BiOWiSH™ TECHNOLOGIES

Fact Sheet

About Enzymes

Introduction

Enzymes have played an important role in many aspects of life since the dawn of time. In fact they are vital to the existence of life itself. Civilizations have used enzymes for thousands of years without understanding what they were or how they work. Over the past several generations science has unlocked some of the mystery of enzymes and has applied this knowledge to make better use of these amazing substances in an ever-growing number of applications. Enzymes play crucial roles in regulating our bodies, digesting our food and converting it to energy and are the basis of every bio-chemical reaction in the human body. Enzymes are also instrumental in producing the food we eat, the clothes we wear and even in producing fuel for our automobiles. Enzymes are also important in reducing both energy consumption and environmental pollution.

BiOWiSH Technologies believes the importance of enzymes in everyday life is one of today's best-kept secrets and has developed a range of products for different applications and challenges we face today which will benefit you and the world we live in.

What do Enzymes do

Let us start with an example. Common white sugar (sucrose) is not only a sweetener for our food but is a form of energy that can be used by our bodies as glucose. Chemically the reaction that takes place is:

$$C_{12}H_{22}O_{11} \; + \; O \qquad CO_2 \; + \; H_2O \; + \; \text{Energy}$$

or

Sucrose + Oxygen   Carbon Dioxide + Water + Energy

Looking at this equation it is reasonable to ask that if this is the chemical reaction, why can a bag of sugar sit on the shelf in your cupboard for years without any discernable conversion to carbon dioxide and water? The sugar is exposed to oxygen (just as it is in the body) and it can be at a similar temperature to normal body temperature without any change.

The truth is that the above reaction is taking place but just extremely slowly.

How is it then that when we consume that sugar it is converted to energy almost instantaneously? The answer is the body produces enzymes that catalyse the reaction. It is the same reaction, just exponentially faster.

So in the body our reaction becomes:

$$C_{12}H_{22}O_{11} \; + \; O \; + \; E_z \qquad CO_2 \; + \; H_2O \; + \qquad \text{Energy}$$

or

Sucrose + Oxygen + Enzyme   Carbon Dioxide + Water + Energy + Enzyme

What are Enzymes?

Enzymes are something you have most likely heard of but cannot quite explain exactly what they are and what they do. In fact, enzymes are fascinating and very important.

You may not be aware of the fact that many different kinds of enzymes exist inside our bodies (scientists have identified 3,000) and that they perform a multitude of functions. **Without enzymes our bodies would cease to exist.**

Technically speaking enzymes are proteins and each enzyme is comprised of a specific sequence of the 20 basic amino acids that make up all proteins.

Enzymes are a component of the chemistry of all biological organisms.

Due to their size enzymes are known as macro-molecules, meaning large molecule. They have a molecular mass ranging from 12,000 to over 1 million mr giving us a glimpse as to just how broad a range there is.

As proteins enzymes are 100% biodegradable, and unless produced through genetic modification they are 100% organic. As a point of interest, enzyme usage is an alternative to GM technology in achieving desired biological outcomes.

You will note from this that the enzyme is not consumed or changed in the reaction. It merely acts as a catalyst to rapidly accelerate the reaction so our body can obtain the energy that is locked up in the sugar. This oxidization of the sugar releases the energy originally from the sun stored through the process of photosynthesis by the sugar cane. This example is characteristic of the role of enzymes.

Enzymes are biological molecules that accelerate chemical reactions. They are central to the existence of life. You cannot digest or absorb food without them. Even sitting, reading, thinking and the beating of your heart involves enzymes. Without enzymes, seeds would not sprout, fruit would not ripen, leaves would not change colour and life on earth would not exist.

Enzymes are bio-chemical catalysts. This means that they make things happen chemically or put simply, they split molecules into component parts or combine smaller molecules into large molecules. Enzymes are highly specialised in that they will perform their catalytic role on in a certain way on a specific type of molecule. This molecule is known as the enzyme's substrate. The substrate binds with the enzyme at a special location on the enzyme known as the active site which is designed to only accept this particular substrate molecule. This specificity is what makes enzymes very targeted in their application and very safe to humans and the environment.

The majority of enzymes (95%) are known as catabolic enzymes in that they divide their substrate into smaller components which are released from the enzyme. The other type of enzyme is anabolic enzymes that combine substrates into larger molecules.

Enzymes carry a vital energy factor needed for every chemical action and reaction that occurs in nature. Enzymes are essential for all metabolic processes, but are not alive themselves

The catalytic role played by enzymes allows bio-chemical reactions to take place in aqueous conditions that are moderate in temperature and neutral in terms of pH levels or acidity.

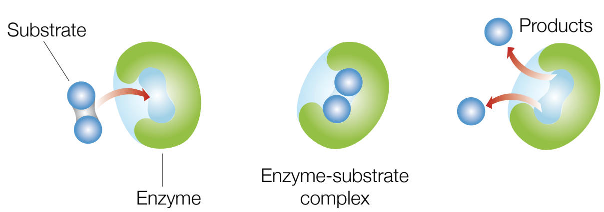
Enzymes are extraordinary catalysts and accelerate bio-chemical reaction speeds. Typical rate enhancements of enzymes are 10^5 to 10^{17}. That is up to 1,000,000,000,000,000,000 times faster than the reaction would take place without the presence of the enzyme. To put that in context a reaction that would take over 31 billion years without a catalyst would be complete in 1 second.

A typical enzymatic reaction can be represented by the following equation:

E + S   ES   EP   E + P

Where E, S and P represent the enzyme, substrate and product. ES is what's called an enzymatic complex and it represents a bonding of the enzyme with the substrate. A second enzymatic complex is then formed after the transformation which is the EP complex or a combination of the enzyme bonded to the product of the transformation. The final step in the reaction is the release of the product from the enzyme. The enzyme is then able to act as catalyst in the next reaction. This process can take place thousands of times a second. A diagrammatic representation of this simple reaction is shown below as an enzyme divides a substrate into 2 component products.

Mechanism of enzyme activity



Often this process is known as a 'Lock and Key' due to the specificity of the enzyme / substrate bond.

The efficiency of enzymes is further enhanced by a transformation of a substrate into a final product being achieved in a number of small steps rather than one large step that would require much more energy to perform. This energy required for a reaction to take place is known as the activation energy. Enzymes as catalysts reduce the activation energy for a given reaction.

This step-by-step process is known as an Enzymatic Cascade and sees the product of one reaction become the substrate for the next and so on. In some cases a transformation from initial substrate to final product can have up to 24 steps. This process not only conserves energy, it also provides an additional degree of safety in that any stage of the transformation can be inhibited and if necessary reversed.

In an enzymatic cascade the products of one reaction before they are utilized as substrates in the next reaction are known as intermediates. These intermediates can often be toxic or odorous. BiOWiSH™ has been formulated to limit the production of intermediates by providing very rapid achievement of full decomposition and enzymatic cascades that bypass the toxic and odorous intermediates.

Where do Enzymes come from

Enzymes are produced naturally by both plants and animals of all types as an essential process of living.

Animals produce enzymes to perform a number of digestive and metabolic tasks. In humans most of our enzyme production takes place in the pancreas and to lesser degree elsewhere in the body including the salivary glands. Similarly plants produce enzymes that assist with their biological processes.

Bacteria

One type of living organism is bacteria. Bacteria are unicellular microorganisms that are found in every habitat on earth. They vary greatly in shape and size and biological function. In a single gram of soil it is estimated a population of 40 million bacteria can be found. It is also estimated that there are 10 times more bacterial cells than human cells in the human body. Bacteria play a very important role in helping us digest our food sources.

Bacteria play a very important role in nature as recycling agents. They are at the core of the decomposition of organic matter which releases nutrients back into the environment where they can be re-used in the formation of new organisms. Without bacteria and their ability to decompose organic matter all the carbon on earth would be locked up in the cells of formerly living organisms.

Bacteria use a form of asexual reproduction known as binary fission where each bacteria cell splits to produce 2 identical copies of itself. Bacteria populations have been shown to be able to double in less than 10 minutes providing for exponential growth in population where 1 bacteria cell can grow to 68 billion cells in 6 hours if environmental conditions remain favourable. Ultimately bacteria replication is restricted due to a limitation in the available substrate or nutrient source.

Most bacteria are aerobic in that they require oxygen (as an electron donor) in their digestion and therefore the elimination of waste products. Some bacteria are known as facultative anaerobes as they are able to utilise electrons other than oxygen in their metabolism. This enables these bacteria to effectively digest waste in environments where there is low oxygen content. This provides a wider range of environments in which the bacteria are effective.

As very simple organisms, bacteria do not possess an internal digestive tract. Bacteria still need to consume nutrients from their environment which they metabolise for life functions and replication. So unlike us humans and most other animals, bacteria digest their food sources external of their own cell walls. This extracellular digestion is achieved by the bacteria excreting enzymes through their cell walls which break down the nutrient source into smaller components that can be absorbed through the bacteria's cell wall. The bacteria then metabolise this nutrient source and excrete by-products.

Full decomposition of matter is achieved when the bacteria waste products are final inert compounds of nature such as water, carbon dioxide, oxygen, hydrogen etc. At the end of their life cycle or when their nutrient source is exhausted the bacteria will themselves be broken down by other bacteria and their enzymes and recycled into nature.

How are Commercial Enzyme Preparations made

Commercial sources of enzymes are obtained from three primary sources animal tissue, plants and microbes (bacteria). These naturally occurring enzymes are quite often not readily available in sufficient quantities for food applications or industrial use. However, by isolating microbial strains that produce the desired enzyme and optimising the conditions for growth, commercial quantities can be obtained. This technique is called fermentation and has been known for more than 3,000 years. Today, this fermentation process is carried out in a contained vessel under tightly controlled conditions. Once fermentation is completed, the microorganisms are destroyed; the enzymes are isolated and further processed for commercial use.

BiOWiSH™ has been developed from naturally occurring microbes found in a particular mangrove environment noted for its ability to sanitise water. As a natural, non-animal formulation, the BIOWISH™ super microbes fight pathogenic microbes, control pollution, help balance the environment and perform a range of useful bio-remediation functions.

BiOWiSH™ is not genetically modified (GM) as it is derived strictly from a pure strain of naturally occurring microbes.

As BiOWiSH™ contains active microbes, it has a significant benefit over products that are purely enzyme based. As identified above, the cocktail of useful microbes that comprise BIOWISH will rapidly multiply to digest the amount of waste product available to it. In this way the BiOWiSH™ product is a truly scalable technology that operates at low dosages and is therefore cost effective.

Being a cocktail product BiOWiSH™ will also digest a very wide range of waste products including fats, oils, grease, carbohydrates, proteins and many chemicals.

BiOWiSH™ is also formulated from microbes known as facultative anaerobes. This ensures that BiOWiSH™ will operate effectively in environments where natural bacteria will not. This can significantly accelerate decomposition times and reduce the need for external energy such as aeration of water or turning of composts.

Co-Enzymes and Co-Factors

Co-enzymes are small organic, non-protein molecules that transport chemical groups from one enzyme to another thereby increasing the rate of catalysis. Some of these chemicals such as riboflavin, thiamine and folic acid are vitamins. Vitamin C is a coenzyme. Unlike the enzymes themselves a coenzyme is consumed or chemically changed during the bio-chemical reaction and therefore needs to be present in sufficient quantity to optimise the effectiveness of an enzyme preparation.

Co-factors are non-protein chemical compounds that are tightly bound to an enzyme. They are required for catalysis to occur. They can be considered ‘helper molecules/ions’ that assist in bio-chemical reactions. Any enzyme without a cofactor is referred to as an apoenzyme and the completely active enzyme (in addition to the cofactor) is called a holoenzyme.

Co-factors are also often referred to as trace elements and include many metal ions such as manganese, iron, cobalt, nickel, copper and zinc.

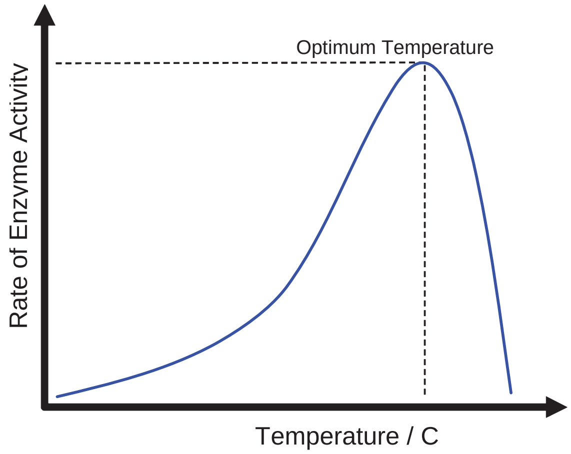
The formulation of BiOWiSH™ contains an optimum blend of co-enzymes and co-factors which are essential to the rapid achievement of full digestion and therefore the elimination of waste, odor and toxins. Natural bacteria decomposition is a step by step process where toxic and odorous intermediate compounds are formed before full decomposition is achieved.

Operating Conditions and Speed of Catalysis

As natural organisms microbes and the enzymes that they produce have a range of environmental conditions that they can tolerate. Temperature, pressure, pH level (acidity), alkalinity, salinity, oxygen, antibiotics, coenzymes, cofactors, chlorine and chemicals can all accelerate, impede or even destroy microbes and enzymes.

During a bio-chemical reaction temperature increases will increase the rate of catalysis up to a specific point at which the enzymes will be broken down into their component amino acids and therefore lose their ability to react with their substrate.

During a bio-chemical reaction temperature increases will increase the rate of catalysis up to a specific point at which the enzymes will be broken down into their component amino acids and therefore lose their ability to react with their substrate. The diagram below shows the relationship between rate of reaction and temperature.



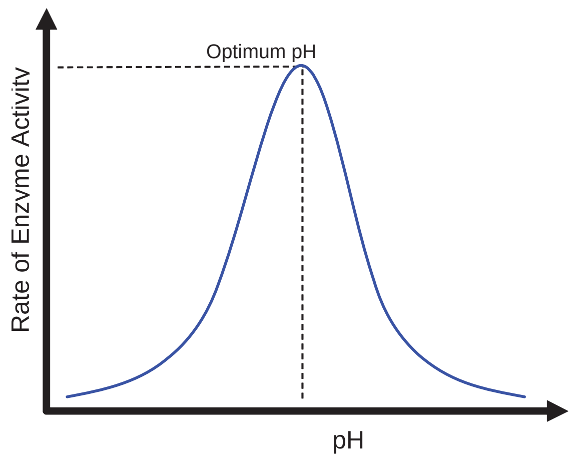
At very low temperatures enzymes are inactive.

The activity of enzymes increases when the temperature increases (and therefore the reactions that they work on, or catalyze, will also become faster). Each enzyme has a special temperature at which it is most active. This is the optimum temperature for that enzyme. Each enzyme has its own optimum temperature. When the temperature is too high, the enzymes are destroyed, or denatured.

For most natural enzymes a rapid destruction occurs as temperatures approach 50°C. BiOWiSH™ has been formulated to tolerate temperatures up to 65°C.

Similarly, the level of acidity in the environment will have a great effect on the ability of microbes and enzymes to operate. This relationship is shown below.

Effect of pH on an Enzyme



The pH that makes the enzyme most active is known as the optimum pH. If the pH is very high (base) or very low (acidic), enzymes can be denatured.

Most enzyme preparations begin to lose effectiveness at pH levels below 4.5 and above 7.5. BiOWiSH™ has been formulated to operate from pH 1.5 to 9.0 providing a much wider range of operative conditions.

The presence of antibiotics and many chemicals can prevent enzyme preparations from working. BiOWiSH™ has been formulated to operate in the presence of many chemicals and can actually remove many chemical contaminants from the environment.

Other factors that affect the speed of enzyme reactions are:

1. the presence of adequate coenzymes
2. the presence of adequate cofactors
3. the concentration of substrate available
4. the concentration or number of enzymes; and
5. the base rate of the particular enzyme on the particular substrate.

Advantages of Enzymes

The use of enzymes frequently results in solutions to problems where no solution currently exists because of their unique ability to break compounds down into their component parts.

In many cases enzymes provide benefits and results that cannot be obtained with traditional chemical treatment and do so with far less negative impact on people and the environment.

Enzymes help with bio-remediation of the cleaning up of all types of waste. Appropriately used they can also assist with reducing energy consumption and even increasing renewable energy sources.

Traditional chemical treatments are generally nonspecific, not always easily controlled and often create harsh conditions. They often produce undesirable side effects and /or waste disposal problems.

The degree to which a desired technical effect is achieved by an enzyme can be controlled through various means, such as dose, temperature and time. Because enzymes are catalysts the amount added to accomplish a reaction is relatively small.

Enzymes are 100% biodegradable and due to their specificity they are unlikely to have any negative impact on any other aspect of the environment.

History of Enzymes

For thousands of years man has been unknowingly harnessing the power of enzymes and using enzymes as our microbial workers. For example in the brewing of alcohol, the rising of yeast to make bread and the production cheese.

The history of enzyme use in cheese making is a good example. Legend has it that the discovery of the conversion of milk to cheese occurred when an Arabian carried milk in a vessel made from a sheep's stomach on the side of a camel. The combination of the enzymes from the sheep's stomach as a catalyst, the motion of the camel and the warm sun on the pouch transformed the milk into cheese. This gave rise to the first enzyme that could be isolated and deliberately harnessed.

The history of modern enzyme technology really began in 1874 when the Danish chemist Christian Hansen produced the first specimen of rennet by extracting dried calves' stomachs with saline solution. Apparently this was the first enzyme preparation of relatively high purity used for industrial purposes.

Even though the action of enzymes has been recognised and enzymes have been used throughout history, it is only recently that their importance has been realised. Enzymatic processes, particularly fermentation, were the focus of numerous studies in the 19th century and many valuable discoveries in this field were made. A particularly important experiment was the isolation of the enzyme complex from malt by Payen and Persoz in 1833. This extract, like malt itself, converts gelatinised starch into sugars, primarily into maltose, and was termed 'diastase'.

Development progressed during the following decades, particularly in the field of fermentation where the achievements by Schwann, Liebig, Pasteur and Kuhne were of the greatest importance. A famous scientific dispute between Liebig and Pasteur concerning the fermentation process caused much heated debate.

Liebig claimed that fermentation resulted from chemical process and that yeast was a nonviable substance continuously in the process of breaking down. Pasteur, on the other hand, argued that fermentation did not occur unless viable organisms were present. The dispute was finally settled in 1897 after the death of both adversaries when the Buchner brothers demonstrated that cell free yeast extract could convert glucose into ethanol and carbon dioxide just like viable yeast cells. In other words, the conversion was not ascribable to yeast cells as such but to their enzymes.

In 1876, William Kuhne proposed that the name ‘enzyme’ be used as the new term to denote phenomena previously known as ‘unorganised ferments’, that is, ferments isolated from the viable organisms in which they were formed. The word itself means ‘in yeast’ and is derived from the Greek ‘en’ meaning ‘in’, and ‘zyme’ meaning ‘yeast’ or ‘leaven’.

Research has rapidly accelerated throughout the 20th century from the year 1926 in which Sumner isolated and crystallised urease proving it was a form of protein to today’s every growing research industry.

Today enzymes are in common place usage in industry, food preparation, environmental management and medicine. Enzymes are still being discovered in nature and their individual properties are still largely a mystery. It is not exaggerating to say that there is still more to learn about enzymes and that enzymes hold the promise to many exciting scientific and medical breakthroughs.

Enzymes have been firmly established as a crucial tool to be harnessed for the betterment of mankind.

Frequently Asked Questions

What is modern Biotechnology?

New developments in biotechnology (modern biotechnology) allow scientists to identify and safely transfer specific genes coding for desired traits from one organism to another.

Why do we need this technology?

The world’s population continues to grow and as it does, it becomes more concentrated in urban areas and increasingly dependent upon modern conveniences. All this contributes to increased demand for food, energy and all other limited natural resources. At the same time this growth puts significant stress on the environment. Modern biotechnology is one tool that can help meet the challenge this growth poses.

Thus far modern biotechnology has contributed to 1) increased food production efficiency 2) reduced pesticide use 3) increased energy efficiency, reduced raw material consumption and water demand in manufacturing processes 4) reduced industrial waste and 5) aided in pollution remediation. Enzymes produced using modern biotechnology contribute to this effort by assuring the availability of safe, pure enzymes that replace harsh chemical processes (reducing energy consumption and environmental burden).

Are enzymes used in medicine?

Many people are familiar with enzymes as digestive aids but enzymes are commonly used throughout modern medicine to diagnose and treat a wide variety of conditions. For example a particular blood marker released during a heart attack is an enzyme. One of the definitive tests to determine if a patient has experienced a heart attack is to test for this enzyme.

A second use of enzymes is in diagnostic tests or assays that use an enzyme that will react with specific compounds, viruses or pathogens. A third use of enzymes is through enzyme inhibition where a drug is developed that will prevent an otherwise naturally occurring reaction. An example of this is enzyme inhibitors which have revolutionized the treatment of HIV.

The final common example of enzyme usage in medicine is though what is known as systemic enzyme therapy. In this case enzymes are used as supplements to the normal diet or even provided intravenously.

Systemic enzyme therapy is used for specific conditions such as cystic fibrosis, cancer, multiple sclerosis, arthritis and other inflammatory or autoimmune diseases. There is a growing body of research and evidence on the benefits of enzyme supplementation to prevent disease.

More Information

Please visit the BiOWiSH Technologies website **www.biowishtechnologies.com** for more information on how this amazing technology works and to see the wide range of products we have created using the BiOWiSH™ technology.

We are constantly developing our range of products and applications using the remarkable properties of our core technology which is both groundbreaking and versatile.

We are also partnering with other product manufacturers to assist them in including BiOWiSH™ in their products.

By making the choice to use one of the core BiOWiSH™ range of products you will soon discover why BiOWiSH™ is one of the most exciting and powerful biotechnologies to emerge this century.

References

1. Pyle GG, et al. "Effect of pretreatment of food gluten with prolyl endopeptidase on gluten-induced malabsorption in celiac sprue." Clin Gastroenterol Hepatol . 2005 Jul;3(7):629-30.
2. Sollid LM, Khosla C. "Future therapeutic options for celiac disease." Nat Clin Pract Gastroenterol Hepatol . 2005 Mar;2(3):140-7.
3. Chang CT, et al. "Potent fibrinolytic enzymes from a mutant of Bacillus subtilis IMR-NK1". J Agric Food Chem . 2000 Aug;48(8):3210-6.
4. Moriya N, et al. "Intestinal absorption of serrapeptase (TSP) in rats". Biotechnol Appl Biochem . 1994 Aug;20(Pt 1): 101-8.
5. Esch PM, et al. "Reduction of post-operative swelling. Objective measurement of swelling of the upper ankle joint in treatment with serrapeptase-a prospective study". Fortschr Med . 1989 Feb 10;107(4):67-8, 71-2.
6. Mazzone A, et al. "Evaluation of Serratia peptidase in acute or chronic inflammation of otorhinololaryngology pathology: a multicentre, double-blind, randomized trial versus placebo". J Int Med Res . 1990 Sep-Oct;18(5):379-88.
7. Selan L, et al. "Proteolytic enzymes: a new treatment strategy for prosthetic infections?" Antimicrob Agents Chemother . 1993 Dec;37(12):2619-21.
8. Suzuki Y, et al. "Dietary supplement of fermented soybean, natto, suppresses intimal thickening and modulates the lysis of mural thrombi after endothelial injury in rat femoral artery". Life Sci . 2003 Jul 25;73(10):1289-98.
9. Modi K, et al. "Effect of coenzyme Q10 on catalase activity and other antioxidant parameters in streptozotocin-induced diabetic rats". Biol Trace Elem Res . 2006 Jan;109(1):25-34.
10. Nakamura S, et al. "Effect of the proteolytic enzyme serrapeptase in patients with chronic airway disease". Respirology . 2003 Sep;8(3):316-20.
11. Yan J, et al. "Reduced coenzyme Q10 supplementation decelerates senescence in SAMP1 mice". Exp Gerontol . 2005 Dec 29.

Biological help for the human race

www.biowishtechnologies.com

GEFSGE9MY03