

# ENZYMES

## A PRIMER ON USE AND BENEFITS TODAY AND TOMORROW

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## **INTRODUCTION**

Enzymes have played an important role in many aspects of life since the dawn of time. In fact they are vitally important 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 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 producing the food we eat, the clothes we wear, even in producing fuel for our automobiles. Enzymes are also important in reducing both energy consumption and environmental pollution.

The Enzyme Technical Association believes the importance of enzymes in everyday life is one of today's best-kept secrets. To help change this, we have assembled three documents that explain, in lay terms, what enzymes do, where they are used in products we encounter every day, and how modern biotechnology is opening doors that will further expand the use of enzymes into exciting new areas.

To find out more read on.

Editor

# **THE ENZYME ADVANTAGE**

## **WHAT ARE ENZYMES AND WHAT DO THEY DO?**

Enzymes are proteins with highly specialized catalytic functions, produced by all living organisms. Enzymes are responsible for many essential biochemical reactions in microorganisms, plants, animals, and human beings.

Enzymes are essential for all metabolic processes, but are not alive. Although like all other proteins, enzymes are composed of amino acids, they differ in function in that they have the unique ability to facilitate biochemical reactions without undergoing change themselves. This catalytic capability is what makes enzymes unique.

Enzymes are natural protein molecules that act as highly efficient catalysts in biochemical reactions, that is, they help a chemical reaction take place quickly and efficiently. Enzymes not only work efficiently and rapidly, they are also biodegradable. Enzymes are highly efficient in increasing the reaction rate of biochemical processes that otherwise proceed very slowly, or in some cases, not at all.

## **WHAT TYPES OF ENZYMES ARE THERE?**

Enzymes are categorized according to the compounds they act upon. Some of the most common include; *proteases* which break down proteins, *cellulases* which break down cellulose, *lipases* which split fats (lipids) into glycerol and fatty acids, and *amylases* which break down starch into simple sugars.

## **HOW ARE ENZYMES USED?**

Enzymes play a diversified role in many aspects of everyday life including aiding in digestion, the production of food and several industrial applications. Enzymes are nature's catalysts. Humankind has used them for thousands of years to carry out important chemical reactions for making products such as cheese, beer, and wine. Bread and yogurt also owe their flavor and texture to a range of enzyme producing organisms that were domesticated many years ago.

### The Human Body

The human body uses thousands of enzymes to carry out a myriad of biochemical processes. One clear example of an enzyme-assisted process is digestion. Enzymes help break down carbohydrates, fats and proteins into

simple compounds that the body can absorb and burn for energy or use to build or repair tissue. These include:

- > Amylase and lipase in saliva break down carbohydrates and fats;
- > Proteases (pepsin) released in the stomach aid in digestion of proteins; and
- > Lipases, amylases, and proteases are secreted in the small intestine and play a pivotal role in completing the digestive process.

### Food Production and Industrial Applications

Since ancient times, enzymes have played an important part in food production. One of the earliest examples of an industrial enzyme use was in the production of whiskey. Today, nearly all commercially prepared foods contain at least one ingredient that has been made with enzymes. Some of the typical applications include enzyme use in the production of sweeteners, chocolate syrups, bakery products, alcoholic beverages, precooked cereals, infant foods, fish meal, cheese and dairy products, egg products, fruit juice, soft drinks, vegetable oil and puree, candy, spice and flavor extracts, and liquid coffee, as well as for dough conditioning, chill proofing of beer, flavor development, and meat tenderizing.

Enzymes also play a significant role in non-food applications. Industrial enzymes are used in laundry and dishwashing detergents, stonewashing jeans, pulp and paper manufacture, leather dehairing and tanning, desizing of textiles, deinking of paper, and degreasing of hides. A brief discussion of some everyday applications is provided in ENZYME APPLICATIONS.

### **HOW ARE ENZYME PREPARATIONS MADE?**

Commercial sources of enzymes are obtained from three primary sources, i.e., animal tissue, plants and microbes. 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 optimizing the conditions for growth, commercial quantities can be obtained. This technique, well known for more than 3,000 years, is called fermentation. Today, this fermentation process is carried out in a contained vessel. Once fermentation is completed, the microorganisms are destroyed; the enzymes are isolated, and further processed for commercial use.

Enzyme manufacturers produce enzymes in accordance with all applicable governmental regulations, including the appropriate federal agencies (e.g., Food

and Drug Administration, United States Department of Agriculture, Environmental Protection Agency, etc.). Regardless of the source, enzymes intended for food use are produced in strict adherence to FDA's current Good Manufacturing Practices (cGMP) and meet compositional and purity requirements as defined in the Food Chemicals Codex (a compendium of food ingredient specifications developed in cooperation with the FDA).

## **THE ADVANTAGES OF ENZYMES**

The use of enzymes frequently results in many benefits that cannot be obtained with traditional chemical treatment. These often include higher product quality and lower manufacturing cost, and less waste and reduced energy consumption. More traditional chemical treatments are generally nonspecific, not always easily controlled, and may create harsh conditions. Often they 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. For example, an enzyme preparation in most food uses is equal to 0.1% (or less) of the product. Enzymes used in food processing are generally destroyed during subsequent processing steps and not present in the final food product.

## **INDUSTRIAL ENZYMES AND THE ENVIRONMENT**

Enzymes can often replace chemicals or processes that present safety or environmental issues. For example, enzymes can:

- Replace acids in the starch processing industry and alkalis or oxidizing agents in fabric desizing;
- Reduce the use of sulfide in tanneries;
- Replace pumice stones for "stonewashing" jeans;
- Allow for more complete digestion of animal feed leading to less animal waste; and
- Remove stains from fabrics. Clothes can be washed at lower temperatures, thus saving energy. Enzymes can be used instead of chlorine bleach for removing stains on cloth. The use of enzymes also allows the level of surfactants to be reduced and permits the cleaning of clothes in the absence of phosphates.

Enzymes also contribute to safer working conditions through elimination of chemical treatments during production processes. For example, in starch, paper and textile processing, less hazardous chemicals are required when enzymes are used.

As our understanding of the function of enzymes has grown, our ability to selectively apply these natural substances to productive uses has continued to grow.

Practical examples of how enzymes impact our everyday life are given in ENZYME APPLICATIONS.

# **ENZYME APPLICATIONS**



# FOOD/FOOD INGREDIENT - APPLICATIONS

## SUGAR SYRUPS FROM STARCH

During the 19<sup>th</sup> century, boiling starch with strong acids like sulfuric acid produced sugar syrups. This harsh process became a predominant method to make a range of starch syrups. However, by the middle of the 20<sup>th</sup> century, enzymes were rapidly supplanting the use of strong acids in the manufacture of sugar syrups.

The use of enzymes provides many advantages, including higher quality products, energy efficiency, and a safer working environment. Processing equipment also lasts longer since the milder conditions reduce corrosion.

In the 1970's, another syrup was developed that closely mimicked the sweetness of sucrose (table sugar). This became known as High Fructose Corn Syrup (HFCS). Although this syrup can be made chemically with sodium hydroxide, the extremely high alkalinity limits the yield since large amounts of byproducts are formed. Because of these limitations, the use of enzymes with greater specificity and mild use-conditions emerged as the production method of choice. Today the production of HFCS is a major industry, which converts large quantities of corn (maize) and other botanical starches to this and other useful sweeteners. These sweeteners are used in soft drinks, candies, baking, jams and jellies and many other foods.

**Environmental Benefits:** Reduced use of strong acids and bases, reduced energy consumption (less greenhouse gas), less corrosive waste, and safer production environment for workers.

**Consumer Benefits:** Sweetener availability and stable prices due to the ability to source from starch as an alternative to sugar cane and sugar beets; consistent, higher quality syrups.

## DAIRY APPLICATIONS

### Cheeses

Rennet, an enzyme mixture from the stomach of calves and other ruminant mammals, is a critical element in cheese making. Rennet has been the principle ingredient facilitating the separation of the curd (cheese) from the whey for thousands of years. Much has been learned about the functional attributes of rennet and other cheese making enzymes since they were first employed. A

purified form of the major enzyme in rennet, chymosin, is produced microbially from genetically modified microorganisms made to contain the gene for calf chymosin and is commercially available today without the need for sacrificing young animals. This chymosin is the same as that isolated directly from calves.

**Environmental Benefit:** Cheese makers are no longer dependent upon enzymes recovered from slaughtered calves, kids and lambs for production of rennet needed for most cheese making. Based on current demand for chymosin, commercial needs for rennet could not be met from animal sources.

**Consumer Benefit:** Plentiful, consistently high quality enzyme (chymosin) is available at an attractive price. This helps assure availability of excellent cheeses at a reasonable cost. Since the enzyme is from a microbe and not a calf, people who follow kosher and vegetarian eating practices can consume cheese.

### **Cheese flavors**

The varied selections of cheeses enjoyed today are due in part to the action of enzymes called lipases. The lipases contribute to the distinctive flavor development during the ripening stage of production.

Lipases are a class of enzymes that act on the butterfat in cheese to produce flavors that are characteristic of different types of cheese. Specific lipases are responsible for the flavors we enjoy in cheeses ranging from the piquant flavor typical of Romano and provolone cheeses to the distinct flavors of blue and Roquefort cheeses.

**Customer Benefit:** Wide variety of flavorful, high quality cheeses.

### **Lactose-free dairy products**

A significant portion of the adult population is unable to consume normal portions of dairy products as they cause gastrointestinal (GI) upset in the form of bloating, gas, or diarrhea, or a combination of GI symptoms.

Lactase, an enzyme that occurs naturally in the intestinal tract of children and many adults, is either absent or not present in sufficient quantity in lactose intolerant adults. Lactase converts the milk sugar found in dairy products, such as milk, ice cream, yogurt, and cheese, to two readily digestible sugars, glucose and galactose. Without adequate lactase, the lactose in the food ferments in the intestine, producing undesirable side effects.

People who historically could not consume dairy products can now enjoy these nutritious foods thanks to the commercial availability of the digestive enzyme, lactase. Many products present in the dairy case today are labeled "lactose-free" as the result of pretreatment of the milk or final product with the enzyme lactase. Additionally, lactase is available at retail for use in treating lactose containing dairy products in the home.

**Consumer Benefit:** Approximately 20-30 percent of US adults are lactose intolerant. These individuals can now enjoy the nutritional benefits and sensory pleasure of dairy products without gastrointestinal side effects by selecting lactose-free or low lactose dairy products or by adding commercially available lactase to dairy products in the home.

## **BAKING APPLICATIONS**

### **Bromate Replacers**

Modern bread production is often reliant upon oxidative compounds that can help in forming the right consistency of the dough. Chemical oxidants such as bromates, azodicarbonamide and ascorbic acid have been widely used to strengthen gluten when making bread. Potassium bromate has also been used for this purpose for many years, as it was the first inorganic compound to be used for improving flour quality. Over the years, bromate has been used to bake bread of a consistently high quality with a high consumer acceptance. Recent studies however, have questioned the use of bromate in bread and its use has been abandoned in many countries around the world.

Enzymes such as glucose oxidase have been used to replace the unique effect of bromate. This way, enzymes can help the baker produce bread that lives up to the quality standards consumers demand.

**Consumer Benefit:** Removal of bromate from the food supply without sacrificing the quality of the bread.

### **Softer Bread Products**

Consumers enjoy soft bread. To ensure high-quality bread, enzymes are often used to modify the starch that in turn keeps the bread softer for a longer period of time.

The staling of white bread is considered to be related to a change in the starch. Over time, the moisture in the starch becomes unbound when starch granules revert from a soluble to an insoluble form. When the starch can no

longer “hold” water, it loses its flexibility and the bread becomes hard and brittle. This results in a subsequent reduction in taste appeal of the bread and it is termed “stale.” By choosing the right enzyme, the starch can be modified during baking to retard staling. The bread stays soft and flavorful for a longer time: 3-6 days.

**Environmental Benefits:** Less waste, better use of raw materials.

**Consumer Benefits:** Better quality bread, less waste due to improved stability.

## **BEVERAGE APPLICATIONS**

### **Low Calorie Beer**

Calorie-conscious consumers can enjoy reduced calorie beer thanks to the use of special enzymes in the brewing process. Major ingredients used in the production of beer include, barley, rice, and other grains. The grains are essential components in the conversion of carbohydrates to alcohol during yeast fermentation. First, simple carbohydrates are converted to alcohol followed by conversion of carbohydrates of increasing complexity, until the desired alcohol-content is achieved. The remaining carbohydrate remains as a component of the finished product. By using enzymes to transform the complex carbohydrates to simpler sugars, the desired alcohol content can be achieved with a smaller amount of added grain. This results in a beer with fewer carbohydrate calories and ultimately, a lower calorie beer.

**Environmental Benefits:** Lower agricultural demand for grains used in brewing.

**Consumer Benefits:** Good tasting, lower calorie beers.

### **Clear fruit juice**

Juices extracted from ripe fruit contain a significant amount of pectin. Pectin imparts a cloudy appearance to the juice and results in an appearance and mouth-feel that many consumers do not find appealing. Pectinases are naturally occurring enzymes that act on pectin yielding a crystal clear juice with the appearance, stability, mouth-feel, taste, and texture characteristics preferred by consumers. While pectinases naturally occur in most fruits used to make juice, the manufacturer often adds more to produce clear juice in a reasonable amount of time.

**Environmental Benefit:** The use of enzymes in juice processing helps assure that the maximum amount of juice is removed from the fruit, thereby reducing waste and controlling costs.

**Consumer Benefit:** Aesthetically pleasing, clear, sediment-free juices from many varieties of fruit are widely available at retail.

## OTHER FOOD APPLICATIONS

### Meat Tenderizing

Some cuts of meat are more tender than others. Meat is mostly protein, indeed a rather complex set of proteins with defined structure(s). The major meat proteins responsible for tenderness are the myofibrillar proteins and the connective tissue proteins. Protease enzymes are used to modify these proteins. In fact, proteases like papain and bromelain have been used to tenderize tougher cuts of meat for many years. This can be a difficult process to control since there is fine line between tender and mushy. To improve this process, more specific proteases have also been introduced to make the tenderizing process more robust.

**Environmental benefits:** Less waste, better use of raw materials.

**Consumer Benefits:** Ability to tenderize tougher cuts of meat thereby making less expensive cuts more attractive menu items.

### Confections

Soft candy and other treats made with sugar, especially soft center candy such as chocolate covered cherries, often have short shelf life because the sugar sucrose contained in the product begins to crystallize soon after the confection is produced. A similar change occurs in soft cookies and other specialty bakery items. An enzyme, invertase, converts the sucrose to two simple sugars, glucose and fructose and thus prevents the formation of sugar crystals that otherwise would severely shorten the shelf life of the product or make some products virtually unavailable at reasonable prices.

**Environmental Benefit:** Enzymes replace hydrochloric acid in the manufacturing process, thereby eliminating the need for harsh chemical processing and thereby reducing risk to the environment. Elimination of a strong acid also provides a safer workplace.

**Consumer Benefit:** Confections and specialty baked goods with excellent mouth-feel and taste characteristics are readily available at reasonable cost thanks to the use of the enzyme, invertase. Soft centers of fine chocolates remain smooth and creamy; Tootsie Rolls stay chewy and soft cookies available on the grocer's shelves rival homemade versions because of this special food enzyme.

## **HOUSEHOLD & PERSONAL CARE APPLICATIONS**

### **Lower Temperature & No Phosphate Clothes Washing**

The global trend has been to reduce wash temperatures and ban phosphates. To compensate for the reduced cleaning ability, detergent manufacturers have turned to enzymes for help and have introduced several classes of enzymes into their products.

A lower wash temperature significantly reduces the energy needed to do a load of laundry. For example, in northern Europe wash temperatures have been reduced from about 90°C (195°F) to 40 - 60 °C (100 to 140 °F). The energy input is dramatically reduced and thanks to enzymes, the same wash performance is maintained.

Also, the reduction in phosphate load to rivers and lakes is believed to lower the human-induced decline of these systems.

**Environmental Benefits:** Reduced phosphate load to rivers and lakes.  
Reduced energy consumption with lower temperature washing.

**Consumer Benefits:** Lower energy bills, less color fading, and longer fabric life due to gentler wash conditions. Wider choice of fabric types due to the lower temperature washing.

### **Milder Dishwashing Detergents**

Automatic dishwashing detergents are formulated to be very alkaline in some countries. This alkalinity is needed to assure the full cleansing of the dishware. Enzymes have replaced harsh chemicals while maintaining the cleaning.

**Environmental Benefits:** Reduced chemical load.

**Consumer Benefits:** More user-friendly and/or safer products due to the reduced alkalinity of the product

### **Contact Lens Cleaner**

When you wear contacts, various naturally occurring proteinaceous and lipid materials from the eye gradually accumulate on the contact lens. Indeed, the eye ducts secrete an enzyme called lysozyme to help keep the eye surface clean. When a contact lens, a foreign object, is introduced onto the eye surface

it interferes with the normal cleansing process. Incorporating protease and lipase enzymes in the lens cleaning system can dramatically enhance removal of this soil that accumulates on the contact lens.

**Environmental Benefits:** Cleansing of contact lenses with use of biodegradable enzymes.

**Consumer Benefits:** Better contact lens washing.



## FOOD AND FEED – DIGESTIVE AIDS

### **Alpha-Galactosidase for Improved Nutritional Value of Legume- and Soy-based Foods**

Enzymes can be used to improve the nutritional quality of food for humans and animals. The full utilization of the potential nutritive value in legume- and soy-based foods is limited by the presence of non-digestible sugars such as raffinose and stachyose. These sugars contain chemical linkages that cannot be broken by the natural enzymes produced by the body. Consequently, the sugars proceed through the digestive tract until reaching the large intestine where they are hydrolyzed by the natural microflora in the intestine. These organisms utilize the sugars that are converted to gas during this metabolism causing discomfort and flatulence.

The enzyme, alpha-galactosidase, is used to convert stachyose and raffinose to simple sugars that are adsorbed by the human digestive tract, thereby preventing the flatulence often caused by legumes such as beans and soy-based foods. This enzyme can be used to hydrolyze raffinose and stachyose during soy processing, during the food preparation process or by addition to the food itself immediately before ingestion.

**Consumer Benefits:** Avoid physical discomfort when consuming soy products, beans, and other legumes that contain poorly digested sugars. Greater acceptance of soy-based foods will allow their full nutritive value to be realized.

### **Reduced Phosphorous Animal Feed**

Poultry and hog feed grains contain phosphorous which is bound to phytic acid. In this form the phosphorous is not available to the animals and is excreted in the animal's waste. Since these animals, like humans, need phosphorous for bone growth and other biochemical processes, the feed suppliers normally add extra phosphorous to the diet. A specific enzyme, phytase releases the bound phosphorous, making it digestible to the chicken or hog. Phytase added to the feed eliminates the need for compensating levels of phosphorous and thus dramatically reduces the phosphorous content of the animal waste.

**Environmental Benefits:** Lower phosphorous animal waste reduces environmental impact on rivers and lakes.

**Consumer Benefits:** Reduced environmental and feed costs helps control consumer prices.

## INDUSTRIAL APPLICATIONS - ENERGY

### Ethanol Fuel from Renewable Resources

Prior to the discovery of petroleum, natural carbohydrates were used for the production of food, clothing and energy. Ethanol fuels can be derived from renewable resources - dedicated agricultural crops such as corn, sugar cane, and sugar beet or from agricultural byproducts such as whey from cheese making and potato processing waste streams. Ethanol can be used as a 100% replacement for petroleum fuels or as an extender. Ethanol can also be utilized in petroleum fuels as a replacement for the toxic oxygenate, Methyl t-Butyl Ether (MTBE).

Enzymes such as alpha-amylase, glucoamylase, invertase and lactase hydrolyze starch, sucrose and lactose into fermentable sugars. The sugars are then fermented with yeast to produce ethanol. The production of grain, oilseed and textile fibers results in a substantial quantity of underutilized agricultural crop residues. Although it is desirable to return some of this cellulosic residue back to the soil, much of this material could be diverted to ethanol. The current best available technology for conversion employs an acid hydrolysis of the biomass into sugars. The enzymatic alternative, using cellulase and hemicellulase, avoids the use of strong acids and results in a cleaner stream of sugars for fermentation and fewer by-products.

**Environmental benefit:** Greater utilization of natural, renewable resources, safer factory working conditions, reduced harmful auto emissions.

**Consumer Benefit:** Safer alternative to MTBE , augments existing supply of liquid fuel, i.e., gasoline.

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EPA is gathering input for rulemaking under the Toxic Substances Control Act to phase out MTBE.

## **INDUSTRIAL APPLICATIONS – TEXTILES**

### **Textiles Processing**

Textile processing has benefited greatly on both environmental and product quality aspects through the use of enzymes. Prior to weaving of yarn into fabric, the warp yarns are coated with a sizing agent to lubricate and protect the yarn from abrasion during weaving. Historically, the main sizing agent used for cotton fabrics has been starch because of its excellent film forming capacity, availability, and relatively low cost. Before the fabric can be dyed, the applied sizing agent and the natural non-cellulosic materials present in the cotton must be removed. Before the discovery of amylase enzymes, the only alternative to remove the starch-based sizing was extended treatment with caustic soda at high temperature. The chemical treatment was not totally effective in removing the starch (which leads to imperfections in dyeing) and also results in a degradation of the cotton fiber resulting in destruction of the natural, soft feel, or hand, of the cotton.

The use of amylases to remove starch-based sizing agents has decreased the use of harsh chemicals in the textile industry, resulting in a lower discharge of waste chemicals to the environment, improved the safety of working conditions for textile workers and has raised the quality of the fabric. New enzymatic processes are being developed (cellulase, hemicellulase, pectinase and lipase), which offer the potential to totally replace the use of other chemicals in textile preparation processes.

**Environmental Benefits:** Lower discharge of chemicals and wastewater and decreased handling of hazardous chemicals for textile workers.

**Consumer Benefits:** Improved fabric quality.

## **Stonewashed Jeans Without Stones**

Traditionally, to get the look and feel of stonewashed jeans, pumice stones were used. However, thanks to the introduction of cellulase enzymes, the jeans industry can reduce and even eliminate the use of stones. Of course, a big driver for the jeans industry is fashion. Enzymes give the manufacturer a newer, easier set of tools to create new looks.

Although many consumers do not want their jeans to look or feel new, they usually do not want them to look worn-out or torn. The pumice stones used to "stonewash" the denim clothes can also over abrade or damage the garment. By using enzymes, the manufacturer can give consumers the look they want, without damaging the garment.

**Environmental Benefits:** Less mining, reduced waste, less energy, less clogging of municipal pipes with stones and stone dust, fewer worn out machines and pipes attributed to stones and stone dust.

**Consumer Benefits:** More fashion choices, longer garment life/wear due to lower damage of original fabric.

## **Yarn Treatment**

In the preparation of cotton yarn for dyeing and garment manufacture, hydrogen peroxide is used to bleach the yarn. Normally, either a reducing agent is used to neutralize the hydrogen peroxide or water is used to rinse out the hydrogen peroxide bleach since it must be removed for proper dyeing. An enzyme, catalase, can be used to breakdown the hydrogen peroxide to water and oxygen. With the use of catalase, the reducing agent can be eliminated or the amount of rinse water can be dramatically reduced, resulting in less polluted wastewater or lower water consumption.

The benefits have been documented in a Life Cycle Analysis. Again, enzymes can help us develop sustainable processes by lowering the environmental impact we humans impose.

**Environmental Benefits:** Reduced chemical load, reduced water consumption, lower energy consumption.

## **INDUSTRIAL APPLICATIONS – LEATHER PROCESSING**

### **Leather Tanning with Enzymes: Dehairing, Bating**

Hides and skins have hair attached to them that must be removed for their use as leather. The conventional way to remove hair from hides is to use harsh chemicals such as lime and sodium sulfide. These chemicals completely dissolve the hair and open up the fiber structure.

With enzyme-assisted dehairing, it is possible to reduce the chemical requirements and obtain a cleaner product and a higher area yield with fewer chemicals in the wastewater. Since the enzyme does not dissolve the hair as the chemicals do, it is possible to filter out the hair, thus reducing the chemical and biological oxygen demand of the wastewater.

Additionally the hides and skins contain proteins and fat between the collagen fibers that must be all or partially removed before the hides can be tanned. To make the leather pliable, it is necessary to subject the hide to an enzymatic treatment before tanning to selectively dissolve certain protein components. This is called bating.

Traditionally, dog or pigeon dung was used as the bating agent. This was a difficult, unreliable and smelly process. Obviously, this was a very unpleasant environment to work in. Since "dung bates" owed their softening effect to the action of a protease enzyme, during the 20<sup>th</sup> century, the Leather Industry has switched over to using bacterial proteases and pancreatic trypsin.

**Environmental Benefits:** Lower chemical load to waste system. Lower odor during processing,

**Consumer Benefits:** Better leather, lower odor process for workers and the factory neighbors.

### **Degreasing of Leather**

Traditionally, the degreasing of sheepskins is done by solvent-extraction using paraffin solvent systems. A new process based on the enzymatic breakdown of fats by a lipase enzyme has been introduced to the leather industry. The enzymatic degreasing process replaces the solvent-based process. Since the enzyme interferes less with the skin structure, the enzymatic process also results in a product with improved quality, for example improved tear strength and more uniform color.

**Environmental Benefits:** Replaces solvent-based system, lowers volatile organic chemical load.

**Consumer Benefits:** Higher quality leather – the improved tear strength should be very meaningful to anyone with leather furniture!

## **INDUSTRIAL APPLICATIONS - PAPER**

### **Helping Papermakers Reduce Their Load on the Environment**

It takes a lot of chemical processing to turn trees into white paper. The pulp and paper industry employs chlorine oxidants to bleach pulp. As a result, chlorine-containing organics, a class of compounds with toxicity concerns, are produced as by-products. The classic problem with chlorine bleaching is that in whitening the paper, papermakers are also left with a waste stream containing a range of chlorinated organic compounds, some of which scientists have demonstrated to be detrimental to our ecosystem.

Enzymes can help papermakers reduce the use of harsh chemicals such as chlorine bleach. Hemicellulase enzymes such as xylanase can enhance the bleaching efficacy allowing a dramatic reduction in the consumption of chlorine. The enzymatic treatment opens up the pulp matrix allowing better penetration of the bleaching chemicals and better extraction or washout of lignin and the associated dark brown compounds.

**Environmental Benefits:** Less chlorine bleach, therefore less chlorinated organics in the waste stream.

**Consumer Benefits:** White paper with lower environmental impact. Cleaner rivers and streams

### **Deinking of Waste Paper**

The basic technology to recycle waste paper is a relatively straightforward process. The cellulosic fibers can readily be separated by repulping and cleaning, and made into new paper. The majority of the fillers and binders used in the original paper can be easily extracted during reprocessing. The residual printing inks and adhesives are the most difficult of the components to remove. Historically, caustic surfactants and large quantities of wash water are used to separate the ink from the cellulosic fibers.

The quantity of chemicals and wash water can be dramatically reduced by the use of the enzymes cellulase and hemicellulase. These enzymes are able to hydrolyze some of the linkages that entrap the ink.

**Environmental Benefits:** Improved deinking creates more opportunity for recycled paper, less chemical discharge to waste streams, less wash water use, decreased load on landfills and a better utilization of natural resources.

## **CONCLUSION**

These examples are just a few of the many ways commercial enzymes touch our lives. They are tools of nature that help provide everyday products in an environmentally conscious manner. Current commercial use of enzymes, together with new applications, will continue to play an important role in maintaining and enhancing the quality of life we enjoy today while protecting the environment for generations to come.



# OPPORTUNITIES THROUGH MODERN BIOTECHNOLOGY

## **MICROORGANISMS – PROTEIN FACTORIES**

During the twentieth century humankind has harnessed microorganisms to produce useful biochemicals including antibiotics, vitamins, amino acids, flavors and colors, as well as specific proteins. Some of these proteins have important medical uses such as insulin, human growth hormone, and blood factors like erythropoietin. Microorganisms have also been domesticated to produce another class of important proteins called enzymes.

Until recently, availability of enzymes, also known as nature's catalysts, have been limited to the quantities that could be produced in the host organism in which they were naturally derived. But today, modern biotechnology techniques developed since 1973, are allowing the development of safe and efficient microbial hosts in which large quantities of many commercially useful enzymes can be produced. In fact, manufacturers have developed a series of proven, safe, microbial hosts for use in the production of several enzymes. Further, enzymes that have not been readily available in adequate quantity can now be produced using this technology. This in turn has opened up important applications beneficial to humankind. Additionally, modern techniques are leading to the development of tailored enzymes with optimized functional properties specific for their intended use. An example of this is the modification in specific proteases so that they work more efficiently in the alkaline environment of detergent formulations. As a result, less of the modified protease is needed to deliver equivalent cleaning power, while using fewer resources during the manufacturing process.

### **WHAT IS MODERN BIOTECHNOLOGY?**

The microbial cell, i.e., a bacterium, yeast, or mold, is the key instrument in many enzyme production processes. To optimize the microbial strains for production of the desired enzyme, the strain's genetic properties are often modified either through natural evolution or through classical breeding and selection techniques; these classical techniques have been used for decades to improve microbial production strains.

Since 1973, more precise methods of genetic modification have been developed. The methods, sometimes termed genetic engineering, are based on processes occurring in nature - the transfer of genes between different cells. When used by scientists to transfer genetic material between cells from the same or different species, microorganisms such as yeasts, molds, and bacteria with new or improved properties for industrial applications can be developed.

In nature, genetic modification of has been occurring since life began. Such genetic changes are generally random, with a natural selection process favoring the change(s) best adapted for survival. Using this process, animal, plant, and microbial breeders have likewise selected individuals within a species with desired characteristics for further propagation. Since such changes are generally random and frequently affect more than one trait, isolation and breeding for a single trait is often not possible. Using the tools of modern biotechnology, modifications can now be made more precisely, and with much less chance of developing unwanted secondary changes that could potentially have undesired effects. In nature, and in our production systems, microbes do not express only single enzymes. Rather, each microbial cell has the genetic machinery to produce many different enzymes. Frequently, only one of these enzyme activities is needed for a specific application and the "side activities" are removed or substantially reduced during the recovery process. Often, these side activities are unwanted, and may even be detrimental to the final use. For example, in the past enzyme preparations used for bread making contained side activities that could negatively affect the dough if the enzyme preparation was not used precisely. By using the techniques of modern biotechnology, it is relatively easy to remove such unwanted side activities. Additionally, scientists are now able to discover and/or evolve enzymes that will catalyze chirally pure compounds for applications including pharmaceutical manufacturing, both greatly reducing unwanted byproduct production as well as making the target product potentially safer and more effective.

## **WHY IS MODERN BIOTECHNOLOGY IMPORTANT?**

By using the tools of modern biotechnology, the enzyme industry has developed safe host organism systems for the production of many enzymes that could not otherwise be produced. These safe host systems have been used since the early 1980's for the production of different enzymes in contained manufacturing facilities. The host organisms and their enzyme products have been tested to demonstrate that they are safe for their intended use; this includes, but isn't restricted to, testing of the organism to demonstrate that it is not pathogenic and does not produce toxins, and testing of the product to demonstrate that it is safe for the intended use, and to determine whether it is an irritant and how likely it is to cause allergies when inhaled. Guidelines for safety assessments of food and food ingredients developed through biotechnology have been prepared by several internationally recognized scientists and expert groups (Organization for Economic Cooperation and Development<sup>1</sup>, Health Canada<sup>2</sup>, Food and Agriculture Organization / World

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<sup>1</sup> Organisation for Economic Cooperation and Development, Safety Evaluation of Foods Derived by Modern Biotechnology, Paris, 1993.

Health Organization<sup>3</sup>, International Life Sciences Institute - Europe, Novel Food Task Force<sup>4</sup>), and specific recommendations for microbially derived food ingredients (IFBC, 1990<sup>5</sup>) and enzyme preparations (Pariza and Foster<sup>6</sup>, Pariza and Johnson<sup>7</sup>, EU SCF<sup>8</sup>, US FDA<sup>9</sup>) have also been published.

Using tools of modern biotechnology, the enzyme industry is continuing to dramatically improve a wide range of production processes with both primary and secondary benefits. Reduced consumption of energy, water, and raw material, while generating less waste and fewer environmental pollutants are among the target benefits of this technology. Additionally, many unavoidable waste materials can be effectively modified, either for recycling as a useful raw material, or as a valuable secondary product.

Since the 1980s, new enzymes have been developed through modern biotechnology for widespread use in many products. Two examples involve laundry detergents and paper production. The use of these new enzymes in detergents enables consumers to remove difficult stains at lower wash temperatures without the use of harsh chemical additives, thus reducing the burden on wastewater systems. Enzymes are simple proteins and thus are fully biodegradable and environmentally sound. The use of new, more robust enzymes in paper processing can significantly reduce the need for chlorine and chlorine dioxide bleach, which ultimately feeds into wastewater streams as chlorinated organic compounds. Without the use of the modern tools of biotechnology, not enough of these enzymes, as well as enzymes in other

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<sup>3</sup> Health Canada, Guidelines for the Safety Assessment of Novel Foods, Food Directorate Publication, Health Protection Branch, Health Canada, Ottawa, 1994.

<sup>4</sup> FAO/WHO. Biotechnology and Food Safety, Report of a Joint FAO/WHO Consultation. FAO Food and Nutrition Paper 61. Rome, Italy. 1996.

<sup>5</sup> Jonas, D.A., Antignac, E., Antoine, J.M., Classen, H.G., Huggett, A., Knudsen, I., Mahler, J., Ockhuizen, T., Smith, M., Teuber, M., Walker, R., and de Vogel, P. The Safety Assessment of Novel Foods, Guidelines prepared by ILSI Europe Novel Food Task Force. Food Chemical Toxicology 34:931-940, 1996.

<sup>6</sup> IFBC (International Food Biotechnology Council). 1990. Chapter 4: Safety Evaluation of Foods and Food Ingredients Derived from Microorganisms in Biotechnologies and Food: Assuring the Safety of Foods Produced by Genetic Modification. Regulatory Toxicology and Pharmacology 12:S1-S196.

<sup>7</sup> Pariza, M.W. and Foster, E.M.. Determining the Safety of Enzymes Used in Food Processing. Journal of Food Protection, 46:5:453-468, 1983.

<sup>8</sup> Pariza, M. W. and Johnson, E. A., Evaluating the Safety of Microbial Enzyme Preparations Used in Food Processing: Update for a New Century, in press Regulatory Toxicology and Pharmacology, 2001

<sup>9</sup> Scientific Committee for Food, Report (27<sup>th</sup> series), Ref. No EUR14181 EN- Guidelines for the presentation of data on food enzymes. p 13 - 22.

<sup>10</sup> US Food and Drug Administration. Enzyme preparations from animal and plant sources; Affirmation of GRAS status as direct food ingredients. Fed. Regist. 60:32904-32912, 1995.

Kessler, D.A., Taylor, M.R., Maryanski, J.H., Flamm, E.L., and Kahl, L.S. The Safety of Foods Developed by Biotechnology. Science 256:1747-1749, and 1832, 1992.

US Food and Drug Administration. Substances generally recognized as safe. Proposed Rule. Fed. Regist. 62:18938-18964, 1997.

industrial applications, can be produced to meet industry demand. Further, these modern tools give us the opportunity to produce enzymes that can replace other processes and/or chemicals that are less environmentally sound and thereby continue to move towards sustainability.

## **WHAT DOES THE FUTURE HOLD?**

There are many enzymes existing in nature with significant potential value to society. However, these enzymes have not been commercially developed because they exist in organisms that cannot easily be used for production or where other components of the organism are unacceptable for the intended use. Isolating and then transferring the genes that code for these enzymes into an organism that can easily be grown and has been demonstrated to be a safe host can eliminate the unacceptable characteristics. The result will be valuable new enzymes, demonstrated to be safe, that are commercially available at affordable prices.

For example, with the modern tools of biotechnology, enzymes from nature can be accessed which are sufficiently robust to be useful at extremes of pH and temperature, and thus hold great promise for replacing certain chemical processes with much cleaner protein-catalyzed processes. Just as exciting, these new enzymes can make the dream of converting waste biomass to useful energy an economic reality.

Overall, the use of modern biotechnology for enzyme production can have a major impact on improving the cost and quality of products we need and want while at the same time working towards sustainability. The vision is to make our currently landfilled waste into productive raw materials, to utilize microbes that can sustain production for long periods because they can reproduce themselves, and to reduce the need for fossil fuels and the waste/pollution they can create. Enzymes will continue to play a very important role in facilitating more environmentally sound production and making production systems more sustainable.

For more information about current enzyme use see ENZYME APPLICATIONS.

# **ENZYMES**

## **Questions & Answers**

## **DEFINITIONS**

### ***What are enzymes?***

Enzymes are complex proteins produced by all living cells. These proteins accelerate specific chemical reactions without undergoing any alteration themselves (i.e., they act as biological catalysts).

### ***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.

### ***What are GMO's?***

GMO is the acronym for "genetically modified organism". It is used to describe organisms (plant, animal or microbial) that have been altered through use of modern biotechnology.

## **WHY MODERN BIOTECHNOLOGY**

### ***Why are GM microorganisms used to produce enzymes?***

Modern biotechnology has proven to be a safe and valuable tool when applied to microorganisms, including microorganisms used to produce enzymes. Modern biotechnology is indispensable in improving enzyme products and assuring adequate supplies of high quality enzyme preparations at reasonable cost. Modern biotechnology has improved enzyme production and enzyme quality in several ways: 1) Increased efficiency of enzyme production resulting in higher yields; 2) Increased enzyme purity through reduction or elimination of side activities; 3) Enhancing the function of specific enzyme proteins, e.g., by increasing the temperature range over which an enzyme is active. The results are better products, produced more efficiently, often at lower cost and with less environmental impact.

### ***How did we deal with these challenges before the advent of modern biotechnology?***

For many generations, mankind has taken advantage of natural selection, crossbreeding practices and, more recently, stress induction to achieve desired characteristics in plant, animal, and microbial species. To a large extent this was

an uncontrolled process where several iterations could be employed to obtain progeny with the best possible overall ratio of desired and undesired characteristics.

Modern biotechnology is a continuation of this long-standing practice. However, it provides a distinct advantage that could not be achieved with traditional techniques. Targeted alteration of the genetic makeup of an organism (including microorganisms) helps assure the desired characteristic is transferred, while avoiding introduction of undesirable characteristics, which often compromised the outcome when traditional genetic techniques were employed.

### ***Why do we need this technology?***

The world's population continues to grow. As it does, it becomes more concentrated in urban areas, and increasingly dependent upon modern conveniences. All this contributes to increase demand for food, energy, and all other limited natural resources. At the same time this growth adds 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).

## **CONSUMER INFORMATION**

### ***What consumer information is available?***

The enzyme industry seeks to educate consumers about the importance of enzymes in everyday life, through media such as the Enzyme Technical Association Primer on the Use and Benefits of Enzymes and this Q & A. While some food products include enzyme labeling, generally, enzymes are not listed on the food label. This is because in most instances enzymes used in processing are added at extremely low levels and are removed or destroyed in the production process. Under these circumstances, as with other substances used as processing aids, listing the enzyme as an ingredient could be construed to be false or misleading since the enzyme would not be present in the purchased food product.



## **SAFETY QUESTIONS**

### ***Are enzyme products produced by biotechnology safe for human consumption?***

It is important that consumers feel confident about the food they buy. Modern biotechnology is therefore subject to strict controls. These are designed to ensure that new genetically modified products are safe to eat and that they pose no new risks to the environment. Food enzymes produced by modern biotechnology or traditional techniques undergo rigorous safety evaluation, and must meet strict safety standards. Modern biotechnology allows highly controlled modification of an organism's genetic makeup to target specific characteristics based on the function of the specific genes involved.

Enzymes used by the food industry come from living organisms but are **not** themselves living. These enzymes are protein molecules, not unlike the thousands of enzymes found in the human body. Food grade enzymes are extracted from safe, well characterized microorganisms. Food grade enzymes are produced and formulated with food grade raw materials using current Good Manufacturing Practices (cGMP) defined by the US Food and Drug Administration.

## **ENVIRONMENTAL QUESTIONS**

### ***How does modern biotechnology impact the environment?***

Modern biotechnology continues to develop new, precise tools to improve a wide range of production processes (e.g., for food, microbial, chemical, agricultural, applications.). These tools offer a broad array of benefits including reduced energy and raw material consumption. The savings includes less waste and fewer environmental pollutants. For example, many enzyme production microorganisms have been improved to produce the desired enzyme(s) more efficiently, using fewer raw materials and less energy than the starting production organism. As populations continue to increase, and standards of living improve, humankind will continue to tax the natural resilience of the environment. Modern biotechnology can help maintain/restore the balance by providing tools, methods, and opportunities to stop or lessen the impact of human activity on the environment. By pinpointing specific changes in microorganisms to direct a defined result, new solutions to existing problems can benefit all.

