrite reacts with the remaining ammonia, anaerobically, as follows:

$NH_4^+ + NO_2^-\ \text{------->}\ N_2 + 2H_2O$ (21)

Anammox is believed to account for a significant portion of the denitrification that occurs world-wide. So far it has been used at a few treatment plants to denitrify strong, warm ammonia containing wastewaters, such as anaerobic digester supernatant. It has the advantage of not requiring a carbon source and using less oxygen. The disadvantage is the difficulty, at least with current knowledge, of starting and controlling the process. The organisms are very slow growing and it may take years to obtain a viable process without using a seed from an existing anammox process. Also special measures must be used to retain the anammox organisms in the process, since they are so slow growing. Fortunately, in slurry reactors they grown in dense, red clumps that can be separated from waste biomass with a cyclone.

A FINAL NOTE

It is possible to balance redox equations by inspection. For some simple reactions, you can obtain the correct answer. For more complicated reactions, one almost always obtains the wrong answer by balancing by inspection. I grade giving partial credit. If one shows the work and applies the rules to systematically balance an equation, and for some reason, the answer is incorrect, perhaps due to an addition error, I give nearly all the credit. If the answer is incorrect because of balancing by inspection, or not showing the work and steps, then there is no partial credit.