

## APPLICATIONS OF CELLULASES – REVIEW

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### ABSTRACT

Cellulases are important enzymes not only for their potential applications in different industries like industries of animal feed, agricultural, laundry & detergent, textile, paper&pulp, wine & brewery, food processing, olive oil extraction. Carotenoid extraction, pharmaceutical & medical sciences, analytical applications, protoplast production, genetic engineering and pollution treatment, but also for the significant role in bio conversion of agriculture wastes into sugar and bio ethanol. This review paper simply assesses about: Enzymes, Cellulose, Cellulases & its types, Cellulase production and detailed study on Applications of cellulases.

**Keywords:** Enzymes, Cellulose, Cellulase.

### INTRODUCTION

Enzymes are biological catalysts which are the most remarkable, highly specialized and energized protein molecules found in every cell and are necessary for life. There are over 2000 known enzymes, each of which is involved with one specific chemical reaction. These proteins and their functions are determined by their shape. They have extraordinary catalytic power, often for greater than that of synthetic or inorganic catalysts.

Enzymes are vital to every biochemical process. They have a high degree of specificity for their substrates. They accelerate chemical reactions tremendously and they function in aqueous solutions under mild conditions and of normal temperatures.

Enzymes like other proteins have molecular weights ranging from about 12,000 to more than 1 million. Some enzymes require no chemical groups for activity, but many require non-protein co-enzymes or co-factors for their catalytic function.

According to International Union of Biochemists 1979, Six classes of enzymes have been designated. They are: 1. Oxido - reductases, 2. Transferases, 3. Hydrolases, 4. Lyases, 5. Isomerases, 6. Ligases. Major sources of industrial enzymes reveal that about 85% are

hydrolases, the remaining 15% are divided among oxidoreductases and isomerases.

Enzymes are widely used in food, detergents, analytical applications, medical sciences, production of chemicals & waste treatment.

### Cellulose

Cellulose is found in plants as micro fibrils (2 – 20nm diameter and 100 – 40,000nm long). These form the structurally strong frame work in the cell walls. Cellulose is mostly prepared from wood pulp.

Cellulose is a linear glucose polymer composed of anhydro glucose units coupled to each other by beta - 1,4 - glucosidic bonds and its most abundant biological compound on terrestrial earth.

Cellulose is an insoluble molecule consisting of residues between 2,000 – 14,000 with some of their properties being somewhat shorter. It forms crystals (Cellulose I<sub>a</sub>) where, intra molecular and intra strand hydrogen bonds hold the network flat allowing the more hydrophobic ribbon faces to the stack. Each residue is oriented 180° to the next with the chain synthesized two residues at a time. The natural crystal is made up from meta stable "Cellulose I" with all the cellulose strands parallel and no inter sheet hydrogen bonding.

Cellulose has many uses as an anticake agent, emulsifier, stabilizer, dispersing agent, thickener and gelling agent, but these are generally subsidiary to its most important use of holding on to water. Water cannot penetrate crystalline cellulose, but dry amorphous cellulose absorbs water becoming soft and flexible.

The degradation of cellulosic materials has gained increasing attention due to its world wide availability and immense potential for transforming them into sugars, fuels and chemical feed stocks.

Enzymatic hydrolysis hold tremendous promise due to the high specificity and production of high yields of glucose without generation of degradation products, unlike acid hydrolysis, utility cost are also low as hydrolysis occurs under mild reaction conditions. Microorganisms that degrade cellulose are both abundant and ubiquitous in nature. The enzymatic hydrolysis of cellulose requires the use of cellulase.

### Cellulase

Cellulase is a class of enzyme that catalyzes the cellulolysis i.e., hydrolysis of cellulose. Cellulase is a multiple enzyme system consisting of endo - 1, 4 - $\beta$ -D - glucanases and exo - 1, 4 - $\beta$ - D - glucanases along with cellobiase ( $\beta$ - D - glucosideglucano hydrolase).

### Types of Cellulases

Fractionation studies on culture filtrate have demonstrated that, there are 'three' major types of enzymes involved in the hydrolysis of native cellulose to glucose, namely:

1. Endo - glucanase
2. exo - glucanase
3.  $\beta$ - glucosidase / cellobiase.

#### 1. Endo - glucanase

Endo - glucanase (endo - $\beta$ - 1, 4 - glucanase, endo - $\beta$ - 1, 4 - D - glucan - 4 - glucanohydrolase, E.C.3.2.1.4) often called CMC ase, hydrolysis carboxyl methyl cellulose or swollen cellulose, due to which there is a rapid decrease in chain length along with a slow increase in reducing groups. Endoglucanase also acts on cellodextrins, the intermediate products of cellulose hydrolysis and converts them to cellobiase and glucose.

#### 2. Exo - glucanase

Exoglucanase (Exo- $\beta$ - 1, 4 - D - glucan - 4 - cellobiohydrolase, E.C. 3.2.1.91) degrades cellulose by splitting off the cellobiost units from the non- reducing end of the chain. Cellobiohydrolase does not degrade cotton rapidly, but can effect considerable saccharification of micro crystalline celluloses.

### 3. $\beta$ - glucosidase / Cellobiase

$\beta$ - glucosidase ( E.C.3.2.1.21) completes the process of cellulose hydrolysis by cleaving cellobiose and removing glucose from the non - reducing ends of oligosaccharides.

Complete degradation of cellulose to glucose requires the synergistic action of all the three components.

### Cellulase production

Successful utilization of cellulosic materials as renewable carbon sources is dependent on the development of economically feasible process technologies for cellulase production. It has been calculated that cellulase production accounts for ~27 - 20% of the cost of ethanol production from lignocellulosic materials. So, to keep the costs down, it is therefore very important to use a substrate that is inexpensive, such as wheat straw, rice bran, green gram husk etc. Large no. of microorganisms are capable of degrading cellulose, fungi, bacteria are the main cellulase producing microorganisms.

### Major steps involved in cellulase production

- Selection of potent strain as cellulose source.
- Selection of waste as substrate, eg. Lignocellulosic agricultural waste.
- Enrichment of lignocellulosic waste in carbon content by process of pre-treatment.
- Fermentation growth of selected strain utilizing the pre-treated lignocellulosic waste.
- Harvesting the biomass cultivated after fermentation.
- Downstream processing of extra cellular enzymes and recovery of cellulase.

From the above steps, it can be observed that, there are three main stages for the production of cellulase. They are 'pre - fermentative stage', where the pre - treatment of substrate and medium preparation is done, followed by 'fermentative stage' for cultivation of organism and finally 'post - fermentative stage' involving downstream processing and product recovery.

### Cellulases Applications

Recent development on bio - chemistry, genetics and protein as well as on the structure function relationships of cellulases including cellulosomes and related enzymes from bacteria and fungi has led to speculation and anticipation of their enormous commercial potential in bio - technology and research. Cellulases have been a potential candidate for research by both academic and industrial research groups. Biotechnological conversion of cellulosic

biomasses potentially sustainable approach to develop novel bioprocesses and products. Cellulase have become the focal biocatalysts due to their complex nature and wide spread industrial applications.

Cellulases were initially investigated several decades back for the bio conversion of the biomass which gave way to research in the industrial applications of the enzyme in Waste management, Bio - ethanol industry, Animal feed industry, Agricultural industry, Laundry&detergent industry, Textile industry, Paper& pulp industry, Wine& Breweryindustry, Food processing industry, Olive oil extraction, Carotenoid extraction, Pharmaceutical & medical sciences, Analytical applications, Protoplast production, Genetic engineering and Pollution treatment.

### **Waste Management**

Lignocellulose is the most abundant plant cell wall component of the biosphere and the most voluminous waste produced by our society. Biomass is the only domestic, sustainable and renewable primary energy resource that can provide liquid transportation fuels. The conversion of cellulosic waste to useful by - products has long been recognized as a desirable endeavour. Disposal of cellulosic municipal solid waste through processes that would also desire energy production are of the particular interest. The benefits would be 'Two fold': Firstly, the amount of cellulose waste would be diminished and its effects on our environment will be reduced and Secondly, the pollutant would be converted to an alternative source of energy to help displace our growing dependence on fossil fuels.

The biological decomposition of organic matter principally to methane and carbon dioxide by 'Anaerobic' digestion is a natural process that occurs readily in solid waste landfills. In natural anaerobic digestion processes some members of the microbial consortia collectively produce fermentable sugars from polysaccharides and others specialize in converting sugars to methane and carbon dioxide. Such mixed fermentations are notoriously difficult to establish and maintain at large scale. The anaerobic biological conversion of the major polymerize components of solid waste requires appropriate microorganisms and hydrolytic enzyme systems. Extra cellular hydrolytic enzymes such as cellulase and lipases have been shown to be effective in the post hydrolysis of anaerobic digester effluent solids.

In conventional technologies for the 'Aerobic' degradation of lignocellulose, lignocellulosic sugars are typically released by thermal

chemical pre- treatment by aerobic enzymatic hydrolysis of chopped or milled biomass.

### **Bio-Ethanol Industry**

Enzymatic saccharification of lignocellulosic materials such as rice bran, sugarcane bagasse, corncob, rice straw, green gram husk, saw dust, and forest residues by cellulases for bioethanol production is perhaps the most popular application currently being investigated. Bioconversion of lignocellulosic materials into useful and higher value products normally requires multistep processes. These processes include; pretreatment (mechanical, chemical, or biological), hydrolysis of the polymers to produce readily metabolizable molecules (e.g., hexose and pentose sugars), bioconversion of these smaller molecules to support microbial growth and/or produce chemical products, and the separation and purification of the desired products. The utility cost of enzymatic hydrolysis may be low compared with acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4-6 and temperature 45-50°C) and does not have corrosion issues.

Technologies are currently available for all steps in the bioconversion of lignocellulosics to ethanol and other chemical products. However, some of these technologies must be improved to produce renewable biofuel and other byproducts at prices, which can compete with more conventional production systems. Not only the recalcitrance of the substrate, but also several other factors that also limit cellulase efficiency during the hydrolysis process including end product inhibition, thermal deactivation of the native protein, nonspecific binding to lignin, and irreversible adsorption of the enzymes to the heterogeneous substrate.

To reduce the enzyme cost in the production of fuel ethanol from lignocellulosic biomass, two aspects are widely addressed: optimization of the cellulase production and development of a more efficient cellulase-based catalysis system. Protein engineering and directed evolution are powerful tools that can facilitate the development of more efficient thermophilic cellulases. Strategies for recycling and re-usage of the enzymes may also be used to reduce enzymatic hydrolysis costs. The recovery of enzymes is largely influenced by adsorption of the enzymes onto the substrate, especially to lignin and enzyme inactivation. There are several reports where the nonspecific and irreversible adsorption of cellulase to lignin has been observed. Besides, there are also reports where the compounds that mimic cellulose or the compounds have high affinity towards lignin

have been used to prevent the adsorption of cellulases to lignin. Moreover, recently Scott and coworkers have filed a US patent (20100221778) on novel lignin-resistant cellulase enzyme, in which linker peptides have been modified to prevent their adsorption onto lignin and enhance the enzyme activity. Among different strategies to recover and reuse the cellulases concentration of the cellulose fraction by ultrafiltration to remove sugars and other small compounds that may inhibit the action of the enzymes and recycling of immobilized enzymes, which enables separation of the enzymes from the process flow. However, the recycling techniques are mostly tested at laboratory scale. Therefore, the ability to scale up the techniques, the robustness, and feasibility still needs to be demonstrated.

### **Animal feed Industry**

Applications of cellulases and hemicellulases in the animal feed industry have received considerable attention because of their potential to improve feed value and performance of animals. Pretreatment of agricultural silage and grain feed by cellulases or xylanases can improve its nutritional value. The enzymes can also eliminate antinutritional factors present in the feed grains, degrade certain feed constituents to improve the nutritional value, and provide supplementary digestive enzymes such as proteases, amylases, and glucanases. For instance, the dietary fiber consists of nonstarch polysaccharides such as arabinoxylans, cellulose, and many other plant components including resistant dextrins, inulin, lignin, waxes, chitins, pectins,  $\beta$ -glucan, and oligosaccharides, which can act as anti-nutritional factor for several animals such as swine. In this case, the cellulases effectively hydrolyse the anti-nutritional factor, cellulose, in the feed materials into easily absorbent ingredient thus improve animal health and performance.

$\beta$ -Glucanases and xylanases have been used in the feed of monogastric animals to hydrolyze nonstarch polysaccharides such as  $\beta$ -glucans and arabinoxylans. Cellulases, used as feed additives alone or with proteases, can significantly improve the quality of pork meat. Glucanases and xylanases reduce viscosity of high fibre rye- and barley-based feeds in poultry and pig. These enzymes can also cause weight gain in chickens and piglets by improving digestion and absorption of feed materials.

Most low-quality feedstuffs contain higher concentrations of cellulose, small amounts of protein and fat and relatively high ash contents when compared with high-quality feedstuffs.

Cellulases can be used to improve silage production for cattle feeding, which involves enhancement of the digestibility of grasses containing large amounts of potentially total digestible nutrients and energy values together with only small amounts of water-soluble carbohydrates. The forage diet of ruminants, which contains cellulose, hemicellulose, pectin, and lignin, is more complex than the cereal-based diet of poultry and pigs. Enzyme preparations containing high levels of cellulase, hemicellulase, and pectinase have been used to improve the nutritive quality of forages. Nevertheless, the results with the addition of enzyme preparations containing cellulase, hemicellulase and pectinase to ruminant diet are somewhat inconsistent.

Animal feedstock production processes generally include heat treatments that inactivate potential viral and microbial contaminants. Application of thermophilic cellulase in feedstock production has the potential to reduce pathogens as well as to enhance digestibility and nutrition of the feed, thereby facilitating a combination of heat treatment and feed transformation in a single step. The cellulases and hemicellulases are responsible for partial hydrolysis of lignocellulosic materials, dehulling of cereal grains, hydrolysis of  $\beta$ -glucans, and better emulsification and flexibility of feed materials, which results in the improvement in the nutritional quality of animal feed. Moreover, these enzymes can cause partial hydrolysis of plant cell wall during silage and fodder preservation.

The use of enzymes in animal nutrition became more important after the prohibition of using some nutritive ionophore antibiotics, which were previously used in the EU countries. There is a large variation in the digestibility of starch origins. The low digestibility of some starches promotes the appearance of some digestive tract diseases because the non-digested and non-absorbed starch reaching the large intestine can act as a substrate for bacterial fermentation supporting the proliferation of some potentially hazardous pathogenic bacteria. Cellulases have a positive effect on the caecal fermentation processes by increasing the production of propionic acid, which act as a bacteriostatic material and thus can decrease the colonization of pathogenic bacteria.

### **Agricultural Industry**

Various enzyme preparations consisting of different combinations of cellulases, hemicellulases, and pectinases have potential applications in agriculture for enhancing growth of crops and controlling plant diseases. Plant or

fungal protoplasts produced using microbial hydrolases can be used to produce hybrid strains with desirable properties. Cellulases and related enzymes from certain fungi are capable of degrading the cell wall of plant pathogens in controlling the plant disease. Fungal  $\beta$ -glucanases are capable of controlling diseases by degrading cell walls of plant pathogens. Many cellulolytic fungi including *Trichoderma* sp., *Geocladium* sp., *Chaetomium* sp., and *Penicillium* sp. are known to play a key role in agriculture by facilitating enhanced seed germination, rapid plant growth and flowering, improved root system and increased crop yields. Although these fungi have both direct (probably through growth-promoting diffusible factor) and indirect (by controlling the plant disease and pathogens) effects on plants, it is not yet clear how these fungi facilitate the improved plant performance. It has been reported that  $\beta$ -1,3-glucanase and N-acetyl-glucosaminidase from *T. harzianum* strain P1 synergistically inhibited the spore germination and germ tube elongation of *B. cinerea*. Moreover, the exoglucanase promoters of *Trichoderma* are used for the expression of the different proteins, enzymes, and antibodies in large amount. The exoglucanase promoters of *Trichoderma* have been used for the expression of chymosin and other proteins: glucoamylase, lignin peroxidase, and laccase. Cellulases have also been used for the improvement of the soil quality. Traditionally straw incorporation is considered an important strategy to improve soil quality and reduce dependence on mineral fertilizers. Many studies have attempted to hasten straw decomposition via microbial routes. Cellulolytic fungi applications such as *Aspergillus*, *Chaetomium*, and *Trichoderma*, and actinomycetes have shown promising results. Exogenous cellulase supplementation accelerated decomposition of cellulose in soil. Therefore, using exogenous cellulase may be a potential means to accelerate straw decomposition and increase soil fertility.

### Laundry and Detergent Industry

Use of cellulases along with protease and lipase in the detergents is a more recent innovation in this industry. Cellulase preparations capable of modifying cellulose fibrils can improve color brightness, feel, and dirt removal from the cotton blend garments. The industrial application of alkaline cellulases as a potential detergent additive is being actively pursued with a view to selectively contact the cellulose within the interior of fibers and remove soil in the interfibril spaces in the presence of the more

conventional detergent ingredients. Nowadays, liquid laundry detergent containing anionic or nonionic surfactant, citric acid or a water-soluble salt, protease, cellulose, and a mixture of propanediol and boric acid or its derivative has been used to improve the stability of cellulases. As most of the cellulose fibers in the modern textile industry enzymes are used increasingly in the finishing of fabrics and clothes are arranged as long, straight chains of some small fibers can protrude from the yarn or fabric. The cellulases are applied to remove these rough protuberances for a smoother, glossier, and brighter-colored fabric.

### Textile Industry

Cellulases are the most successful enzymes used in textile wet processing, especially finishing of cellulose-based textiles, with the goal of improved hand and appearance. Traditional stonewashing of jeans involves amylase-mediated removal of starch coating (desizing) and treatment (abrasion) of jeans with pumice stone (1-2 kg/pair of jeans) in large washing machines. Cellulases have been successfully used for the biostoning of jeans and biopolishing of cotton and other cellulosic fabrics. During the biostoning process, cellulases act on the cotton fabric and break off the small fiber ends on the yarn surface, thereby loosening the dye, which is easily removed by mechanical abrasion in the wash cycle. The advantages in the replacement of pumice stones by a cellulose-based treatment include less damage of fibers, increased productivity of the machines, and less work-intensive and environment benign.

While the bio-polishing is usually carried out during the wet processing stages, which include desizing, scouring, bleaching, dyeing, and finishing. The acidic cellulases improve softness and water absorbance property of fibres, strongly reduce the tendency for pill formation, and provide a cleaner surface structure with less fuzz. Cellulase preparations rich in endoglucanases are best suited for biopolishing enhancing fabric look, feel, and color without needing any chemical coating of fibers. The action of cellulases removes short fibers, surface fuzziness, creates a smooth and glossy appearance, and improves color brightness, hydrophilicity and moisture absorbance, and environmentally friendly process.

Similarly, endoglucanase activity-rich cellulase is also proved better for biofinishing. Most cotton or cotton-blended garments, during repeated washing, tend to become fluffy and dull, which is mainly due to the presence of partially detached microfibrils on the surface of garments. The use of cellulases can remove



these microfibrils and restore a smooth surface and original color to the garments. The use of cellulase also helps in softening the garments and in removal of dirt particles trapped within the microfibril network.

Interestingly, there are several reports where the performance of the whole cellulase preparations was quite different from the enzyme rich in endoglucanase activity, and that the latter offered better performance in applications where losses in fabric strength and weight were minimum. Depilling/cleaning and/or ageing effects are the result of the synergistic action of cellulases and mechanical action, simultaneously or sequentially. Attempts have also been made via cellulases treatment to improve the dimensional stability of cellulosic fabrics and to upgrade the surface and dyeing properties of bleached cotton, mercerized cotton, and cotton/polyester blend fabric (50/50), using the pad-wet batch technique, followed by subsequent washing under mechanical action.

#### **Paper & Pulp Industry**

Interest in the application of cellulases in the pulp and paper industry has increased considerably during the last decade. The mechanical pulping processes such as refining and grinding of the woody raw material lead to pulps with high content of fines, bulk, and stiffness. While in contrast, biomechanical pulping using cellulases resulted in substantial energy savings (20–40%) during refining and improvements in hand-sheet strength properties.

Mixtures of cellulases (endoglucanases I and II) and hemicellulases have also been used for biomodification of fiber properties with the aim of improving drainage and beatability in the paper mills before or after beating of pulp. Mansfield et al. studied the action of a commercial cellulase preparation on different fractions of Douglas fir kraft pulp and observed that the cellulase treatment decreased the defibrillation reducing the fibre coarseness. While endoglucanases have the ability to decrease the pulp viscosity with a lower degree of hydrolysis, cellulases have also been reported to enhance the bleachability of softwood kraft pulp producing a final brightness score comparable to that of xylanase treatment.

Cellulases alone, or used in combination with xylanases, are beneficial for deinking of different types of paper wastes. Most applications proposed so far use cellulases and hemicellulases for the release of ink from the fiber surface by partial hydrolysis of carbohydrate molecules. It has been postulated

that improvements in dewatering and deinking of various pulps result in the peeling of the individual fibrils and bundles, which have high affinity for the surrounding water and ink particles. The main advantages of enzymatic deinking are reduced or eliminated alkali usage, improved fiber brightness, enhanced strength properties, higher pulp freeness and cleanliness, and reduced fine particles in the pulp. Moreover, deinking using enzymes at acidic pH also prevents the alkaline yellowing, simplifies the deinking process, changes the ink particle size distribution, and reduces the environmental pollution. Although enzymatic deinking can lower the need for deinking chemicals and reduce the adverse environmental impacts of the paper industry, the excessive use of enzymes must be avoided, because significant hydrolysis of the fines could reduce the bondability of the fibers.

Interestingly, the use of cellulases in improving the drainage has also been pursued by several mills with the objective to increase the production rate. Enzyme treatments remove some of the fines or peel off fibrils on the fiber surface and dissolved and colloidal substances, which often cause severe drainage problems in paper mills. In this aspect, cellulases have shown considerable improvement in the overall performance of paper mills. Enzymatic treatment also destabilizes the lipophilic extractives in the filtrates and facilitates their attachment to thermo-mechanical pulping fibers. These enzymes are also used in preparation of easily biodegradable cardboard, manufacturing of soft paper including paper towels and sanitary paper and removal of adhered paper.

#### **Wine and Brewery Industry**

Microbial glucanases and related polysaccharides play important roles in fermentation processes to produce alcoholic beverages including beers and wines. These enzymes can improve both quality and yields of the fermented products. Glucanases are added either during mashing or primary fermentation to hydrolyze glucan, reduce the viscosity of wort, and improve the filterability.

In wine production, enzymes such as pectinases, glucanases, and hemicellulases play an important role by improving color extraction, skin maceration, must clarification, filtration, and finally the wine quality and stability.  $\beta$ -Glucosidases can improve the aroma of wines by modifying glycosylated precursors. Macerating enzymes also improve pressability, settling, and juice yields of grapes used for wine fermentation. A number of commercial enzyme

preparations are now available to the wine industry. The main benefits of using these enzymes during wine making include better maceration, improved color extraction, easy clarification, easy filtration, improved wine quality, and improved stability.

Beer brewing is based on the action of enzymes activated during malting and fermentation. Malting of barley depends on seed germination, which initiates the biosynthesis and activation of  $\alpha$ - and  $\beta$ -amylases, carboxypeptidase, and  $\beta$ -glucanase that hydrolyze the seed reserves. In an earlier study, researchers observed that endoglucanase II and exoglucanase II of the *Trichoderma* cellulase system were responsible for a maximum reduction in the degree of polymerization and wort viscosity.

Significant and reproducible improvements in grape pressability, settling rate, and total juice yield were achieved using a combination of macerating enzymes. Such improvements were noticeable only with a correct balance of pectinases, cellulases, and hemicellulases. Using three varieties (Soave, Chardonnay, and Sauvignon) of white grapes, reports show that, the performance of Cytolase 219 (mixture of cellulase, pectinase, and xylanase) in wine making and 10–35% increase in the extraction of the first wine must, a 70–80% increase in the must filtration rate, 50–120 minutes decrease in pressing time, 30–70% decrease in must viscosity, 20–40% saving of energy during cooling of fermenter, and a significant improvement in wine stability. A range of improved enzymes like cellulase and pectinase that would be exogenously added to the process are expected to enhance the productivity of existing brewing processes in future.

### Food processing industry

Cellulases have a wide range of potential applications in food biotechnology as well. The production of fruit and vegetable juices requires improved methods for extraction, clarification, and stabilization. Cellulases also have an important application as a part of macerating enzymes complex (cellulases, xylanases, and pectinases) used for extraction and clarification of fruit and vegetable juices to increase the yield of juices. The use of macerating enzymes increases both yield and process performance without additional capital investment. The macerating enzymes are used to improve cloud stability and texture and decrease viscosity of the nectars and purees from tropical fruits such as mango, peach, papaya, plum, apricot, and pear. Texture, flavor, and aroma properties of fruits and vegetables can be improved by reducing excessive bitterness of citrus fruits by

infusion of enzymes such as pectinases and  $\beta$ -glucosidases. Enzyme mixtures containing pectinases, cellulases, and hemicellulases are also used for improved extraction of olive oil. Use of macerating enzymes not only improves the cloud stability and texture of nectars and purees, but also decreases their viscosity rapidly. Thus, the macerating enzymes, composed of mainly cellulase and pectinase, play a key role in food biotechnology, and their demand will likely increase for extraction of juice from a wide range of fruits and vegetables. Furthermore, infusion of pectinases and  $\beta$ -glucosidases has also shown to alter the texture, flavor, and other sensory properties such as aroma and volatile characteristics of fruits and vegetables.

### Olive Oil extraction

In recent years, extraction of olive oil has attracted the interest of international market because of its numerous health claims. Extraction of olive oil involves (1) crushing and grinding of olives in a stone or hammer mill; (2) passing the minced olive paste through a series of malaxeurs and horizontal decanters; (3) high-speed centrifugation to recover the oil. To produce high-quality olive oil, freshly picked, clean, and slightly immature fruits have been used under cold pressing conditions. However, high yields have been obtained with fully ripened fruit, when processed at higher than ambient temperatures, but this resulted in oil with high acidity, rancidity, and poor aroma. Hence, an improved method for the extraction of high-quality olive oil was needed to meet the growing consumer demand. The commercial enzyme preparation, Olivex (a pectinase preparation with cellulase and hemicellulase from *Aspergillus aculeatus*), was the first enzyme mixture used to improve the extraction of olive oil. Furthermore, the use of macerating enzymes increased the antioxidants in extravirgin olive oil and reduced the induction of rancidity. The main advantages of using macerating enzymes during olive oil extraction are (1) increased extraction (up to 2 kg oil per 100 kg olives) under cold processing conditions; (2) better centrifugal fractionation of the oily must; (3) oil with high levels of antioxidants and vitamin E; (4) slow induction of rancidity; (5) overall improvement in plant efficiency; (6) low oil content in the waste water. Likewise, the macerating enzymes could play a prominent role in the extraction of oils from other agricultural oilseed crops.

These enzymes can also be used during olive paste malaxation. The presence of collateral activities of a cellulase and hemicellulase nature

of the enzymatic formulation guarantees a rapid and intense disintegration of the cell walls and membranes of the olive fruits, thereby favoring the passage of noble substances (particularly the polyphenols and aromatic precursors) into the final product. It is also used to decrease olive paste viscosity in olive oil production and to intensify the process of extracting the polyphenolic substances contained in the olive fruit. It is necessary to underline that the selected enzymes are naturally present inside the olive fruit, but they are strongly deactivated during the critical pressing step, probably because of oxidation phenomena. So, the replacement of these enzymes is expected to be appropriate in relation with the role they play in determining the final product quality.

### **Carotenoid Extraction**

Carotenoids are the main group of coloring substances in nature being responsible for many plant colors from red to yellow. There is a continuously growing market for carotenoids as food colorants due to their desirable properties, such as their natural origin, null toxicity, and high versatility, providing both lipo- and hydrosoluble colorants with colors ranging from yellow to red. In addition, provitamin A activity, a role in lipid oxidation, and anticarcinogenic properties are very important biological functions of these pigments.

Usually a combination of cellulolytic and pectinolytic enzymes accelerates the rate of hydrolysis for achieving complete liquefaction. Cellulase randomly splits cellulose chains into glucose whereas commercial pectinase preparations from *Aspergillus niger* have pectinesterase (PE), polygalacturonase (PG), and pectin lyase (PL) activity. The use of pectinase and cellulase enzymes disrupts the cell wall of orange peel, sweet potato and carrot, and releases the carotenoids in the chloroplasts and in cell fluids. These pigments remain in their natural state still bound with proteins. This bonded structure prevents pigment oxidation and also affects color stability, whereas solvent extraction dissociates the pigments from the proteins and causes water insolubility and ease of oxidation.

### **Pharmaceutical & Medical Sciences**

Considerable research in this area is being done and newer applications are being discovered, such as the treatment of enzyme deficiencies. Enzymes are also used as anti-tumor or anti-microbial agents, treatment of blood clots and herniated discs. Effect of enzyme cellulase is as digestive aid i.e. cellulase digests fiber. It helps in the remedy of digestive problems such as

'malabsorption'. Since, humans poorly digest cellulose fiber, taking a digestive enzyme product, like Digestin, that contains cellulase enzymes is not only necessary, but also vital for healthy cells.

### **Analytical Applications**

Use of cellulases in various aspects of chemical analysis has been a growing interest. The principal analytical application of enzymes in the diagnostics and food analysis.

### **Protoplast Production**

For overcoming interspecific incompatibility, protoplast combination method is a proper procedure for making a new plant with desired traits. For this purpose, protoplast preparation is a first and important step. Hence, experimental results show that protoplast yield was significantly affected to the maximum extent by the enzyme cellulase along with pectinase.

### **Genetic Engineering**

Combining different types of enzymes and genetically engineering new enzymes that work together to release both hemicellulosic sugars and cellulosic sugars. Biotechnological advances, especially in genetic engineering of commercially useful enzyme producing strains are playing an important role in solving some of the bottlenecks that occur in production and will be vital in securing cellulosic ethanol as a competitive liquid fuel for the future by using cellulase.

### **Pollution Treatment**

Environmental pollution is growing more & more due to the indiscriminate and frequently deliberate release of hazardous, harmful substances. Research efforts have been devoted to develop new, low cost, low technology, eco-friendly treatments capable of reducing and even eliminating pollution in the atmosphere, the hydrosphere and soil environments. Among biological agents, enzymes have a great potentiality to effectively transform and detoxify polluting substances because they have been recognized to be able to transform pollutants at a detectable rate are potentially suitable to restore polluted environments. Enzymes are capable of transforming some classes of pollutants into innocuous products.

Several substances with high polluting potential are present in the environment & affect soil, sediments, water, air, microbial organisms, plants, animals & humans. The origins and sources of pollution are different, industrial activities such as mining and metal processing,



petrochemical and industrial complexes, industry effluent, chemical weapons production, pulp & paper industries, dye industries, anthropogenic activities such as traffic, agricultural practices and others. Pollutants may affect the health of humans, animals and environments for several causes.

Therefore, a growing interest is being devoted to the search of effective remediation technologies for partial or total recovery of polluted sites. Several methodologies have been applied for remediation of polluted systems, of which 'two' basic strategies have been utilized: engineering and biological ones. Bioremediation appear now as appealing technologies being based on their enzymatic set.

The use of enzymatic proteins may represent a good alternative for overcoming most disadvantages related to the use of microorganisms. The most representative enzymatic classes in the remediation of polluted environments are oxidoreductases, hydrolases i.e., Cellulases and amylases.

#### CONCLUSION

It was estimated that, the world sale of industrial enzymes have already reached a market value of 1.6 billion US of which cellulase and allied enzymes occupy a significant position. Microbes are an attractive topic of interest for the production of cellulases and hemicellulases due to their immense potential for cellulase production, enzyme complexity and extreme habitat variability. Microbial cellulases are preferred for their vast industrial applicability and relatively lower cost of production. In fact the craze for these cellulaseenzymes is increasing day to day worldwide for their use in waste management, food processing, pharmaceuticals, pulp&paper and other industries. More and more research works are resulting into improved scientific knowledge along with the success of meeting the growing demands of the cellulase and related enzymes for generation of environment friendly textiles, detergents, bio-pulping and bio-alcohols. Moreover, it is opening new avenues for utilization of various agro-wastes and organic pollutants as a source of renewable energyinstead of dumping them to cause environmental degradation. In near future newer knowledge of excellent cellulolytic and hemi-cellulolytic systems and adoption of different biotechnological strategies will certainlybringsgreat prospect in the field of industrial greenchemistry.

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#### REFERENCES

1. Sharada R, Swathi K, Venkateswar S, AnandRao M and Narsi Reddy M. Optimization of cultural conditions for cellulase production by *Aspergillusniger* (MTCC 2196) using submerged fermentation. *Biosciences Biotechnology Research Asia*. 2009;6(1):289-292.
2. Sharada R, Venkateswarlu G, NarsiReddy M, Venkateswar S and Anand Rao M. Production of Cellulase by Solid State Fermentation. *IJPRD*. 2012;4(4):224-230.
3. Sharada R, Venkateswarlu G, Venkateswar S and Anand Rao M. Production of Cellulase – A Review. *IJCBS*. 2013;3(4):1070-1090.
4. Anupama and Ravindra P. Value added food: Single cell protein. *Biotechnol Adv*. 2000;18(6): 459-479.
5. Anuppama and Ravindra P. Studies on production of single cell protein by *Aspergillusniger* insolid state fermentation of rice bran. *Braz Arch BiolTech*. 2001a;44:79-88.
6. Edward A Bayer, Raphael Lamed and Micheal E Himmel. The Potential of Cellulases and Cellulosomes for Cellulosic Waste Management. *Current Opinion in Biotechnology*. 2007;18:237-245.
7. Ajoy K Sarkar and Nolan Etters J. Enzymatic hydrolysis of cotton fibers:Modeling using an empirical equation. *The Journal of cotton science*. 2004;8:254-260.
8. ArturCavaco-Paulo. Mechanism of cellulase action in textile processes. *Carbohydrate polymers*. 1988;37:273-277.
9. Braz J Demuner, Nei Pereira Junior and Adelaide MS Antunes. Technology prospecting on enzymes for the pulp and paper industry. *J Technol Manag Innov*. 2011;6(3).
10. William R Kenealy and Thomas W Jeffries. Enzyme processes for pulp and paper: A review of recent developments.Chapter 12.
11. Ramesh Chander Kuhad, Rishi Gupta and Ajay Singh. Microbial cellulases and their industrial applications. *Enzyme research*. 2011;10.

12. Mai C, Kües U and Militz H. Biotechnology in the wood industry. Applied Microbiology and Biotechnology. 2004;63:477-494.
13. Singh A, Kuhad RC and Ward OP. Industrial application of microbial cellulases, in Lignocellulose Biotechnology. Future Prospects. 2007;345-358.
14. Akhtar M. Biochemical pulping of aspen wood chips with three strains of *Ceriporiopsis subvermispora*. Holzforschung. 1994;48:199-202.
15. Pere J, Puolakka A, Nousiainen and Buchert J. Action of purified *Trichoderma reesei* cellulases on cotton fibers and yarn. Journal of Biotechnology. 2001;89(2-3):247-255.
16. Bhat MK. Cellulases and related enzymes in biotechnology. Biotechnology Advances. 2000;18(5):355-383.
17. Dienes D, Egyházi A and Réczey K. Treatment of recycled fiber with *Trichoderma* cellulases. Industrial Crops and Products. 2004;20(1):11-21.
18. Mansfield SD, Wong KKY, De Jong E and Saddler JN. Modification of Douglas-fir mechanical and kraft pulps by enzyme treatment. Tappi Journal. 1996;79(8):125-132.
19. Pere J, Siika-aho M, Buchert J and Viikari L. Effects of purified *T. reesei* cellulases on the fiber properties of kraft pulp. Tappi Journal. 1995;78(6):71-78.
20. Suominen P and Reinikainen T. Foundation for biotechnical and industrial fermentation research. In Proceedings of the 2nd Symposium on *Trichoderma reesei* Cellulases and Other Hydrolases (TRICEL '93). 1993;8, Espoo, Finland.
21. ChanderKuhad R, Mehta G, Gupta R and Sharma KK. Fed batch enzymatic saccharification of newspaper cellulose improves the sugar content in the hydrolysates and eventually the ethanol fermentation by *Saccharomyces cerevisiae*. Biomass and Bioenergy. 2010;34(8):1189-1194.
22. Kibblewhite RP, Bawden AD and Brindley CL. TMP fiber and fines qualities of 13 radiata pine wood types. APPITA. 1995;48:367-377.
23. Kuhad RC, Gupta R, Khosa YP and Singh A. Bioethanol production from *Lantana camara* (red sage): pretreatment, saccharification and fermentation. Bioresource Technology. 2010;101(21):8348-8354.
24. Stork G and Puls J. Changes in properties of different recycled pulps by endoglucanase treatments. In Proceedings of the International Conference on Biotechnology in the Pulp and Paper Industry: Recent Advances in Applied and Fundamental Research. E. Srebotnik and K. Mesner. 1996;1:145-150.
25. Karnis A. The role of latent and delatent mechanical pulp fines in sheet structure and pulp properties. Paperi ja Puu-Paper Timber. 1995;77:491-497.
26. Kantelinen A, Jokinen O and Sarkki ML. Effects of enzymes on the stability of colloidal pitch. In Proceedings of the 8th International Symposium on Wood and Pulp Chemistry. 1995;1:605-612.
27. Buchert J, Oksanen T, Pere J, Siika-aho M, Suurnakki A and Viikari L. Applications of *Trichoderma reesei* enzymes in the pulp and paper industry in *Trichoderma* & *Gliocladium*-Enzymes, G. F. Harman and C. P. Kubicek. of Biological Control and Commercial Applications. 1998;2:343-363.
28. Salonen SM. Method for manufacturing paper or cardboard and product containing cellulose. US patent 4980023, 1993.
29. Hsu JC and Lakhani NN. Method of making absorbed tissue from recycled waste paper. US patent 6413363, 2002.
30. Sharyo M, Sakaguchi H and Ohishi M. Method of making sanitary paper from chemical pulp using a single component cellulase that does not contain cellulose-building domain. US patent 6468391, 1978.
31. Hebeish A and Ibrahim NA. The impact of frontier sciences on textile industry. Colourage. 2007;54:41-55.
32. Karmakar M and Ray RR. Current trends in research and application of microbial cellulases. Research Journal of Microbiology. 2011;6(1):41-53.
33. Uhlig H. Industrial Enzymes and Their Applications. John Wiley & Sons, New York, NY, USA, 1998.

34. Galante YM, DeConti A and Monteverti R. Application of Trichoderma enzymes in food and feed industries in Trichoderma and Gliocladium Enzymes, G. F. Harman and C. P. Kubicek, Eds of Biological Control and Commercial Applications, Taylor & Francis, London, UK, 1998;2:311-326.
35. Sreenath HK, Shah AB, Yang VW, Gharia MM and Jeffries TW. Enzymatic polishing of jute/cotton blended fabrics. Journal of Fermentation and Bioengineering. 1996;81(1):18-20.
36. Ibrahim NA, El-Badry K, Eid BM and Hassan TM. A new approach for biofinishing of cellulose-containing fabrics using acid cellulases. Carbohydrate Polymers. 2001;83(1):116-121.
37. Baker JO, McCarley JR and Lovett R. Catalytically enhanced endocellulase Cel5A from *Acidothermus cellulolyticus*. Applied Biochemistry and Biotechnology A. 2005;121(1-3):129-148.
38. Cortez JM, Ellis J and Bishop DP. Using cellulases to improve the dimensional stability of cellulosic fabrics. Textile Research Journal. 2002;72(8):673-680.
39. Sukumaran RK, Singhania RR and Pandey A. Microbial cellulases production, applications and challenges. Journal of Scientific and Industrial Research. 2005;64(11):832-844.
40. Kuhad RC, Gupta R and Khosa YP. Bioethanol production from lignocellulosic biomass: an overview in Wealth from Waste. B. Lal, Ed., Teri Press, New Delhi, India, 2010.
41. Kuhad RC, Singh A and Eriksson KE. Microorganisms and enzymes involved in the degradation of plant fiber cell walls. Advances in Biochemical Engineering Biotechnology. 1997;57:45-125.
42. Gupta R, Khosa YP and Kuhad RC. Evaluation of pretreatment methods in improving the enzymatic saccharification of cellulosic materials. Carbohydrate Polymers. 2011;84:1103-1109.
43. Ghosh P and Singh A. Physicochemical and biological treatments for enzymatic/microbial conversion of lignocellulosic biomass. Advances in Applied Microbiology. 1995;39:295-333.
44. Wyman CE, Dale BE, Elander RT, Holtzapfel M, Ladisch MR and Lee YY. Coordinated development of leading biomass pretreatment technologies. Bioresource Technology. 2005;96(18):1959-1966.
45. Kuhad RC and Singh A. Lignocellulose biotechnology: current and future prospects. Critical Reviews in Biotechnology. 1993;13(2):151-172.
46. Mosier N, Wyman C and Dale B. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology. 2005;96(6):673-686.
47. Yang and Wyman CE. Effect of xylan and lignin removal by batch and flow through pretreatment on the enzymatic digestibility of corn stover cellulose. Biotechnology and Bioengineering. 2004;86(1):88-95.
48. Taniguchi M, Suzuki H, Watanabe D, Sakai K, Hoshino K and Tanaka T. Evaluation of pretreatment with *Pleurotus ostreatus* for enzymatic hydrolysis of rice straw. Journal of Bioscience and Bioengineering. 2005;100(6):637-643.
49. Lee D, Yu AHC and Saddler JN. Evaluation of cellulase recycling strategies for the hydrolysis of lignocellulosic substrates. Biotechnology and Bioengineering. 1995;45(4):328-336.
50. Singh A, Kumar PKR and Schugerl K. Adsorption and reuse of cellulases during saccharification of cellulosic materials. Journal of Biotechnology. 1991;18(3):205-212.
51. Bernardez TD, Lyford K, Hogsett DA and Lynd LR. Adsorption of *Clostridium thermocellum* cellulases onto pretreated mixed hardwood, Avicel, and lignin. Biotechnology and Bioengineering. 1993;2(7):899-907.
52. Yang B and Wyman CE. Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose. Biotechnology and Bioengineering. 2004;86(1):88-95.
53. Yang B and Wyman CE. BSA treatment to enhance enzymatic hydrolysis of cellulose in lignin containing substrates. Biotechnology and Bioengineering. 2006;94(4):611-617.

54. Kumar R and Wyman CE. Effect of additives on the digestibility of corn stover solids following pretreatment by leading technologies. *Biotechnology and Bioengineering*. 2009;102(6):1544–1557.
55. Scott BR, St-Pierre P, Lavigne J, Masri N, White TC and Tomashek JJ. Novel lignin-resistant cellulase enzymes. US patent: 20100221778, 2010.
56. Tu M, Chandra RP and Saddler JN. Evaluating the distribution of cellulases and the recycling of free cellulases during the hydrolysis of lignocellulosic substrates. *Biotechnology Progress*. 2007;23(2):398–406.
57. Dourado F, Bastos M, Mota M and Gama FM. Studies on the properties of Celluclast/Eudragit L-100 conjugate. *Journal of Biotechnology*. 2002;99(2):121–131.
58. Bamforth CW. Current perspectives on the role of enzymes in brewing. *Journal of Cereal Science*. 2009;50(3):353–357.
59. Canales AM, Garza R, Sierra JA and Arnold R. The application of beta-glucanase with additional side activities in brewing. *MBAA Technical Quarterly*. 1998;25:27–31.
60. Oksanen J, Ahvenainen J and Home S. Microbial cellulase for improving filterability of wort and beer. In *Proceedings of the 20th European Brewery Chemistry Congress*. 1985;419–425, Helsinki, Finland.
61. Minussi RC, Pastore GM and Duran N. Potential applications of laccase in the food industry. *Trends in Food Science and Technology*. 2002;13(6-7):205–216.
62. De Carvalho LMJ, de Castro IM and da Silva CAB. A study of retention of sugars in the process of clarification of pineapple juice (*Ananas comosus*, L. Merrill) by micro- and ultra-filtration. *Journal of Food Engineering*. 2008;87(4):447–454.
63. Baker RA and Wicker L. Current and potential applications of enzyme infusion in the food industry. *Trends in Food Science and Technology*. 1996;7(9):279–284.
64. Youn KS, Hong JH, Bae DH, Kim SJ and Kim SD. Effective clarifying process of reconstituted apple juice using membrane filtration with filter-aid pretreatment. *Journal of Membrane Science*. 2004;228(2):179–186.
65. Rai P, Majumdar GC, Das Gupta S and De S. Effect of various pretreatment methods on permeate flux and quality during ultrafiltration of mosambi juice. *Journal of Food Engineering*. 2007;78(2):561–568.
66. Grassin C and Fauquembergue P. Fruit juices in *Industrial Enzymology*. T. Godfrey and S. West, Eds., 226–264, MacMillan Press, London, UK, 2nd edition, 1996.
67. Humpf HU and Schreier P. Bound aroma compounds from the fruit and the leaves of blackberry (*Rubus laciniata*, L.). *Journal of Agricultural and Food Chemistry*. 1991;39(10):1830–1832.
68. Marlatt C, Ho CT and Chien M. Studies of aroma constituents bound as glycosides in tomato. *Journal of Agricultural and Food Chemistry*. 1992;40(2):249–252.
69. Dhiman TR, Zaman MS, Gimenez RR, Walters JL and Treacher R. Performance of dairy cows fed forage treated with fibrolytic enzymes prior to feeding. *Animal Feed Science and Technology*. 2002;101(1–4):115–125.
70. Godfrey T and West S. *Textiles in Industrial Enzymology*. Macmillan Press, London, UK, 2nd edition, 1996;360–371.
71. Ali S, Hall J and Soole KL. Targeted expression of microbial cellulases in transgenic animals in *Carbohydrate Bioengineering*, S. B. Petersen, B. Svensson, and S. Pedersen, Eds., vol. 10 of *Progress in Biotechnology*. Elsevier, Amsterdam, The Netherlands, 1995;279–293.
72. Shrivastava B, Thakur S, Khasa YP, Gupte A, Puniya AK and Kuhad RC. White-rot fungal conversion of wheat straw to energy rich cattle feed. *Biodegradation*. 2011;22(4):823–831.
73. Graham H and Balnave D. Dietary enzymes for increasing energy availability in *Biotechnology in Animal Feeds and Animal Feedings*, R. J. Wallace and A. Chesson, Eds., VHC, Weinheim, Germany. 1995;296–309.
74. Lewis GE, Hunt CW, Sanchez WK, Treacher R, Pritchard GT and Feng P. Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage-based diet fed to beef steers.

- Journal of Animal Science. 1996;74(12):3020-3028.
75. Paulo AC and Gubitz GM. Textile Processing with Enzymes, Woodhead, Cambridge, UK, 2003.
  76. Cowan WD. Animal feed in Industrial Enzymology, T. Godfrey and S. West, Eds., 360-371, Macmillan Press, London, UK, 2nd edition, 1996.
  77. Pazarlioglu NK, Sarişik M and Telefoncu A. Treating denim fabrics with immobilized commercial cellulases *Process Biochemistry*. 2005;40(2):767-771.
  78. Pascual JJ. Recent advances on early weaning and nutrition around weaning. In Proceedings of the 2nd Meeting of COST 848 Working Groups. 2001;4:31-36, Hungary.
  79. Fortun-Lamothe L, Gidenne T, Debray L and Chalaye F. Intake regulation, performances and health status according to feeding strategy around weaning in Proceedings of the Second Meeting of COST 848 Working Group 4, 40-41, Hungary, 2001.
  80. Chet I, Benhamou N and Haran S. Mycoparasitism and lytic enzymes in *Trichoderma* and *Gliocladium*—Enzymes, G. F. Harman and C. P. Kubicek, Eds. Biological Control and Commercial Applications. Taylor & Francis, London, UK. 1998;2:327-342.
  81. Bailey BA and Lumsden RD. Direct effects of *Trichoderma* and *Gliocladium* on plant growth and resistance to pathogens in *Trichoderma* & *Gliocladium*—Enzymes, G. F. Harman and C. P. Kubicek, Eds. Biological Control and Commercial Applications. Taylor & Francis, London, UK, 1998;2:327-342.
  82. Harman GE and Björkman T. Potential and existing uses of *Trichoderma* and *Gliocladium* for plant disease control and plant growth enhancement in *Trichoderma* and *Gliocladium*, C. P. Kubicek and G. E. Harman, Eds. Taylor and Francis, London, UK, 1998;2:229-265.
  83. Harman GE and Kubicek CP. *Trichoderma* and *Gliocladium*: Enzymes. Biological Control and Commercial Applications, Taylor & Francis, London, UK, 1998;2.
  84. Lorito M, Hayes CK, Di Pietro A, Woo SL and Harman GE. Purification, characterization, and synergistic activity of a glucan 1,3-beta-glucosidase and an N-acetyl-beta-glucosaminidase from *Trichoderma reesei*,” *Phytopathology*. 1994;84(4):398-405.
  85. Harkki A, Uusitalo J, Bailey M, Penttilä M and Knowles JKC. A novel fungal expression system: secretion of active calf chymosin from the filamentous fungus *Trichoderma reesei*. *Biotechnology*. 1989;7(6):596-603.
  86. Penttilä M. Heterologous protein production in *Trichoderma reesei*. *Trichoderma & Gliocladium—Enzymes*, G. F. Harman and C. P. Kubicek, Eds. Biological Control and Commercial Applications. Taylor & Francis, London, UK, 1998;2:365-382.
  87. Saloheimo M and Niku-Paavola ML. Heterologous production of a ligninolytic enzyme: expression of the *Phlebiaradiata* laccase gene in *Trichoderma reesei*. *Biotechnology*. 1991;9(10):987-990.
  88. Saloheimo M, Barajas V, Niku-Paavola ML and Knowles JKC. A lignin peroxidase-encoding cDNA from the white-rot fungus *Phlebiaradiata*: characterization and expression in *Trichoderma reesei*. *Gene*. 1989; 85(2):343-351.
  89. Ortiz Escobar ME and Hue NV. Temporal changes of selected chemical properties in three manure—amended soils of Hawaii. *Bioresource Technology*. 2008;99(18):8649-8654.
  90. Tejada M, Gonzalez JL, García-Martínez AM and Parrado J. Application of a green manure and green manure composted with beet vinasse on soil restoration. effects on soil properties. *Bioresource Technology*. 2008;99(11):4949-4957.
  91. Bowen RM and Harper SHT. Decomposition of wheat straw and related compounds by fungi isolated from straw in arable soils. *Soil Biology and Biochemistry*. 1990;22(3):393-399.
  92. Tiwari VN, Pathak AN and Lehri LK. Effect of plant waste incorporation by different methods under uninoculated and inoculated conditions on wheat crops. *Biological Wastes*. 1987;21(4): 267-273.
  93. Abdulla HM and El-Shatoury SA. Actinomycetes in rice straw decomposition *Waste Management*. 2007;27(6):850-853.



94. Fontaine S, Bardoux G, Benest D, Verdier B, Mariotti A and Abbadie L. Mechanisms of the priming effect in a Savannah Soil amended with cellulose. *Soil Science Society of America Journal*. 2004;68(1):125-131.
95. Han W and He M. The application of exogenous cellulase to improve soil fertility and plant growth due to acceleration of straw decomposition. *Bioresource Technology*. 2010;101(10): 3724-3731.
96. De Faveri D, Aliakbarian B, Avogadro M, Perego P and Converti A. Improvement of olive oil phenolics content by means of enzyme formulations: effect of different enzyme activities and levels. *Biochemical Engineering Journal*. 2000;41(2):149-156.
97. Fantozzi P, Petruccioli G and Montedoro G. Trattamenti con additive enzimatiche di paste di olivasottoposteadestrazione per pressioneunica: influenze delle cultivars, dell'epoca di raccolta e dellaconservazione. *Grasse*. 1977;54:381-388.
98. Ranalli A, Pollastri L, Contento S, Lucera L and Del Re P. Enhancing the quality of virgin olive oil by use of a new vegetable enzyme extract during processing. *European Food Research and Technology*. 2003;216(2):109-115.
99. Chiacchierini E, Mele G, Restuccia D and Vinci G. Impact evaluation of innovative and sustainable extraction technologies on olive oil quality. *Trends in Food Science and Technology*. 2007;18(6):299-305.
100. Çinar I. Effects of cellulase and pectinase concentrations on the colour yield of enzyme extracted plant carotenoids. *Process Biochemistry*. 2005;40(2):945-949.
101. Ory R and Angelo AJSt. *Enzymes in Food and Beverage Processing*, American Chemical Society, Washington, DC, USA, 1977.
102. Fennema OW. *Food Chemistry*, Marcel Dekker, New York, NY, USA, 1985.
103. Bassi R, Pineau B, Dainese P and Marquardt J. Carotenoid-binding proteins of photosystem II. *European Journal of Biochemistry*. 1993;212(2):297-303.
104. Saranraj P, Stella D and Reetha D. Microbial cellulases and its applications - A review. *International J of Biochem and Biotech Sci*. 2012;1:1-12.