Anaerobes

An **anaerobic organism** is any organism that does not require oxygen for growth and even dies in its presence.

- **Obligate anaerobes** will die when exposed to atmospheric levels of oxygen.
- **Facultative anaerobes** can use oxygen when it is present.
- **Aerotolerant** organisms can survive in the presence of oxygen, but they are anaerobic because they do not use oxygen as a terminal electron acceptor.

Microaerophiles are organisms that may use oxygen, but only at low concentrations (low micromolar range); their growth is inhibited by normal oxygen concentrations (approximately 200 micromolar). Nanaerobes are organisms that cannot grow in the presence of micromolar concentrations of oxygen, but can grow with and benefit from nanomolar concentrations of oxygen.

Obligate anaerobes may use fermentation or anaerobic respiration. In the presence of oxygen, facultative anaerobes use aerobic respiration; without oxygen some of them ferment, some use anaerobic respiration. Aerotolerant organisms are strictly fermentative. Microaerophiles carry out aerobic respiration, and some of them can also do anaerobic respiration.

There are many chemical equations for anaerobic fermentative reactions.

Fermentative anaerobes

Fermentative anaerobic organisms mostly use the lactic acid fermentation pathway:

$$C_6H_{12}O_6 + 2\ ADP + 2\ phosphate \rightarrow 2\ lactic\ acid + 2\ ATP$$

The energy released in this equation is approximately 150 kJ per mol, which is conserved in regenerating two ATP from ADP per glucose. This is only 5% of the energy per sugar molecule that the typical aerobic reaction generates.

Plants and fungi (e.g., yeasts) generally use alcohol (ethanol) fermentation when oxygen becomes limiting:

$$C_6H_{12}O_6 + 2\ ADP + 2\ phosphate \rightarrow 2\ C_2H_5OH + 2\ CO_2 + 2\ ATP$$

The energy released is about 180 kJ per mol, which is conserved in regenerating two ATP from ADP per glucose.

Anaerobic bacteria and archaea use these and many other fermentative pathways, e.g., propionic acid fermentation, butyric acid fermentation, solvent fermentation, mixed acid fermentation, butanediol fermentation, Stickland fermentation, acetogenesis or methanogenesis.

Some anaerobic bacteria produce toxins (e.g., tetanus or botulinum toxins) that are highly dangerous to higher organisms, including humans.

Obligate anaerobes

Obligate anaerobes, which live only in the absence of oxygen, **do not possess the defenses that make aerobic life possible and therefore cannot survive in air**. The excited singlet oxygen molecule is very reactive. Therefore, superoxide must be removed for the cells to survive in the presence of oxygen.

Obligate (strict) anaerobes die in presence of oxygen due to the absence of the enzymes superoxide dismutase and catalase which would convert the lethal superoxide formed in their cells due to the presence of oxygen. Instead of oxygen, obligate anaerobes use alternate electron acceptors for respiration such as sulfate, nitrate, iron, manganese, mercury, and carbon monoxide. The energy yield of these respiratory processes is less than oxygen respiration, and not all of these electron acceptors are created equally.

The most favorable (after oxygen) is sulfate. In marine sediments this leads to large amounts of sulfate reduction, which most of us are familiar with as the rotten egg smell and black material that can be found just a few centimeters below the sediment surface.

Next in line is nitrate, then the metal ions, and lastlly a zone of methanogenesis is found. Very little energy is obtained from methanogeneis and vast amounts of substrate need to be turned over to make a living.

Bacteroides and *Clostridium* species are examples of non-spore forming and spore-forming strict anaerobes, respectively.

Why strict anaerobic bacteria die, when exposed to oxygen?

The aerobes can survive in the presence of oxygen only by virtue of an elaborate system of defenses. Without these defenses, key enzyme systems in the organisms fail to function and the organisms die. Obligate anaerobes, which live only in the absence of oxygen, do not possess the defenses that make aerobic life possible and therefore cannot survive in air.

During growth and metabolism, oxygen reduction products are generated within microorganisms and secreted into the surrounding medium. The superoxide anion, one oxygen reduction product, is produced by univalent reduction of oxygen:

$O_2^{e-} \rightarrow O_2^-$

It is generated during the interaction of molecular oxygen with various cellular constituents, including reduced flavins, flavoproteins, quinones, thiols, and iron-sulfur proteins. The exact process by which it causes intracellular damage is not known; however, it is capable of participating in a number of destructive reactions potentially lethal to the cell. Moreover, products of secondary reactions may amplify toxicity. For example, one hypothesis holds that the superoxide anion reacts with hydrogen peroxide in the cell:

$O_2^- + H_2O_2 \rightarrow OH^- + OH^{\cdot} + O_2$

This reaction, known as the Haber-Weiss reaction, generates a free hydroxyl radical (OH·), which is the most potent biologic oxidant known. It can attack virtually any organic substance in the cell. A subsequent reaction between the superoxide anion and the hydroxyl radical produces singlet oxygen (O_2*), which is also damaging to the cell:

$O_2^- + OH \rightarrow OH + O_2^*$

The excited singlet oxygen molecule is very reactive. Therefore, superoxide must be removed for the cells to survive in the presence of oxygen.

Most facultative and aerobic organisms contain a high concentration of an enzyme called superoxide dismutase. This enzyme converts the superoxide anion into ground-state oxygen and hydrogen peroxide, thus ridding the cell of destructive superoxide anions:

$2O_2^- + 2H^+ \xrightarrow{\text{Superoxide Dismutase}} O_2 + H_2 O_2$

The hydrogen peroxide generated in this reaction is an oxidizing agent, but it does not damage the cell as much as the superoxide anion and tends to diffuse out of the cell. Many organisms possess catalase or peroxidase or both to eliminate the H_2O_2. Catalase uses H_2O_2 as an oxidant (electron acceptor) and a reductant (electron donor) to convert peroxide into water and ground-state oxygen:

$H_2O_2 + H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2$

Peroxidase uses a reductant other than H_2O_2:

$H_2O_2 + H_2R \xrightarrow{Peroxidase} 2H_2O + R$

One study showed that facultative and aerobic organisms lacking superoxide dismutase possess high levels of catalase or peroxidase. High concentrations of these enzymes may alleviate the need for superoxide dismutase, because they effectively scavenge $H_2\,O_2$ before it can react with the superoxide anion to form the more active hydroxyl radical. However, anaerobic bacteria lacks superoxide dismutase and catalase enzyme.

*****