



Why Bacteria are not Enzymes, and other Essentials

By James M. Barbaree, Ph.D., and James E. Harless

Many products today contain bacteria or enzymes that can clear blocked pipes, break down oil or grease and remediate the environment. To appreciate and understand the potential of these products, we need basic information about bacteria and enzymes to be aware of their benefits and uses.

Historically, we have been using bacteria and other enzyme-producing microorganisms to our advantage for many years. Within the food industry, we have created wines that are made by the enzymatic actions of microbes on grapes, and in grounds care we mulch our grass and leaves by allowing the microbes to break them down to a usable fertilizer.

In the environment, we use the action of microbes to degrade our sewage, and even in environmental pollution, like oil spills, microbes are used as a partial measure to break down the oil that pollutes water.

HISTORY OF ENZYMES

The first enzyme to be purified in crystalline form was urease, extracted from the jack bean, in 1926 by Dr. James Sumner of Cornell University. This enzyme hydrolyzes (a chemical process that uses the molecules of water to break down chemical bonds) urea molecules to carbon dioxide and ammonia. Today, some carpet cleaning products contain bacteria that have the ability to produce urease that is intended to target urine present in some soiled rugs.

Increased awareness of the natural action of microbes and enzymes emerged in the 1970s. Cumulative data showing the toxic effects of compounds such as PCBs, DDT and detergents caught our attention.

These compounds are recalcitrant, meaning that they accumulate and are not broken down by microbes into essential elements. It became obvious that, as an industrial society, we needed to start producing pesticide and detergent compounds that could be attacked by microbes and degraded over a period of time, or we would slowly poison ourselves.



An example of a success story is the change in the formulation of detergents that are composed of branching chemical structures to ones that have straight chemical chains, which are more easily biodegraded by microorganisms.

Bacteria, Not Enzymes

Bacteria are not enzymes. Like all living cells, bacteria produce and use enzymes to break down or assimilate (put together) chemical compounds necessary for cell survival. Some scientists refer to bacteria as "sacks of enzymes."

Enzymes are termed "biological catalysts" because they are chemicals (mainly protein) that bind with another chemical (referred to as the substrate) that is then broken down. Because of their enzymes, bacterial organisms vary in their abilities to withstand environmental conditions and break down compounds.

Some of these organisms can withstand temperatures of 212 degrees Fahrenheit or minus 200 F. Some live in extremely acidic or alkaline conditions, and some are able to use oil (hydrocarbons) or lipids (fat) for food. These survival characteristics are governed by the bacteria's ability to make and use various enzymes.

Factors that are important for enzyme action alone, or for bacterial action via enzymes, are temperature, pH (degree of acidity or alkalinity) and solubilization of the substrates. Most bacteria are active in an environment of 90 to 100 F with a neutral pH.

Pure enzymes vary in pH requirements and stability (see table). When pure enzymes are added to a building environment such as a drain, the enzyme is usually short-lived due to the fragile nature of the enzyme under those conditions. The solubility of the substrate can be improved by the presence of surfactants (detergent-like substances) added to the enzyme cleaning solution.

PURE ENZYMES

An example of the beneficial action of an enzyme is the production of lipase in the presence of cooking grease. Under the appropriate conditions, bacteria release lipase enzymes that bind to lipids, which may have been rendered soluble by surfactants.

A lipid is a complicated structure that usually contains fatty acids that are bound to a basic glycerol structure. The lipase enzyme attacks the lipid, releasing the fatty acids. These fatty acids can then be broken down to smaller compounds by enzymes from other bacteria or from the same bacterium.



This same bacterium, or other bacteria, may produce protease enzymes (that break down protein from the meat that is present in the grease) and amylase enzymes (that break down starch from the flour that is present). The grease, which is a combination of lipids, proteins and starch, is broken down to more soluble compounds that flow more readily.

Pure enzyme solutions are made by extracting enzymes from animal tissues, the broth in which microorganisms grow or from lysed microbial cells. The stabilization of enzymes is sometimes difficult because they have to be packaged at the correct pH level with a chelating agent, which protects the enzymes from binding to certain metals and becoming inactive.

Some enzymes are offered in commercial products to help clear drains or partially break down proteins. The disadvantage in using enzyme solutions alone is that pure enzymes will disintegrate in a short period of time, while bacteria are capable of colonizing and will reproduce to produce more enzymes.

PACKAGED' BACTERIA

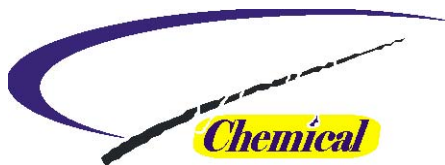
To "package" bacteria for use, they have to be put into a dormant state to withstand some harsh environmental conditions, such as temperature variations. The packaging is accomplished by drying (but not killing) them or by selecting bacteria that can become dormant in a spore stage.

The drying process stops enzyme activity and makes the cells stable until they are rehydrated. This product containing bacteria is a powder that we sprinkle onto the liquid. Once in the liquid, the bacteria are rehydrated, which activates their enzymes and stimulates the bacteria to begin feeding on the nutrients (the soil that we are trying to remove from the surface).

Bacterial spores are very resistant to temperature variations and acidic or alkaline environments. Spore-forming bacteria are grown in a way that causes the formation of spores. The spores are harvested and packaged dry or in a specially formulated liquid.

The main spore-forming species found in grease-cleaning products is *Bacillus*. When the *Bacillus* spores are added to a favorable environment (possibly a grease trap) the spores germinate into vegetative (growing) cells that activate enzyme systems and begin feeding on the available nutrients.

Bacteria must be given time to degrade soil compounds, so simply wiping a surface with a bacterial solution that degrades oils would not be very effective. However, the necessary temperature, pH, solubilized substrate and other miscellaneous factors must also be present for bacterial growth.



| Examples of Purified Enzymes | | | | |
|-------------------------------------|---|-------------------------|--|---------------|
| Enzyme | Substrate | Optimum pH Range | Stability | Source |
| Lipoxidase | Polyunsaturated | 6.5-7.0 | 1-2 years Fatty Acids freeze-dried & stored at 39°F | Tissue |
| Lipase | Insoluble forms of Glycerol and long- chain fatty acids | 7.5-8.0 | Extremely labile; 1 year if dry and stored at 39°F | Tissue |
| Phospho-lipase C | Lecithin (a complex fat) | 7.0-7.6 | 6 Months at 39°F | Bacteria |
| Cellulase | Cellulose | 4.2-5.2 | 1 year freeze dried and stored at 39°F | Fungi |
| Urease | Urea | 6.5-7.0 | When freeze-dried and stored at 39°F, loses 5% activity per month | Bacteria |

HAZARDS

The degree of health hazard while using enzyme or bacterial products is probably very low. However, one concern may be the breathing of aerosols of bacteria sprayed onto surfaces. Most *Bacillus* species don't produce disease, but some of the other bacteria that may be removed from the surface due to the action of the enzyme or bacteria solutions have the potential to cause disease.

An example of harmful bacteria is *Pseudomonas aeruginosa*, which can be found in dust, soil and a variety of natural environments. Under the appropriate conditions it can affect humans, especially through skin lacerations or the respiratory tract of immunocompromised individuals.

Today, bacterial products with enzymes are being developed that might replace the toxic actions of strong acids and surfactants. Scientists who are on the cutting edge of technology are genetically engineering microbes to optimize the capabilities and effectiveness of the enzymes that they produce. Desirable genes from environmentally-unstable microbes are being transferred to other microbes that are more resistant to environmental changes and easier to grow.

Commercial manufacturers, the environment and, eventually, the end user will benefit as the technology grows.

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