GENERAL INFORMATION ABOUT ENZYMES AND Terraroads®

Nature of Enzymes

Enzymes are protein molecules are joined into long polypeptide chains of amino acids. These chains are coiled (preformed) in a specific manner to form structures or shapes that enable the enzymes to act as catalysts. This action may happen sequentially or in a step-by-step fashion, or, once initiated, proceed in a parallel

fashion with many events taking place simultaneously. Enzymes are present in all of the cells of all living things. They control all functions and processes of all living things. One of their best-known functions is the control of metabolic processes in both animals and plants. The unique characteristic of enzymes is that they function as catalysts to speed up chemical reactions up to a billion-fold without being consumed or permanently altered by the process. The word "catalyst" was derived from the Greek word "katalusis", which means to dissolve or loosen. Catalysts help to accelerate change in the materials to form new chemical structures. Many materials change their forms over a period of time, but transformations that would have taken years to accomplish can be completed within fractions of a second when the appropriate catalyst is added.

In a controlled environment enzymes will continue to cause the desired catalytic reaction to occur until essentially all of the material(s) being converted by the process have been converted. They will not interact with or otherwise affect any materials in the controlled environment that they have not been designed to affect. Unlike toxic inorganic catalysts such as acids, bases, metals and metal oxides, most enzymes demonstrate great specificity, reacting with only one or a small group of closely related chemical compounds; thus, sometimes a "family" or group of several enzymes is required for efficient catalytic function. Some enzymes depend on the presence of coenzymes for their function. For the enzyme to continue to be effective, its three-dimensional molecular structure must be maintained (usually in water). X-ray crystallography is used to analyze the structure of enzymes when they can be sufficiently purified. Over 1000 different enzymes have been identified to date, and the exact sequence of amino acids (subunits of a protein) has been determined for many proteins since 1967, when the first such determination was made. It is believed that enzymes function by attaching the substrate molecule to a specific molecular site, so that the electrostatic forces of nearby atoms sharply reduce the energy needed to cleave or reform the appropriate chemical bonds.

One way to describe the enzyme function is to say that the substrate molecule fits into an active site in the enzyme molecule somewhat like a key fits into a lock, thus enabling a reaction to occur. As described above, the enzyme's structure is modified temporarily, but continually reformats itself during the reaction so that it will be available to repeat the same task over and over. This behavior can be observed in the use of enzymes in bioremediation, where the enzymes facilitate the growth of indigenous bacteria in the soil to digest the petroleum contaminants.

Enzymes are extremely efficient catalysts. For example, the enzyme catalase is so efficient that one enzyme molecule can catalyze the breakdown of five million molecules of hydrogen peroxide to water and oxygen in one minute. They come from natural biological systems and when they are degraded, the amino acids of which they are made can be readily absorbed back into nature. Enzymes work only on organic materials, which includes fruit, vegetables, meats, soil, cotton, leather, wood and all types of hydrocarbon compounds. Both the usable products and the waste of most enzymatic reactions are non-toxic and readily broken down into harmless organic residue. Industrial enzymes can be produced in an ecologically sound way where the waste sludge is recycled as a non-toxic fertilizer.

Enzymes work best at atmospheric pressure and in moderate conditions with respect to temperature and acidity (pH). Most of the enzymes that we normally encounter in our environment function optimally at a temperature of 30- 70°C and at pH values, which are near the neutral point (pH 7). For specific technical applications, specific enzymes have been isolated and reproduced in commercial quantities to work at higher temperatures, higher pressures, higher or lower levels of pH, etc.; however, no enzyme can withstand temperatures above 100°C for sustained periods. Enzyme processes require very little external energy and do not need special equipment that is resistant to heat, pressure or corrosion. Enzymes are very well suited for a wide range of industrial applications (including many not yet developed) due to their efficiency, ability to be designed for very specific action, the mild conditions in which they work and their high biodegradability.

Specificity of Enzymes

One of the properties of enzymes that makes them so important as diagnostic and research tools is the specificity they exhibit relative to the reactions they catalyze. A few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity:

* **Absolute specificity-the enzyme will catalyze only one reaction**
* **Group specificity-the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups**
* **Linkage specificity-the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure**
* **Stereochemical specificity-the enzyme will act on a particular steric or optical Isomer.**

Though enzymes exhibit great degrees of specificity, cofactors may serve many apoenzymes. For example, nicotinamide adenine dinucleotide (NAD) is a coenzyme for a great number of dehydrogenase reactions in which it acts as a hydrogen acceptor.

Among them are the alcohol dehydrogenase, malate dehydrogenase and lactate dehydrogenase reactions used to produce beer, wine and cheese.

Naming and Classification

Except for some of the originally studied enzymes such as pepsin, rennin, and trypsin, most enzyme names end in "ase". The International Union of Biochemistry (I.U.B.) initiated standards of enzyme nomenclature, which recommend that enzyme names. indicate both the substrates acted upon and the type of reaction catalyzed. Under this system, the enzyme uricase is called urate: O_2 oxidoreductase, while the enzyme glutamic oxaloacetic transaminase (GOT) is called L-aspartate: 2-oxoglutarate aminotransferase.

Enzymes can be classified by the kind of chemical reaction catalyzed:

1. Addition or removal of water-

- **Hydrolases-these include esterases, carbohydrases, nucleases, deaminases, amidases, and proteases**
  - **Hydrases such as fumarase, enolase, aconitase and carbonic anhydrase**

1. Transfer of electrons-

- **Oxidases**
  - **Dehydrogenases**

1. Transfer of a radical-

- **Transglycosidases--of monosaccharides**
- **Transphosphorylases and phosphotnutases - of a phosphate group**
- **Transaminases-of amino group**
- **Transmethylases-of a methyl group • Transacetylases-of an acetyl group**

4. Splitting or forming a C-C bond • Desmolases

5. Changing geometry or structure of a molecule • Isomerases

1. Joining two molecules through hydrolysis of pyrophosphate bond in ATP or other tri-phosphate

- **Ligases**

Development of Commercial/Industrial Applications

Virtually all industrial enzymes originate from microorganisms in plants or in the soil, such as fungi or yeasts. Just one microorganism can contain over a thousand different enzymes. Several months of

experimenting by trial and error in the laboratory are needed to isolate the best microorganism for producing a particular type of enzyme. The

isolated microorganism can then be modified by chemical or radiological means to produce the desired enzyme at high yields. After the microorganism has been identified and modified, it is "cooked" in huge fermentation tanks where it produces the desired enzyme in large quantities. This process makes it possible to produce enzymes economically in virtually unlimited quantities.

Currently, a significant portion of the U.S. Gross National Product (GNP) is generated by products, which utilize catalytic chemistry. These products range from plastics, paints and detergents, to foods and beverages. The potential future applications of enzyme technology for commercial and industrial applications appear to be unlimited due to the fact that (1) enzymes exist in several million varieties in nature and (2) there has been limited research on the use of enzymes to solve commercial and industrial needs.

Terraroads Enzymes

Terraroads is a method of stabilizing soil by mixing together soil, water and Terraroads (a crop plant biomass microorganism-expressed enzyme composition) to form a mixture; causing the mixture to be shaped into a selected structure whether it be road base, bricks, block, tile or board; and compacting the structure. Terraroads is a crop plant biomass microorganism-expressed enzyme composition that includes a ureolytic enzyme, or such a composition that includes an enzyme expressed by a ureolytic microorganism and using the soil as a nitrate ion source. Terraroads can be formed such as a road base, brick, block, board, tile, or paver.

The electrostatic characteristics of soil particles are factors in soil-Terraroads (enzyme) interactions. Terraroads (enzymes) may lower the dipole moments of water molecules and dissociation may then occur to form a hydroxyl (-) ion and a hydrogen (+) ion. The hydroxyl ion in turn may dissociate into oxygen and hydrogen, and the hydrogen atom of the hydroxyl ion may be transformed into a hydronium ion. The hydronium ion can accept or reject either positive or negative charges, depending on the circumstances. Terraroads compositions may be adsorbed by the clay lattice in soil, and then released upon a complete cycle of exchange with metal cations such as sodium and potassium. Terraroads compositions may initially cause the clay lattice to expand and then to tighten. It may also be absorbed by colloids, enabling enzyme molecules to be transported through soil electrolyte media. Terraroads compositions may also may help soil bacteria to release hydrogen ions, resulting in an acidic pH gradient at the surfaces of clay particles, which may assist in breaking up the structure of the clay and facilitating its compaction.

Terraroads is a multi-enzyme which includes the ureolytic enzyme microorganisms. Urea is converted by certain microorganisms into ammonium carbonate, releasing ammonium cations which are then converted by certain microorganisms into nitrite ions and then into nitrate ions. Nitrate ions are taken up as a nutrient by certain plants. Ammonium nitrate, ammonium sulfate, ammonium carbonate and ammonium bicarbonate as examples, are direct sources of ammonium cations for in situ conversion into nitrate ions. Ammonium nitrate is additionally a direct source of nitrate ions. Ammonia, as another example, ionizes into ammonium ions. Terraroads uses a fermentation process which includes incubation of selected microorganisms together with a crop plant biomass under conditions suitable for growth of the microorganisms. The selected microorganisms may then collectively express such an enzyme in a harvestable amount and concentration. Selected microorganisms may further express a plurality of enzymes. Terraroads uses an active ureolytic enzyme which converts urea into cationic ammonium ions. These enzymes remain enzymatically active in the enzyme composition

Terraroads may include one or more of the following types of enzymes: urease, hydrolase, oxidoreductase, transferase, lyase, aspartase, l-glutaminase, amidohydrolase, dehydrogenase, acid phosphatase, alkaline phosphatase, arylsulfatase, betaglucosidase, amylase, catalase, alkaline phosphomonoesterase, phosphodiesterase, deaminase, invertase, cellulase, protease, asparaginase, amidase, chitinase, lipase, carbohydrase, phenoloxidase, peroxidase, laccase, lipase, aminopeptidase, and glucose oxidase. Terraroads utilizes a termite and ant enzymes.

Terraroads enzyme composition may be formulated with or without addition of surfactants. Terraroads uses enzymes with a pH within a range of between about 7 and about 8. Terraroads composition including a nitrate ion source. Urea may be converted by the enzymes or by microorganisms in the soil, into ammonium ions. Urea may be added to one or more of the following forms: ammonium ions (NH_4^+), nitrate ions (NO_3^-), and urea ($CO(NH_2)_2$.

Glossary of Terms

The following is a simplified definition of some of the terms used in this brochure. Bacteria: Primitive single-celled organisms that are not compartmentalized by internal cellular membranes. In Bioremediation, microscopic living organisms that can act on polluted or contaminated soil or water by metabolizing the organic matter in sewage, oil spills, etc.

Biodegradable: Any substance, which can be readily decomposed by bacterial activity. Bioremediation: Use of living organisms to clean up spills or remove other pollutants from soil, water, or wastewater; use of organisms such as non-harmful insects to remove agricultural pest or counteract diseases of trees, plants or garden soil.

Biotechnology: Techniques that use living organisms or parts of organisms to (i) produce a variety of products (from medicines to

industrial enzymes), (ii) improve the health of plants and animals, or (iii) develop microorganisms to perform specific tasks such as removing toxins from bodies of water, acting as pesticides, etc.

Contaminant: Any physical, chemical, biological, or radiological substance or matter that has an adverse effect on air, water, or soil.

Enzyme: These are proteins that form long polypetide chains of amino acids~ They function as catalysts to increase the speed of chemical reactions in all living systems (plant or animal).

EP A~ The Environmental Protection Agency, a U.S. Governmental agency with jurisdiction over air and water pollution, solid-waste disposal, pesticide registration, toxic substances, the setting of radiation standards, noise control, etc.

Fermentation: Process in which cellular energy is generated from the breakdown of nutrient molecules where there is no net change in the oxidation state of the products as compared with that of the reactants.

In-Situ: This refers to a process, like Bioremediation, which occurs in the original place or site where the materials being processed are located naturally.

PCB: Any of a family of highly toxic compounds now banned in the U.S., which were formerly used in industry and manufacturing processes.
Polypeptide: One of a group of amides formed from amino acids.

Proprietary Enzyme Products: The Company's unique blend of enzymes and other organic materials (such as energy sources) to form a "group" or "family" of organisms that will accomplish a specific commercial or industrial task or process.
R&D: Research and Development.