

# BAE 204: Introduction to Environmental and Ecological Engineering

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# Preface

This is the first attempt at a textbook for BAE 204. It will be updated as class proceeds.

## Author

François Birgand is an Associate Professor of biogeochemistry and ecological engineering at North Carolina State University, in Raleigh, NC, USA. His research interests aim to provide solutions to improve the quality of water in streams and rivers. Practically, his research focuses on improving the treatment efficiencies of streams, wetlands, soils or woodchip bioreactors. Much of this research is based upon principles this textbook is trying to provide for students of all ages.



# Chapter 1

## Introduction

You can label chapter and section titles using `{#label}` after them, e.g., we can reference Chapter 1. If you do not manually label them, there will be automatic labels anyway, e.g., Chapter `??`.

Figures and tables with captions will be placed in `figure` and `table` environments, respectively.

```
par(mar = c(4, 4, .1, .1))
plot(pressure, type = 'b', pch = 19)
```

Reference a figure by its code chunk label with the `fig:` prefix, e.g., see Figure 1.1. Similarly, you can reference tables generated from `knitr::kable()`, e.g., see Table 1.1.

```
knitr::kable(
  head(iris, 20), caption = 'Here is a nice table!',
  booktabs = TRUE
)
```

You can write citations, too. For example, we are using the **bookdown** package (Xie, 2018) in this sample book, which was built on top of R Markdown and **knitr** (Xie, 2015).

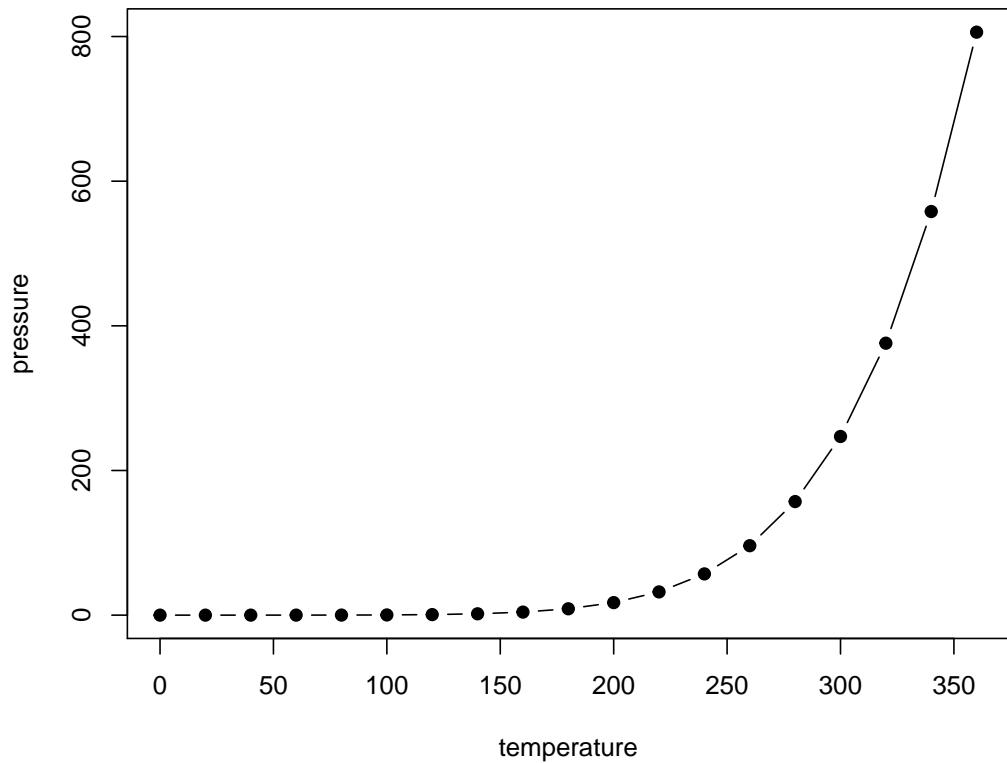


Figure 1.1: Here is a nice figure!

Table 1.1: Here is a nice table!

Sepal.Length	Sepal.Width	Petal.Length	Petal.Width	Species
5.1	3.5	1.4	0.2	setosa
4.9	3.0	1.4	0.2	setosa
4.7	3.2	1.3	0.2	setosa
4.6	3.1	1.5	0.2	setosa
5.0	3.6	1.4	0.2	setosa
5.4	3.9	1.7	0.4	setosa
4.6	3.4	1.4	0.3	setosa
5.0	3.4	1.5	0.2	setosa
4.4	2.9	1.4	0.2	setosa
4.9	3.1	1.5	0.1	setosa
5.4	3.7	1.5	0.2	setosa
4.8	3.4	1.6	0.2	setosa
4.8	3.0	1.4	0.1	setosa
4.3	3.0	1.1	0.1	setosa
5.8	4.0	1.2	0.2	setosa
5.7	4.4	1.5	0.4	setosa
5.4	3.9	1.3	0.4	setosa
5.1	3.5	1.4	0.3	setosa
5.7	3.8	1.7	0.3	setosa
5.1	3.8	1.5	0.3	setosa

# Chapter 2

## Life's secrets for capturing energy

Much of the environmental and ecological engineering challenges are about dealing with too many suspended sediments and too many nutrients in surface and ground waters, and the consequences of too much organic matter and reduced compounds in aquatic ecosystems. We have also seen that these three challenges are inter-connected and related. Nutrients and organic matter are related because the former contain the needed atoms necessary to build living cell and organism structures, corresponding to *living organic matter*, while the latter generally refers to *dead organic matter* and/or the assemblage of molecules which at one point were part of a living organism.

This definition of the linkage between the two is hardly satisfying, however... When one looks at where the electrons are allocated on the important atoms, a much more unifying scheme appears. This chapter describes this unifying scheme.

### 2.1 The five fundamental requirements of life

Before we dive down to the electron level, let us make sure we recognize that it is possible to simplify why there is, or not, life on our planet and the universe. Such a list includes:

- the presence of liquid water
- available nutrients that can fulfill the need for the six most numerous and important atoms that build most of our cell and organism structure: C, H, O, N, S, P. Without the commas comes this delightful acronym *CHONSP* which generations of students have come to love
- a source of energy, which in most cases is the form of solar or chemical energy
- electrons acceptor(s), without which chemical energy cannot be released
- and a suitable temperature range (about -5°C to +50°C) because of course most of living organisms will not live for very long outside this range

There are many other requirements for life to occur, e.g., the ability to reproduce or even to die, but for what we are interested, this is a satisfying list. In this chapter, we will address the source of energy and what secret living organisms have found to store energy. Primary producers, which include most of chlorophyll containing plants from algae to angiosperms (flowering plants), have the ability to use solar radiations as a source of energy. An entire chapter is dedicated to this marvel.

However, even for primary producers, solar radiations cannot be the sole source of energy, otherwise they would die at night... And for the rest of living organisms, solar radiations are just not a source of energy (merely a source of 'bien-être' or vitamin D for humans....!). So obviously, life has had to find a solution so that energy would be available for all conditions of light and temperature on earth.

The *first secret of life* is the ability to **store energy in the form of high energy electrons onto organic molecules**. Having a source of energy is always a good thing. However, energy can only be released if there

is an outlet for it, otherwise it stays as potential energy. Practically, this means that the energy stored on organic molecules can and is only released when the electrons go from the high potential to a lower one, or in others words from *electron donors* (most of the time from organic molecules) to *electron acceptors*.

This is *the second secret* of life on earth: on our planet exists this miracle molecule, O<sub>2</sub>, which acts as an extremely powerful electron acceptor. So you have to imagine O<sub>2</sub> less as a *gentle acceptor of electrons* that organic matter would be kind enough to *donate*, but more like a very *aggressive electron seeker* and any organic matter located close to oxygen runs the risk to be **oxidized**, that is to lose its electrons. Sometimes, I like to refer to O<sub>2</sub> as the *electron kleptomaniac*. So in oxygenated environments such as earth's atmosphere and most water bodies, living organisms' only concern is to have potential energy available in the form of high energy electrons stored onto organic molecules, because this energy can easily be released at any time thanks to the very oxidizing agent O<sub>2</sub>. Chapter 3 goes into the details of how this energy is released and transferred in cells.

## 2.2 Electron allocation onto CHONSP

First, among the 6 atoms that form CHONSP, and this is true for all atoms except for noble gases which are stable monatomic atoms, none of them exist as monatomic atoms: they always form bonds with other atoms to form molecules. Among the CHONSP, three of them are homonuclear diatomic molecules, i.e., they can form molecules of two atoms of the same chemical element: H<sub>2</sub> (dihydrogen, although it is most often referred to simply as hydrogen, which can be quite confusing), O<sub>2</sub> (dioxygen, although oxygen is also most often used, unfortunately), and N<sub>2</sub> (dinitrogen, which name is generally properly applied). Obviously, they can also bind to other elements, which is what we are about to see.

If indeed, on our planet floats a very oxidizing agent in the form of O<sub>2</sub>, then the stable state of all other molecules should be where most other elements have lost their electrons to oxygen. And there ought to be techniques to see where the electrons are allocated on molecules.

Chemists have created the **oxidation state (OS)** or **oxidation number** indicator which quantifies this electron allocation to some extent, but we find it to be rather confusing for our purpose of allocating or view where the electrons are Stored in molecules. More discussion and information on Oxidation State is available in the glossary of this book.

We must first lock in the electronegativity concept. Electronegativity is a chemical property which describes how well an atom can attract an electron to itself. In the periodic table in Figure 2.1, first observe that our CHONSP all belong to the non-metallic atoms.

Second, all atoms are more or less electronegative. The most electronegative atom is F, fluorine. Electronegativity decreases towards the left and the bottom of the table as illustrated in Figure 2.2. For our CHONSP, ElecNeg<sub>O</sub> > ElecNeg<sub>N</sub> > ElecNeg<sub>S</sub> > ElecNeg<sub>C</sub> > ElecNeg<sub>H</sub> > ElecNeg<sub>P</sub>.

If you look carefully in Figure 2.2, you will see that the electronegativity of oxygen is 3.44, which is a lot greater than that of N (3.04), and the difference between N and C and S, is about 0.5. In other words, all the CHNSP stand no chance next to O: in any molecule which may involve CHNSP, the O atom will attract the electrons for itself. For example, in the H<sub>2</sub>O molecule, one can see in the Lewis dot Structure below that by applying the electronegativity rule, the O atom has all the electrons for itself, while the 2 H, have none for themselves. Let us make sure that there is no confusion between the octet rule and the electronegativity. Atoms assemble into molecules so that their octet rules be fulfilled. So in the H<sub>2</sub>O molecule, yes each H shares an electron with the O and reciprocally. But, the electronagativity tells us which atom really has the electrons for itself. And that atom is O.

We realize that the electron allocation can be quite confusing or a bit artificial at first, but we shall see in Chapter 4 that it is fully corroborated with oxidation and reduction processes.

*This chapter is still under construction*

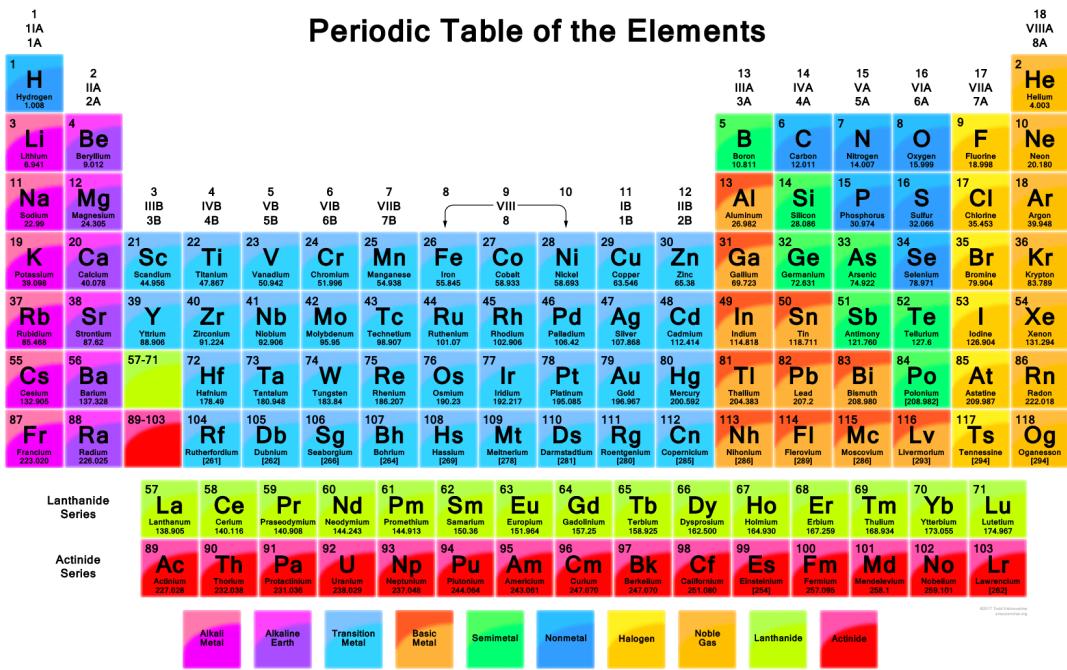
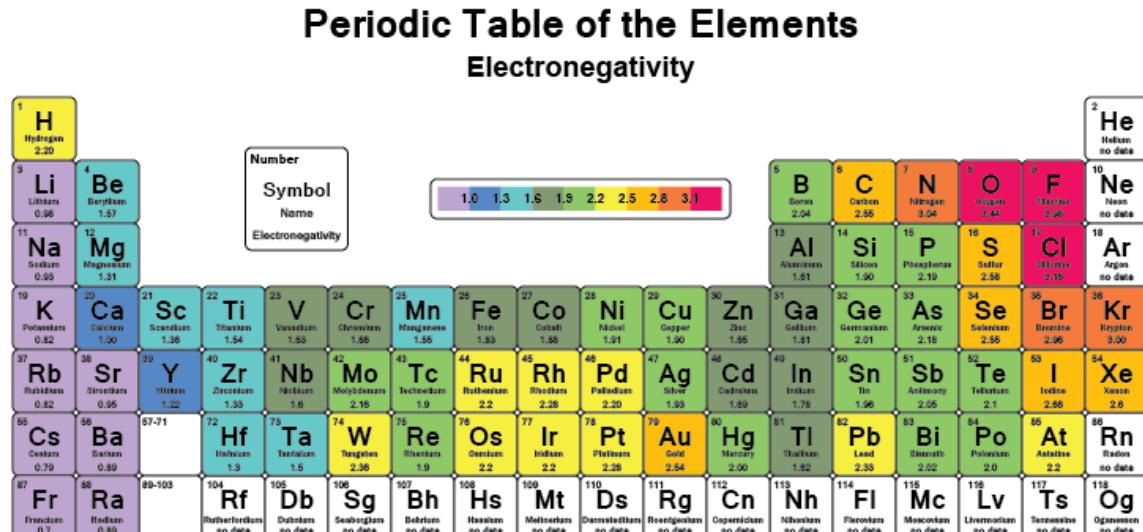


Figure 2.1: Vibrant Periodic Table With 118 Elements. Obtained freely from <https://scienzenotes.org/periodic-table-pdf-2/>



Lanthanide Series	57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu
Actinide Series	89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

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sciencenotes.org

Figure 2.2: Periodic table of the elements with electronegativities shown. Obtained freely from <https://sciencenotes.org/list-of-electronegativity-values-of-the-elements/>

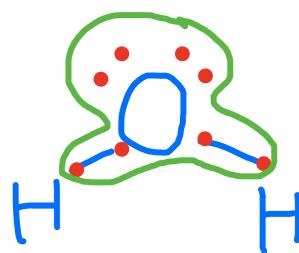


Figure 2.3: Electron allocation on the water molecule following the electronegativity rule

# Chapter 3

## Aerobic and anaerobic respiration

### 3.1 Generating energy: transfer of electrons

Us humans have the freedom to go about as we wish. All we need is to eat, drink, breath, and eliminate what we do not need. We could take our automobile cars as another example of bodies able to move about as long as they are fed some fuel. A lot of energy is realized from combustion of fuel or wood. In fact, provided that this energy be funneled into some very smart mechanical systems (e.g., combustion engines), and the energy power delivered is largely superior to any power any human could output. However, the energy consumed is also phenomenal and much of it is lost through heat. Life has found a way to be a lot more parsimonious with energy spending, which makes living organisms to be a lot more energy efficient than combustion in the long run: the *respiration* process.

But in both the cases of combustion or of respiration, energy is liberated or transferred to ATP, respectively, when **electrons are transferred** from an **electron donor** to an **electron acceptor**. Not surprisingly, life and its diversity as we know it, has been able to develop on our planet because primary producers have been able to transfer and store solar energy into a chemical energy, where electrons are effectively stored onto organic molecules, and, because dioxygen O<sub>2</sub>, the most powerful electron acceptor, is freely floating in our atmosphere.

But, then if we ourselves are nothing but a lot of organic matter, therefore a very large source of electrons, and if we leave in an oxygenated environment, how come we are not going into combustion? Obviously this applies to all living organisms. Some of you might think that it is because of the water. But then why would not a very dry piece of paper, or leaves, not automatically catch on fire? The answer lays in two words: activation energy.

Electrons do not readily transfer from an electron donor to an electron acceptor because of the activation energy barrier which must be overcome. Activation energy is the level of energy necessary to be overcome for a reaction to carry on. On the figure below borrowed from Wikipedia, the energy difference between the peaks and the final energy level corresponds to the activation energy E<sub>a</sub>

In normal conditions, this energy is preventing a reaction to proceed. This is why wood or most organic matter do not go into combustion or decomposition on their own. Nitroglycerin is notoriously unstable and the activation energy for electrons to be transferred to O<sub>2</sub> is very small, hence its propensity to explode and its danger. However, the activation energy can be overcome with enough heat brought near an electron donor in the form of a flame or a spark. Not much heat is necessary for natural gas (CH<sub>4</sub>) for combustion to take place, i.e., for transfer of electrons from the carbon to oxygen, releasing twice the volume of gases:



hence the explosive nature of this reaction.

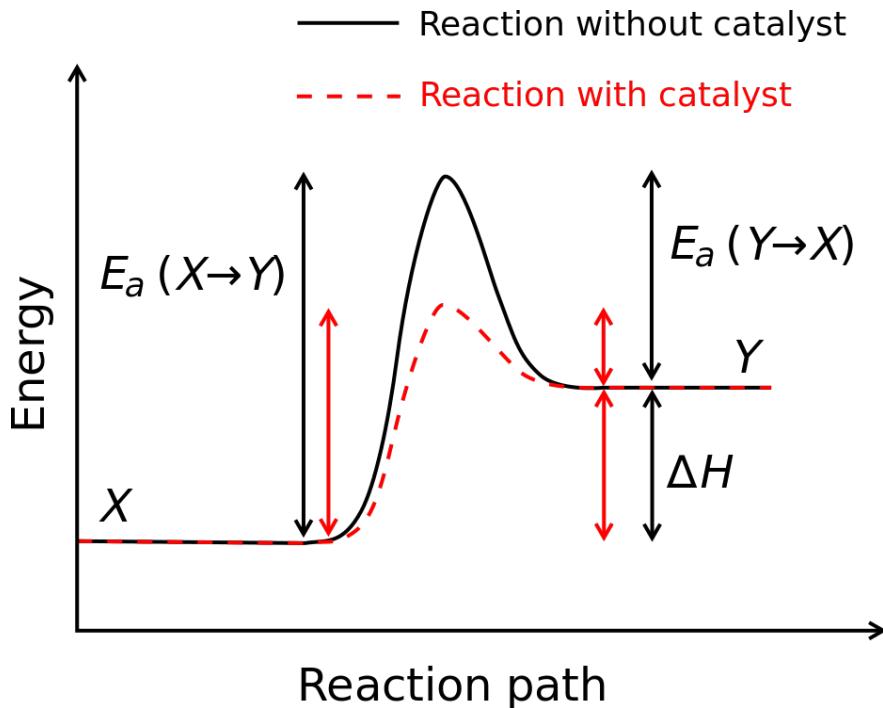


Figure 3.1: Activation Energy ( $E_a$ ) needed for a reaction to proceed.  $(E_a)$  can be lowered thanks to a catalyst, and in the case of the respiration chain of living organisms, many of them. Figure borrowed from Wikipedia, Copyrighted free use, <https://commons.wikimedia.org/w/index.php?curid=779552>

## 3.2 Generating energy without combustion

Now because none of us and all the living organisms with us, *burn* to generate energy, there must be other systems to manage to liberate energy, and, there must be ways to have much lower activation energy. Life has found several mechanisms to optimize the transfer of energy from organic matter to the a magic molecule: ATP or Adenosine TriPhosphate.

## 3.3 ATP or the energy currency of the cell

There are many ways of transferring energy. To heat a house in the western world, we most often have a centralized heating (and sometimes cooling) system, where the heat is generated, e.g., in a furnace, and then transferred to the rest of the house via pipes and the like. The equivalent system might be for mammals the blood that gets re-oxygenated with the lungs, before it delivers oxygen throughout the body.

At the cellular level, however, ATP or Adenosine TriPhosphate is created throughout the cell near the equivalent of the little furnaces: mitochondria. Needless to say, there is no combustion with the mitochondria, and yet transfer of electrons and energy delivered. The energy is transferred from the organic matter to ATP, and ATP being a relatively small molecule, can easily reach all metabolic processes, usually operated by proteins, which need energy to proceed (to overcome the activation energy mentioned above).

The key to storing energy is in the bond between the phosphate, which are often referred to as high-energy bonds. High-energy phosphate bonds are pyrophosphate bonds, acid anhydride linkages formed by taking phosphoric acid derivatives and dehydrating them. As a consequence, the hydrolysis of these bonds is exergonic under physiological conditions, releasing energy. As ATP releases its energy by the break of the pyrophosphate bond, ATP thus becomes ADP (Adenosine Diphosphate) and releases a phosphate in the

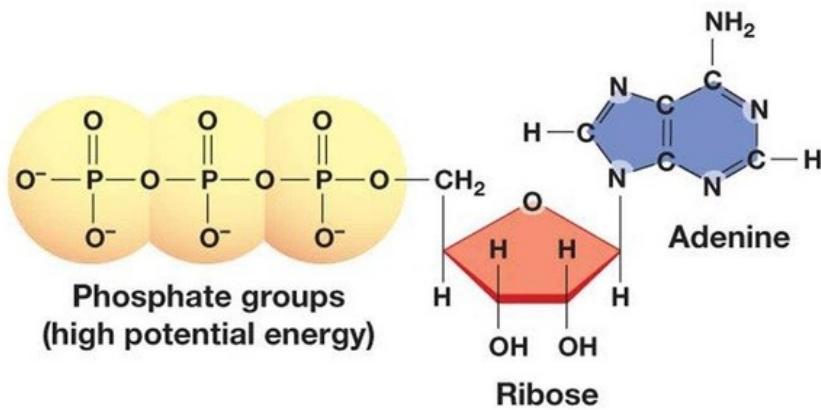


Figure 3.2: ATP molecular structure containing adenosine (= adenine + ribose) and the three phosphate linked together with a pyrophosphate energy rich bond. Copyright Pearson Benjamin Cummings

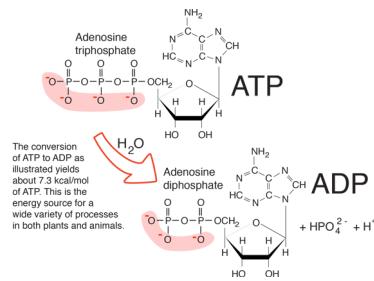


Figure 3.3: Release of energy ATP hydrolyzed into ADP. <http://hyperphysics.phy-astr.gsu.edu/hbase/Biology/atp.html>

meantime, like in figure 3.3. A common representation of this reaction is  $\text{ATP} \Rightarrow \text{ADP} + \text{P}_i$  with the  $\text{P}_i$  referring to as a phosphate.

Now that we have allocated electrons on atoms and molecules, you easily understand that the number of electrons on the outside of the triplet  $\text{PO}_3 - \text{O} - \text{PO}_2 - \text{O} - \text{PO}_2$  is huge. In his fantastic book, Degens (1989) suggests that

- because of the plethora of electrons on the triphosphate part of ATP,
- because the tetrahedral phosphate molecules are linked together by one of the corners of each tetrahedron (figure 3.4),
- and because of the  $\pi$  electron bonds (double bond) which tends to repulse each other (Degens, 1989),

the triphosphate molecule can only be in constant movement, which 'maintains the animated world' (Degens, 1989).

## 3.4 The ATP manufacture

If ATP freely releases energy for a reaction to proceed in the cell, as ATP becomes  $\text{ADP} + \text{Pi}$ , there must be energy at one point to 'manufacture' or recharge the ATP molecule in the first place. We have seen that much of the energy in the cell is transferred thanks to the ATP molecule. So could we just make ATP from

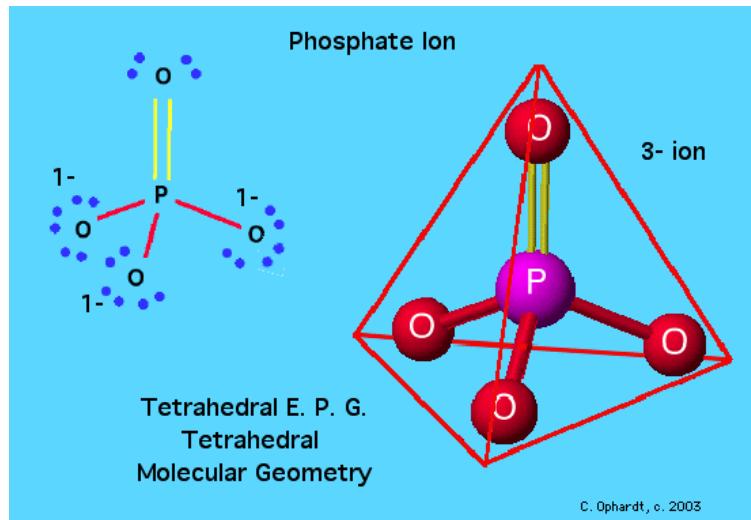


Figure 3.4: Tetrahedral configuration of each of the phosphate molecule which when linked together supposedly are in constant movement

the energy liberated by ATP??? The answer is no, obviously... The energy to create ATP has to come from elsewhere, otherwise it just cannot work....!!!

Life has found a wonderful way to do that thanks to proton flows. Yes, that is correct, a proton flow. ATP is literally formed from an ADP and a phosphate and assembled thanks to a protein called ATP synthase, or ATPase, which seems to be activated or powered almost mechanically, very much like a water mill, by a proton flow (Figure 3.5). The ‘head’ of the ATPase, which looks a bit like a ‘mushroom hat’, referred to as the F<sub>O</sub> region, rotates as a result of the proton flow and allows the phosphorylation of ADP. It is thus fair to call the ATPase a ‘proton mill’. You may want to look at this very nice concise document on ATP synthase on Wikipedia, which provides more detailed information and shows quite nicely how the mechanical force of the H<sup>+</sup> flow is thought to allow P<sub>i</sub> to be attached to an ADP. This part of the energy recovery is referred to as **phosphorylation**.

So, this mechanical force is what generates the synthesis of ATP from ADP, or again **phosphorylation**. So this solves that part of the ATP synthesis. Now, another mechanism must be responsible for the formation of the proton flow.

### 3.5 Creating a proton gradient as a source of proton flow

Until now, I have not even mentioned where all this takes place. We are about to see where this happens. But first, let us take an analogy to better understand the proton flow responsible for the synthesis of ATP. Flow of matter just does not happen on its own, it only happens as a result of a **gradient** between a compartment and another. Using the water mill analogy, the reason why there is release of energy in the form of kinetic energy of the water is because there is a drop in elevation, and therefore in potential energy between the ‘compartments’ upstream and downstream the mill. To increase the hydraulic gradient, men throughout the world have built dams to create compartments and increase the hydraulic gradient between upstream and downstream the mill. So in the water mill analogy, the hydraulic gradient is maintained thanks to the dam which creates compartments, and to the continuous arrival of new water upstream and to the leaving of water downstream. So three things are necessary for the release of kinetic energy (which in the end is going to power the rotation of a mill wheel, and in the old days would grind wheat or corn grain):

- a system to compartmentalize water,
- a supply of water, and,

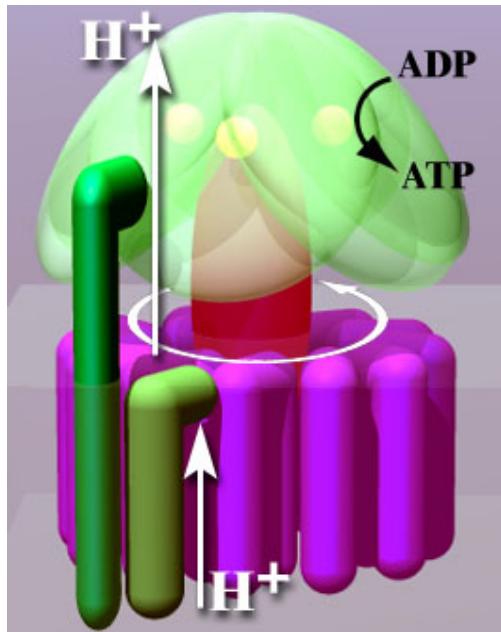


Figure 3.5: Artist representation of the ATP synthase powered, almost mechanically by proton flows. By The original uploader was Asw-hamburg at German Wikipedia - Transferred from de.wikipedia to Commons by Leyo using CommonsHelper., CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=8993938>

- an outlet for the water so that it does not accumulate.

By analogy, the formation of ATP corresponds to the grain grinding; the proton flow corresponds to the water flowing that activates the mill wheel, and in our case the proton flow activates the ATPase. We now need to address the three parts of the system needed to have a proton gradient and flow:

- a compartment system,
- a proton supply system, and,
- a system to use protons so the gradient is maintained.

### 3.5.1 A compartment to accumulate protons

We saw earlier that the double phospholipid layer made the cell membrane. Well if you close a membrane on itself you will make a compartment or a balloon. It turns out that if you look at a typical prokaryotic cell and at organelles in a eukaryotic cell (Figure 3.6), there are compartments formed between a cell wall and the plasma membrane for prokaryotic cells, and between two double layer phospholipid membranes for the eukaryotic cells. So in reality, it is a bit as if one were to create a space between two balloons.

At the end though, there exists for prokaryotic cells and mitochondria (and most organelles for eukaryotic cell) an inter-membrane space, which effectively creates compartments to accumulate ‘things’. The double phospholipid membrane is so strong and so tight that it is *proton-tight*. In other words, if somehow protons accumulate in this inter-membrane space, protons cannot leave through the membrane, they can only leave through designated areas. And yes, you guessed right, the designated area for protons to leave the inter-membrane space is the ATP synthase! So to take another analogy, you can imagine an inner tube full of air under pressure, air can only leave through the valve. And if you were to put a little fan in front of the valve as you are releasing air, the fan would rotate. Imagine that this is essentially what happens: the ATPase is both the valve and the fan as it literally rotates with the proton flow.

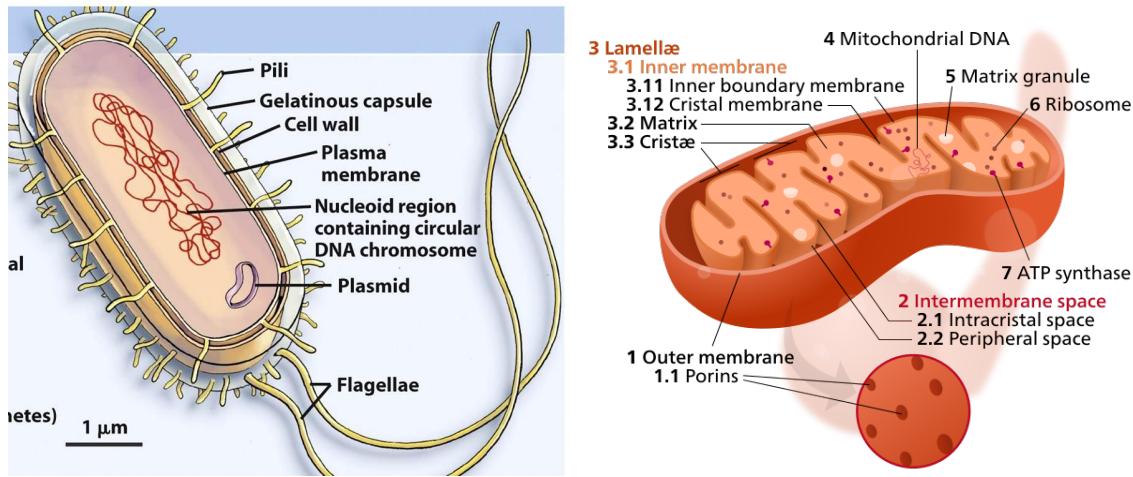


Figure 3.6: Artist representation of a prokaryotic cell and a mitochondrion from a eukaryotic cell showing the intermembrane spaces: between the cell wall and plasma membrane for the prokaryotic cell, and, between the outer and inner membranes for the mitochondrion. <https://micro.magnet.fsu.edu/cells/animals/animalmodel.html> and By Kelvinsong - Own work, CC0, <https://commons.wikimedia.org/w/index.php?curid=27715320>

### 3.5.2 A supply of protons for the intermembrane space: proton pumps

Now, the next natural question is that in the case of an inner tube, somebody pumped some air into the tube to put it under pressure. Similarly, there must be a system that pumps protons into the inter-membrane space. And yes indeed, you guessed it right, there are proton pumps that do the job. But again, these pumps must be powered by some sort of energy.

Let us pause for a second. We have seen in section 3.1 that generally speaking, energy is generated from the transfer of electrons. But until now, we have not even mentioned electrons: only ATP and protons...? This is where electrons come in: **energetic electrons transported onto electron transfer molecules are the ones that power the proton pumps!** It is time to take a bird's eye view again. Energy liberation exists when electrons are transferred from an electron donor to an electron acceptor. Life has found a way to capture this energy in the form of ATP, which allows transportation of energy to the needed places in the cell. So the energy transfer in the cell does not have to be totally instantaneous like combustion would be, and it can be spent exactly where it needs to be spent.

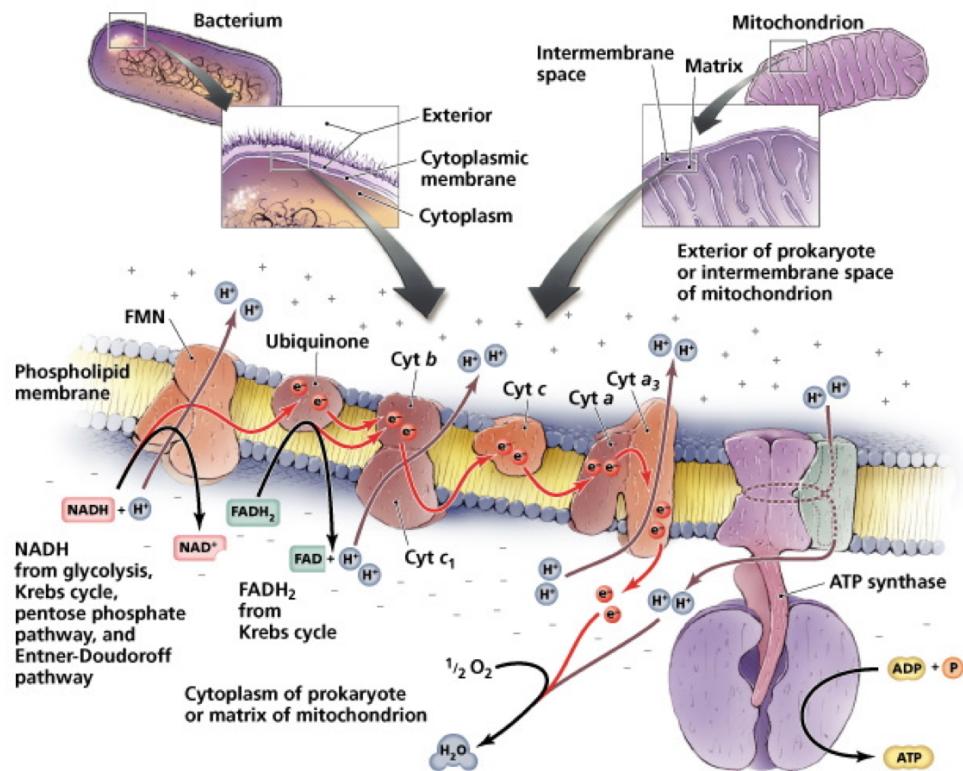
Yet, the production of ATP is in fact generated by an electron transfer, but indirectly.

- No, electrons are **NOT** transferred from organic matter to ATP.
- Yes, electrons are *ultimately* transferred from organic matter to an electron acceptor, which in the case of aerobic respiration is  $O_2$ .

But just like the generation of ATP is indirectly linked to the electron transfer, the transfer of electrons from organic matter to the ultimate electron acceptor is also indirect. A set of molecules called **electron transfer molecules** are intermediate carrier of electrons and are the ones which power the proton pumps as represented in figure 3.7 below.

### 3.5.3 Electron transfer molecules that power the proton pumps

The two main electron transfer molecules in the respiration process are called NAD (Nicotinamide Adenine Dinucleotide; figure 3.8) and FAD (flavine adenine dinucleotide; figure 3.9). These two nucleotides have the



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Figure 3.7: Artist representation of the ATP synthesis powered by a proton flow, itself powered by a proton gradient, itself produced thanks to proton pumps powered by energy rich electrons carried by electron transfer molecules. Obtained from 2006 Pearson Education

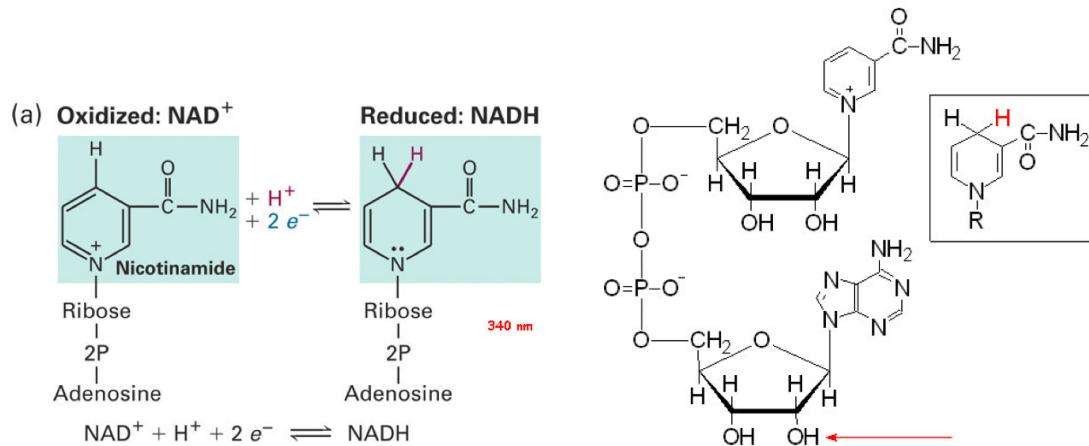


Figure 3.8: Molecular formula of Nicotinamide Adenine Dinucleotide (NAD) in both oxidized and reduced states

ability to be reduced (= gain electrons) and oxidized (= lose electrons), and because they are mobile, to carry electrons from the cell (bacteria) or mitochondrion (eukaryot) cytoplasm to the proton pumps.

### 3.6 Transfer of electrons from the Organic Carbon to an electron acceptor

So this answers the question of how the proton pumps are powered, but it is now time to look at the global fate of electrons: where do they come from and where do they end up? Actually, we already know the global answers to these questions: the electrons are stored unto the C, N, and S of organic molecules and they are eventually accepted by an electron acceptor; in the case of aerobic respiration,  $\text{O}_2$  is the ultimate electron acceptor.

Before there might be any confusion, in the aerobic respiration process that involves organic molecules as electron donors (we will see that inorganic molecules can also be electron donors), strangely enough, the only atom which donates electron is **carbon** while the nitrogen and sulfur atoms keep all their eight electrons. Specialized microbes, called *lithotrophs*, literally ‘feed on stone’, which means that their source of electron is from an inorganic molecule, have the ability to use ammonium and hydrogen sulfide as electron donor (8 electrons available for ‘donation’) in their aerobic respiration chains. So all this to say that in aerobic respiration that involves organic matter, **only** the carbon atom donates its electrons. The organisms which use organic matter as electron donors are called *organotrophs*. We, as mammals and humans, are *organotrophs*.

#### 3.6.1 Oxygen reduction

Let us start from the end of the process: the reduction of oxygen into  $\text{H}_2\text{O}$ , as represented on figure 3.7 above. As the NAD and FAD are powering proton pumps (which names include FMN, ubiquinone, cytochrome a, b, and c see figure 3.7), the electrons lose some of their energy and migrate within the phospholipid membrane from the proton pumps towards the ATPase. Because of the involvement of many different proteins involved in this transport, this is referred to as the **electron transfer chain**. Because the NAD and the FAD molecules are oxidized (they have given up their electrons), and because at the ATPase location, ADP gains a phosphate to become ATP, the whole process is referred to as **oxidative phosphorylation**. At the confluence between the ATPase and the cytochrome  $a_3$ , the reaction (3.2) occurs:

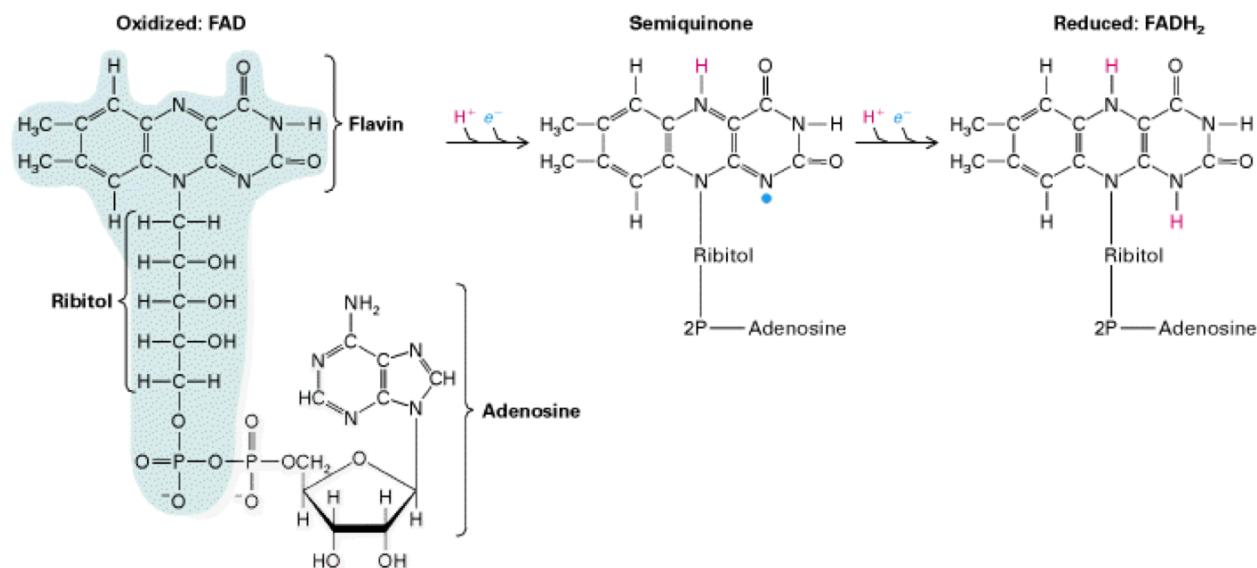


Figure 3.9: Molecular formula of Flavine Adenine Dinucleotide (FAD) in both oxydized and reduced states



This is the place where the electrons are accepted!!! So now you know exactly where all this is happening! Is not that wonderful? So the electrons are accepted by O<sub>2</sub> (remember on a molecule of O<sub>2</sub>, each Oxygen atom only has 6 electrons for itself, so each can accept two more), and these electrons reduce oxygen into H<sub>2</sub>O. This has several consequences:

- the reduction of O<sub>2</sub> also consumes 4 H<sup>+</sup>, which solves the necessity for an outlet for protons as they flow out of the inter-membrane through the ATPase, which was the third condition to maintain a proton gradient
- the reduction of O<sub>2</sub> obviously consumes electrons, which also provides an outlet for the electrons. In order words, if no electron acceptors are available, then the electrons at the proton pumps have no outlet, so the proton pumps are stalled, which in turn halts the maintaining of a proton gradient, which eventually stops the production of ATP.

### 3.6.2 Electron fate from organic matter to electron transfer molecules

The fate of electrons from organic matter or glucose to the electron transfer molecules involves two processes called glycolysis and the Krebs or citric acid cycle. Biology majors have to learn in details all the steps and the name of the molecules involved in these chain reactions. This is beside our point for our class. Instead I want you to understand and know that these reactions involve

- transfer of electrons from organic carbon onto electron transfer molecules
- the release of carbon atoms which have lost all their electrons, therefore releasing C in the form of CO<sub>2</sub>.
- the hydrolysis of glucose molecules to pyruvate, a C3 molecule (a 3 carbon organic molecule) during the glycolysis, which then loses CO<sub>2</sub> as it enters the citric cycle
- the acetyl-coA, a co-enzyme is key to incorporate organic carbon in the Krebs cycle.

In figure 3.10 below which summarizes all the aerobic respiration pathways, you can see that most of the ATP is formed thanks to the proton flow, and that electron transfer molecules are involved in all steps.

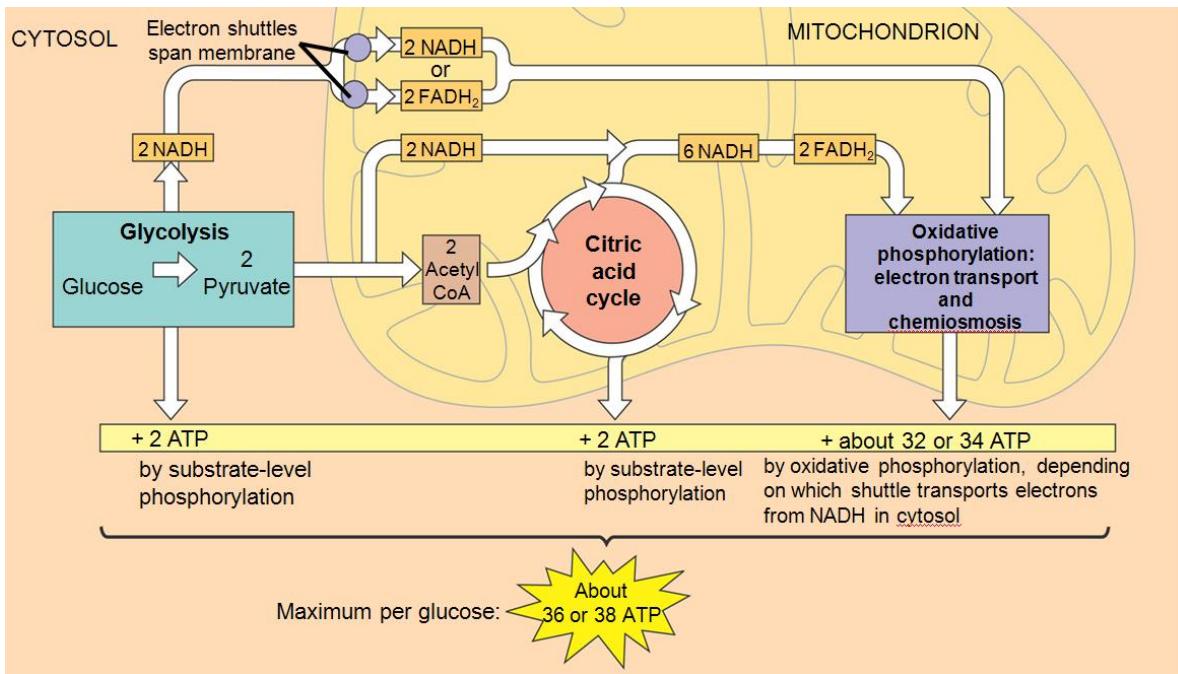


Figure 3.10: Summary of cellular respiration with the ATP and electron transfer budget. Obtained from <https://cdn.thinglink.me/api/image/847806852426104839/1240/10/scaletowidth>

This 3.10 diagram does not show where the CO<sub>2</sub> are produced so I invite to see, in addition to the notes given in class, that CO<sub>2</sub> is released as pyruvic acid enters in the figures 3.12 and 3.11 below.

### 3.7 Summary of respiration

We have purposely presented the molecular processes of respiration starting from the end and moving back to the beginning of the respiration chain. We can summarize the respiration process again in that order:

- the goal of respiration is to transfer energy initially stored as high energy electrons on organic molecules to ATP, the energy currency of the cell
- The production of ATP itself has to come from a different source of energy than that liberated by ATP itself
- ATP is synthesized by the phosphorylation of ADP by the ATP synthase, itself powered by a proton flow from the inter-membrane space to the cytoplasm space
- the proton flow is powered by a proton gradient between the inter-membrane space and the cytoplasm space
- This gradient is made possible thanks to
  - a proton-tight compartment corresponding to the inter-membrane space
  - a supply of protons from the cytoplasm to the inter-membrane space, fed by proton pumps
  - an outlet for the protons flowing out and powering the ATPase ('the proton mill'), which combined with the reduction of oxygen form water molecules
- The proton pumps are themselves powered by the oxidation of electron transfer molecules (NAD and FAD), which carry high energy electrons from the Krebs or citric cycle to the electron transfer chain
- The electron transfer molecules accept the electrons, or are reduced during glycolysis, before and during the Krebs cycle as organic carbon are oxidized or lose their electrons and release CO<sub>2</sub>.

The other way to present respiration is to say that:

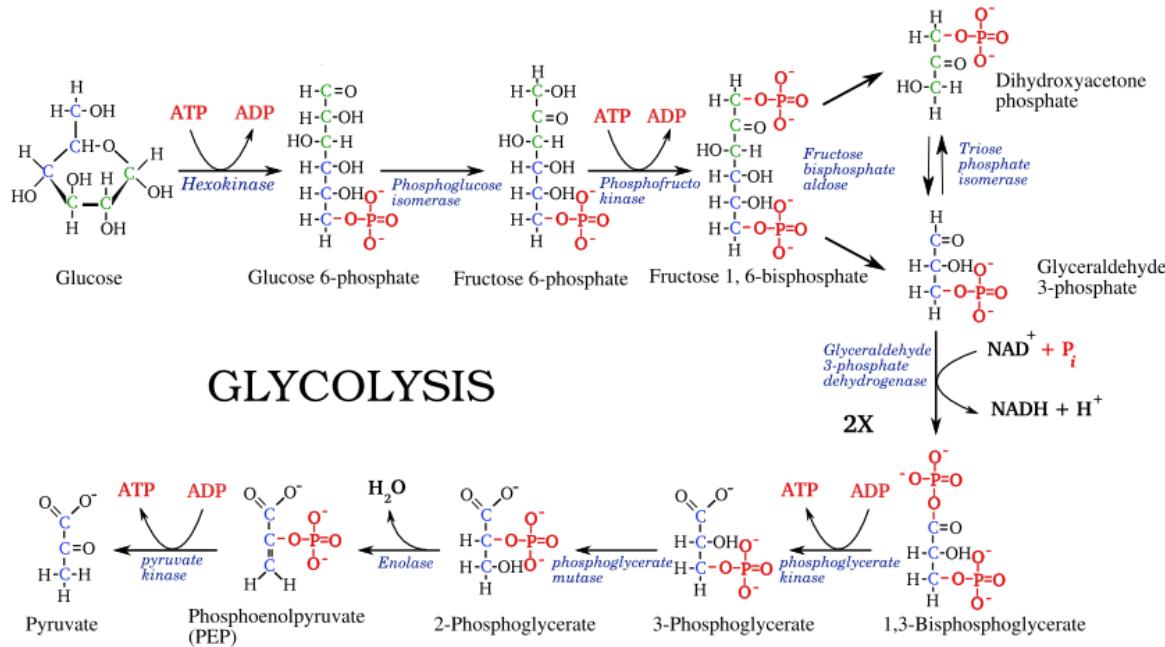


Figure 3.11: Glycolysis pathway. Image

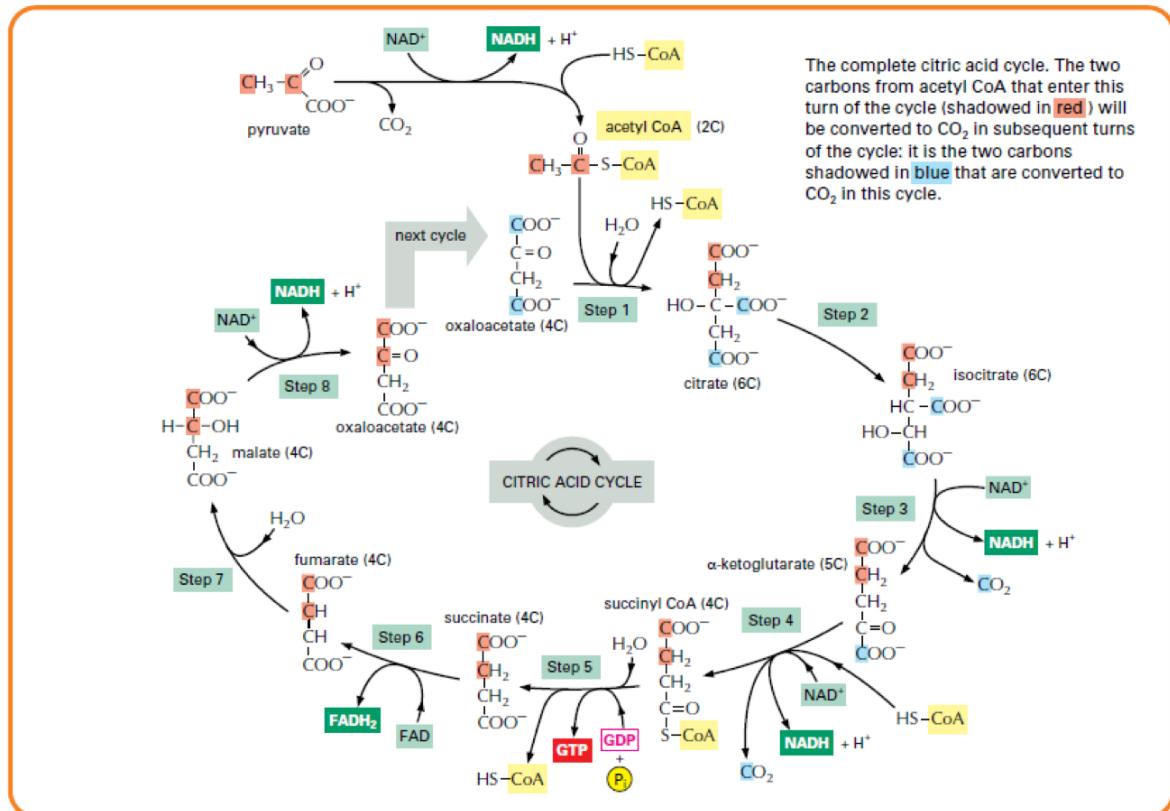


Figure 3.12: Krebs or citric acid cycle pathway.

- Energy rich electrons stored on organic molecules are transferred onto electron transfer molecules during glycolysis, before and during the Krebs cycle
- The high energy electrons thus transported power proton pumps placed in the inner membrane of the cell for unicellular organisms or of the mitochondrion for eukaryotic cells
- These pumps feed a supply of protons to the inter-membrane space which, because this space is proton-tight, and because protons outlets are limited to the ATP synthase, creates an accumulation of protons in this space
- The accumulation of protons creates a proton gradient between the inter-membrane space and the cytoplasm, which generates a proton flow at designated ‘holes’ in the membrane
- The protons flow out the inter-membrane through the ATP synthase which can be approximated by proton canals and almost mechanically turn the ATPase head which acts as a ‘proton mill’
- This proton mill catalyzes the phosphorylation of ADP into ATP
- The proton gradient is maintained possible because the protons flowing out of the inter-membrane space are combined with electrons reducing oxygen into water
- The electron flow is maintained thanks to the same oxygen reduction, which in turns allows electron transfer molecules to be oxidized so that then can take their ‘proton load’ again.
- Overall respiration consists in transferring high energy electrons from organic molecules to oxygen, and by doing so creating a proton gradient which in turn is the main driver for the formation of ATP in the cell.

Both summaries present the same story but have their own logic. Other important subtleties of respiration include:

- In the case organic molecules are the source of electrons, only the carbon atom donates electrons, the amine and thiol radicals being eliminated as a by-product DO NOT donate their electrons in this respiration system. This is admittedly a bit weird as the the amine and thiol groups still have 8 electrons to donate each.

## 3.8 Respiration electron flow schemes

### 3.8.1 Linkages between respiration and microbial processes

These few paragraphs and pages were necessary but we now need to connect them with some of the practical consequences that concern ecological engineering.

- The electron donors are the fuel for all microbial processes involved in the substrate of ‘treatment systems’.
- The byproduct of the loss of electrons originally stored on the electron donor will be a source of inorganic molecules that matter
- When the electron acceptor is  $O_2$ , this results in an oxygen demand which may have consequences on the overall oxygen level and may lead to anaerobic conditions, where other electron acceptors come in play
- We try to take advantage of the anaerobic conditions to dissipate or strip excess nutrients, e.g.  $NO_3^-$

### 3.8.2 Respiration schemes

So we thought we could provide a nice concise summary of respiration to highlight the points that matter for environmental and microbial considerations in the form of *respiration schemes*, still maintaining some of the important drivers of the formation of ATP. In the first respiration scheme in Figure 3.13 below, the transfer of electrons is represented by the *blue wavy line*. Electrons are transferred from an electron donor to an electron acceptor. The consequences of the transfer are represented in *thicker light blue arrows*. The first consequence of the transfer of electrons is the powering of proton pumps, which in turn create a proton gradient in the inter-membrane space, which result into proton flow, which gives the necessary energy to

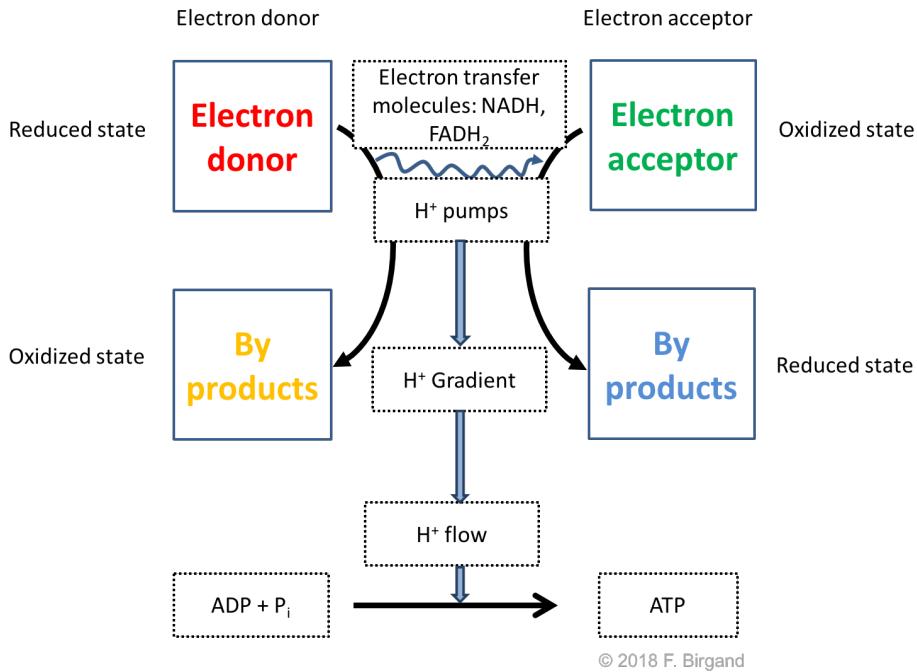


Figure 3.13: Generic respiration scheme illustrating the flow of electrons from an electron donor to an electron acceptor, the by-products of the respective oxidation and reduction, and the consequences on proton gradients and flow, which ultimately help catalyse the formation of ATP

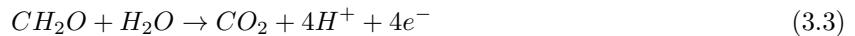
catalyze the phosphorylation of ADP into ATP. The products of oxidation, reduction, and phosphorylation are represented in black arrows.

The respiration scheme is obviously an oversimplified representation of what happens as described above, but generally holds true. Some ATP are generated during glycolysis and in association with the Krebs cycle, but represent 4 out of a total of 38, or about 10% of the total. So it is probably fair to simplify things to represent the majority of the processes (here 90%). The beauty of this scheme is that it applies to just about all types of electron donors and acceptors.

### 3.8.3 Aerobic respiration schemes for organotrophs

In the next scheme, we take the classic example of aerobic respiration, where glucose is considered as the electron donor, and O<sub>2</sub> the electron acceptor.

Using the generic carbohydrate CH<sub>2</sub>O as a source of electrons, it is possible to express the donation of electrons in equation (3.3) (redox half reaction):



Organisms which use *organic* molecules as electron donors are referred to as organotrophs, hence the title. We shall see later that some microorganisms are able to use inorganic molecules as their electron donors. In our case (Figure 3.14), the byproduct of glucose as the carbon atoms lose all their electrons becomes CO<sub>2</sub> as shown in equation (3.3).

In turn, the electrons are accepted by O<sub>2</sub> following equation (3.4)



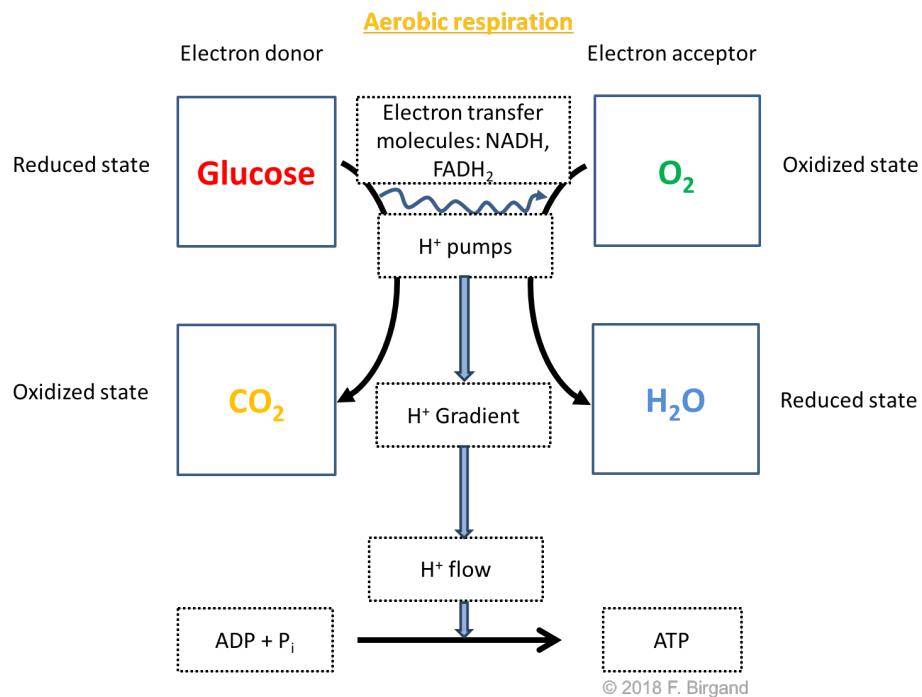


Figure 3.14: Aerobic respiration scheme illustrating the flow of electrons from glucose to  $O_2$ , the by-products of the respective oxidation and reduction being  $CO_2$  and  $H_2O$ , and  $H_2O$ . The consequences of the transfer of electrons on proton pumps, gradients and flow, which ultimately help catalyse the formation of ATP, do not change from the generic respiration scheme above

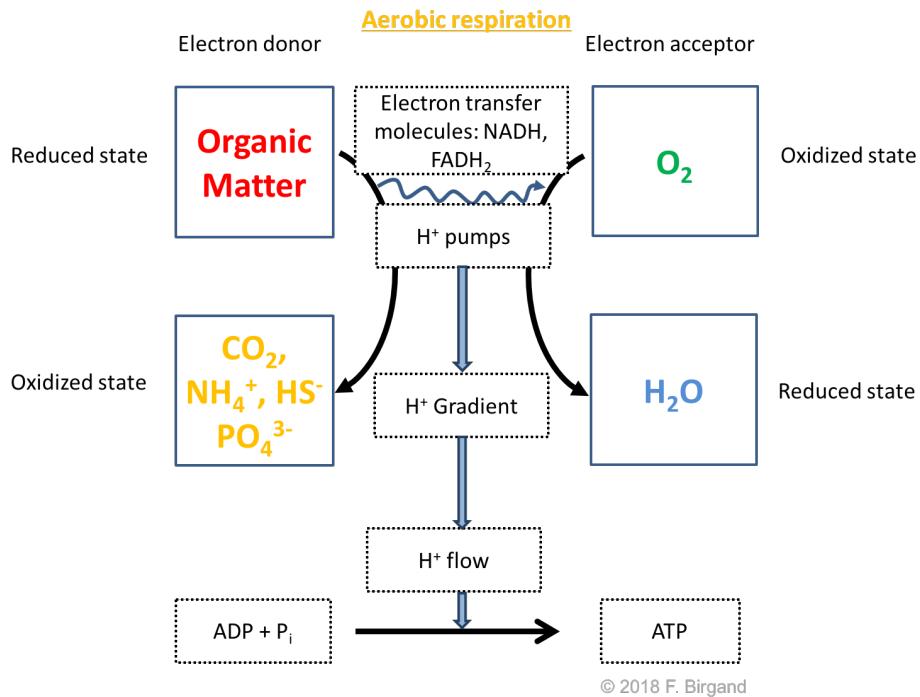
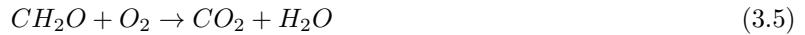


Figure 3.15: Aerobic respiration scheme illustrating the flow of electrons from organic matter to  $O_2$ , the by-products of the oxidation of OM include ammonium, hydrogen sulfide and phosphate this time

The combination of donating electrons from glucose (equation (3.3)), or organic matter illustrated in Figure 3.15, combined with the accepting of these electrons by  $O_2$  (equation (3.4)), results in the combined overall summary of aerobic respiration:



The third scheme (Figure 3.15), the generic term organic matter is used to show that in reality, not only glucose can be part of the electron transfer chain, but essentially all types of organic molecules, as represented in Figure 3.16. So the only difference with the previous scheme with glucose only, are the by-products of the catabolism of organic matter:  $CO_2$ ,  $NH_4^+$ ,  $HS^-$ , and  $PO_4^{3-}$ .

The important thing to notice here is that the N and S atoms, which in *organic molecules* possess 8 electrons for themselves, or are fully reduced, **stay reduced** as byproducts of respiration. This might appear odd as mentioned before, because there are electrons available for another oxidation to take place. Lithotroph micro-organisms are specialized in taking advantage of these available electrons, as we shall see later. The important message here is that you can see that respiration processes essentially breakdown large molecules into small inorganic ones, and that in the case of respiration for organotrophs, the only atom to lose its electrons is carbon.

If we think about this apparent oddity, N and S are a lot more electronegative than C, so in the organotroph cell, it is a lot easier for oxygen to strip electrons from carbon than it is from N and S. It might be worth taking a hydraulic analogy: in a soil, pore water will tend to drain through the area of a soil that has the highest hydraulic conductivity, or use the path of least resistance, totally bypassing areas of lower hydraulic conductivity. Chemically, it is just a lot easier to obtain electrons and energy out of the reduced carbon, and a lot harder to obtain them from amine (-NH<sub>2</sub>) and thiol (-SH) radicals; this is why N and S stay in a reduced form as byproducts of organotrophic respiration. Again, several group of lithotrophs specifically target the electrons on ammonium and on Hydrogen sulfide, and use  $O_2$  as their electron acceptor.

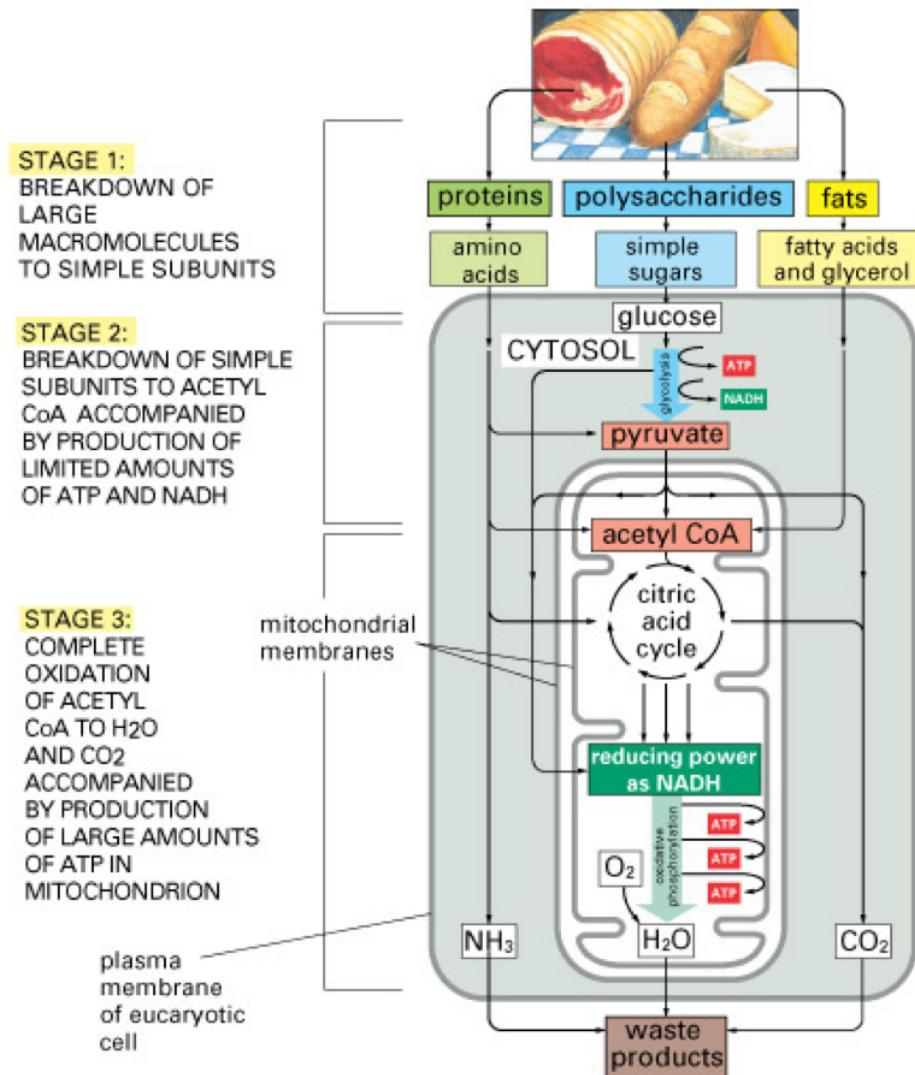


Figure 3.16: Oxidative phosphorylation of organic molecules of carbohydrates but of other molecular families as well. Figure obtained from Essential Cell Biology from Garland Science

In the next chapter, we will introduce all the respiration processes, aerobic and anaerobic, and performed by organotrophs and lithotrophs.



# Chapter 4

## The classic redox sequence of wetland soils

In the previous chapter, we introduced quite a few details on the molecular functioning of respiratory processes. We took aerobic respiration as our model to determine that most of the ATP produced in the cell is principally due to a proton flow from the inter-membrane space to the cytoplasm, for microbial cells and within the mitochondria for eukaryotic cells (Figure 3.10). The respiration schemes provide a very handy method to summarize the important drivers and consequences of respiration: the electron donors, the electron acceptors, and the byproducts of both oxidation and reduction. In this chapter we will use the same respiration schemes to explore the processes at play in wetland soils and the consequences on concentration gradients and movement of molecules of importance in environmental and ecological engineering.

### 4.1 The theoretical vertical sequence of respiratory processes in wetland soils

For this we will use a theoretical wetland soil to illustrate our point. Such theoretical soil has ‘enough’ organic matter content for microbial respiration to take place throughout its profile, ‘enough’ of other sand, silt, and clay and all the minerals that accompany them, including iron and manganese oxides. Let us assume that this theoretical soil, is sufficiently moist and aerated for microbial aerobic respiration to take place throughout the soil profile. Let us then assume that this soil is suddenly flooded, and let us explore the consequences of this. Respiratory processes are going to occur on a temporal sequence, which in fact will be mirrored by vertical sequence of processes in the wetland sediment.

### 4.2 An aerobic layer near the soil-water interface

At first, all micro-organisms are going to use all the oxygen that might be present in the pore space. But because pore space is now getting filled with water, it is possible that the amount of oxygen available for microorganisms might change. And yes, indeed, water does not have nearly the capacity to provide oxygen as air does for several reasons:

- at 15°C in water there are about 10 mg of O<sub>2</sub> in one liter of water. Comparatively, in one liter of air, there is about 300 mg of O<sub>2</sub>, or **30 times** more. How does one calculate this? At standard conditions, 1 liter of air at 21% oxygen possesses 0.21 L of oxygen. Since for these conditions, 1 mole of gas occupies 22.4 L, simply divide 0.21/22.4, to arrive at 0.0094 moles of oxygen. Then the mass of oxygen in 1 liter of air is  $0.0094 \times 32 \text{ g/mole} = 300 \text{ mg}$ .

- the diffusivity (which quantifies the ability of elements to move about) of O<sub>2</sub> in air is 0.176 cm<sup>2</sup>/s while that of O<sub>2</sub> in water is  $2.10 \times 10^{-5}$  cm<sup>2</sup>/s, or more than **8,000 times smaller** (Wikipedia contributors, 2017)

In other words, it is good to remember that **the amount of oxygen available in water compared to air is about 30 less, and that oxygen in water moves almost 10,000 slower than in water.**

So, one can clearly see that the potential supply of oxygen from flooded porewater is thus a lot more limited than in aerated pore space. Now, where is the potential source for supply of oxygen for our recently flooded soil? The answer is the atmosphere. For a real flooded wetland soil, another source of O<sub>2</sub> might be photosynthesis from algae and aquatic vegetation during the day. But in all cases, most of the oxygen needed for microbial aerobic respiration of our new flooded soil will have to travel the distance corresponding to the thickness of the water column, and, the linear distance within the porous medium of the soil, which can be quite *tortuous*. This property of the soil/sediment has been factored in by researchers and been called *tortuosity*.

**In summary**, the supply of oxygen to bacteria in a flooded soil is limited because of four factors:

- there is about 30 times less O<sub>2</sub> in water than there is in the same volume of air
- the O<sub>2</sub> diffusive transport capacity in water is about 10,000 times smaller than that in air
- O<sub>2</sub> has to travel from the atmosphere to the soil through the thickness of the water column, and
- travel through the tortuous path of the soil porous medium

In addition to these four rules, which apply to all aquatic systems, the velocity of water matters very much in the reaeration process, which corresponds to factor 1 above: reaeration is much higher for streams than for stagnant waters. In other words, the stagnant water above our flooded wetland soil example, is another factor, compared to streams, which further limits the supply of oxygen to the aerobic bacteria in the flooded soil.

Not surprisingly, this supply is just too limited compared to the demand. As a result, most of our recently flooded soil bacteria consumes all the O<sub>2</sub> and the only part of the sediment that might have a little bit of oxygen is the area at the soil-water interface. This is what it illustrated in Figure 4.2 below.

Animation summarizing the formation of aerobic and anaerobic layers of a theoretical flooded wetland soil due to the imbalance between bacterial respiratory oxygen demand and oxygen supply through the water column

Because the O<sub>2</sub> demand exceeds the O<sub>2</sub> supply from the atmosphere and the water column, an O<sub>2</sub> concentration gradient forms from the soil-water interface down. This concentration gradient, in turn, drives a downward movement of oxygen from the water column into the sediment down to the depth where there is no more concentration gradient. This depth defines the beginning of the anaerobic zone of the sediment, and above, the aerobic layer of the sediment.

### 4.3 Respiration in the anaerobic zone of the soil

What happens to all the microbes in the anaerobic zone of the sediment? Certainly the exclusively aerobic microbes just do not survive, but most bacteria are facultative aerobes. In other words, they can switch from aerobic to anaerobic respiration. Let us state this again: in the anaerobic zone of the sediment, only **unicellular microorganisms are able to survive** and have had to adapt their respiration to still be able to produce ATP for their metabolism, but using electron acceptors *other than O<sub>2</sub>*.

It turns out that thermodynamics dictate that not all electron acceptors can generate the same amount of energy when they strip electrons from their electron donors. As a result, one can classify electron acceptors in decreasing order from the most to the least oxidizing, and the list of preferred electron acceptors goes as such:

- nitrate or NO<sub>3</sub><sup>-</sup>

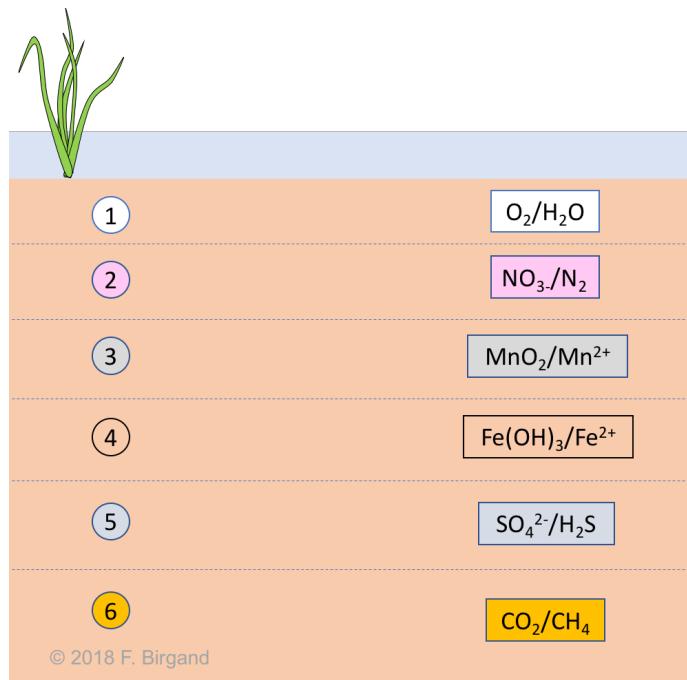


Figure 4.1: Theoretical spatial layering of wetland soils corresponding to the electron acceptor available, \*not too long after flooding\*. In each layer, the oxidizing and the reduced forms are illustrated as oxidizing/reduced. \*Not to scale\*

- Manganese oxide ( $MnO_2$ ) or Mn IV
- Iron oxides/hydroxyde or Fe III
- sulfate or  $SO_4^{2-}$
- Carbon dioxide or  $CO_2$

Although very different microbes are involved at the different stages, the apparent *demand* for electron acceptor, in our theoretical wetland soil, can be described as a *temporal* sequence of events: oxygen is the preferred electron acceptor; when  $O_2$  is all used,  $NO_3^-$  will be used as the preferred electron acceptor; when all the  $NO_3^-$  is used, the next most powerful electron acceptor is  $MnO_2$  (also referred to Mn IV; IV corresponds to its oxidation state), which is present in a solid or mineral form in soils; when all the  $MnO_2$  is used, then iron oxide or hydroxyde, which are also in the solid phase (also referred to Fe III, III corresponds to its oxidation state) will be used as the preferred electron acceptor; when all the Fe is used, then  $SO_4^{2-}$  is used as the next preferred electron acceptor, and then when finally all the other electron acceptors have been all used,  $CO_2$  can be the ultimate electron acceptor...!

Finally, to this temporal sequence corresponds a theoretical *spatial* sequence or layers where each of the electron acceptor essentially defines a soil layer, with the layers are organized with depth from the most to the least oxidizing electron acceptor as represented in Figure 4.1

It is now time to present the respiration processes in each of the *redox* layer.

## 4.4 A denitrification layer below the aerobic layer

The next most powerful or oxidizing electron acceptor after  $O_2$  is  $NO_3^-$ . We have seen in the previous chapters that the N atom in nitrate has zero electron for itself, hence its ability to accept electrons. Just like for aerobic respiration, nitrate reduction just does not happen on its own. Facultative anaerobic bacteria called *denitrifiers* take advantage of the electrons available on organic matter and of nitrate to accept them

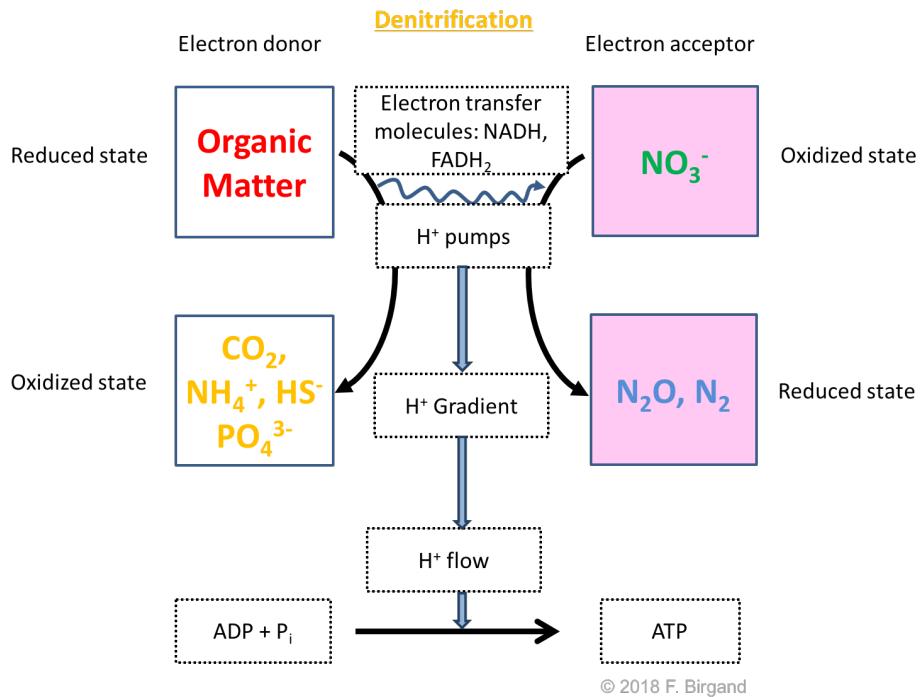
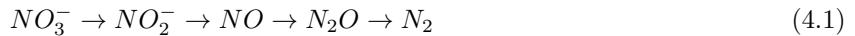


Figure 4.2: Respiration scheme for heterotrophic denitrification

to generate their energy. The denitrification of this theoretical wetland soil is referred to as heterotrophic denitrification because the source of carbon for these denitrifiers is also the source of electron, as OM is the source of both. On a side note, there are autolithotrophic denitrifiers, which use pyrite FeS<sub>2</sub> as their source of electrons, and have to find their carbon from another source than OM.

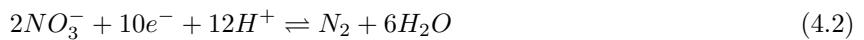
There are two possible byproducts of nitrate reduction: N<sub>2</sub>, which is the inert gas that makes 78% of our atmosphere, and N<sub>2</sub>O, which is a potent greenhouse gas. The byproduct of the oxidation of OM are the same as the ones in aerobic heterotrophic respiration, i.e., CO<sub>2</sub>, NH<sub>4</sub><sup>+</sup>, HS<sup>-</sup>, and PO<sub>4</sub><sup>3-</sup>.

In reality, you will find in textbooks that denitrification involves not a direct reduction of nitrate into N<sub>2</sub> or N<sub>2</sub>O, but rather a sequence of reductions, summarized in equation (4.1), where nitrite, nitrogen monoxide, and nitrous oxides are intermediate products:



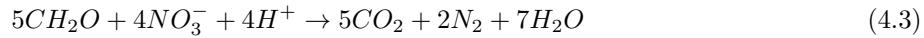
Nitrous oxide is thus evidence of an incomplete denitrification. Because denitrification currently is the one mechanism, which removes nitrogen from the aqueous phase as gaseous byproduct, it currently ranks as the most effective ways to treat excess nitrogen in water. Entire research programs are dedicated to the development of methods and treatment systems to optimize this process. One of the active research areas is about finding ways to have denitrification go all the way to the N<sub>2</sub> stage to minimize the production of N<sub>2</sub>O.

Because this is a reduction process, and because nitrate is *not assimilated* in the denitrifier cells, a very short and good definition of denitrification is the *microbially mediated dissimilatory reduction of nitrate into dinitrogen*. Nitrate reduction (= gains electrons) in denitrification is illustrated in the redox half reaction (4.2):



If you look carefully, you can see that to have the half-reaction balanced, 10 electrons were added for 2 nitrate molecules, or 5 electrons per nitrate molecule. This should ring a bell to you! Remember when we allocate electrons on N for dinitrogen and nitrate molecules, each N for dinitrogen has 5 electrons for itself, and the N of nitrate has 0 electrons for itself. So nitrogen needs to acquire 5 electrons to go from nitrate to dinitrogen. And guess what? This is exactly what half-reaction (4.2) shows! So now, if you were not convinced of the importance of the electron allocation in the early chapters, maybe you see its use now.

The overall transfer of electrons from the organic matter to  $\text{NO}_3^-$ , can be written as the combination of equation (4.2) and equation (3.3), to yield, let us admit it a rather complicated equation:



A more complete and more descriptive definition of denitrification is

Denitrification refers to the dissimilatory reduction, by essentially aerobic bacteria, of one or both of the ionic nitrogen oxides (nitrate,  $\text{NO}_3^-$ , and nitrite,  $\text{NO}_2^-$ ) to the gaseous oxides (nitric oxide,  $\text{NO}$ , and nitrous oxide,  $\text{N}_2\text{O}$ ), which may themselves be further reduced to dinitrogen ( $\text{N}_2$ ).

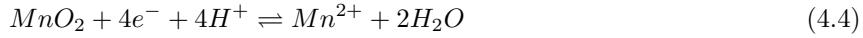
The nitrogen oxides act as terminal electron acceptors in the absence of oxygen. The gaseous nitrogen species are major products of these reductive processes. (Knowles, 1982)

Denitrification is thought to be inhibited by the presence of oxygen, and thus only occurs in our theoretical wetland soil, below the aerobic layer. However, denitrification only occurs if there is nitrate present as electron acceptor. Therefore, for denitrification to proceed, there must be a supply of nitrate that can compensate the demand due to denitrification. The only place from where nitrate can be supplied, is the water column, and, possibly, the aerobic layer of the sediment where nitrification can take place, as we shall see later.

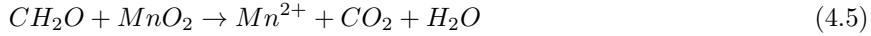
Following the same analysis as that of aerobic respiration, the demand for nitrate creates a **downward flux** of nitrate from the water column down. Because of the transport distance for nitrate from the water column and through the aerobic layer of the sediment, the supply tends to be lower than the demand. This suggests that at one point in depth there will be no more nitrate as they are consumed faster than they can diffuse downward. Nitrates thus diffuse through layer 1, and both diffuse and are consumed as electron acceptors in layer 2 in Figure 4.1. Again, because the demand exceeds the supply, nitrates cannot move further down than the bottom of layer 2. Denitrification is thus restricted to layer 2.

## 4.5 Manganese and Iron oxides reductions

Below the denitrification layer, microbes have to find new electron acceptors for their respiration. After  $\text{O}_2$ , which is a gas,  $\text{NO}_3^-$ , which is a dissolved anion, the next elements which serve as electron acceptors are solids: Manganese and Iron oxides. They will serve as electron acceptors, only if soil minerals containing Mn and Fe oxides are present. In our theoretical wetland soil, the assumption is that these oxides are present. In layer 3 (Figure 4.1),  $\text{MnO}_2$  serve as the electron acceptor following the general redox half-reaction (4.4), and respiration scheme illustrated in Figure 4.3.



The overall transfer of electrons from the organic matter to  $\text{MnO}_2$ , i.e., the combination of equation (4.4) and equation (3.3) can be written as:



While the electron donor is immobile, the reduction of  $\text{MnO}_2$  (equation (4.4)) produces  $\text{Mn}^{2+}$  ions, which are dissolved, mobile in water, and can diffuse following concentration gradients. In our original hypothesis

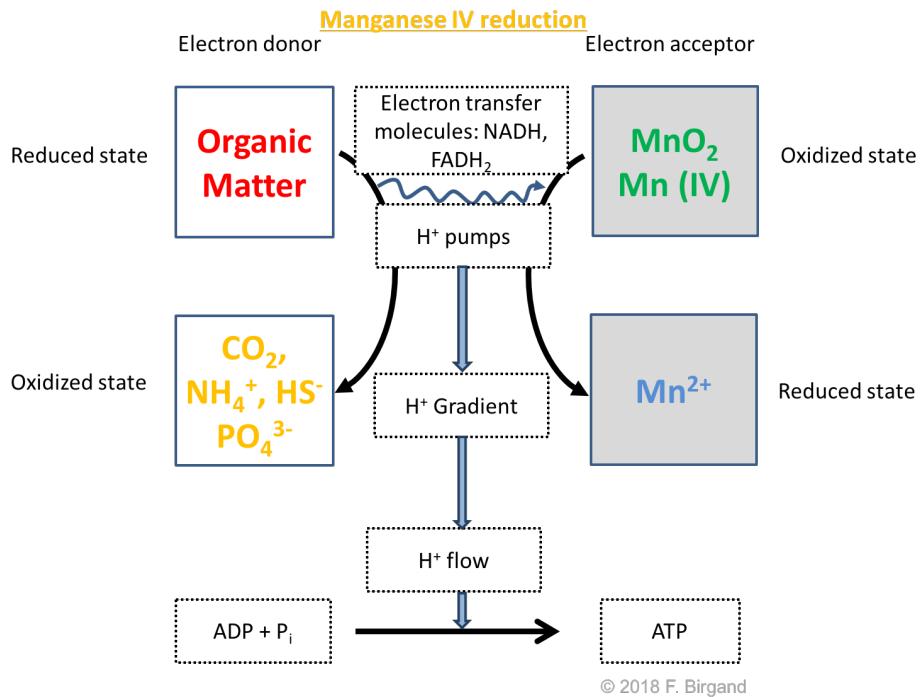


Figure 4.3: Respiration scheme for heterotrophic Manganese oxide reduction

of the sudden flooding of the wetland soil, MnO<sub>2</sub> oxides would be reduced from layer 3 down. The Mn<sup>2+</sup> ions produced in these layers, and the lack of Mn<sup>2+</sup> ions in layers 1 and 2, would create a concentration gradient, which would generate an **upward flux** of Mn<sup>2+</sup>, this time, from layers 3 to 6 into layers 2 and 1. The fate of Mn<sup>2+</sup> ions as they reach the aerobic layer is discussed below.

After the MnO<sub>2</sub> oxides are reduced, the iron oxides and hydroxide minerals will similarly serve as electron donors following the general equation (4.7), and respiration scheme illustrated in Figure 4.4. The reduction of an iron hydroxide Fe(OH)<sub>3</sub> has been added to show that in reality, Fe<sup>3+</sup> never exists as such, but almost always as iron oxides or hydroxides. Many forms of iron oxides exist in soils, hence the choice of choosing iron hydroxide Fe(OH)<sub>3</sub> in equation (4.7). Fe<sup>3+</sup>, Fe(OH)<sub>3</sub>, and other iron oxides are referred to as **ferric iron** or Fe(III), because their oxidation state is 3. Ferric iron generally has an orange rusty color.



The overall transfer of electrons from the organic matter to ferric iron can be written as the combination of equation (4.7) and equation (3.3) to yield:



Similarly to Mn<sup>2+</sup> ions, Fe<sup>2+</sup> ions, or **ferrous iron**, or Fe(II) is a dissolved iron which is mobile in water, and can diffuse following concentration gradients. For the same reasons explained above for Mn<sup>2+</sup>, an upward concentration gradient between zones 4 to 6 and layers 3 to 1 is going to appear and the Fe<sup>2+</sup> ions will tend to diffuse **upward** through layers 3 and 2. The fate of Fe<sup>2+</sup> ions as they reach the aerobic layer is discussed below.

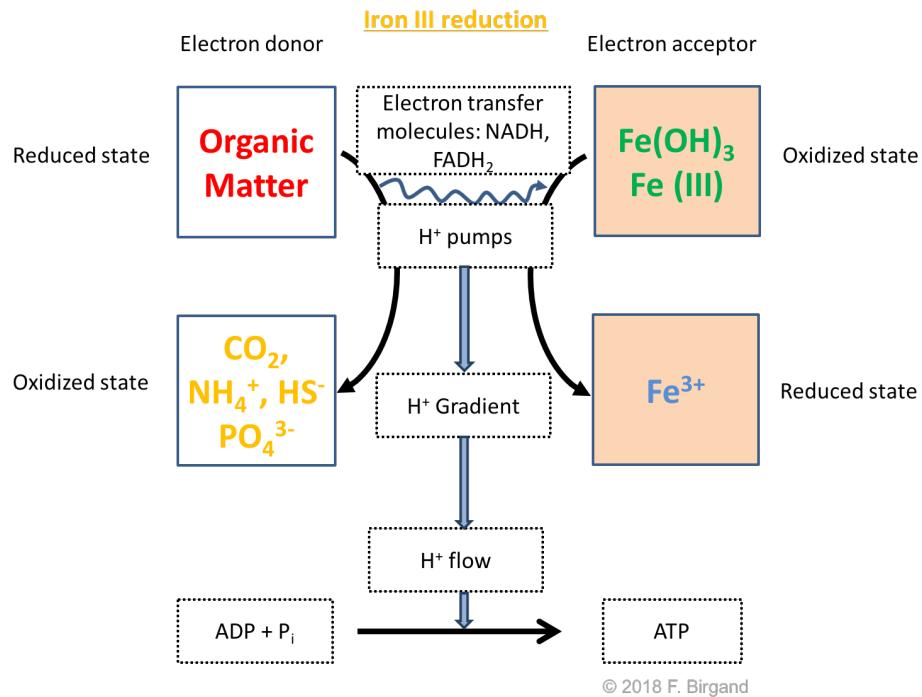


Figure 4.4: Respiration scheme for heterotrophic Iron oxide reduction

This vertical spatial sequence of layers 3 and 4 presented here probably only applies not too long after the theoretical wetland soil has been flooded. Indeed, over the long periods, the supply of ferric iron and manganese oxides will run out, as the only supply is in immobile mineral forms. So over long periods, layers 3 and 4 do not exist. Is the case of a net downward water infiltration, the  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  ions diffuse upward all the way into the aerobic layer, and accumulate there for reasons illustrated below. In the more likely case of small but real net downward flux of water, the dissolved ions will leach out of the soil profile. The consequences are that poorly drained soils then to leach out their iron and manganese, which is referred to as *iron and manganese depletion*. This is the reason for the grey color of hydric soils (Figure 4.5).



Figure 4.5: Example of pale bluish gray redox depletions. Note the faint rusty orange concentration distributed throughout the soil matrix. Reproduced with permission © 2012 Nature Education All rights reserved.

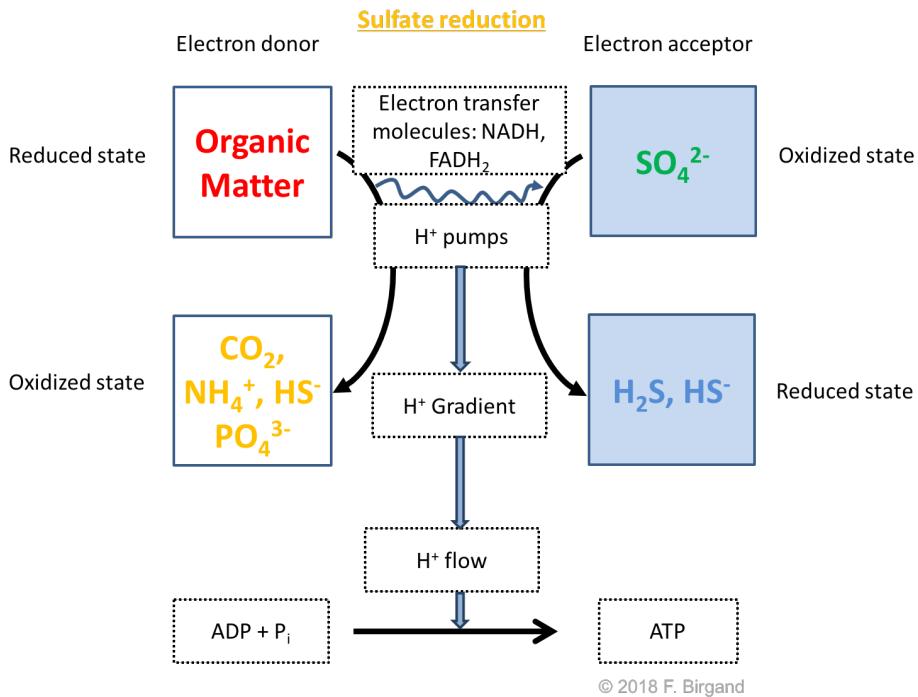
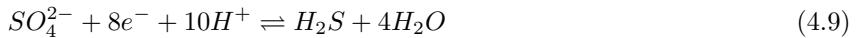


Figure 4.6: Respiration scheme for heterotrophic sulfate reduction

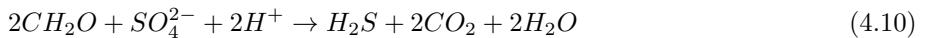
## 4.6 Sulfate reduction

After all previous electron acceptors have been used, sulfate becomes the next electron acceptor. As we have seen before, the sulfur atom on SO<sub>4</sub><sup>2-</sup> has zero electrons for itself and it therefore can accept electrons following redox half-reaction equation (4.9).



Here again, to balance the half-reaction, 8 electrons were needed. And by now, this should not surprise you because you now remember that the electron allocation rules tell you that on sulfate and dihydrogen sulfide, the S atom respectively has 0 and 8 electrons for itself. So the reduction of sulfate into dihydrogen sulfide requires the addition of 8 electrons, and guess what? Half-reaction (4.9) confirms just that.

While the electron donor is still organic matter as shown in Figure 4.6, the overall transfer of electron from the OM to sulfate can be written as:



Typical concentrations of sulfate in ground- and stream waters are between 1 and 10 mg SO<sub>4</sub><sup>2-</sup>/L. In our theoretical wetland soil profile, sulfate that might be originally present in the porewater will be reduced in layers 5 and 6 of Figure 4.1. For the same reasons invoked for oxygen and nitrate, the potential source of supply for sulfate for the sulfate reducing layer is all the sulfate that might be present in the layers above and the water column. The demand for sulfate in layer 5 will create a downward concentration gradient which will generate a downward diffusive movement of sulfate down to layer 5. And again, because of all the diffusion distance, the supply of SO<sub>4</sub><sup>2-</sup> is limited and does not match the demand. The imbalance between the sulfate supply and demand will limit the diffusion of sulfate to the bottom of layer 5, below which there will be no more sulfate.

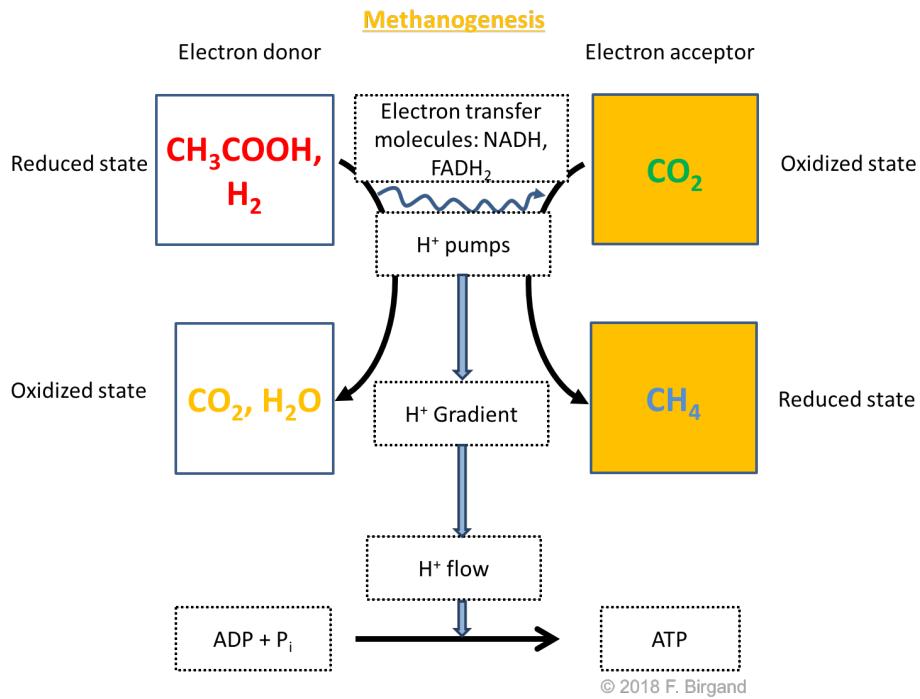


Figure 4.7: Respiration scheme for the heterotrophic Carbon dioxide reduction or methanogenesis

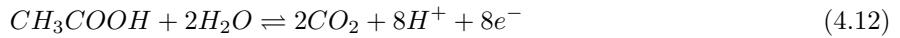
## 4.7 The methanogenesis oddity

The last redox reactions to take place at the bottom of our theoretical wetland soil uses CO<sub>2</sub> as the electron acceptor. Interestingly, the generic organic matter is no longer the electron donor, but is replaced by two byproducts of fermentation processes: H<sub>2</sub> and acetic acid CH<sub>3</sub>COOH as illustrated in Figure 4.7.

The fermentation products are beyond the scope of this class so, we will not come back to that, but it is important to recognize that H<sub>2</sub> and CH<sub>3</sub>COOH can donate electrons as shown in these half-reactions:



and



## 4.8 First summary on the electron acceptor chain in wetland soils

- except for methanogenesis, organic matter always serves, for organotrophs, as the electron donor. Moreover, it is **exclusively** the Carbon of the OM which provides the electrons.
- as a result, the byproducts of the oxidation of the OM when the organic carbons lose their electrons are always the same: CO<sub>2</sub>, NH<sub>4</sub><sup>+</sup>, H<sub>2</sub>S/HS<sup>-</sup>, and PO<sub>4</sub><sup>3-</sup> (but for methanogenesis). This means that at one point, all of these four (five if one counts both H<sub>2</sub>S and HS<sup>-</sup>, their exact proportion depends on pH, see details in the glossary) molecules will accumulate where they are produced unless they are used by another process

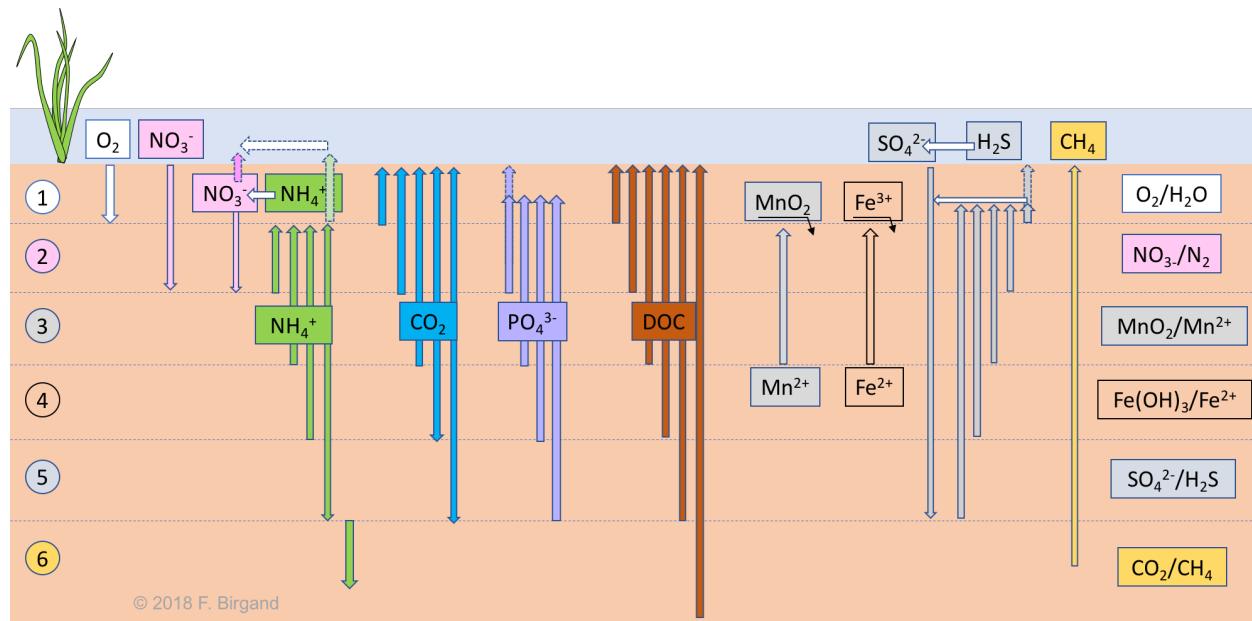


Figure 4.8: Diffusion fluxes of electron acceptors and all other soil diagenesis processes of a theoretical layered wetland soil

- To the contrary, the byproducts of the reduction of the electron acceptors are variable and depend on the electron acceptor. In the case of incomplete denitrification, and in the case of methanogenesis, N<sub>2</sub>O and CH<sub>4</sub> are significant greenhouse gases.

## 4.9 Supply and demand of electron acceptors and of the byproducts of Organic Matter oxidation

Now that we have established the reduction processes of the different electron acceptors at play, let us look at the consequences of the demands and the supplies associated with the respiratory processes.

### 4.9.1 Demands drive downward fluxes of dioxygen, nitrate and sulfate

In the Figure 4.8 below, the general directions of the fluxes of electrons acceptors and byproducts of respiration are illustrated for our theoretical wetland soil. The processes taken together is sometimes referred to as soil or sediment **diagenesis** and all the processes in there are then collectively referred to as **diagenetic processes**.

The demands for dioxygen, nitrate, and sulfate in their respective layers, lower their concentrations compared to the overlying water and layers, hence the formation of concentration gradients, which then drive downward diffusive fluxes of these electron acceptors to their respective layers. These fluxes are represented as downward arrows in Figure 4.8.

Now, it is the imbalance between the supply from above and the demand from below, that explain why the downward diffusion does not go beyond the bottom of the respective layers 1, 2, and 5 in Figure 4.8. The limitation of supply has been described for dioxygen in section 4.2. The diffusivity, distance, and tortuosity of the soil pores also apply for nitrate and sulfate, and explain why the demand is generally not met by the supply.

#### 4.9. SUPPLY AND DEMAND OF ELECTRON ACCEPTORS AND OF THE BYPRODUCTS OF ORGANIC MATTER OXIDATION

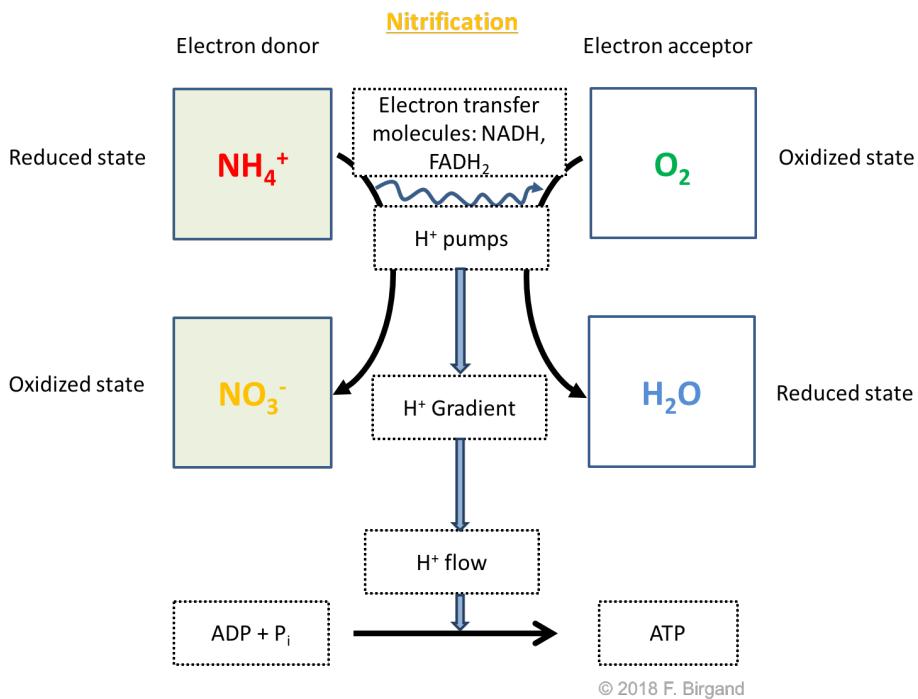


Figure 4.9: Respiration scheme for nitrification

#### 4.9.2 Supply of byproducts of organic matter oxidation

For every respiration process described above (except for methanogenesis), the byproducts are:  $\text{CO}_2$ ,  $\text{NH}_4^+$ ,  $\text{H}_2\text{S}/\text{HS}^-$ , and  $\text{PO}_4^{3-}$ . This suggests that in every single layer, there is a supply of all four of these byproducts. Inevitably, this will create an upward concentration gradient, which will then be followed by an upward flux of these four molecules in the sediment.

Phosphate will thus dissolve upward, at least until it reaches the aerobic layer. There it might encounter soil mineral oxides where it might bind, as we shall see in future chapters, hence the tips of the arrows not reaching the soil-water interface. However, it is possible that, if the aerobic layer is very thin and the mineral oxides are scarce in this thin layer, phosphates may diffuse all the way into the water column, hence the dotted arrow.

#### 4.9.3 Nitrification

Similarly, because of ammonium production in all the layers, but that of methanogenesis, ammonium will diffuse upward, until it reaches the aerobic layer. Remember that the nitrogen atom on the ammonium carries 8 electrons for itself, so it potentially carries a lot of energy. And yes, you guessed right, microbes called **nitrifiers** take advantage of these electrons and use **ammonium as their electron donors**, and use  $\text{O}_2$  as their electron acceptor. Because the electron donor this time is not an organic molecule, nitrifiers are called lithotrophs. Nitrification is represented in Figure 4.8 as the horizontal white arrow to the left. The nitrification respiration schemes is summarized in Figure 4.9.

Depending on the thickness of the aerobic layer and the availability of dioxygen, all or only a portion of the ammonium may get nitrified as ammonium moves upward, hence the dotted arrow going all the way to the water column. As nitrate is now produced in the aerobic layer of the soil, then the concentration gradient may sway upward or remain downward, depending on the nitrate concentration in the water column, hence the upward nitrate dotted arrow in Figure 4.8. You may also notice that a dotted white arrow pointing the

left has been added to illustrate nitrification which tends to readily occur in the water column, often thanks to nitrifiers attached at the soil-water interface.

In reality nitrification involves two stages: the oxidation of ammonium into nitrite, performed by ammonium oxidizing bacteria, of which two important genera are *Nitrosomonas* and *Nitrosococcus*, and then the oxidation of nitrite into nitrate, performed by *Nitrobacter* and *Nitrospira* bacteria (Equation (4.13)). But as far as we are concerned, both steps tend to occur almost simultaneously, and nitrite is thermodynamically unstable, and as a result very little tends to accumulate, either in soil or sediment.



#### 4.9.4 Gas bubble formation

Among the last two OM oxidation byproducts, CO<sub>2</sub> and H<sub>2</sub>S are gases. We saw earlier, that in reality the balance between H<sub>2</sub>S and HS<sup>-</sup> depends on the pH. In rather organic soils, which most treatment wetland soils are, the pH tends to be rather acidic, often below 6.5. So it is fair to represent the H<sub>2</sub>S/HS<sup>-</sup> couple as H<sub>2</sub>S rather than HS<sup>-</sup> (see H<sub>2</sub>S/HS<sup>-</sup> Figure), hence the choice of H<sub>2</sub>S in Figure 4.8 and the use of H<sub>2</sub>S below.

So in the end, if the summation of partial pressure of these and all other dissolved gases exceeds 1 atm + the hydraulic head, gas bubble will form and migrate upward. But because of surface tension forces, gas bubbles tend to get rather easily trapped in wetland soils and sediment. Hence the release of wetland gases when somebody or something disturbs the sediment, as we have seen in lab. To these two gases, one should add the production of CH<sub>4</sub> in the methanogenesis layer, which will readily ‘join’ the gas bubbles and the ride with them. The dissolved fraction will also tend to move upward because of the concentration gradient. Interestingly, methane does not oxidized very well in normal aerobic conditions of wetland soils, and will therefore tend to diffuse all the way up to the water column as illustrated in Figure 4.8.

Because of the demand for CO<sub>2</sub> in the methanogenesis layer, downward arrows have been added for layers 4 and 5 in Figure 4.8. The downward diffusion would only apply for the dissolved CO<sub>2</sub> as the gaseous form would obviously tend to move upward.

#### 4.9.5 Oxidation of upward moving reduced sulfur

Similarly to ammonium, and although a good proportion of H<sub>2</sub>S will end up in the gas phase, a still significant amount will stay in solution and will diffuse upward until it reaches the aerobic layer. Very similarly to ammonium, H<sub>2</sub>S still carries 8 electrons, which can be used for respiration provided that a strong enough oxidizer be present. In the aerobic layer of the sediment, H<sub>2</sub>S will be oxidized back into sulfate following the respiration scheme in Figure 4.10

The bacteria taking advantage of the electrons on sulfur of H<sub>2</sub>S are called **sulfur oxidizing bacteria**. There are different from the **sulfur oxidizing bacteria** which use OM as their electron donors, and sulfate as their electron acceptors. The oxidation of H<sub>2</sub>S has been summarized by the white horizontal arrow in both the aerobic layer of the soil and the water column. Sulfate produced can then diffuse back downward to layer 5 in Figure 4.8.

#### 4.9.6 Oxidation of upward moving Mn<sup>2+</sup> and Fe<sup>2+</sup>

The last direct reduction byproducts of respiration processes include Mn<sup>2+</sup> and Fe<sup>2+</sup>. Because they are being produced in layers 3 and below, an upward concentration gradient will be created, followed by an upward movement of Mn<sup>2+</sup> and Fe<sup>2+</sup>. In Figure 4.8, both of them have been represented as starting to move up from layers 4. In all logic, Mn<sup>2+</sup> and Fe<sup>2+</sup> should start moving upward as low as layer 6 because our original hypothesis was that soil was suddenly flooded, so in the temporal sequence of electron acceptors,

#### 4.9. SUPPLY AND DEMAND OF ELECTRON ACCEPTORS AND OF THE BYPRODUCTS OF ORGANIC MATTER OXIDATION

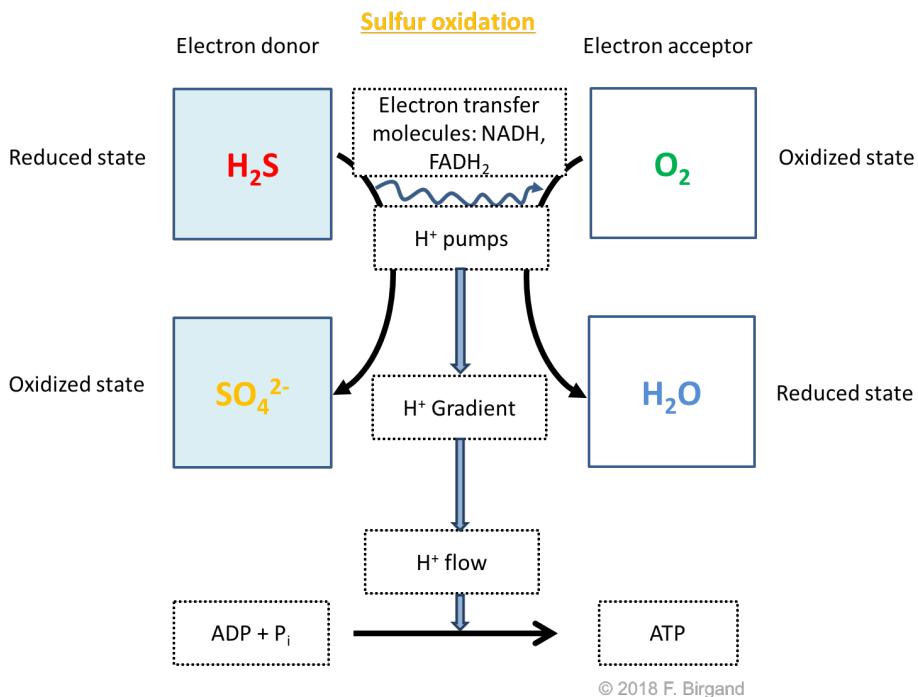


Figure 4.10: Respiration scheme for hydrogen sulfide oxidation

before sulfate carbon dioxide reduction conditions would prevail in Layers 5 and 6, respectively, the Mn and Fe oxides will serve as electron acceptors, and Mn<sup>2+</sup> and Fe<sup>2+</sup> will then be produced.

The more important fact is the fate of both Mn<sup>2+</sup> and Fe<sup>2+</sup> as they reach the aerobic layer. Because they are both reduced, they potentially have one electron to give, and yes, some bacteria have specialized in the ability to oxidize these ions. These bacteria are referred to as manganese and iron oxidizing bacteria. The respiration scheme corresponding to iron oxidation is illustrated in Figure 4.11 below.

As both Mn<sup>2+</sup> and Fe<sup>2+</sup> get oxidized in the aerobic layer of the sediment, they form Mn and Fe oxides, which are solids and precipitates with the other soil minerals, hence the underlying and downward pointing arrows under the MnO<sub>2</sub> and Fe<sup>3+</sup> in Figure 4.8. The precipitation of iron oxides or hydroxides can be quite visually spectacular sometimes as reduced groundwater seeps out into the open. Iron can get very quickly oxidized and form rusty biofilms.

##### 4.9.7 Moving of Dissolved Organic Carbon

The last but not least of the processes at play in diagenesis is the formation and diffusion of Dissolved Organic Carbon in poorly or anoxic soils. This is the subject of a future chapter.

This concludes this long chapter on the importance respiration processes occurring in wetland soils.

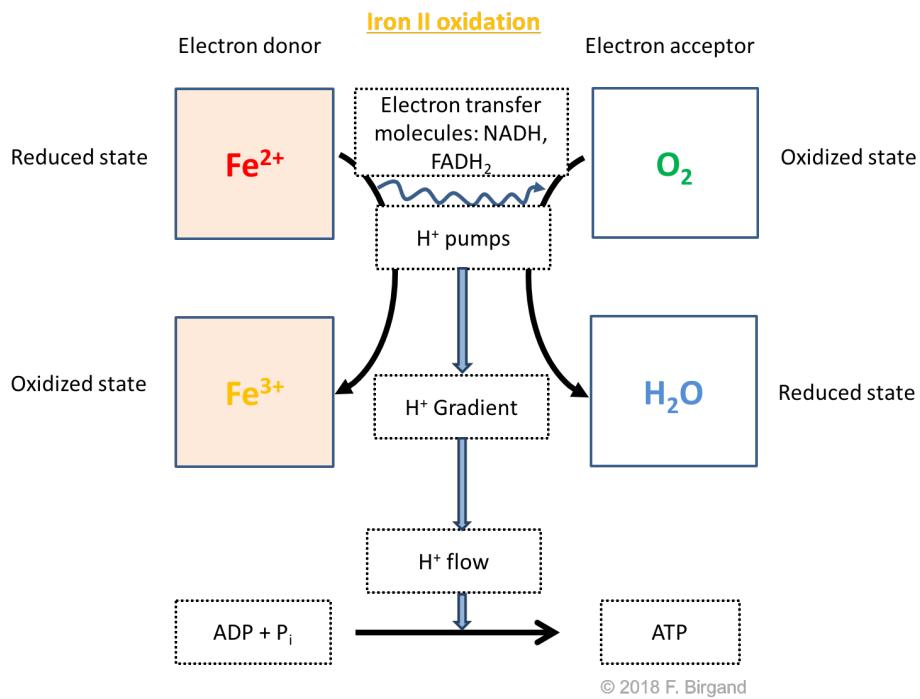


Figure 4.11: Respiration scheme for iron oxidation



Figure 4.12: Picture of an 'iron seep' in Goldsboro, NC, as reduced groundwater gets oxidized in contact with air

# Chapter 5

## Glossary

This glossary is meant to assemble terms that we routinely use in Environmental Sciences and Engineering and which are expected to be mastered by students taking BAE 204 at NC State university. They are ordered in alphabetical list for better retrieval and look up.

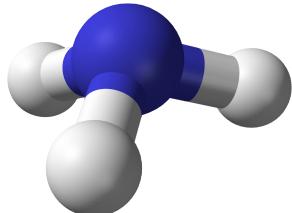
### 5.1 A

#### 5.1.1 Aerobic respiration

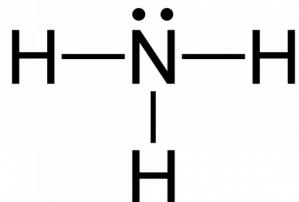
#### 5.1.2 Anaerobic respiration

#### 5.1.3 Ammonia

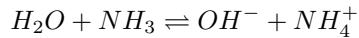
- Ammonia is a colorless gas with a characteristic pungent smell
- Formula:  $NH_3$



- Ammonia 3D shape:

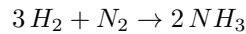


- Lewis dot structure:
- Number of electron N has for itself following electronegativity rule: eight
- $NH_3$  can only be an **electron donor**
- Because N has so many electrons to be potentially donated, ammonia is generally unstable in an aerobic environment. As a result, it tends to trace quantities in nature
- When dissolved in water, and depending on the pH of the solution, ammonia converts to ammonium following the reaction:



- Production:

- Because of its many uses, ammonia is one of the most highly produced inorganic chemicals. Dozens of chemical plants worldwide produce ammonia. Consuming more than 1% of all man-made power, ammonia production is a significant component of the world energy budget.
- In 2014, about 88% of the ammonia produced was used for fertilizing agricultural crops
- Modern ammonia-producing plants generally depend on the Haber-Bosch process which consists into reducing dinitrogen into ammonia



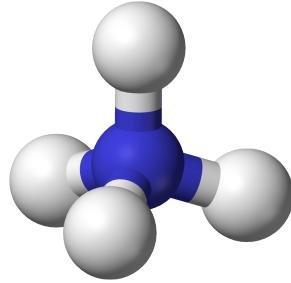
- Consumption:

- Ammonia is directly or indirectly the precursor to most nitrogen-containing compounds. Virtually all synthetic nitrogen compounds are derived from ammonia.
- 

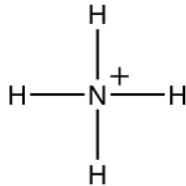
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### 5.1.4 Ammonium

- Ammonium is the most reduced inorganic nitrogenous cation (positively charged).



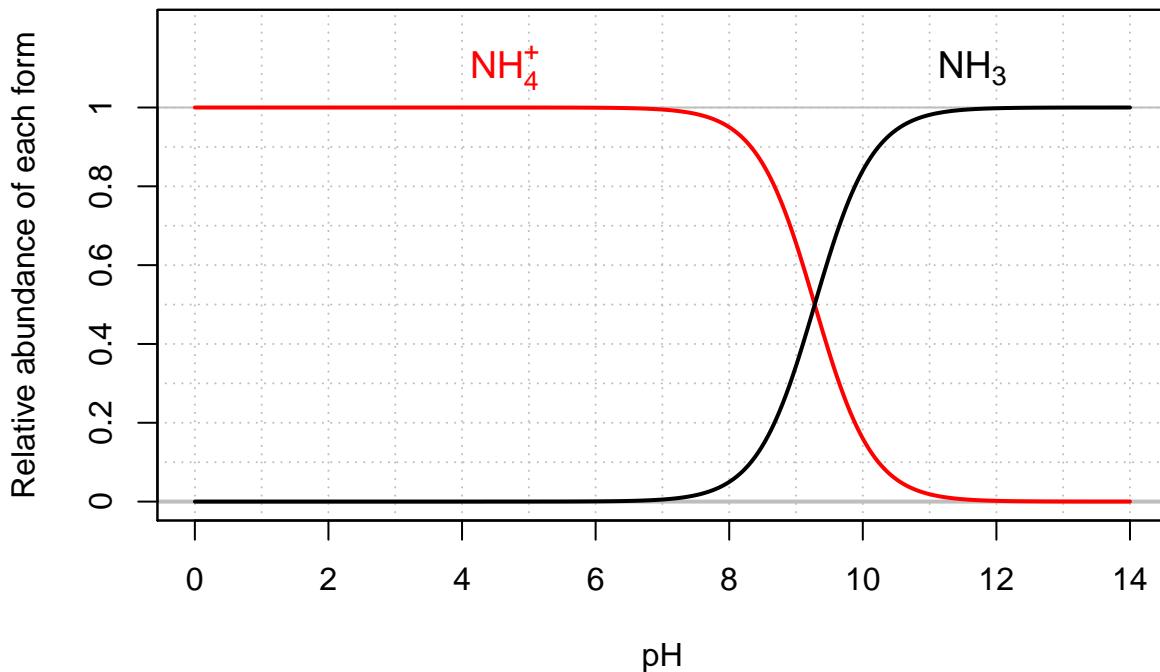
- Ammonium 3D shape:



- Lewis dot structure:
- Number of electron N has for itself following electronegativity rule: eight
- It is formed by the protonation of ammonia following the reaction:



- The relative abundance of ammonium vs ammonia depends on the pH of the solution. See figure below

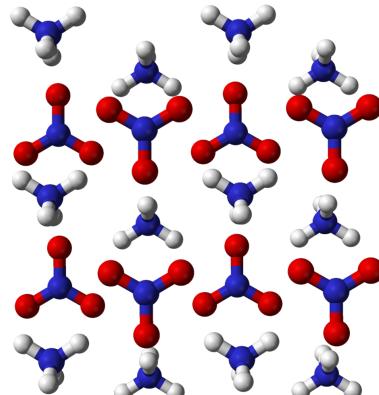


- Because in most natural aqueous environments, pH is below 8, ammonium tends to be the preponderant form.
- Production:
  - In nature, ammonium is a waste product of the mineralization of organic molecules
  - It is added as fertilizer on soils as ammonium nitrate
- Health hazard:
  - Ammonia vapor has a sharp, irritating, pungent odor that acts as a warning of potentially dangerous exposure
  - Exposure to very high concentrations of gaseous ammonia can result in lung damage and death
- Drinking water standard:

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### 5.1.5 Ammonium nitrate

- Ammonium nitrate is a chemical compound, the nitrate salt of the ammonium cation
- It is a white crystal solid and is highly soluble in water.
- Formula:  $\text{NH}_4\text{NO}_3$



- 3D shape:
- It is predominantly used in agriculture as a high-nitrogen fertilizer
- Its other major use is as a component of explosive mixtures used in mining, quarrying, and civil construction
- Production:
  - Ammonium nitrate does exist naturally in mines of the Atacama desert in Chile but globally nearly all ammonium nitrate is now produced synthetically
  - Byproduct of all respiration processes. Ammonium ions are a waste product of the metabolism of animals. In fish and aquatic invertebrates, it is excreted directly into the water. In mammals, sharks, and amphibians, it is converted in the urea cycle to urea, because urea is less toxic and can be stored more efficiently. In birds, reptiles, and terrestrial snails, metabolic ammonium is converted into uric acid, which is solid and can therefore be excreted with minimal water loss.
- Consumption/utilization:
  - It is used as a fertilizer, because it tends to release inorganic nitrogen slowly in soil. Applied as a surface fertilizer, it penetrates the soil with rainfall infiltration. Highly soluble, the nitrate anion becomes readily available to plant roots, although it is susceptible to leaching below the root system into the shallow and deep groundwater. The ammonium cation tends to adsorb to soil particles and is thus not as susceptible to leaching. Ammonium can be directly uptaken by plant roots, which thermodynamically makes sense, although because in most soils aerobic conditions are preponderant, nitrate tends to be the ion uptaken most often. Soil bacteria in the aerobic zone of the soil will oxidize adsorbed ammonium into nitrate, which then becomes available for plant roots. The whole chain of events slows the release of inorganic nitrogen to crops and thus makes for more effective fertilizers.
- Health hazard
  - No direct known health hazard

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### 5.1.6 Anoxic waters

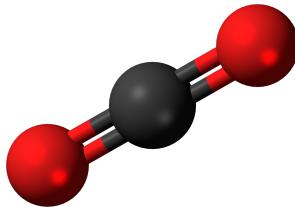
- Anoxic waters are areas of sea water, fresh water, or groundwater that are depleted of dissolved oxygen and are a more severe condition of hypoxia (Wikipedia)
- Anoxic waters result from an **IMBALANCE** between oxygen supply and demand

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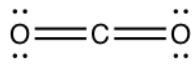
## 5.2 C

### 5.2.1 Carbon dioxide

- Carbon dioxide is a colorless gas which density is 50% higher than that of dry air.
- Formula:  $CO_2$



- Carbon dioxide 3D shape:



- Lewis dot structure:
- Number of electron C has for itself following electronegativity rule: zero
- $CO_2$  can only be an electron acceptor
- Production:
  - oxidation of C in all organic molecules
  - Almost all respiratory processes on earth (some respiration does not involve oxidation of C)
  - Combustion of all Carbon-based fuel
- Consumption:
  - Photosynthesis
    - \* Atmospheric carbon dioxide is **the primary carbon source for life on Earth**
  - Calcite precipitation in the oceans
- Ecological Significance:
  - Greenhouse Gas, which serves as reference for all other GHG
  - Concentration in the atmosphere ~380 ppm on the rise, or 0.38%, or a partial pressure of 0.38 atm

concentrations of carbon dioxide in the atmosphere from hundreds of thousands of years ago through 2015, measured in parts per million (ppm). The data come from a variety of historical ice core studies and recent air monitoring sites around the world. Each line represents a different data source (EPA, 2016)

- In reality, concentrations are not stable, and vary widely in time and in space at the next two videos nicely show.
- The next one results from the model simulations

Youtube video of  $CO_2$  modeled seasonal variations

- The following one is the combination of both models and observations

Youtube video of  $CO_2$  modeled and observed seasonal variations

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### 5.2.2 Carbonates

- After carbon dioxide dissolves in water, it will combine with water to form carbonic acid ( $H_2CO_3$ ).

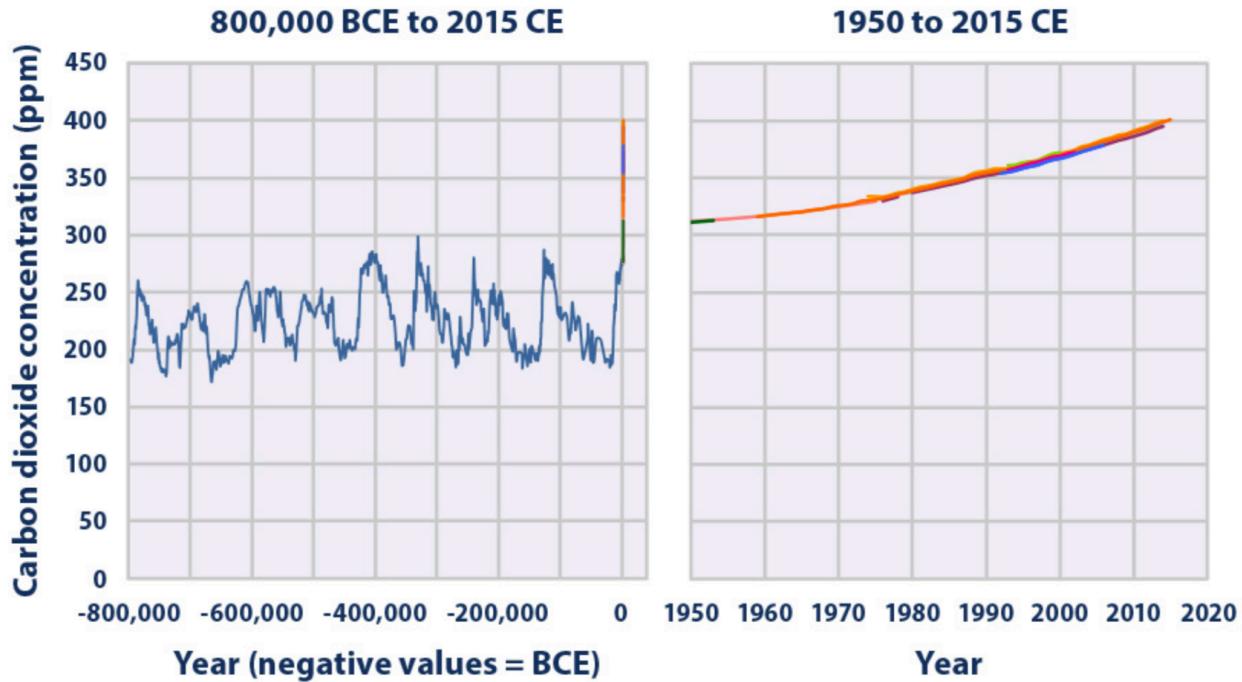
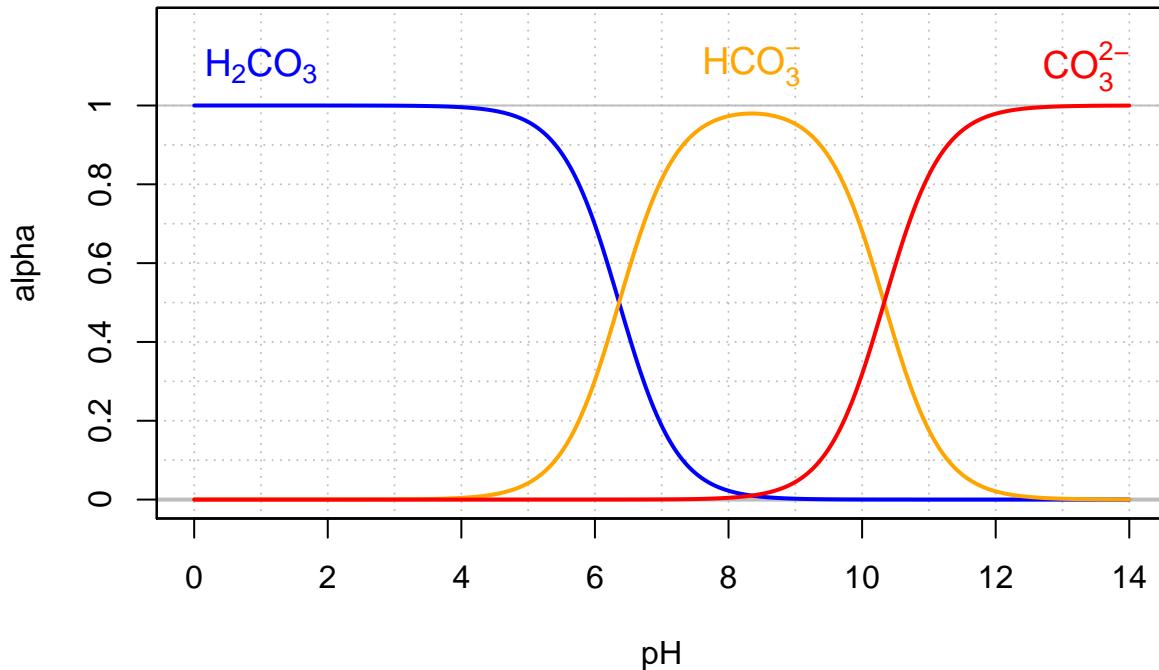


Figure 5.1: Carbon Dioxide variations through ancient and modern times

- Carbonate serves as **the carbon source** for aquatic vegetation
- Carbonic acid can then dissociate into bicarbonate ( $HCO_3^-$ ) and carbonate ( $CO_3^{2-}$ )



- In an environment not open to the atmosphere (or where direct exchange with the atmosphere is very limited like in stream or wetland sediment), the preponderant form depends on the pH and can be calculated as illustrated on the graph below.



- In sea water, Carbonate can combine with  $\text{Ca}^{2+}$  to form Calcium Carbonate ( $\text{CaCO}_3$ ), which precipitates out of solution. In other words, calcium carbonate formation is a sink for carbonate, and ultimately from  $\text{CO}_2$  addition from the atmosphere to increased  $\text{CO}_2$  concentrations in the atmosphere.
- Carbonates are thus a great pH buffer in aquatic environments

### 5.2.3 Catabolism

- Reactions involving the breaking down of organic substrates, typically by oxidative breakdown, to provide chemically available energy (e.g. A TP) and/or to generate metabolic intermediates used in subsequent anabolic reactions (De Bolster, 1997).
- Synonyms:
  - Aerobic and anaerobic *respirations* which use organic molecules as electron donors, are synonyms of catabolism.
  - In soil science another synonym of catabolism is *mineralization*, which refers to the decomposition or oxidation of the chemical compounds in organic matter releasing the nutrients contained in those compounds into soluble inorganic forms that may be plant-accessible (Wikipedia contributors, 2018c).

## 5.3 D

### 5.3.1 Denitrification

- The *microbially mediated dissimilatory reduction of nitrate into dinitrogen*
- see denitrification in chapters for more details

### 5.3.2 Dihydrogen sulfide

- see hydrogen sulfide

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## 5.4 E

### 5.4.1 Eutrophication

- Definitions:
  - ‘an increase in the rate of supply of organic matter to an ecosystem’ (Nixon, 1995)
  - ‘is the enrichment of a water body with nutrients, usually with an excess amount of nutrients’ (Wikipedia)
  - ‘the enrichment of water by nutrients, especially nitrogen and/or phosphorus, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of water concerned’ (Anonymous, 1991b)
  - ‘the enrichment of water by nitrogen compounds causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of water concerned’ (Anonymous, 1991a)

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## 5.5 G

### 5.5.1 Greenhouse gases (*GHG*)

A greenhouse gas is a gas in an atmosphere that absorbs and emits radiant energy within the thermal infrared range. This process is the fundamental cause of the greenhouse effect. The primary greenhouse gases in Earth’s atmosphere are **water vapor**, **carbon dioxide**, **methane**, **nitrous oxide**, and **ozone**. Without greenhouse gases, the average temperature of Earth’s surface would be about  $-18^{\circ}\text{C}$  ( $0^{\circ}\text{F}$ ), rather than the present average of  $15^{\circ}\text{C}$  ( $59^{\circ}\text{F}$ ). In the Solar System, the atmospheres of Venus, Mars and Titan also contain gases that cause a greenhouse effect. (Wikipedia)

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## 5.6 H

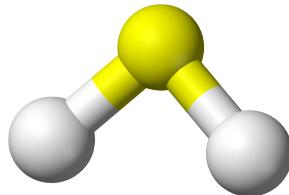
### 5.6.1 Haber-Bosch process

- Haber-Bosch process

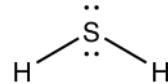
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### 5.6.2 Hydrogen Sulfide

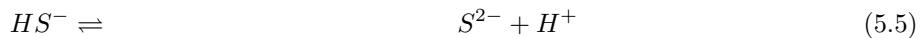
- It is a colorless gas with the characteristic foul odor of rotten eggs.
- It is very poisonous, corrosive, flammable and acidic in nature.
- Formula:  $\text{H}_2\text{S}$



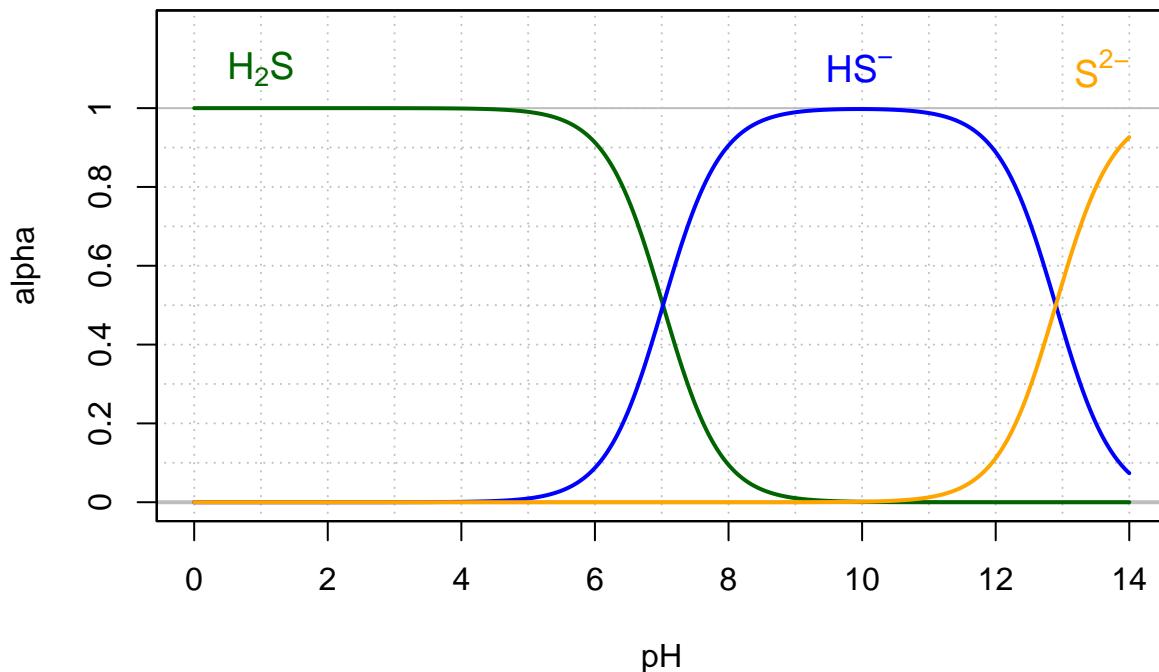
- hydrogen sulfide #D shape:



- Lewis dot structure:  $\begin{array}{c} \cdot\cdot \\ \text{S} \\ \backslash \quad / \\ \text{H} \quad \text{H} \end{array}$
- Number of electron S has for itself following electronegativity rule: eight
- $H_2S$  can only be an electron donor
- Unstable under aerobic conditions, will readily be oxidized into sulfate
- $H_2S$  is a polyprotic acid which can lose up to 2 protons in water, depending on the pH.



- The figure below suggests that at pH found in most streams (4.5 to 8),  $H_2S$  is either preponderant or corresponds to at least 20% of all sulfide forms.  $H_2S$  being a highly volatile product, it explains why we can easily smell and detect it in most conditions in streams.



- Production:
  - Hydrogen sulfide often results from the microbial breakdown, or mineralization, of organic matter in anaerobic conditions, such as may exist in swamps and sewers. When happening in sediment, this is referred to as sediment diagenesis
- Consumption:

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## 5.7 L

### 5.7.1 Lithotrophs

- Lithotrophs are a diverse group of organisms using inorganic substrate (usually of mineral origin) to obtain reducing equivalents for use in biosynthesis (e.g., carbon dioxide fixation) or energy conservation (i.e., ATP production) via aerobic or anaerobic respiration. Known chemolithotrophs are exclusively microorganisms. (Wikipedia contributors, 2018b)

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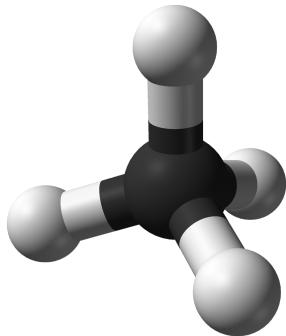
### 5.7.2 Limiting factor

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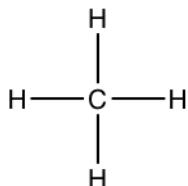
## 5.8 M

### 5.8.1 Methane

- Under normal conditions for temperature and pressure, methane is a colorless, odorless gas main constituent of natural gas, and the simplest alkane
- Formula:  $CH_4$



- Methane 3D shape:



- Lewis dot structure of methane:
- Number of electron C has for itself following electronegativity rule: eight
- $CH_4$  can only be an electron **donor**
- Because methane has so many electrons to give, it will easily ‘burn’ in normal atmosphere (provided that ignition  $T^\circ\text{C}$  be reached, e.g., by a spark), liberating large quantities of heat (55.5 MJ/kg). The electrons are transferred from the carbon to the oxygen atoms following two redox half-reactions to obtain the overall reaction:

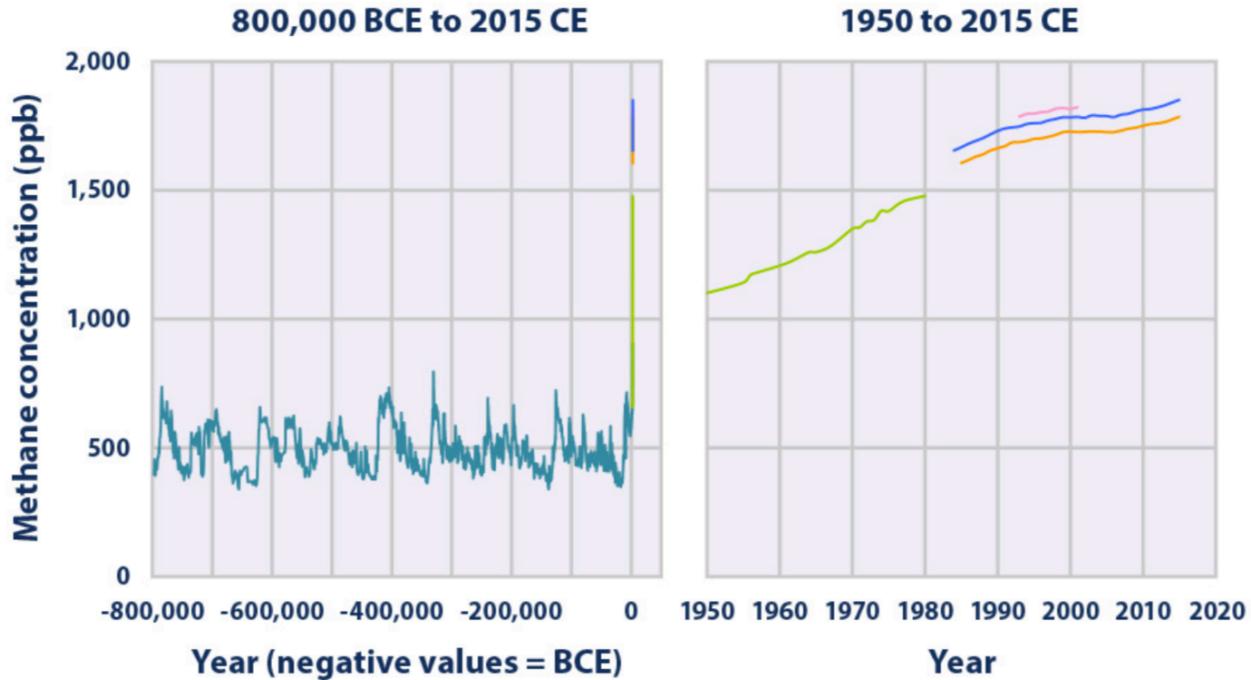
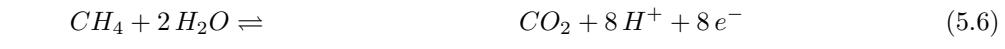


Figure 5.2:



(5.8)

(5.9)



- Production:
- Consumption:
- Ecological significance:

Concentrations of methane in the atmosphere from hundreds of thousands of years ago through 2015, measured in parts per billion (ppb). The data come from a variety of historical ice core studies and recent air monitoring sites around the world. Each line represents a different data source(EPA, 2016)

- Health effects:

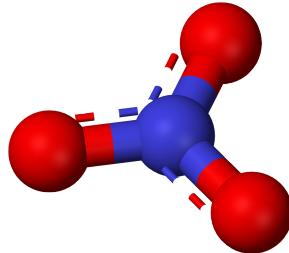
### 5.8.2 Mineralization

- see catabolism

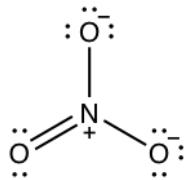
## 5.9 N

### 5.9.1 Nitrate

- Nitrate is the stable inorganic nitrogenous anion in oxidized water
- Formula:  $NO_3^-$



- Nitrate 3D shape:



- Lewis dot structure of nitrate:
- Number of electron N has for itself following electronegativity rule: zero
- $NO_3^-$  can only be an electron acceptor
- $NO_3^-$  is technically the conjugate base of nitric acid  $HNO_3$ , but the  $pK_A$  of the reaction is at a theoretical pH of -1.38. In other words, for the pH of most natural waters ( $4.5 < \text{pH} < 8$ ),  $HNO_3$  is totally insignificant.
- Production:
  - from the complete oxidation of inorganic nitrogenous molecules which include ammonia, ammonium, nitrite
  - from the mineralization and complete oxidation of amine radicals in organic molecules
- Consumption:
  - **Uptake** from microbes, plants, and algae for their anabolism, which consists in building complex organic molecules from inorganic ones.
    - \* Uptake, assimilation, anabolism, immobilization are all synonymous terms to express the fact that the N atom is immobilized, at least temporarily in organic molecules.
    - \* Because N is assimilated in organic molecules during uptake/anabolism, and because N gains electrons in the process (it is thus **reduced**), we refer to nitrate uptake as **assimilatory nitrate reduction**.
  - **Denitrification**: under anaerobic conditions, nitrate is used by facultative anaerobes as electron acceptor to generate ATP in their respiration chain. The two major end products of denitrification are gases, namely dinitrogen ( $N_2$ ) and nitrous oxide ( $N_2O$ ), which leave the aqueous environment. As such, nitrate is not assimilated by any bacteria and denitrification is therefore, as opposed to uptake, referred to as **dissimilatory nitrate reduction** into dinitrogen ( $N_2$ ) and nitrous oxide ( $N_2O$ ).
- Ecological significance:
  - Because of assimilation and denitrification processes, the overall nitrate concentrations in rivers tends to diminish from the catchment headwaters to the receiving bodies such as estuaries and

coastal areas. As a result, inorganic nitrogen has naturally been in very short supply in these coastal water bodies, and nitrate and traditionally been the nutrient limiting aquatic productivity there. Very much like with phosphate, algae have adapted to be able to grow in very low concentrations. Anthropogenic activities, and agriculture in particular, have largely increased the loads and concentration of nitrate reaching estuaries, to the point where nitrate is no longer the limiting factor. As a result, excess nitrate is one of the reasons for the global and persistent presence of algal blooms in estuaries and coastal waters.

- Health hazard:

- There is a heated debate about the health hazard that nitrate might pose. Some argue that if anything, there might be beneficial effects, while others argue that there are evidence of cancers linked to excess nitrate absorption. Unfortunately, arguments on both sides might not be totally independent of militantism and lobbies.
- The only consensus everybody seems to agree upon is the Blue Baby syndrome, or methemoglobinemia. Methemoglobinemia is an unusual and potentially fatal condition in which hemoglobin is oxidized to methemoglobin and loses its ability to bind and transport oxygen, hence the cyanosis (blue appearance) usually visible on fingers, toes, and lips. Nitrate reduced to nitrite in the body of humans and animals enters the body stream where it seems to directly oxidize oxyhemoglobin to methemoglobin-peroxide complex.



**Picture of a Blue Baby from syndromespedia.com/blue-baby-syndrome.html**

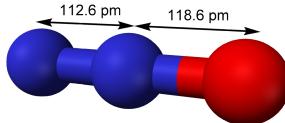
- Drinking water standards

- Although there is still a heated debate whether or not nitrate does have detrimental health effects, the World Health Organization has provided maximum concentration guidelines of 50 mg/L as nitrate (World Health Organization, 2011). These guidelines have been enacted in hard laws in the US and in Europe. The 50 mg/L as nitrate equates 11.2 mg  $NO_3\text{-N}/\text{L}$  and in the US, the drinking water standard is **10 mg  $NO_3\text{-N}/\text{L}$** .

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## 5.9.2 Nitrous Oxide

- Commonly known as laughing gas
- Nitrous oxide has significant medical uses, especially in surgery and dentistry, for its anesthetic and pain reducing effects.
- Formula:  $N_2O$



- Nitrous Oxide 3D shape:



- Number of electron N has for itself following electronegativity rule:

- the first one on the left has 5
  - the middle N has 3

- $\text{N}_2\text{O}$  can be both an electron acceptor and an electron donor

- Production:

- $\text{N}_2\text{O}$  is produced due to bacterial processes (over 90%) and anthropogenic processes such as burning of fossil fuel

- \* The two main bacterial processes are *nitrification* and *denitrification*

- \* Accounting that human activities have enhanced both nitrification and denitrification processes, it is estimated that overall, about 2/3rd of  $\text{N}_2\text{O}$  production is natural, and about 1/3rd is human enhanced

- Consumption:

- Because of all the electrons stored on the two N atoms ( $5 + 3 = 8$ ), nitrous oxide is a potential electron donor and bacteria can use it for their respiration processes

- Ecological significance:

- Powerful Greenhouse Gas, 298 times that of  $\text{CO}_2$  (EPA)

- Concentration in the atmosphere  $\sim 0.0003$  ppm or  $\sim 325$  ppb on the rise

Concentrations of nitrous oxide in the atmosphere from hundreds of thousands of years ago through 2015, measured in parts per billion (ppb). The data come from a variety of historical ice core studies and recent air monitoring sites around the world. Each line represents a different data source)(EPA, 2016)

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## 5.10 O

### 5.10.1 Oligotrophication

- ‘a decrease in the rate of supply of organic matter to an ecosystem’ (Nixon, 2009)

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### 5.10.2 Oxidation

- Oxidation is the **loss of electrons** by a molecule, atom, or ion.
- The term oxidation was first used by Antoine Lavoisier to signify reaction of a substance with oxygen. Much later, it was realized that the substance, upon being oxidized, loses electrons, and the meaning was extended to include other reactions in which electrons are lost.

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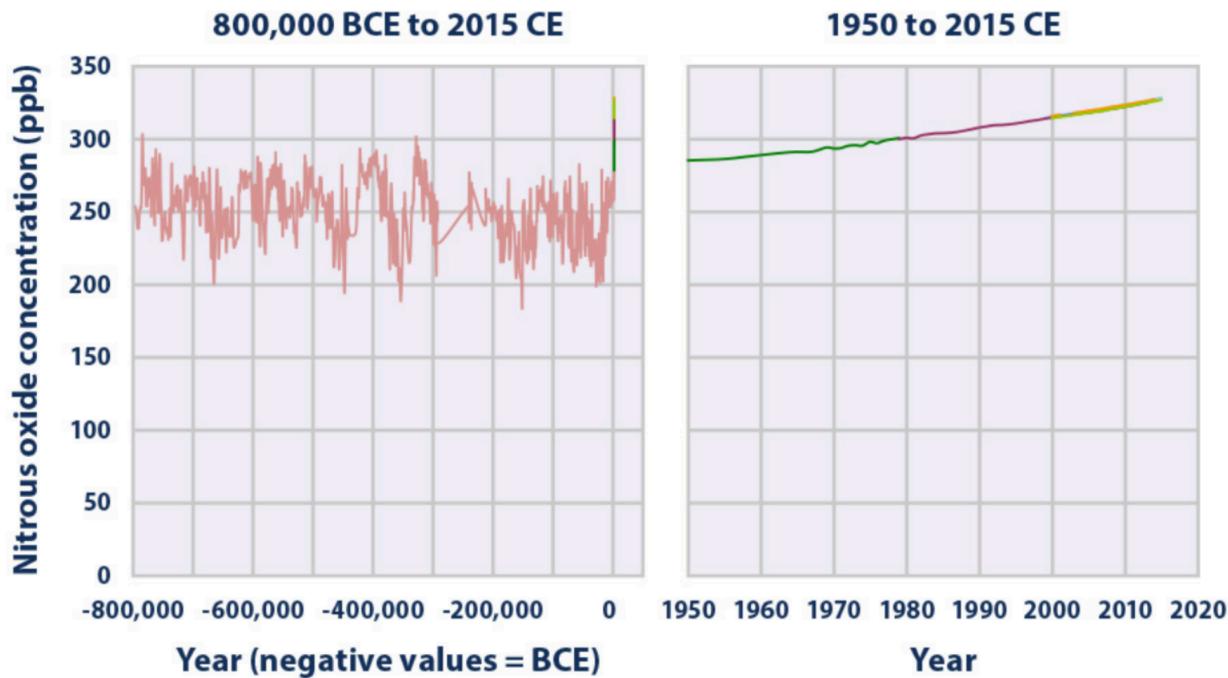


Figure 5.3:

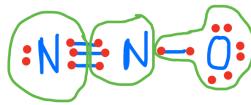
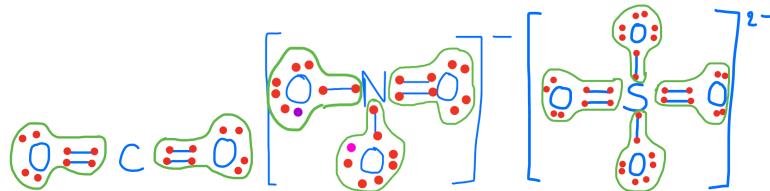
Figure 5.4: Electron allocation on each of the N atom for the dinitrogen molecule  $N_2$ 

### 5.10.3 Oxidation state

- The oxidation state ( $OS$ ), or sometimes referred to as the oxidation number, quantifies the number of electrons that an atom has gained (expressed as ***negative*** charge value) or lost (expressed as ***positive*** charge value) compared to the number of valence electrons it has in its free form. As a result, it can be zero, positive or negative.
- For metal ions, the  $OS$  corresponds to its charge
- The *change* in oxidation state is a very powerful tool to understand the redox processes involved in the environment. But  $OS$  is less than ideal when comparing the absolute number of electrons one element *has for itself*
- For example, the nitrogen atom  $N$ , has 5 valence electrons. In the dinitrogen molecule  $N_2$ , the Lewis dot structure suggests that each atom share 3 electrons with the other but essentially have 5 electrons for themselves as in Figure 5.4

As a result, the number of electron on each atom equals the number of valence electrons on the free form of  $N$ , hence  $OS = 0$ . Now in the nitrous oxide case in Figure 5.5, the nitrogen atom to the left  $OS = 0$ , but for the middle Nitrogen atom,  $OS = -2$ , as two electrons were stripped by oxygen.

The problem with the oxidation state indicator is that it is a relative number, and not absolute. The electron allocation indicator that quantifies the number of electrons each atom has for itself is more absolute and thus comparable. For example, the inorganic molecule with one C atom stable in an oxidized environment is  $CO_2$ , the inorganic molecule with one N atom stable in an oxidized environment is  $NO_3^-$ , and the inorganic

Figure 5.5: Electron allocation on each of the N atom for the N<sub>2</sub>O moleculeFigure 5.6: Electron allocation on each of the C, N, and S atom for the CO<sub>2</sub>, nitrate and sulfate molecules

molecule with one S atom stable in an oxidized environment is  $SO_4^{2-}$ . All three atoms have zero electrons for themselves as oxygen has ‘stolen’ them (Figure 5.6). So they are all potential electron acceptors that can accept up to 8 electrons. In a way they are very similar.

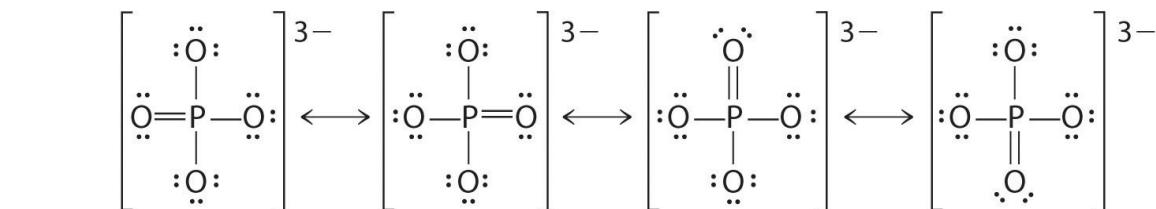
But if one calculates the oxidation states for each of the C, N, and S atoms, for these three molecules, one would find  $OS_C = -4$ ,  $OS_N = -5$ , and  $OS_S = -6$ ... We believe this can be very confusing and prefer to use the electron allocation concept as a more absolute indicator.

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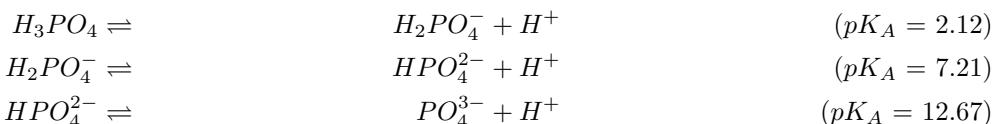
## 5.11 P

### 5.11.1 Phosphate

- Phosphate is an inorganic chemical and a salt-forming anion of phosphoric acid
- Formula:  $PO_4^{3-}$



- Phosphate is one of the anions of the polyprotic acid (i.e., which can liberate several protons  $H^+$ )
- The conjugate bases of phosphate are:
- All conjugate bases are related through the set acid-base chemical equilibria:



- The preponderant form of phosphate in a solution also depends on the pH following this relationship:

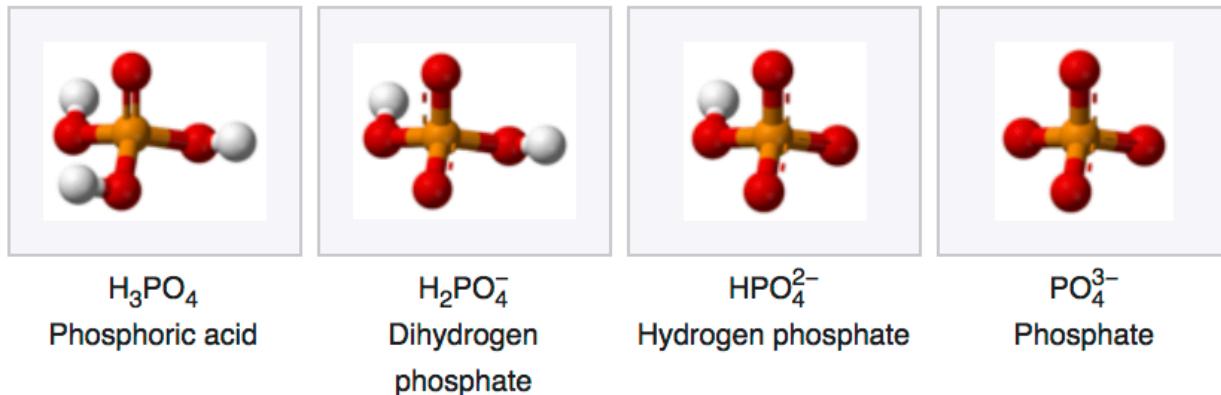
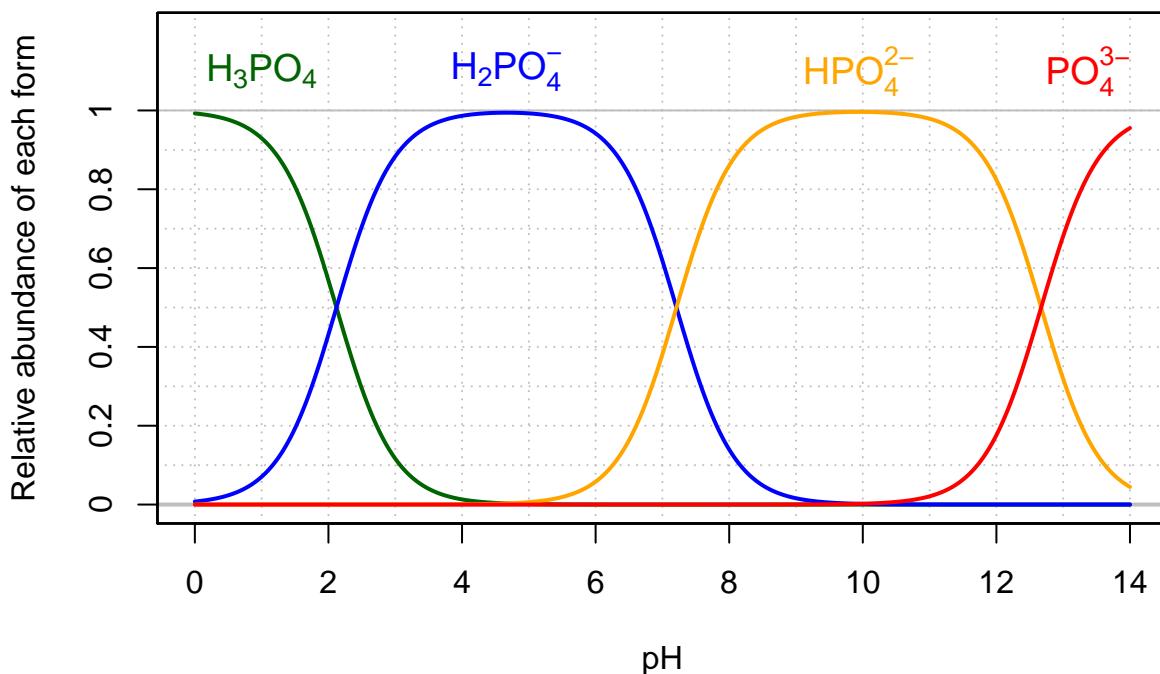


Figure 5.7:



- Production:
  - Phosphorus in general and phosphate in practice has remained one of the nutrients limiting the most plant productivity on our planet
  - Phosphates are the naturally occurring form of the element phosphorus, found in many phosphate minerals
  - Phosphate minerals are mined to obtain phosphorus for use in agriculture and industry
  - The largest global producer and exporter of phosphates is Morocco.
  - Within North America, the largest deposits lie in the Bone Valley region of central Florida, the Soda Springs region of southeastern Idaho, and the coast of North Carolina (near Aurora).
- Consumption:
  - Uptake from all primary producer including plants and algae
  - Phosphate can also be immobilized by bacteria
  - In food industry, phosphates help baked goods rise, they act as emulsifiers in processed cheese and canned soup, they add flavor to cola and color to frozen french fries. They also can be added to meat, poultry and seafood to help the protein bind more water, making it juicier after freezing and reheating.

- Ecological significance:
  - Phosphorus as phosphate naturally is the most limiting factor for primary productivity for land and aquatic plants. Because it tends to bind to particles, phosphates have accumulated with sediment particularly in coastal areas, where phosphate can become available again to algae through sediment diagenetic processes. As a result, phosphate is generally not considered the most limiting factor for algae in estuaries and coastal environment. However, it does tend to be the limiting nutrient in freshwater receiving bodies such as lakes and reservoirs.
  - Excess phosphate in freshwater receiving bodies has been shown to be the nutrient causing some major eutrophication problems throughout the planet

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## 5.12 R

### 5.12.1 Reactive nitrogen

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### 5.12.2 Redox

- Redox (short for reduction–oxidation reaction) is a chemical reaction in which the oxidation states of atoms are changed. Any such reaction involves both a reduction process and a complementary oxidation process, effectively allowing the electron transfer processes.

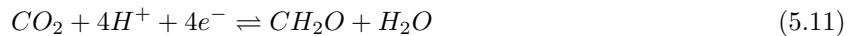
### 5.12.3 Redox couple

- redox couples, commonly noted as ‘Ox/Red’ refer to the two forms that an element might take depending on its oxidation state
- Ox in ‘Ox/Red’ refers to as the *oxidizer* or oxidizing agent, as it has the capability to make other elements **lose** their electrons.
- Red in ‘Ox/Red’ refers to as the *reducer* or reducing agent, as it has the capability to **give** electrons to other elements
- Common redox couples which matter in environmental and ecological engineering include:
  - CO<sub>2</sub>/CH<sub>2</sub>O
  - O<sub>2</sub>/H<sub>2</sub>O
  - NO<sub>3</sub><sup>-</sup>/N<sub>2</sub>
  - NO<sub>3</sub><sup>-</sup>/NH<sub>4</sub><sup>+</sup>
  - NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup>
  - MnO<sub>2</sub>/Mn<sup>2+</sup>
  - Fe<sup>3+</sup>/Fe<sup>2+</sup>
  - SO<sub>4</sub><sup>2-</sup>/H<sub>2</sub>S
  - CO<sub>2</sub>/CH<sub>4</sub>

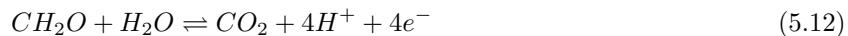
### 5.12.4 Redox half-reactions

- A half reaction is either the oxidation or reduction reaction component of a redox reaction. A half reaction is obtained by considering the change in oxidation states of individual substances involved in the redox reaction (Wikipedia contributors, 2018a)

- Redox half reactions illustrate the transfer of electrons from the oxidized form of an element to a reduced form of an element.
- Half-reactions are often used as a method of balancing redox reactions
- To derive half-reactions,
  - the first convention is that the oxidizer is on the left of the equation, and the reducer on the right
  - one must first equilibrate all elements other than O and H
  - then one balances for O, by adding  $H_2O$  on the appropriate side of the half reaction
  - then one adds the appropriate number of  $H^+$  to balance the H
  - Finally one adds the appropriate number of  $e^-$  to balance the charge. The number of  $e^-$  must correspond to the change in oxidation state
- The half-reactions corresponding to the redox couples above are:
- $CO_2/CH_2O$



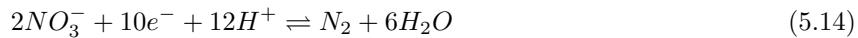
- $CH_2O$  here is a generic formula to represent the electron donating capacity of OM. This equation might be misleading as it corresponds to the OM donating 4 electrons in the respiration schemes, but because it is written using the ox/red convention, is the opposite of the intuitive direction. So a more intuitive half-reaction might be, and is effectively what is used to write the full redox reaction:



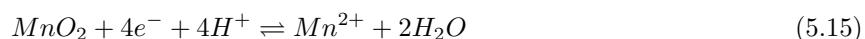
- $O_2/H_2O$



- $NO_3^-/N_2$



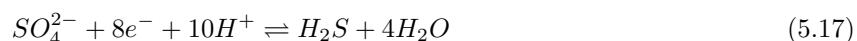
- $MnO_2/Mn^{2+}$



- $Fe^{3+}/Fe^{2+}$



- $SO_4^{2-}/H_2S$



- $CO_2/CH_4$



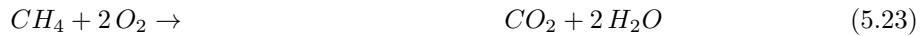
- a redox reaction **always** involves two half reactions where the electrons are transferred from the lowest redox potential couple to the highest redox potential couple. For example, the combustion of methane in oxygen involves the two  $O_2/H_2O$  and  $CO_2/CH_4$  couples. The half-reaction of the highest redox potential couple goes on top, and that of the lowest potential at the bottom, but this time written as red/ox like in the equation below:




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(5.21)

(5.22)



The electrons cancel out, and in this case the protons as well. So the combustion of methane, which is very simple at first glance, really involves a transfer of electrons, which the half-reactions reveal.

### 5.12.5 Reduction

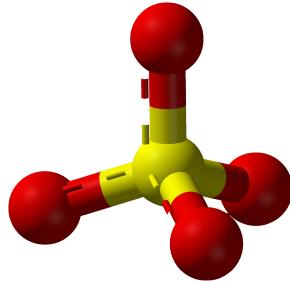
- Reduction is the *gain* of electrons by a molecule, atom, or ion.

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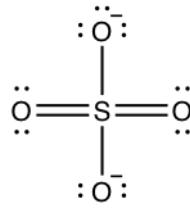
## 5.13 S

### 5.13.1 Sulfate

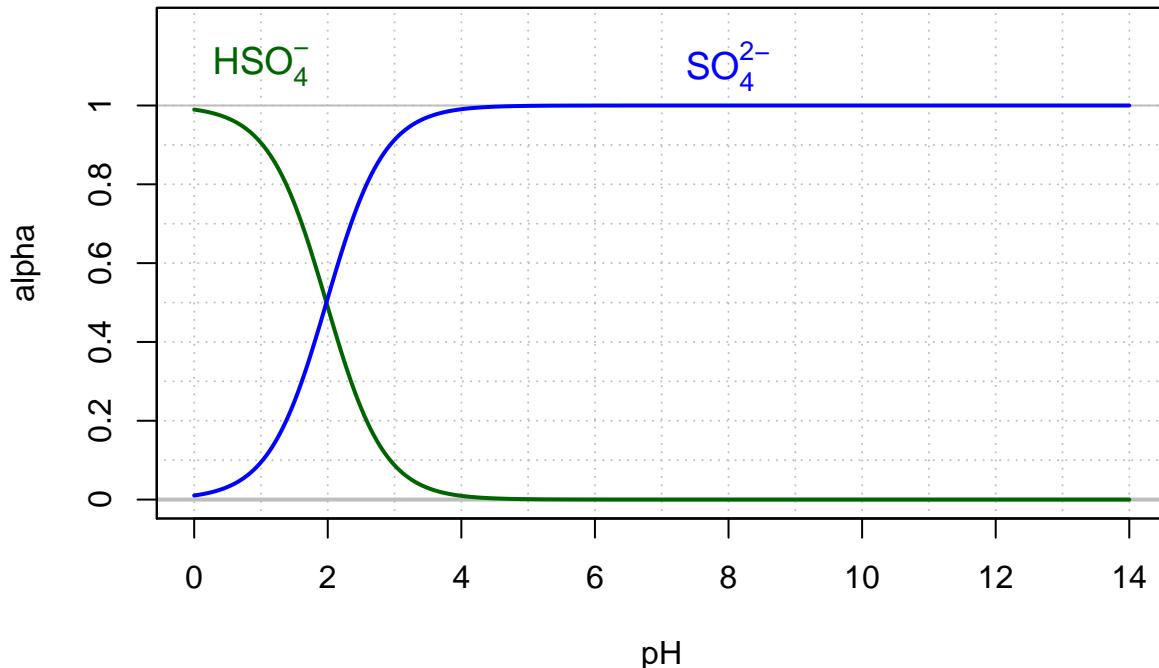
- Sulfate is the inorganic sulfur anion stable in oxidized water
- Formula:  $SO_4^{2-}$



- Sulfate 3D shape:



- Lewis dot structure of nitrate:
- Number of electron S has for itself following electronegativity rule: zero
- $SO_4^{2-}$  can only be an *electron acceptor*
- $SO_4^{2-}$  is the conjugate base of Hydrogen sulfate  $HSO_4^-$ . The figure below shows that for pH normally measured in surface water and streams (4.5-8),  $SO_4^{2-}$  is the truly preponderant form. We therefore generally omit to mention  $HSO_4^-$  as a chemical form that plays any significant role.



- During their anabolism, primary producers uptake sulfate, but the sulfur atoms can be incorporated into amino-acids only after sulfur has been reduced, or gained 8 electrons to be in a thiol ( $-SH$ ) form.
- Production:
- Consumption:
- Ecological significance:
- Health effects:

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## 5.14 T

### 5.14.1 Trophic names

- Depending on the source of carbon, the source of electrons, and the source of energy, organisms have been qualified and called differently.

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- Depending on the source of ***carbon***

#### Autotrophs

- **Autotrophs** obtain their carbon needs, on their own, hence *Auto*.
- In other words, they obtain their carbon from  $CO_2$  and carbonates
- Just about all green plants and algae are autotrophs

#### Heterotrophs

- **Heterotrophs** obtain their carbon needs, from *others*, hence *Hetero*.
- In other words, they obtain their carbon from organic matter, some of which might also serve as a source of electrons
- All animals are heterotrophs, and most bacteria are too.

- Depending on the source of *electrons*

### Lithotrophs

- **Lithotrophs** obtain their reducing power or their high energy electrons from inorganic molecules, or *mineral matter*. Literally, *rock eaters*.
- In other words, they obtain their electrons from molecules or atoms like  $\text{NH}_4^+$  or  $\text{Fe}^{2+}$ .
- ALL Lithotrophs are unicellular microorganisms
- In the examples above, nitrifiers and iron oxidizing bacteria are lithotrophs

### Organotrophs

- **Organotrophs** obtain their reducing power or their high energy electrons from *organic* molecules, hence the *organo*
  - In other words, they obtain their electrons from organic matter
  - All pluricellular organisms are organotrophs
- 

- Depending on the source of *energy*

### Phototrophs

- **Phototrophs** obtain their energy primarily through sunlight.
- In reality, at night, phototrophs become chemotrophs as their cellular respiration is based upon oxidizing organic matter. But they are autosufficient. They capture solar energy and store it in a chemical form thanks to photosynthesis, and used that stored energy later on.
- Just about all green plants are phototrophs.

### Chemotrophs

- **Chemotrophs** obtain their energy as energy previously stored in a chemical form.
- Just about all pluricellular organisms are chemotrophs, even the phototrophs, which turn into chemotrophs at night