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INDUSTRIAL AND BIOTECHNOLOGICAL APPLICATIONS OF LIGNOLYTIC ENZYMES

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Abstract-

Lignolytic enzymes are plays a crucial role in the global carbon cycle. The demand for application of lignolytic enzymes complexes of white rot fungi in industry and biotechnology is ever increasing due to their use in a variety of processes. Lignolytic enzymes have potential applications in a large number of fields, including the chemical, fuel, food, agricultural, paper, textile, cosmetic industrial sectors and more. This lignolytic system of white-rot fungi is also directly involved in the degradation of compounds and dyes. The applications of lignolytic enzymes of basidiomycetes within different industrial and biotechnological area. Among the different existing oxidant enzymes, laccases have been subject of intensive research in the last decades due to their low substrate specificity. The use of laccases in the industry is growing very fast, since besides to decolorize textile effluents, laccases are used to bleach textiles, modify the surface of fabrics and synthetic dyes. Therefore, laccase based processes might replace the traditionally high chemical, energy and water consuming textile operations.

Key words: *Lignolytic, Whit-rot fungi, Laccase, Textile industry, Waste water treatment*

INTRODUCTION

The green oxidation technologies have increased the interest in the use of enzymes to replace the conventional non-biological methods. Among the different existing oxidant enzymes, laccases benzenediol oxygen oxidoreductases; [EC1.10.3.2] have been subject of intensive research in the last decades becausethey have the following properties: low substrate specificity, do not need the addition or synthesis of a low molecular weight cofactor, as their co-substrate oxygen is usually present in their environment, most laccases are extracellular enzymes, making the purification procedures very easy, they generally exhibit a considerable level of stability in the extracellular environment, the inducible expression of laccases in most fungal species also contributes to their easy applicability in biotechnological processes. *Yoshida* (1883) first described laccase in 1883 when he extracted it from the exudates of the Japanese lacquer tree *Rhus vernicifera*, from which the name laccase was derived and was characterized as a metal-containing oxidase by *Bertrand* (1895). This makes it one of the oldest enzymes ever described. Laccases have also been detected in insects [1] and bacteria [2] but they are especially abundant in white-rot fungi, which are the only microorganisms able to degrade the whole wood components Figure-1

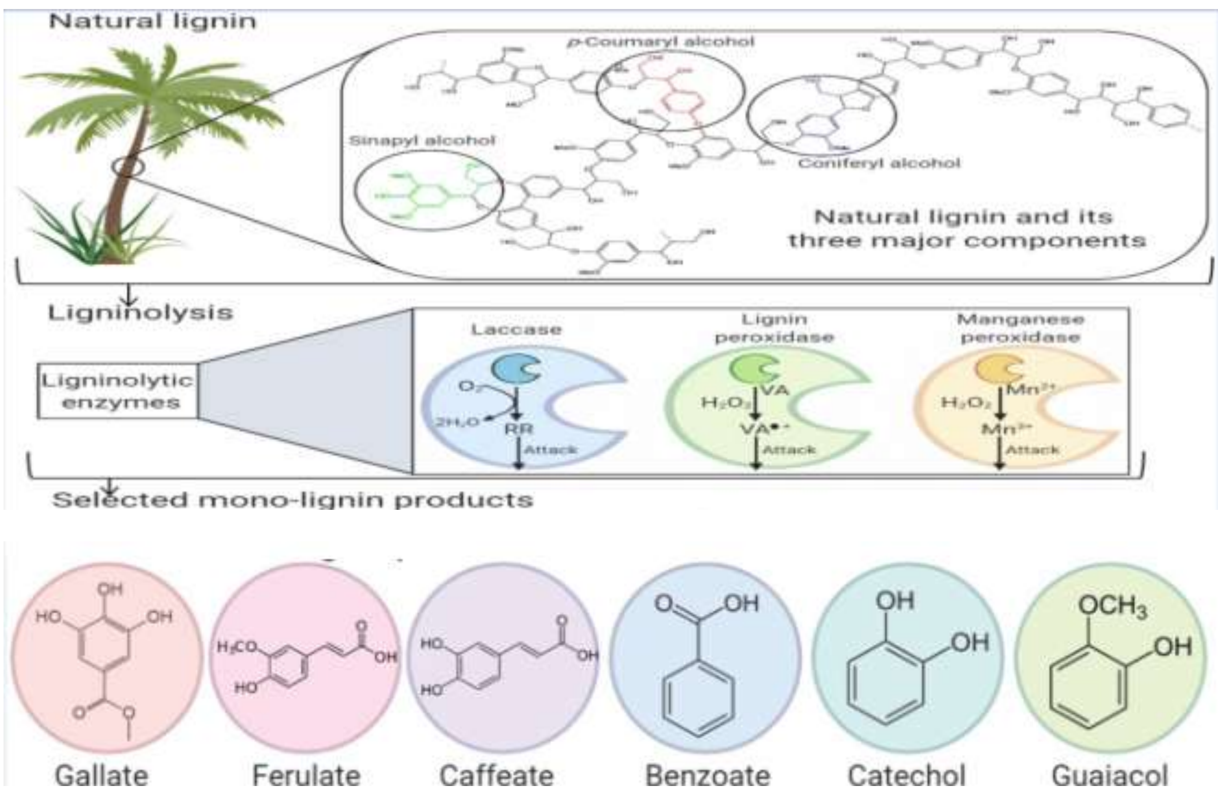


Figure-1: Natural lignin and its three major components

Laccases catalyze one electron oxidation of a wide range of inorganic and organic substances, coupled with electron reduction of oxygen to water [3]. The molecular mass of the monomer ranges from about 50 to 100 kDa [4-6]. An important feature of fungal laccases is a covalently linked carbohydrate moiety (10-45%), which may contribute to the high stability of the enzyme [7]. For the catalytic activity, a minimum of four copper atoms per active protein unit is needed. The copper atoms differ in their light absorbance and electron paramagnetic behaviour (Table 1).

Cu type	Cu atoms/ protein	EPR signal	Features	Coordination	Functions
1.	1	+ Paramagnetic	Blue Cu^{2+} absorbance at 610 nm (oxidation), redox potential +785 mV	2 His, 1 Cys, 1Leu	Substrate oxidation (one electron step)
2.	1	+ Paramagnetic	Non-blue Cu^{2+} (affinity to azide, fluoride, cyanide)	Trinuclear center	Reoxidation of type 1 Cu^{2+} Stabilisation of an H_2O_2 intermediate
3.	2	- Diamagnetic	Spin coupled Cu^{2+}-Cu^{2+} pair Absorbance at 330 nm (oxidation)	8 His	O_2 reduction by enzyme oxidation (four electron transfer)

Table-1: Status of copper in fungal laccases three types of copper have been distinguished

Laccases have relatively lower redox potential (450-800 mV) compared to those of ligninolytic peroxidases (>1 V), so it was initially thought that laccases would only be able to oxidise phenolic substrates [8]. However, the range of substrates oxidised by laccases can be increased through a mediator involved reaction mechanism. Mediators are low molecular weight compounds that are easily oxidised by laccases producing, in some cases, very unstable and reactive cationic radicals, which can oxidise more complex substrates before returning to their original state. Theelectrons taken by laccases are finally transferred back tooxygen to form water [9-10]. Enzymes are proteins with highly specialized catalytic functions, producedby 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 [11], theydiffer 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.

TYPES OF ENZYMES

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

USESES OF ENZYMES

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. Human kind 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 arange of enzyme producing organisms that were domesticated many years ago.

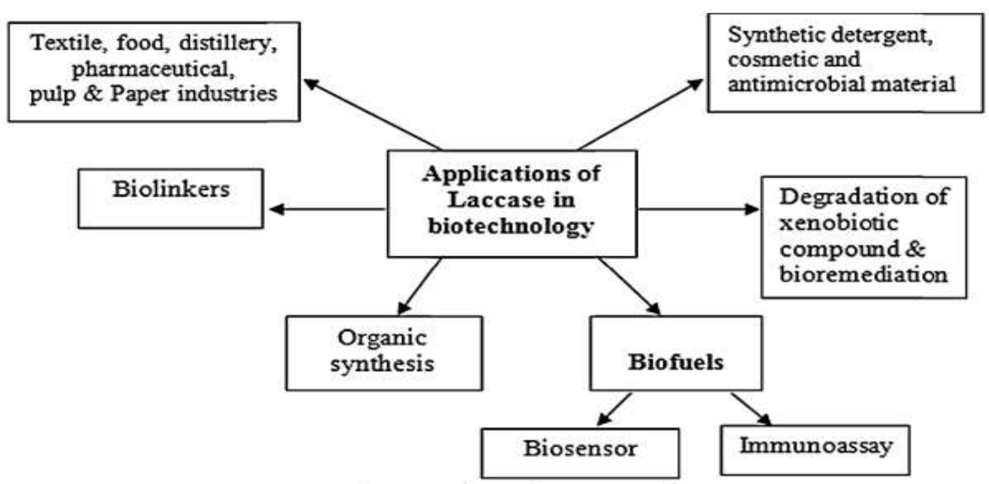
HUMAN BODY

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 [12], fats and proteins into simple compounds that the body can absorb and burn for energy or use to buildor repair tissue. These include:

1. Amylase and lipase in saliva break down carbohydrates and fats;
2. Proteases (pepsin) released in the stomach aid in digestion of proteins;and
3. 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 every day applications is provided in enzyme applications figure-2.



ENZYME PREPARATIONS

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 [13]. 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, EnvironmentalProtection 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 enzymesare 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 lessanimal 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 [14]. Enzymes also contribute to safer working conditions through elimination ofchemical 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.

FOOD/FOOD INGREDIENT APPLICATIONS

SUGAR SYRUPS FROM STARCH

During the 19th 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 20th 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 ofchoice. Today the production of HFCS is a major industry [15], 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.

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 theprinciple ingredient facilitating the separation of the curd (cheese) from the whey for thousands of years. Much has been learned about the functional attributes ofrennet and other cheese making enzymes since they were first employed. A purified form of the major enzyme in rennet, chymosin, is produced microbiallyfrom 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.

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 ofblue 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 enjoythese 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 sensorypleasure 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 [16]. This way, enzymes can help the baker produce bread that lives up to the quality standards consumers demand.

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 longerperiod of time.

The staling of white bread is considered to be related to a change in thestarch. 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 duringbaking to retard staling. The bread stays soft and flavorful for a longer time: 3-6days.

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 productionof 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 [17]. The remaining carbohydrate remains as a component of thefinished 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. 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 naturallyoccurring 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 amountof time.

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.

Confections: Soft candy and other treats made with sugar, especially soft center candy such a 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 strongacid 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 ^{o}C (195^{o}F) to 40-60 ^{o}C (100 to 140 ^{o}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.

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.

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.

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 absorbed 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.

Reduced Phosphorous Animal Feed: Poultry and hog feed grains contain phosphorous which is bound to phyticacid. 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.

INDUSTRIAL APPLICATIONS

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 cellulose and hemi cellulose, avoids the use of strong acids and results in a cleaner stream of sugars for fermentation and fewer by products.

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 (cellulose, hemicelluloses, pectinase and lipase), which offer the potential to totally replace the use of other chemicals in textile preparation processes.

Stone washed Jeans without Stones: Traditionally, to get the look and feel of stonewashed jeans, pumice stones were used. However, thanks to the introduction of cellulose 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 theywant, 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 machinesand pipes attributed to stones and stone dust.

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.

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 thechemicals 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 anenzymatic 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 wasa 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 20th century, the Leather Industry has switched over to using bacterial proteases and pancreatic trypsin.

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 volatileorganic chemical load.

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 aschlorine 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.

Deinking of Waste Paper: The basic technology to recycle waste paper is a relatively straightforwardprocess. The cellulosic fibers can readily be separated by repulping and cleaning, and made into new paper. The majority of the fillers and binders usedin 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 bythe 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

This group of enzymes is highly versatile in nature and they find application in a wide variety of industries. The biotechnological significance of these enzymes has led to a drastic increase in the demand for these enzymes in the recent time. Ligninolytic enzymes are promising to replace the conventional chemical processes of several industries. Thus, there is a broad field of investigation that is almost entirely open to new findings and it is quite reasonable to propose that many new applications will be found in the near future. These examples are just a few of the many ways commercial enzymes touch our lives. They are tools of nature that help provide every day 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.

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