MICROBIAL CELLULASES AND THEIR APPLICATIONS IN TEXTILE PROCESSING

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Abstract:

Basic and applied research on microbial cellulases has not only generated significant scientific knowledge but has also revealed their enormous potential in biotechnology.At present, cellulases and related enzymes are used in food, brewery and wine, animal feed, textile and laundry, pulp and paper industries, as well as in agriculture and for research purposes. Textile processing is a growing industry that traditionally has used a lot of water, energy and harsh chemicals. Due to the ever-growing costs for water and energy worldwide investigations are carried out to substitute conventional chemical textile processes by environment-friendly and economically attractive bioprocesses using enzymes. Enzymes are specific and fast in action and small amounts of enzyme often savelarge amounts of raw materials, chemicals, energy and water. Indeed, the demand for these enzymes is growing more rapidly than ever before, and this demand has become the driving force for research on cellulases and related enzymes. Conventional chemical processes are generally severe and fibre damage may occur. However, enzymes are characterized by their ability to operate under mild conditions. As a result processes may take place without additional harm to the fibre. Enzymes are also readily biodegradable and therefore potentially harmless and environmentally friendly. This work represents a review of various properties of enzymes and applications of cellulases in textile processing.

Key words: Microbial Cellulases, Bio-stoning, Bio-polishing, Eco-Friendly Characteristics, Water and Energy Saving.

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1.Introduction:

Enzymes are biological catalysts. A catalyst is any substance which makes a chemical reaction go faster, without itself being changed. Enzymes are very much the same except that they can be easily denatured by some means. All enzymes are made of protein and because they are sensitive to heat, pH and heavy metal ions. Unlike ordinary catalysts, they are specific to one chemical reaction. An ordinary catalyst may be used for several different chemical reactions, but an enzyme only works for one specific reaction. Enzymes must have the correct shape to do their job. Enzymes change their shape if the temperature or pH changes, so they have to have the right conditions.

In the conventional textile processing, the grey fabric has to undergo a series of chemical treatments before it turns into a finished fabric. This includes desizing, scouring, mercerization, bleaching and washing. The chemicals used for all these steps are quite toxic.

The use of enzymes in the textile industry is an example of white industrial biotechnology, which allows the development of environmentally friendly technologies in fibre processing and strategies to improve the final product quality. The consumption of energy and raw-materials, as well as increased awareness of environmental concerns related to the use and disposal of chemicals into landfills, water orrelease into the air during chemical processing of textiles are the principal reasons for the application of enzymes in finishing of textile materials [O'Neill et al. 1999].

Cellulases are inducible enzymes synthesized by a large diversity of microorganisms including both fungi and bacteria during their growth on cellulosic materials (Table 1) [Kubicek 1993; Sang-Mok and Koo 2001].

Table 1 –Major microorganisms employed in cellulase production

Microorganisms	
Major group	Genus and Species
Fungi	Soft rot fungi Aspergillusniger; A. nidulans; A. oryzae; A. terreus; Fusariumsolani; F. oxysporum; Humicolainsolens; H. grisea; Melanocarpusalbomyces; Penicilliumbrasilianum; P. occitanis; P.

	decumbans; Trichodermareesei; T. longibrachiatum; T. harzianum; Chaetomiumcellulyticum; C. thermophilum; Neurosporacrassa; P. fumigosum; Thermoascusaurantiacus;Mucorcircinelloides; P. janthinellum; Paecilomycesinflatus; P. echinulatum; Trichoderma atrovirid
	Brown rot fungi Coniophora puteana; Lanzites trabeum; Poria placenta; Tyromyces palustris; Fomitopsis sp.
	White rot fungi Phanerochaete chrysosporium; Sporotrichum thermophile; Trametes versicolor; Agaricus arvensis; Pleurotus ostreatus; Phlebia gigantea
Bacteria	Aerobic bacteria Acinetobacter junii; A. amitratus; Acidothermus cellulolyticus; Anoxybacillus sp.; Bacillus subtilis; B. pumilus; B. amyloliquefaciens; B. licheniformis; B. circulan; B. flexus; Bacteriodes sp.; Cellulomonas biazotea; Cellvibrio gilvus; Eubacterium cellulosolvens; Geobacillus sp.; Microbispora bispora; Paenibacillus curdlanolyticus; Pseudomonas cellulosa; Salinivibrio sp.; Rhodothermus marinus
	Anaerobic bacteria Acetivibrio cellulolyticus; Butyrivibrio fibrisolvens; Clostridium thermocellum; C. cellulolyticum; C. acetobutylium; C. papyrosolvens; Fibrobacter succinogenes; Ruminococcus albus
Actinomycetes	Cellulomonas fimi; C. bioazotea; C. uda; Streptomyces drozdowiczii; S. lividans; Thermomonospora fusca; T. curvata

These microorganisms can be aerobic, anaerobic, mesophilic or thermophilic. Among them, the genera of Clostridium, Cellulomonas, Thermomonospora, Trichoderma, andAspergillus are the most extensively studied cellulose producer [Sunand Cheng 2002; Sukumaran et al. 2005; Kuhad et al. 2010].

Cellulases have been commercially available for morethan 30 years, and these enzymes have represented a targetfor both academic as well as industrial research [Singh 1999; Singh et al. 2007].

In the textile industry especially in the apparel sector, cotton, the king of fibres, is widely used because of its superior properties. Cotton and cotton-blended fabrics are subjected to various wet processing treatments to enhance its value. The conventional methods of wet processing of cotton lead to a number of pollution hazards. A number of environmental regulations are to be fulfilled to safeguard the natural resources and this has led to growth in the use of enzymes in the textile industry.Cellulose is the most abundant and renewable biopolymer on earth and dominating waste material from agriculture [Bhat M.K. and Bhat S. 1997].

Currently, most commercial cellulases (including β-glucosidase) are produced by Trichoderma species andAspergillus species[Cherry and Fidantsef 2003;Esterbauer et al. 1991; Kirk et al. 2002].Cellulases derived from the fungus, Trichoderma reesei, is widely used in textile finishing, since it gives higher yield in industrial production. In addition to cellulases originating from the above fungus, those originating from Humicola insolens can also degrade cotton cellulose efficiently, and they find extensive application in biostoning of denim fabric [Cavaco-Paulo1998;Schulein 1997; Campos et al. 2000;Andreaus et al. 2000].Cellulasesare used in the textile industry for cotton softening anddenim finishing; in the detergent market for color care,cleaning, and anti-deposition; in the food industry formashing; and in the pulp and paper industries for deinking,drainage improvement, and fiber modification [Cherry and Fidantsef 2003; Kirk et al. 2002]. Acid cellulase, when used in biopolishing, offers a number of benefits such as improvement in pill resistance, cooler feel, brighter luminosity of colours and softness, and at the same time the treatment results in certain adverse effects like loss in weight and strength.

Thecellulase market is expected to expand dramaticallywhen cellulases are used to hydrolyze pretreatedcellulosic materials to sugars, which can be fermentedto commodities such as bioethanol and biobasedproducts on a large scale [Cherry and Fidantsef 2003;Himmel et al.1999; van Beilen and Li 2002].

2. Literature Review:

2.1Cotton fiber and his structure:

Cotton, the seed hair of plants of the genus Gossypium, is the purest form ofcellulose readily available in nature. It has many desirable fibre propertiesmaking it an important fibre for textile applications.Cotton is the most important of the raw materials for the textile industry. The cotton fibre is a single biological cell with a multilayer structure The layers in the cell structure are,from the outside of the fiber to the inside,cuticle, primary wall, secondarywall, and lumen. These layers are differentstructurally and chemically [Boylston and Hebert 1995; Jeffries et al.1992].The primary and secondary walls havedifferent degrees of crystallinity, aswell as different molecular chain orientations.The cuticle, composed of wax,proteins, and pectins, is 2.5% of thefiber weight and is amorphous. Theprimary wall is 2.5% of the fiberweight, has a crystallinity index of30%, and is composed of cellulose.The secondary wall is 91.5% of the fiberweight, has a crystallinity index of70%, and is composed of cellulose.The lumen is composed of protoplasmicresidues [Li and Hardin 1997]. Cotton fibres have a fibrillar structure.The whole cotton fibre contains 88 to96.5% of cellulose, the rest are noncellulosicpolysaccharides constituting up to 10% of the total fibre weight[Hartzell and Hsieh1998; Buchert and Pere 2000]. The primary wall in mature fibres is only 0.5-1 µm thick and contains about 50% of cellulose. Noncellulosic constituents consist of pectins, fats and waxes, proteins and natural colorants. The secondary wall, containing about 92-95% cellulose, is built of concentric layers with alternatic shaped twists. The layers consist of densely packed elementary fibrils, organized into micro fibrils and macro fibrils. They are held together by strong hydrogen bonds. The lumen forms the centre of the fibers. Cotton is composed almost entirely of the polysaccharide cellulose. Cotton cellulose consists of crystalline fibrils varying in complexity and length and connected by less organized amorphous regions with an average ratio of about two-thirds crystalline and one-third non-crystalline material, depending on the method of determination [Morton and Hearle 1997].The chemical composition of cellulose is simple, consisting of an hydro glucose units joined by β-1,4-glucosidic bonds to form linear polymeric chains. The chain length, or degree of polymerization (DP), of a cotton cellulose molecule represents the number of an hydro glucose units connected together to form the chain molecule. DP of cotton may be as high as 14 000, but it can be easily reduced to 1000–2000 by different purification treatments with alkali [Nevell 1995]. According to Battista (1950), the crystalline regions probably have a DP of 200 to 300. Correspondingly, the molecular weight

(MW) of cotton usually lies in the range of 50,000–by 1,500,000 depending on the source of the cellulose [Sundquist 1983].

2.2 Cellulose in agricultural wastes

Cellulose is considered as one of the most important sources of carbon on this planet and its annual biosynthesis by both land plants and marine algae occurs in many tones per annum. Numerous agricultural residues generated due to diverse agricultural practices and food processing such as rice straw, yam peels, cassava peels, banana peels among others represents one of the most important energy resources. The major components of these are cellulose and hemicellulose (75-80%) while lignin constitutes only 14%.Agriculture wastes contain a high proportion of cellulosic matter which is easily decomposed by a combination of physical, chemical and biological processes. The bunch consists of 70 moisture and 30% solid[Thambirajah et al. 1995]. Lignin is an integral cell wall constituent, which provides plant strength and resistance to microbial degradation [Sherif et al. 2005]. The recognition that environmental pollution is a worldwide threat to public health has given rise to a new massive industry for environmental restoration. Biological degradation, for both economic and ecological reasons, has become an increasingly popular alternative for the treatment of agricultural, industrial, organic as well as toxic waste. These wastes have been insufficiently disposed leading to environmental pollution.

Recycling of agricultural residue can be achieved naturally and artificially by microorganisms.Aerobic organisms such as fungi, bacteria, and some anaerobic organisms have been shown to be able to degrade some constituents of these residues.

Cellulases are important enzymes not only for their potent applications in different industries, like industries of food processing, animal feed production, pulp and paper production , and in detergent and textile industry, but also for the significant role in bioconversion of agriculture wastes in to sugar and bioethanol.

2.3Cellulases

Cellulases are hydrolytic enzymes that catalyse the breakdown of cellulose to smaller oligosaccharides and finally glucose. Cellulases are enzymes which hydrolyze the β-1,4-glycosidic linkage of cellulose and synthesized by microorganisms during their growth on cellulosic materials [Lee and Koo 2001; Nallankilli 1992]. The complete enzymatic hydrolysis of cellulosic materials needs different types of cellulase; namely endoglucanase, (1,4-D-glucan-4-glucanohydrolase; EC 3.2.1.4), exocellobiohydrolase (1,4-D-glucan glucohydrolase; EC 3.2.1.74) and glucosidase (D-glucoside glucohydrolase; EC 3.2.1.21). The commercially available cellulases are a mixture of enzymes: endogluconases, exogluconases and cellobiases. Endogluconases or endocellulases cleave bonds along the length of cellulose chains in the middle of the amorphous region.Cellobiohydrolases or exocellulases start their action from the crystalline ends of cellulose chains, producing primarily cellobiose. The application of cellulases in textile processing started in the late 1980s with denim finishing. Currently, in addition to biostoning, cellulases are also used to process cotton and other cellulose-based fibres.

Enzymatic process to hydrolyze cellulosic materials could be accomplished through a complex reaction of these various enzymes. Two significant attributes of these enzyme-based bioconversion technologies are reaction conditions and the production cost of the related enzyme system. Therefore, worldwide there has been many research works focused on obtaining new microorganisms producing celluloytic enzymes with higher 105 specific activities and greater efficiency [Johnvesly et al. 2002].

Cellulases are usually classified by the pH range in which they are more effective and, accordingly, acidcellulase, neutral cellulase and alkaline cellulase, commercial products are available.

2.3.1 Acid Cellulases

Acid cellulases are class of enzymes that act at pH 3.8-5.8 and in the temperature range of 30-60 °C. However, they can be further classifiedbased on pH sensitivity. The three different types of acid cellulases and their corresponding operating pHare: standard acid cellulases: pH 4-6.5, modified acid cellulases : pH 5-6.5 and endo-enriched acidcellulases : 4.5-6.5. Acid cellulases in general, are more active on cotton cellulose compared to the neutral cellulases, hence aneffective surface etching is obtained in shorter time. However, severe decomposition may occur if notproperly controlled and it may result in lower tear and busting strength of the treated fabrics. Moreover, acidcellulases have a relatively short shelf life, not exceeding 2-3 months. The stability is low when storagetemperature exceeds 700 Fahrenheit. This may lead to inconsistent storage results during summer months.

Besides these, acid cellulases extract substantial amount of dyestuff from the fabric, particularly indigo dyes(this property is exploited in denim washing) which may in turn deposit on the white portion of the fabric. Thisphenomenon is known as back staining.

2.3.2 Neutral Cellulase

Cellulase enzymes which act at pH 6.0-7.0 and in the temperature range of 40-55 □C are termed as neutral cellulases. They are less reactive and therefore require longer treatment timecompared to the former class. Strength loss and back staining problems are less with this class. Neutralcellulose powders have a longer shelf life and good thermal stability.

2.3.3 Alkali Cellulase

Alkali stable cellulase can be incorporated in household detergent formulations for effective stain removal.

2.4 Microbial fermentation for cellulase production

Cellulases are inducible enzymes which are synthesized by microorganisms during their growth on cellulosic materials [Lee and Koo 2001].Various bacteria, actinomycetes and filamentous fungi produce extra cellular cellulases when grown on cellulosic substrates though many actinomycetes have been reported to have less cellulase activity than moulds. Investigations on the extracellular cellulases of fungi have been concentrated mainly on Trichoderma sp. and studies on other mesophilic fungi suggested the possibility that other cellulase systems could be utilized for the hydrolysis of cellulose [Darmwal et al. 2009].

Microbial production of cellulases utilizes submerged fermentation technology (SmF) and the widely studied organisms used in cellulase production isTrichoderma reesei. Carbon sources in majority of commercial cellulase fermentations are cellulosic biomass including straw, rice or wheat bran, bagasse, paper industry waste and various other cellulosic residues [Belghith et al. 2001; Reczey et al. 1996; Shen and Xia 2004]. The major technical limitation in fermentative production of cellulases remain the increased fermentation times with a low productivity. Solid-state fermentation (SSF) for production of cellulases is rapidly gaining interest as a cost effective technology. Tengerdy (1996) indicated that there was about a tenfold reduction in the production cost in SSF than SmF.Pandey et al. (1999) describes the technology for industrial cellulase production on SSF. Milalaet al. (2009) used rice husk, millet straw, guinea corn stalk and sawdust as fermentation feed substrate for the evaluation of cellulase activity secreted by Aspergillus candidus.

2.5 Applications of cellulases

Cellulases were initially investigated several decades back for the bioconversion of biomass which gave way to research in the industrial applications of the enzyme in animal feed, food, textiles and detergents and in the paper industry. Microbial cellulases find applications in various industries asshown in Table 2.

2.5.1 Applications of cellulases in textile industry

Cellulases have achieved their worldwide success in textile and laundry because of theirability to modify cellulosic fibres in a controlled and desired manner, so as to improve thequality of fabrics. Although, cellulases were introduced in textile and laundry only a decadeago, they have now become the third largest group of enzymes used in these applications [Xia and Cen 1999]. Bio-stoning and bio-polishing are the best-known current textile applications of cellulases(Table 3).Cellulases are used in the bio- stoning of denim garments for producing softness and the faded look of denim garments replacing the use of pumice stones which were traditionally employed in the industry [Belghith et al. 2001;Bhat 2000].Denim is heavy grade cotton. In this dye is mainly adsorbed on the surface of the fibre. That is why fading can be achieved without considerable loss of strength. They act on the cellulose fiber to release the indigo dye used for coloring the fabric producing the faded look of denim. Humicola insolens cellulase is most commonly employed in the equally good cellulases are utilized for digesting off the small fiber ends protruding from the fabric resulting in a better finish [Cortez et al. 2001].Cellulases have also been used in softening defibrillation , and in processes for providing localized variation in the color density of fibers [Galante&Formantici 2003].

Cellulases are the most successfulenzymes used in textile wet processing, especially finishingof cellulose-based textiles, with the goal of improved handand appearance [Hebeish and Ibrahim 2007; Karmakarand Ray 2011]. In traditional process sodium hypochlorite or potassium permanganate was used called as pumice stones. Disadvantage of these method are as follows:

- Pumice stones cause large amount of Back-staining;
- Pumice stones are required in very large amount;
- They cause considerable wear and tear of machine.

Traditional stonewashing of jeansinvolves 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.Cellulaseshave been successfully used for the biostoning of jeans andbiopolishing of cotton and other cellulosic fabrics. Duringthe biostoning process, cellulases act on the cotton fabricand break off the small fiber ends on the yarn surface,thereby loosening the dye, which is easily removed bymechanical abrasion in the wash cycle. The advantages in thereplacement of pumice stones by a cellulose-based treatmentinclude less damage of fibers, increased productivity of themachines, and less work-intensive and environment benign[Sukumaran et al. 2005; Singh et al. 2007; Uhlig 1998; Galante et al. 1998].

Biopolishing is a finishing process that improves fabric quality by mainly reducing fuzziness and pilling property of cellulosic fibre. The objective of the process is elimination of micro fibrils of cotton through the action of cellulose enzyme.

Table 2: Applications of cellulases in various industries

Industry	Applications
Agriculture	Plant pathogen and disease control; generation of plant and fungalprotoplasts; enhanced seed germination andimproved root system; enhanced plant growth and flowering; improved soil quality; reduced dependence onmineral fertilizers
Bioconversion	Conversion of cellulosic materials to ethanol, other solvents, organic acids and single cell protein, and lipids;production of energy-rich animal feed; improved nutritional quality of animal feed; improved ruminantperformance; improved

	feed digestion and absorption; preservation of high quality fodder
Detergents	Cellulase-based detergents; superior cleaning action without damaging fibers; improved color brightness anddirt removal; remove of rough protuberances in cotton fabrics; antiredeposition of ink particles
Fermentation	Improved malting and mashing; improved pressing and color extraction of grapes; improved aroma of wines;improved primary fermentation and quality of beer; improved viscosity and filterability of wort; improved mustclarification in wine production; improved filtration rate and wine stability
Food	Release of the antioxidants from fruit and vegetable pomace; improvement of yields in starch and proteinextraction; improved maceration, pressing, and color extraction of fruits and vegetables; clarification of fruitjuices; improved texture and quality of bakery products; improved viscosity fruit purees; improved texture,flavor, aroma, and volatile properties of fruits and vegetables; controlled bitterness of citrus fruits
Pulp and Paper	Coadditive in pulp bleaching; biomechanical pulping; improved draining; enzymatic deinking; reduced energyrequirement; reduced chlorine requirement; improved fiber brightness, strength properties, and pulp freenessand cleanliness; improved drainage in paper mills; production of biodegradable cardboard, paper towels, andsanitary paper
Textile	Biostoning of jeans; biopolishing of textile fibers; improved fabrics quality; improved absorbance propertyof fibers; softening of garments; improved stability of cellulosic fabrics; removal of excess dye from fabrics;restoration of colour brightness
Others	Improved carotenoids extraction; improved oxidation and colour stability of carotenoids; improved olive oilextraction; improved malaxation of olive paste; improved quality of olive oil; reduced risk of biomass waste;production of hybrid molecules; production of designer cellulosomes

Table 3 Cellulases in textile and laundry biotechnology

Enzyme	Function	Application	Reference
Cellulase, preferably neutraland endoglucanase rich	Removal of excess dye fromdenim fabrics; soften the cottonfabrics without damaging the	Bio-stoning of denim fabrics; production of highquality and environmentallyfriendly washing powders	Galante et al.,1998; Godfrey,1996; Uhlig, 1998

	fibre		
Cellulase, preferably acid andendoglucanase rich	Removal of excess microfibrils from the surface of cotton and non-denim fabrics	Bio-polishing of cotton and non-denim fabrics	Galante et al.,1998; Godfrey,1996; Kumar et al.,1994, 1996
Cellulase, preferably endoglucanase rich	Restoration of softness and colour brightness of cotton fabrics	Production of high qualityfabrics	Galante et al.,1998; Godfrey,1996; Kumar et al.,1994

The acidic cellulases, when used in biopolishing, offers a number of benefits such as improve softness and water absorbance property of fibres,strongly reduce the tendency for pill formation, and providea cleaner surface structure with less fuzz [Sreenath et al. 1996].

The main characteristics imparted to the fabric during Biopolishing treatment are as follow:

- Cleaner surface is obtained conferring a cooler feel;
- Lustre is obtained as a side effect;
- Fabric obtains softer feel;
- Tendency of the fabric to pill ends.

These disadvantages lead to give rise the process of use of enzymes.

3. Future Directions:

The progress in biotechnology of cellulases and related enzymes is truly remarkable and attracting worldwide attention. Currently, cellulases, hemicellulases and pectinases arewidely used in food, brewery and wine, animal feed, textile and laundry, paper and pulp industriesas well as in research and development. Some of these applications prefer one or two selected components of cellulase, hemicellulase or pectinase, while others require mixturesof cellulases, hemicellulases and pectinases for maximum benefit.

Hence, to meet the growing demand for cellulases and related enzymes and to realize their full potential in biotechnology and research, continued multidisciplinary research on basic and applied aspects is vital. These developments together with improved scientific knowledge are expected to pave the way for a remarkable success in the biotechnology of cellulases and related enzymes in the 21st century.

Today, white biotechnology is geared towards creating new materials and biobased fuels from agricultural waste and providing alternative biobased routes to chemical processes. These efforts could lead to the development of improved enzymes such as amylases, hemicellulasesorcellulases that could be used in the textile industry. The possibility of leveraging innovations over industries could lead to new opportunities for biobased textile processes. Enzymes can be used in order to develop environmentally friendly alternatives to chemical processes in almost all steps of textile fibre processing. There are already some commercially successful applications, such as amylases for desizing, cellulases and laccases for denim finishing, and proteases incorporated in detergent formulations. New enzymes with high specific activity, increased reaction speed, and tolerance to more extreme temperatures and pH could result in development of continuous processes for bioscouring or biofinishing of cellulosic fibres. Development of other processes in the future could also expand the use of enzymes on natural fibers into use on man-made fibers such as nylon and polyester.

The use of enzymes not only make the process less toxic (by substituting enzymatic treatments for harmful chemical treatments) and eco-friendly, they reduce costs associated with the production process, and consumption of natural resources (water, electricity, fuels), while also improving the quality of the final textile product.

There is still considerable potential for new and improved enzyme applications in future textile processing. Advances in enzymology, molecular biology and screening techniques provide possibilities for the development of new enzyme-based processes for a more environmentally friendly approach in textile industry. It seems that in the future it will be possible to do every process using enzymes.

4. Conclusion:

Fungi are the main cellulase producing microorganism and Aspergillus and Trichoderma are the main fungal genera that were used for commercial production of cellulase. Therefore the ability of microorganisms to synthesize high amount of extra cellular exoglucanase within a relatively short period of time, utilizing agro wastes that would otherwise cause environmental pollution, could be used for rapid and commercial production of cellulose.

The use of various enzymes is in the early stages of development but their innovative applications are increasing and spreading rapidly into all areas of textile processing. The textile industry can greatly benefit from the expanded use of these enzymes as non-toxic, environmentally friendly compounds.

Enzymes are a sustainable alternative to the use of harsh chemicals in industry and reduce energy and water consumption, and chemical waste production during manufacturing processes. Cellulases are being commercially produced by several industries globally and are widely being used in food, animal feed, fermentation, agriculture, pulp and paper, and textile applications. With modern biotechnology tools, especially in the area of microbial genetics, novel enzymes and new enzyme applications will become available for the various industries.

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