BASIC DEFINITIONS

BIOTECHNOLOGY :
The science of the selection and the application of living microorganisms for purposes useful for mankind and the environment.

BACTERIA :
Any of a group of diverse and ubiquitous prokaryotic, single-celled microorganisms.

SPORE :
The dormant or inactive stage that some bacteria can temporarily assume.

ENZYME : (a/k/a non-living chemical catalyst)
A protein produced by living organisms (i.e. bacteria) that function as catalysts in assisting chemical processes such as digestion or degradation of organic waste.

> **NOTE :** While bacteria metabolize a wide variety of organic material, enzymes are substrate specific. For example :
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> **Protease** enzymes catabolizes (“breaks down”) protein
> **Amylase** enzyme breaks down starch and carbohydrate
> **Lipase** enzyme breaks down fat and grease
> **Cellulase** enzyme breaks down cellulose
> **Urease** enzyme breaks down urea

AEROBIC BACTERIA :
Bacteria that require the presence of oxygen to live and function.

ANAEROBIC BACTERIA :
Bacteria that do not require the presence of oxygen to survive – they are capable of living and functioning in the absence of oxygen.

FACULTATIVE BACTERIA :
Bacteria that are capable of living and functioning **either** in the presence of oxygen, **or** in the absence of oxygen.

BIODEGRADATION SUMMARY

Introducing selected, naturally-occurring, non-pathogenic, bacteria (which produce enzymes) to organic waste is a safe, environmentally responsible method of augmenting and accelerating nature's own biodegradation process.

In the natural environment, both bacteria and the enzymes play a significant part in biodegradation -- Bacteria produce the enzymes essential for metabolizing the food source (organic waste) into energy necessary for further growth of the living organism. The enzymes facilitate the phase of metabolism in which complex compounds are broken into simpler ones (catabolism). This in turn speeds the process of converting the food source into an available energy supply for the bacteria.

A reasonable period of time after introducing Calfarme's specially selected bacteria into water medium containing organic waste, the spores will vegetate (go from dormant to active), produce specific enzymes, and degrade or digest the available organic waste. The introduced microorganisms are capable of exponential growth – they can double in number every twenty to thirty minutes. The by-products of this bacteria / enzyme activity are H_2O and CO_2.

By adding Calfarme's strains of *Bacillus* bacteria to organic waste, the following beneficial results can be achieved :

- reduction of BOD (Biological Oxygen Demand)
- reduction of COD (Chemical Oxygen Demand)
- reduction of SS (Suspended Solids)

Foul odours and noxious gases are reduced, by eliminating their source (organic waste)

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Calfarme's bacterial concentrates are not genetically engineered or altered; they are naturally-occurring – found in soil and water. They were carefully selected because they are :

- safe and stable
- non-pathogenic
- non-toxic
- facultative (capable of growth with or without oxygen)
- vigorous enzymes producers
- logarithmic reproduction, every 30 minutes.

FATE OF INTRODUCED BACTERIA AND ENZYMES

This is a brief explanation of the ecology and fate of introduced microorganisms and enzymes. It is by no means complete, but it is intended to provide a basis for addressing those concerns.

Microorganism in the genus Bacillus are ubiquitous. That is, they can be found essentially everywhere. They belong to a group of microorganisms that form endospores.

The primary habitat for Bacillus sp. is the soil. In this environment, they remain inactive until a suitable substrate (food source) becomes available. Usually these substrates include large molecules from the remains of plants and animals. The large molecules, like proteins, lipids, and carbohydrates are suitable substrate for Bacillus sp. because these strains excrete enzymes which break these molecules into smaller, more soluble compounds.

The process of reducing in size the larger compounds is similar to what occurs at a municipal sewage treatment plant and is important on several levels. To the bacterial microflora, this is important because these smaller compounds are now available for absorption into and use by the cell. On the community level, this process is especially important because it initiates the degradation process. Finally, on a global scale, this process plays a vital role in the global recycling of carbon and nitrogen.

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When placed in a suitable environment (like a drain or a septic) and applied in high numbers, our Bacillus species will proliferate, excrete enzymes, and break down the large organic matter into smaller usable compounds. However, when they are removed from this suitable environment, they can no longer be as metabolically active (ie., their growth rates are slower).

There are least two reasons for this reduction in metabolic activity. First, the down stream or open water nutrient concentration is at least 100-1000 times less concentrated than a typical clogged drain or septic system. Second, changes in the physical (i.e. temperature) and chemical (i.e. pH, salt concentration) conditions reduce the microorganisms capacity to grow. Once downstream, the metabolically inactive cell has several possible fates.

First, because the cell may lyse (break open) or be used as a food source by other organisms (predators), the cell may die. Second, this vegetative cell may become incorporated into the sediment. In this case, the growth of the bacteria is reduced to a fraction (approx. 1/2000th) of the optimal rate, dependent on the amount of waste material (food source) found in the sediment. Last, if the proper conditions are met, the vegetative cell may undergo a complex series of structural changes to form an endospore.

The endospore will remain dormant, until a suitable environment exists where it will convert back to a vegetative cell. This would occur when the endospore reaches a residential or municipal waste treatment facility. In the last situation, the introduced bacteria becomes incorporated into an environment that already contains many species of bacilli, and will continue to clean up nature's waste.

Enzymes are a very specialized class of proteins. They catalyze, or increase, the rate of a chemical reaction. It is not unusual that an enzyme will cause a reaction to occur one million times faster than normal. Every organism produces a large variety of enzymes, most of which are produced in small quantities and are usually involved in internal cellular processes. However, some microorganisms produce large quantities of enzymes that are excreted by the cell. Bacilli are one group of microorganisms known for this ability.

Microbial enzymes are often produced commercially for use in a wide variety of industries. These industries include the baking, paper, food, starch, pharmaceutical, meat, textile, and laundry industries. Products that use enzymes in their manufacturing include bread, syrup, glucose, meat tenderizing and detergents.

The use of enzymes are advantageous because they act very fast, are not used up in a reaction, and are very specific for the type of reaction that they catalyze. However, because enzymes are very sensitive to change in their physical and chemical environment, they are relatively short lived. A change in temperature, pH, or salt concentration will denature (or destroy) the enzyme.

In addition, enzymes are very sensitive to biological activity. Since enzymes are mainly protein (rich in nitrogen and sulfur) they are substrates (food source) for bacterial growth. Bacteria make use of these molecules by secreting enzymes (proteases) that degrade (among other proteins) enzymes. The enzyme fragments are absorbed into and used by the growing bacteria. These characteristics, of quick action followed by a reduction of activity, are good for our products because they give a quick start to the degradation process, create a situation where the bacteria can proliferate, and are not harmful when transported down stream.

In summary, both introduced bacteria and introduced enzymes have several characteristics in common that will determine their fate in the environment and address our concerns about the fate. First, both are naturally occurring and can be isolated or identified in the environment. If they do survive down stream, they would continue to help clean up waste byproducts. Second, both are sensitive to physical and chemical changes in the environment (nature's way of population control). Lastly, both are acted upon by microbial activity (predators).