

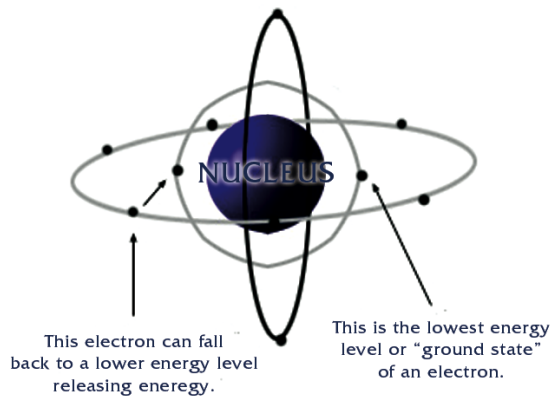
How Oxygen Kills Pathogens

Higher life forms depend on oxygen to create energy for the cells. But there are unicellular microorganisms that fear oxygen because of its ability to also destroy life. This process is called "oxidation" and relies on oxygen's unique ability to attract (or "receive") electrons from other atoms and molecules.

Surrounding the nucleus of atoms are electrons that spin in orbits. When an orbit lacks a set of "paired electrons", that orbit will make every effort to attach itself to another atom, or group of atoms, so that the orbiting electrons become more stable. These atoms may even "steal" electrons from other atoms or molecules. (Molecules are a group of two or more atoms jointed together).

To help visualize the remarkable potential of electrons, imagine yourself spinning a golf ball around yourself and that this golf ball is connected to a spring. The golf ball represents the

electron and the place where you are standing is where the atom's nucleus would be. If you spin the golf ball around you in a constant speed, the electron will be at the same distance from you, spinning at the same speed you are turning. If you start to spin harder, by putting more energy into your spin, you'll notice that the golf ball will move away from you and the spring will expand. Likewise, if you spin more slowly, the golf ball will be closer to you and the spring will contract. In the same way, with changes in energy, an electron can occupy a different orbit around its nucleus.



The smallest of these orbits represents the lowest energy that the electron can possess. This lowest energy state is known as the "ground state." If the electron absorbs energy of the right amount, (such as visible, infrared (heat), or ultraviolet light,) the electron can jump to a higher orbit or "energy level" in the atom. With the electron in a higher orbit, the atom is said to be in the "excited state." At this point, the electron can fall back to a lower energy orbit or even the ground state. As it falls one orbit at a time, it emits a certain amount of energy, which may also be in the form of light, heat, or so on.

It is this remarkable movement and exchanging of electrons on an atomic and subatomic basis that actually serves as a defense mechanism for our immune system as well as a process to control dangerous microorganisms that cause every known disease on the planet today.

How Unicellular Organisms React to the Presence of Oxygen:

Unicellular organisms fall into four general categories that describe how these microbes react to the presence of oxygen:

AEROBES are microorganisms that require the presence of oxygen to live and reproduce themselves. Strict aerobes cannot survive in the absence of oxygen and produce energy only by oxidative phosphorylation. (Oxidative phosphorylation is a biochemical process in cells. It is the final metabolic pathway of cellular respiration in which energy, as ATP, is created in the cell's mitochondria.)

AEROTOLERANT ANAEROBES are microorganisms that do not require the presence or oxygen to live and reproduce, and are not destroyed if oxygen is present. They generate ATP only by fermentation and have mechanisms to protect themselves from oxygen.

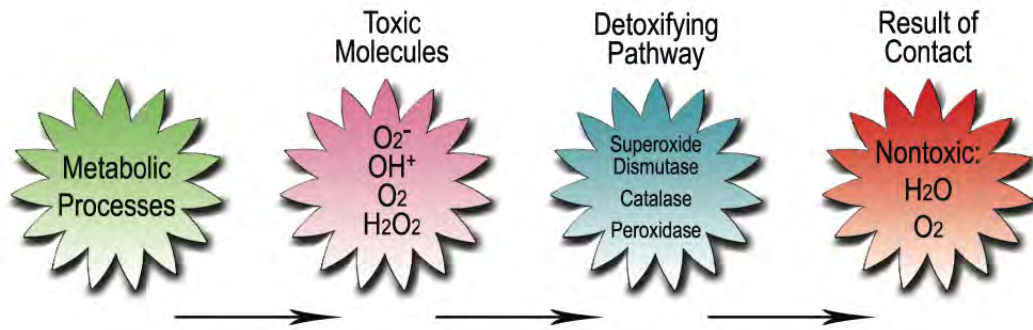
STRICT ANAEROBES, in most cases, generate their energy by fermentation or by anaerobic respiration and are always killed in the presence of oxygen. These organisms are also called "obligate anaerobes". Obligate anaerobes vary greatly in their sensitivity to oxygen. Extremely oxygen-sensitive anaerobes, such as spirochetes and some *Clostridium* species, cannot tolerate even 0.5% oxygen. Thus, oxygen is toxic for them.

FACULTATIVE ANAEROBES prefer to grow in the presence of oxygen, using oxidative phosphorylation, but can grow in an anaerobic environment using fermentation.

The most virulent and destructive pathogens that affect mankind generally fall into the "strict anaerobe" category. They include bacteria like *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Clostridium botulinum* and *Escherichia coli*. Viruses include *Mycobacterium bovis*, *Herpesviridae* and *Influenza A virus/Orthomyxoviridae*.

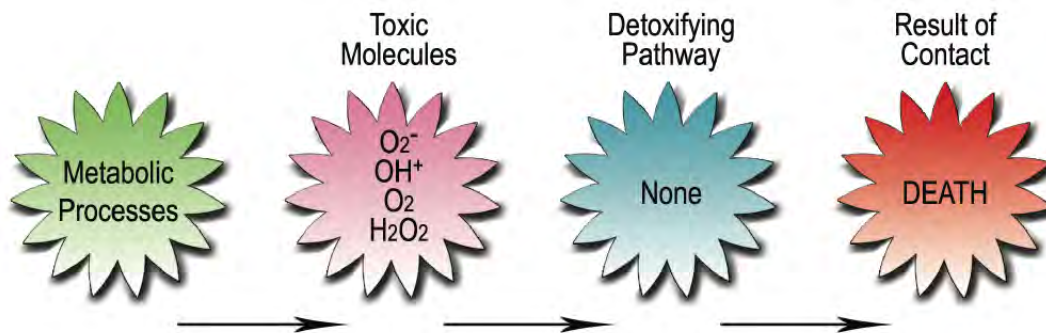
Oxygen has a tendency to form very reactive by-products, (including hydrogen peroxide [H₂O₂] and O₂-superoxide,) inside a cell. These by-products create havoc by reacting with protein and DNA, thus inactivating them. Cells that are able to live in the presence of oxygen have enzymes, (like Superoxide Disutase, Catalase and Peroxidase,) that help them cope with H₂O₂ and O₂- and thus are not destroyed by the presence of oxygen.

Oxygen's anti-microbial mechanisms are not completely understood. It is known that the cell envelopes surrounding many pathogen's, like bacteria, are made up of polysaccharides and proteins. In gram-negative pathogenic organisms, fatty acid alkyl chains and helical lipoproteins are present. In acid-fast bacteria, such as *Mycobacterium tuberculosis*, one third to one half of the capsule is composed of complex lipids, (esterified mycolic acid, in addition to normal fatty acids), and glycolipids (sulfolipids, lipopolysaccharides, mycosides, trehalose mycolates).



ABOVE: Aerobic organisms possess enzymes that deactivate oxygen so that reactive toxic molecules containing oxygen do not damage the cells.

It is this high lipid content of the cell walls of these pathogenic bacteria that may explain their sensitivity, and eventual destruction, when exposed to oxygen molecules. Oxygen molecules penetrate these cellular envelopes and affect the cytoplasmic integrity of these pathogenic organisms. In addition, oxygen disrupts the metabolic activity of these disease-causing cells.



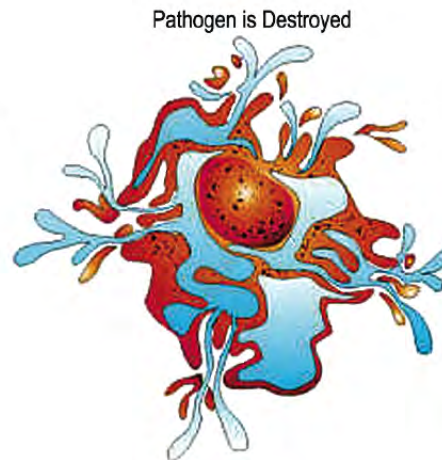
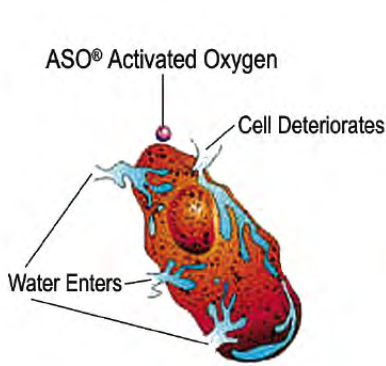
ABOVE: Unlike aerobic organisms, anaerobic organisms do not possess enzymes that are able to deactivate oxygen. Thus, reactive toxic molecules containing oxygen, damage the cells' structural integrity, stop the metabolic processes, and bring about cellular destruction and death.

As mentioned above, the outer cytoplasmic membranes of unicellular pathogens are composed of lipids, proteins, and lipoproteins. These membranes act as a diffusion barrier for water, ions and nutrients. Research indicates that the membranes are actually a lipid matrix containing randomly distributed globular proteins that penetrate through the lipid bilayer.

Oxygen reacts with the unsaturated fatty acids of the lipid layer in cellular membranes, forming hydro-peroxides. There is a synergistic effect with cellular- formed H_2O_2 . Lipid peroxidation products include alkoxy and peroxy radicals, singlet oxygen, ozonides, carbonides, carbonyls, alkanes and alkenes.

Oxygen disrupts the integrity of the bacterial cell envelope through oxidation of the phospholipids and lipoproteins. In fungi, oxygen inhibits cell growth at certain stages. With viruses, the oxygen damages the viral capsid and disrupts the reproductive cycle by disrupting the virus-to-cell contact with peroxidation. The weak enzyme coatings on cells that make them

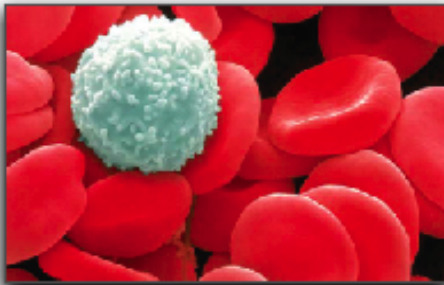
vulnerable to invasion by viruses make them susceptible to oxidation and elimination from the body, which then replaces them with healthy cells.



Basically, oxygen disorganizes membrane permeability so that the organism's nucleic acids and cations leak out and the cell dies.

In addition, oxygen destroys pathogens in a number of different ways: oxygen short-circuits the processes by which pathogens create energy; oxygen disturbs the structure of the bacterial cell wall; oxygen also interferes with the production of essential proteins.

The Body's Immune System Defends Itself Using Oxygen:



The body's immune system utilizes oxygen's powerful oxidizing potential on pathogens in another remarkable way. When a pathogen infects the body, the body recognizes this invasion and sends a host of warrior cells to attack and destroy this unwanted guest. We call these soldiers "white blood cells" but the scientific term is "phagocyte".

LEFT: A white blood cell (phagocyte) in the blood stream surrounded by red blood cells.

When a pathogen is in close proximity to a phagocyte, some sort of signal, (the nature of which is still not clearly understood,) triggers the phagocyte digestion (ingestion) process. Ingestion involves the encircling of the target pathogen with the phagocytic membrane so that the pathogen is actually taken inside the cytoplasm of the phagocyte. It is engulfed in a membrane vesicle called a phagosome.

This process requires ATP (energy created by "oxygen"). Contact between a pathogen and a phagocyte also changes the phagocyte's metabolism from an aerobic respiratory process to anaerobic fermentative process, with lactic acid being the final end product. This increase in lactic acid in the phagocyte lowers the pH of the cytoplasm, including the phagolysosome, and this enhances the activity of many of the degradative enzymes present.

The phagosome, containing the microorganism, migrates into the cytoplasm and soon collides with a series of lysosomes and forms a phagolysosome. When the membranes of the phagosome and lysosome meet, the contents of the lysosome explosively discharge, releasing a large number of toxic reactive oxygen macromolecules, as well as other compounds, into the phagosome. The killing processes inside the phagolysosome are confined to the organelle of the



phagolysosome, and this protects the cytoplasm of the phagocyte from these toxic activities.

LEFT: A phagocyte attacks pathogens in the blood stream.

Several minutes after phagolysosome formation, the first detectable effect on the microorganism is that it loses its ability to reproduce. Inhibiting macromolecular synthesis occurs sometime later and most pathogenic organisms are dead 10 to 30 minutes after ingestion.



ASO[®] As a Biocidal Agent:

Extensive independent laboratory tests using ASO[®] in varying strengths has confirmed its ability to kill microorganisms. Tests conducted by Nelson Laboratories demonstrated ASO[®]'s killing affect on Staphylococcus aureus, Salmonella choleraesuis, Pseudomonas aeruginosa, Escherichia Coli, Candida albicans, Aspergillus niger and Aspergillus flavus. SGS U.S. Testing Company completed minimum inhibitory tests using ASO[®] that demonstrated it controlled Escherichia coli O157:H7, Pseudomonas aeruginosa, Salmonella choleraesuis and Staphylococcus aureus.

Research conducted By Dr. Michael Yoshimura, Ph.D. at California Polytechnic State University, School of Biological Sciences, Phytopathology Department, indicated that ASO[®] effectively prevented the germination, or growth of bacteria, and plant pathogens, (especially the pathogen Alternaria blight, on Zinnia seeds.)

Research done by the University of Minnesota's Agronomy and Soils Department indicated that when ASO[®] was used as a soil drench on celery and parsley seeds, these crops had a higher germination rate. In addition, when used as a biocidal agent on sugar beet seedlings, the ASO[®] was more effective in controlling Aphenomyces, (a soil borne disease that attacks sugar beets,) than a standard treatment called Tachigaren, The ASO[®] also helped prevent frost damage on sugar beet seedlings.

Another study conducted at California Polytechnic State University, Food Processing Department, tested ASO[®]'s ability, as a soaking agent, to reduce mold and yeast colonies on Cat's Claw, a botanical used in the nutritional industry derived from the bark of the Uncaria tomentosa tree grown in Brazil. The tests indicate clearly that ASO[®] is very effective when used as a sanitizing disinfectant on the bark reducing the colony counts by as much as 90% after five minutes of contact.

CONCLUSION:

Oxygen has been shown to be an effective antimicrobial agent by killing pathogenic anaerobic organisms on a cellular level. This is accomplished using a number of metabolic pathways including the destruction of the microorganism's cytoplasmic integrity and disrupting the cell's metabolic reproductive cycle.

ASO®, which contains a high level of polyatomic oxygen, has been successfully used, in both laboratory and field settings, to kill anaerobic pathogens.

Note: copies of the above studies are available upon written request to qualified researchers.

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This information is not intended to treat, cure, diagnose or prevent any disease or medical condition. Always consult with a medical professional before taking any dietary supplement, especially if pregnant, nursing or taking prescription medications.

*These statements have not been evaluated by the F.D.A.