# Lignocellulose Biotechnology

## Future Prospects

*Editors*

**R.C. Kuhad • Ajay Singh**

**I.K. International**

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# Foreword

It gives me immense pleasure to write the foreword for *Lignocellulose Biotechnology: Future Prospects* edited by Ramesh Chander Kuhad and Ajay Singh. My interest in lignocellulose biotechnology started in early seventies, when it was realized that if Indian livestock have to produce to this optimal level the only source of carbohydrates was from plant cell wall through the crop residues. They have to become a staple diet of all farm livestock as they are the only source of carbohydrates available to them. The crop residues can only become available source of energy if microbes can be used to release carbohydrates after breakdown of lignin ring in the plant cell wall.

This initial interest in the area of renewable energy resources and agro-industrial waste utilization became intense as new tools of biotechnology became available. Energy is the most fundamental requirement in our life. Due to increasing use of the conventional energy sources such as fossil fuels, hydro and nuclear energy, the supply of such resources is likely to be limited in future. The potential to use lignocellulosic biomass for energy production derives from its position as the most abundant and renewable organic material in the biosphere, accounting for approximately 50% of world biomass. Potential feedstock may include food crops, crop residues and woody biomass. The low cost and chemical composition of crop residues make them attractive as feedstock for production of energy, food, feed and industrial chemicals.

It is difficult to accurately estimate the quantities of agricultural waste and crop residues produced worldwide. According to an estimate of Food and Agriculture Organization, about three billion tons of cereal straws are produced annually in the world. Annual volumes of wastes from pulse crops, oilseeds and plantation crops amount to an almost a billion tons per annum. Sugarcane bagasse can also be a desirable feedstock for the production of industrial chemicals and bioethanol. Brazil and India are the world's two largest sugarcane growers with production of 300 and 285 million tons per year, respectively. Estimates of total residues produced in the US are 375 million dry tons per year. This estimate only includes corn, small grains, sorghum, rice and sugarcane crop residues and does not include soybean and cotton field residues which are difficult to collect.

However, there are numerous challenges in dealing with any waste processing technology. Since type and availability of agricultural and forestry wastes vary with geographic region, climate, environmental conditions, agricultural and processing practices, generic processes for bioconversion of lignocellulosic wastes to industrial products must be versatile and robust as well as cost effective. The main factor that affects the biotechnology of commodity products is the high cost of current processing technology rather than the cost of raw materials. Challenges to development of a cost competitive process may be grouped in terms of converting lignocellulose into reactive intermediates by overcoming the recalcitrance and converting reactive intermediates into useful products, using all the sugars and byproduct value recovery of non-sugars.

*Lignocellulose Biotechnology: Future Prospects*, discusses a wide range of topics related to the fundamental and applied aspects of lignocellulose utilization, processing and biotechnology. The book contains a range of topics including biodiversity of lignocellulolytic microorganisms and their enzymes, molecular biology relevant to biodegradation, characterization of lignocellulolytic enzymes, bioconversion of cellulosic material to produce enzymes, animal feed, bioethanol and xylitol and industrial applications of cellulases and xylanases. Chapters dealing with industrial applications address current biotechnological approaches in lignocellulose bioconversion to value added products.

The book has contributions from the scientists from different disciplines including microbiology, biochemistry, molecular biology, genetics and biochemical engineering with diverse backgrounds; universities, government laboratories and industry. The editors Prof. Kuhad and Singh have done a commendable job in bringing together a range of excellent papers from these experts in the field of lignocellulose biotechnology and shaping them to provide a unique piece of work in the form of this book. I am confident that the book should prove to be very useful to the students, teachers, scientists and engineers in the disciplines of biotechnology, life sciences, microbiology, biochemistry, and environmental sciences and engineering.

**Prof. P.N. Bhat**
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# Preface

The residues from agricultural crops and agro- and forest-based industrial processes are abundant and along with grasses and weeds constitute the major part of the biomass available on earth representing an important energy and material resource. Life on earth depends on photosynthesis, resulting in production of plant biomass having cellulose as the major component. Lignocellulose, the major component of biomass, makes up about half of the matter produced by photosynthesis. The carbon cycle is closed primarily as a result of the action of cellulose-utilizing microorganisms present in soil and the guts of animals. The plant biomass consists of three types of polymer—cellulose, hemicellulose, and lignin—that are strongly bonded by non-covalent forces and by covalent cross-linkages. In nature, cellulose, lignocellulose and lignin are major sources of plant biomass, and therefore their recycling is indispensable for the carbon cycle. Each polymer is degraded by a variety of microorganisms through production of hydrolytic and oxidative enzymes that work synergistically.

Several biological methods for utilizing or recycling of lignocellulosic biomass have been suggested, including composting and their use as raw material for the production of ethanol as an alternative combustible seem to be the most economically feasible. The general use of alternative, environment-friendly technologies that introduce lignocellulose-degrading enzymes at different stages of pulp and paper manufacture as a pretreatment to pulping (biopulping), bleaching (biobleaching), or wastewater treatment has allowed considerable electrical power savings and a reduction of pollutants in the waste water from these industries. In addition, pretreatment of agricultural wastes with ligninolytic fungi enables their use as raw material for paper manufacturing. The use of microorganisms or their enzymes to enhance the de-inking of recycled fibres and the release of toners from office wastes is another promising field that is under research.

Many research groups worldwide are involved in studying different aspects of natural biodegradation of these compounds. Our current knowledge is quite advanced regarding enzymology, physiology, biochemistry, and molecular biology of lignocellulolytic microbes. Bioprocesses utilizing enzymes and microorganisms are being explored for their biotechnological applications. Microbiological conversion of lignocellulosic residues to various industrially useful products like hydrolytic and oxidative enzymes, organic acids, ethanol, fine chemicals, microbial lipids, protein and animal feed can be achieved through the efficient utilization of all the three fractions of biomass—cellulose, hemicellulose and lignin. Production of ethanol and other alternative fuels from lignocellulosic biomass can reduce urban air pollution, decrease the release of carbon dioxide in the atmosphere, and provide new options for agricultural wastes utilization and recycling. Biopulping and biobleaching are leading to cleaner and more efficient pulp and paper manufacture.

Despite the progress achieved, more effort is needed for lignocellulosic enzymes and/or microorganisms to have significant industrial impact.

*Lignocellulose Biotechnology : Future Prospects* addresses a wide range of topics related to the basic, applied and biotechnological aspects of lignocellulose bioconversion. The book is divided into four major sections: (1) Overview of Lignocellulose Biotechnology; (2) Lignocellulolytic Microorganisms and Enzymes; (3) Lignocellulose Bioconversion; and (4) Biotechnological Applications. Topics include lignocellulose resource management, diversity of lignocellulolytic microorganisms, fundamental and applied aspects of lignocellulolytic enzymes and bioconversion of lignocellulosic residues for production of enzymes, feed and chemicals. Several chapters cover applications of lignocellulolytic microbes and enzymes in food, pulp and paper chemical and textile industries.

The book contains contributions from leading scientists with diverse backgrounds—universities, institutions and industry who are involved in the microbiological, molecular, biochemical and biotechnological aspects of lignocellulose biodegradation and utilization research. This book should be of immense use to the students of biotechnology, microbiology, biochemistry, environment sciences, food sciences and animal feed sciences. This book will certainly be of equal interest to the teachers, scientists and engineers, whether in academia, industry or government directly involved in the research or want to learn about biomass utilization and lignocellulosic biotechnology.

We are grateful to all the authors for their excellent contributions.

**Ramesh Chander Kuhad**
**Ajay Singh**

# Contents

# PART I

## OVERVIEW OF LIGNOCELLULOLYTIC BIOTECHNOLOGY

# 1

# Lignocellulolytic Microorganisms, their Enzymes and Possible Biotechnologies based on Lignocellulolytic Microorganisms and their Enzymes

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Lignocellulose, the major component of biomass, makes up about half of the matter produced by photosynthesis. The plant biomass consists of cellulose, hemicellulose and lignin, which are strongly bonded by non-covalent cross linkages. Each of the lignocellulosic component is degraded by a variety of microorganisms through production of various hydrolytic and oxidative enzymes. The research advancement about lignocellulolytic microorganisms and their enzymes have proved their potential in animal feed, biotransformation, bioremediation, production of chemicals, stabilization of food beverages and paper manufacture etc. This chapter deals with diversity of lignocellulolytic microorganisms and their enzymes. The possible biotechnologies based on these organisms and enzymes are also discussed.

## INTRODUCTION

Lignocellulosics in the form of agricultural and forestry residues are the most abundant and inexhaustible or renewable natural resource. There are great possibilities for employing biotechnology if the lignocellulosics is taken as the raw material. Lignocellulose is the major structural component of woody plants and non-woody plants such as grass. It consists of lignin, hemicellulose and cellulose. The chemical properties of the components of lignocellulosics make them a substrate of enormous biotechnological value. Improvement in many processes related to lignocellulose biotechnology has appeared in the past few years.

The huge amounts of residual plant biomass considered "waste", which is generally burnt, can potentially be converted into different value added products including biofuels, chemicals, and cheap carbon sources for fermentation, improved animal feeds and human nutrients by cellulose-degrading

microorganisms. Enzymes produced by these microbes also have significant applications in various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture. Microbial degradation of lignocellulosic residues and potential biotechnological applications are discussed in this chapter.

## Composition of Lignocellulosic Biomass

The composition of lignocellulosic biomass varies; the major components being cellulose (35-50%), followed by hemicellulose (20-35%) and lignin (10-25%) (Sun and Cheng, 2002). Proteins, essential oils and ash make up the remaining fraction. The structure of these materials is very complex and native biomass is generally resistant to enzymatic hydrolysis. Cellulose fibres are embedded in a lignin–polysaccharide matrix. Xylan may play a significant role in the structural integrity of cell walls by both covalent and non-covalent associations (Saha, 2003). The composition of hardwoods and softwoods are significantly different. The lignin content of softwoods is generally higher than that of hardwoods, whereas hemicellulose content of hardwoods is higher than the softwoods.

The cellulose molecule is a homopolymer of glucose units linked with β-1,4-glucosidic units, although the true repeating stereochemical unit of cellulose is cellobiose (β-1,4-D-glucosyl-D-glucose). Glucose and cellodextrins are the products when cellulose is hydrolyzed (Gilbert *et al.,* 1983). The cellulose molecule is a polymer with a degree of polymerization of up to about 15,000. The degree of crystallinity of cellulose may vary from 0% (amorphous) to approximately 100% (Beguin *et al.,* 1994). Crystalline cellulose is highly resistant to microbial attack and enzymatic hydrolysis, whereas amorphous cellulose is degraded at a much faster rate (Eriksson *et al.,* 1990).

Hemicelluloses are easily hydrolyzed short chains of branched heteropolysaccharides composed of both hexoses and pentoses (Whistler *et al.,* 1970). D-Xylose and L-arabinose are the major constituents of the pentosans (xylans), while D-glucose, D-galactose and D-mannose are the constituents of the hexosans (mannans). The major hemicellulose components in softwood are mannan-based, and those in hardwood are xylan-based. The principal sugar components of these hemicellulose heteropolysaccharides are: D-xylose, D-mannose, D-glucose, D-galactose, L-arabinose, D-glucuronic acid, 4-O-methyl-D-glucuronic acid, D-galactouronic acid, and to a lesser extent, L-rhamnose, L-fucose and various O-methylated sugars. They usually have degree of polymerization of 100 to 200 (Kuhad *et al.,* 1997a).

The mannan hemicelluloses, galactoglucomannans and glucomannans, in softwoods and hardwoods, are both branched heteropolysaccharides. Their backbones are built up of 1, 4-linked β-D-glucopyranose and β-D-mannopyranose units. The mannose and glucose units in the backbone are partially substituted in C-2 and C-3 positions by acetyl groups (Eriksson *et al.,* 1990).

Xylan is the most abundant hemicellulose and it comprises 15-30% of annual plants, 20-25% of hardwoods, and 7-12% of softwoods. Xylans have homopolymeric backbone chains of 1, 4-linked β-D-xylopyranose units. Besides xylose, xylans may contain arabinose, glucuronic acid or its 4-O-methyl ether, and acetic, ferulic, and *p*-coumaric acids. The frequency and composition of the branches are dependent on the source of xylan. The backbone consists of *O*-acetyl, α-L-arabinofuranosyl, α-1, 2-linked glucuronic or 4-O-methylglucuronic acid substituents (Kuhad *et al.,* 1997a).

Lignins are highly branched polymeric molecules consisting of phenyl-propane-based monomeric units linked together by different types of bonds, including alkyl-aryl, alkyl-alkyl, and aryl-aryl ether bonds. The molecular weight of lignins may be 100 kDa or more. The relative proportions of three cinamyl alcohol precursors incorporated into lignin, i.e., *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, vary not only with the plant species but also with the plant tissues and location of the lignins within the plant cell wall. Lignin is found in the highest concentration in the middle lamella, but is most abundant in the secondary walls of the vascular plants. The hydrolysable linkages in lignins are suggested to be of two types: β-aryl ether and α-aryl ether (Adler, 1977). The predominant β-aryl ether type bond is more resistant to cleavage. Under mild hydrolytic conditions, the cleavage of the ether bond is exclusively restricted to the α-aryl ether type (Kirk *et al.,* 1987).

Plant cell walls contain three kinds of structural proteins, which differ in amino acid composition are hydroxyproline-rich glycoproteins, glycine-rich proteins and proline-rich proteins, have been found in plants (Cassab and Varner, 1988). All are considered to be important structural components of plant cell walls.

Plant cell walls also contain extraneous materials, including extractives and non-extractives (Ladisch *et al.,* 1983). The extractives can be broadly divided into three groups, i.e., terpenes (isoprene polymers), resins (fatty acids, alcohol, resin acids) and phenols (tannins). In addition, low molecular weight carbohydrates, alkaloids, gums, and various other cytoplasmic constituents are present (Akin *et al.,* 1995). The non-extractives make up 0.2-0.8% of the dry weight and include inorganic components such as silica, carbonates, oxalates and non-cell wall substances such as starch, pectin and protein (McDonald, 1969).

## Microorganisms and their Lignocellulolytic Enzymes

A diverse spectrum of lignocellulolytic microorganisms, mainly fungi and bacteria have been isolated and identified over the years and this list still continues to grow rapidly. Despite the impressive collection of lignocellulolytic microorganisms only a few have been studied extensively. The exact mechanism by which lignocellulose is degraded enzymatically is still not fully understood but significant advances have been made to gain insight into the lignocellulolytic microorganisms, their genes and various enzymes involved in the process.

## Microorganisms Producing Cellulose-degrading Enzymes

A large number of microorganisms including fungi, bacteria and actinomycetes have the ability to produce cellulose-degrading enzymes. But few of them produce all the necessary enzymes for degradation of crystalline cellulose. Fungi are the most studied organisms with respect to degradation of cellulose and production of cellulolytic enzymes. Different categories of microorganisms with lignocellulolytic activity are briefly described in this section. Classification of cellulases capable of hydrolyzing 1, 4-β-glucosidic bonds is provided in Table 1.

**Table 1. Classification of cellulases as enzymes capable of hydrolyzing 1,4-b-glucosidic bonds**

| *Type and origin of the enzyme* | *EC number* | *Family* |
|---|---|---|
| Fungal endo-1,4-β-mannanases, and aerobic and anaerobic bacteria endo-glucanases | 3.2.1.78<br>3.2.1.4 | 5/A1 |
| Endoglucanases of actinomycetes, and aerobic and anaerobic bacteria, as well as nematodes | 3.2.1.4 | 5/A2 |
| Exo-1,3-β-glucanases;<br>Endoglucanases/1,3-1,4-β-glucanases and<br>1,3-β-glucanases (yeast, *Clostridium*) | 3.2.1.58<br>3.2.1.4/73<br>3.2.1.39 | 5/A3 |
| Endoglucanases and mannanases of actinomycetes, aerobic and anaerobic bacteria, and anaerobic fungi | 3.2.1.4<br>3.2.1.78 | 5/A4 |
| Endoglucanases of filamentous fungi and aerobic bacteria | 3.2.1.4 | 5/A5 |
| Endo-1,6-β-glucanases | 3.2.1.75<br>3.2.1.123 | 5 |

Soft-rot fungi mainly degrade the polysaccharides. The best known of these producing a complete set of cellulases is *Trichoderma viride* (Bisaria *et al.,* 1989). Other well-known fungi causing soft-rots are *Aspergillus niger*, *Chaetomium cellullolyticum*, *Fusarium oxysporium*, *Neurospora crassa*, *Penicillium pinophilum* (Singh and Hayashi, 1995).

Brown-rot fungi causing brown rot in plants degrade cellulose rapidly but the enzyme system seems to operate differently from those of soft-rot fungi and white-rot fungi. *Poria placenta*, *Lanzites trabeum*, *Tyromyces palustris* and *Coniophora puteana* are brown-rot fungi among the most studied for their cellulolytic activities (Eriksson *et al.,* 1990).

White-rot group of fungi cause white-rot in plants is rather heterogeneous, but these fungi have, in common, the ability to degrade lignin as well as other lignocellulosic components (Eriksson, 1981). The most studied white-rot fungi were *Phanerochaete chrysosporium* that was first isolated from wood chip piles. Other important white-rot fungi are *Sporotrichum thermophile* and *Coriolus versicolor*.

Rumen fungi secrete cellulases, often described as a complex of enzymes that, by acting together, solubilize cellulose efficiently (Wood, 1991). Some fungal species of relevance are *Sphaeromonas communis*, *Neocallimastrix frontalis*, *N. patriciarum* and *Piromyces communis*.

Cellulolytic enzyme systems of bacteria are not directly comparable to those of fungi. Bacteria often produce cellulases in small amounts and degradation of cellulose seems to take place by a cluster of multi-enzyme complexes, which are difficult to disrupt without loss of total activity as well as of the individual components (Lamed *et al.,* 1993; Rabinovich, 2002a). The most studied bacteria with respect to their cellulase systems are species of *Clostridium*, *Cellulomonas*, *Bacillus* and *Pseudomonas*. Other bacteria producing cellulosome like entities include *Acetovibrio cellulolyticus*, *Bacterioides cellulosolvens*, *Butyrivibrio fibrisolvens*, *Fibriobacter succinogenes*, *Ruminococcus albus*, *R. flavefaciens* and *Thermomonospora curvata* (Lamed *et al.,* 1993).

Extracellular cellulases of actinomycetes degrade cellulose by mechanisms similar to that of fungi, i.e., non-associated enzymes excreted into the culture medium. Some of important members of actinomycetes responsible for the degradation of cellulose are mesophilic species of *Streptomyces* and thermophilic species of *Thermomonospora* and *Thermoactinomyces* (Stutzenberger *et al.,* 1986; Calza *et al.,* 1985).

## Microorganisms Producing Hemicellulose-degrading Enzymes

Hemicellulose-degrading system is widespread as they are produced by fungi, bacteria from terrestrial and marine environments, rumen microorganisms, yeasts and marine algae. Fungi, yeasts and some bacteria secrete hemicellulolytic enzymes extracellularly, although cell wall bound and intracellular hemicellulolytic enzymes have also been reported (Dekker, 1985; Kuhad *et al.,* 1997a). Table 2 lists some important microorganisms producing hemicellulose-degrading enzymes. Only a few yeast species such as some species of *Aureobasidium*, *Cryptococcus* and *Trichosporon* are known to produce xylan-degrading enzymes and are therefore identified as xylanase producers (Eriksson *et al.,* 1990).

**Table 2. Important microorganisms producing hemicellulose-degrading enzymes**

| *Enzyme* | *Bacteria* | *Fungi* |
|---|---|---|
| β-1,4-Xylanases | *Bacillus pumilus* | *Aspergillus niger* |
| | *Bacillus subtilis* | *Fusarium oxysporum* |
| | *Clostridium acetobutylicum* | *Aspergillus wentii* |
| | *Cellulomonas uda* | *Trichoderma koningii* |
| | *Streptomyces xylophagus* | *Neurospora crassa* |
| β-1,4-Xylosides | *Clostridium thermocellum* | *Aspergillus niger* |
| | *Bacillus pumilus* | *Corticium rolfsii* |
| | *Acetovibrio cellulolyticus* | *Penicillium wortmanni* |
| | | *Trichoderma reesei* |
| α-Arabinosidase | *Streptomyces pupurascens* | *Aspergillus niger* |
| | *Bacillus subtilis* | *Corticium rolfsii* |
| | *Ruminococcus albus* | *Trichoderma reesei* |
| α-Glucuronidase | | *Trichoderma reesei* |
| | | *Agaricus bisporus* |
| | | *Pleurotus ostreatus* |
| Esterases | *Fibrobacter succinogenes* | *Aspergillus niger* |
| | *Bacteroides cellulosolvens* | *Aspergillus phoenicus* |
| | *Costridium thermocellum* | *Trichoderma reesei* |
| β-1,4-Mannanases | *Aerobacter aerogenes* | *Aspergillus niger* |
| | *Bacillus subtilis* | *Thielavia terrestris* |
| | *Caldocellum saccharolyticum* | *Trichoderma reesei* |
| | *Streptomyces lividans* | *Paecilomyces variotii* |
| β-1,4-Mannosidase | *Bacillus subtilis* | *Aspergillus niger* |
| | | *Aspergillus awamori* |
| | | *Thielavia terrestris* |
| | | *Polyporus sulphureus* |
| α-Galactosidase | *Bacillus subtilis* | *Aspergillus niger* |
| | *Cellulomonas sp.* | *Sclerotium rolfsii* |
| | | *Aspergillus tamarii* |
| | | *Mortierella vinacea* |

## Microorganisms Producing Lignin-degrading Enzymes

Lignin is degraded to different extents by different microorganisms, of which wood-rotting fungi are the most effective, white rot fungi in particular (Eriksson *et al.,* 1990).

Soft-rot fungi efficiently degrade wood polysaccharides but degrade lignin slowly and incompletely (Janshekar *et al.,* 1983). They have been observed more commonly on hardwoods than on softwoods.

Some soft-rot fungi with lignin degrading ability are *Chaetomium globosum*, *Daldinia concentrica*, *Lecythophora hoffmannii*, *Petrillidium boydii* and *Pialophora mutabilis*.

Brown-rot fungi prefer coniferous wood. These fungi cause extensive degradation of the polysaccharides and only a limited degradation of lignin. These are some brown-rot fungi, which have the capability of degradation of lignin *Fomitopsis pinicola*, *Gleophyllum trabeum*, *Poria placenta*, *Lentinus lepideus*, *Pholiota adipose* and *Tyromyces palustris* (Kuhad *et al.,* 1997a).

White-rot fungi are the most effective wood-rotting fungi. These predominanly degrade wood from deciduous trees but coniferous wood trees are also degraded (Eriksson *et al.,* 1990). Most white-rot fungi degrade wood by a simultaneous attack on the lignin, cellulose and hemicelluloses, but a few are rather specific lignin degraders (Blanchette *et al.,* 1992). There are a number of white-rot fungi studied for the ligninolytic activity, some of which are *Coriolus versicolor*, *Cyathus bulleri*, *Phlebia radiata*, *Pycnoporus sanguineus*, *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *Cereporiopsis subvermispora* and *Pleurotus eryngii*.

Bacteria generally cause a low percentage of mineralization of lignin in lignocellulosic materials. A number of gram-negative bacterial species belonging to the genera *Pseudomonas*, *Xanthomonas*, *Acinetobacter*, *Achromobacter*, *Agrobacterium*, *Aerobacter* and *Erwinia* (Zimmerman, 1990).

Actinomycetes have been reported to mineralize lignin successfully, although not as fast or comprehensively as white-rot fungi. The degradation of lignin results in the release of lignin-rich, water-soluble fragments called acid precipitable polymeric lignin (APPL) (Eriksson *et al.,* 1990). Some of the most active lignin degraders among actinomycetes are *Streptomycetes badius*, *S. cyaneus* and *Thermomonospora mesophila* (Kuhad *et al.,* 1997a).

## Biodegradation of Lignocellulosic Biomass

Of the three components, lignin is the most recalcitrant to degradation whereas cellulose is more resistant to hydrolysis as compared to hemicellulose. Alkaline and acid hydrolysis methods have been used to degrade lignocellulose. Weak acids tend to remove lignin but result in poor hydrolysis of cellulose whereas strong acid treatment occurs under relatively extreme corrosive conditions of high temperature and pH, which necessitate the use of expensive equipment.

Microorganisms such as fungi and bacteria both secrete cellulases, endoglucanases and exoglucanases, which act synergistically to degrade cellulose. Endoglucanases hydrolyze β-1,4-glucosidic bonds in a random fashion over a cellulose chain. As a result, there is a rapid decrease in chain length and a slow increase in reducing end groups. Cellobiohydrolases degrade cellulose by splitting off cellobiose from the non-reducing end of the chain (Rabinowich *et al.,* 2002b). β-Glucosidases, secreted by cellulolytic organisms, catalyze the hydrolysis of water-soluble cellodextrins as well as alkyl- and aryl-β-D-glucosides.

Endoxylanase, the most widely studied and best-characterized xylanolytic enzyme, attacks the xylan backbone to produce both substituted and non-substituted shorter oligomers, xylobiose and xylose. β-xylosidase is employed to convert oligomers to xylose and acts in concert with endoxylanases, α-glucuronidase, α-arabinosidase and acetyl-xylan esterase to achieve total hydrolysis of xylans to monosaccharides (Biely, 1985). Endomannases hydrolyze β-D-mannose and a series of mannose oligosaccharides of DP 2-6 (Ishihara, 1980). Table 3 lists important hemicellulose degrading enzymes.

**Table 3. The major hemicellulases and their classification**

| *Enzymes* | *Substrates* | *EC number* | *Family* |
|---|---|---|---|
| Exo-β-1,4-xylosidase | β-1,4-Xylooligomers xylobiose | 3.2.1.37 | GH 3<br>39<br>43<br>52<br>54 |
| Endo-b-1,4xylanase | β-1,4-Xylan | 3.2.1.8 | GH 5<br>8<br>10<br>11<br>43 |
| Exo-β-1,4-mannosidase | β-1,4-Mannooligomers mannobiose | 3.2.1.25 | GH 1<br>2<br>5 |
| Endo-β-1,4-mannanase | β-1,4-Mannan | 3.2.1.78 | GH5<br>26 |
| Endo-α-1,5-arabinanase | α-1,5-Arabinan | 3.2.1.99 | GH43 |
| α-L-arabinofuranosidase | β-Arabinofuranosyl (1→ 2) or (1→ 3) xylooligomers α-1,5-arabinan | 3.2.1.55 | GH3<br>43<br>51<br>54<br>62 |
| α-glucuronidase | 4-O-Methyl-α-glucuronic acid (1→ 2) xylooligomers | 3.2.1.139 | GH 67 |
| α-galactosidase | α-Galactopyranose (1→ 6) mannooligomers | 3.2.1.22 | GH 4<br>27<br>36<br>57 |
| Endo-galactanase | β-1,4-Galactan | 3.2.1.89 | GH 53 |
| β-Glucosidase | β-Glucopyranose (1→ 4) mannopyranose | 3.2.1.21 | GH 1<br>3 |
| Acetyl xylan esterases | 2-or 3-o-Acetyl xylan | 3.1.1.72 | CE 1<br>2<br>3<br>4<br>5<br>6 |
| Acetyl mannan esterase | 2-or 3-O-Acetyl mannan | 3.1.1.6 | CE 1 |
| Ferulic and p-coumaric acid esterases | 2-or 3-O-Acetyl mannan | 3.2.1.73 | CE 1 |

Lignin peroxidase (ligninase) catalyzes a large variety of reactions, e.g. cleavage of β-O-4 ether bonds and $C_\alpha - C_\beta$ bonds in dimeric lignin model compounds, the basis for the depolymerization reactions catalyzed by LiP. The enzyme also catalyzes decarboxylation of phenylacetic acids, oxidation of aromatic $C_\alpha$-alcohols to $C_\alpha$-oxo compounds, hydroxylation, quinone formation, and aromatic ring opening (Haemmerli *et al.,* 1987). Manganese peroxidase is involved in the oxidation of phenols and phenolic ring structures (Wariishi *et al.,* 1992). Laccase catalyzes demethoxylation reactions of terminal phenolic units. It can also degrade β-dimers and β-O-4 dimers via α-oxidation of alkyl-aryl cleavage (Youn *et al.,* 1995), and $C_\alpha - C_\beta$ cleavage. Laccase has also been shown to catalyze the cleavage of aromatic rings in a similar way to LiP (Kawai *et al.,* 1998). Other enzymes of importance in lignin degradation are

$H_2O_2$-producing enzymes, NAD(P)H:quinone oxidoreductase, aryl alcohol dehydrogenase and probably also, CDH(CBQ). Lignin-degrading enzymes are shown in Table 4.

**Table 4. Ligninolytic enzymes**

| *Enzyme* | *Substrate* | *EC number* |
|---|---|---|
| Lignin peroxidase | Veratryl alcohol<br>$H_2O_2$ | 1.11.1.14 |
| Manganese peroxidase | Vanillyl acetone<br>Phenol red<br>3-methyl-2-benzothiazoline (MBTH) and 3-dimethylaminobenzoic acid (DMAB)<br>$H_2O_2$ | |
| Laccase | Syringaldehyde<br>2,2'-azino-bis-(3-ethylbenzothiazoline)-6-sulphonate (ABTS)<br>MBTH and DMAB | 1.10.3.2 |
| Intracellular and extracellular $H_2O_2$-producing enzymes | | |
| Intracellular enzymes | | |
| Glucose-1-oxidase, glucose-2-oxidase, methanol oxidase and fattyacyl-CoA oxidase | Glucose<br>Methanol | |
| Extracellular enzymes | | |
| Glyoxal oxidase | | |
| Aryl-alcohol oxidase | Aromatic alcohols | |
| Oxidoreductases | | |
| NAD(P)H:quindone oxidoreductase | 2,6-dichlorophenol-indophenol (DCPIP)<br>3-methyl-5-t-butyl-benzoquinone | |
| Aryl alcohol dehydrogenase | (MTBBQ) Cellobiose | |
| CDH(CBQ) | Cytochrome c'Cellobiose | |

## Biotechnological Applications

In general the bioprocessing technology of raw materials or their constituents into bioproducts entails three steps: process design, system optimization and model development. Processing involves the use of biocatalysts, whole microorganisms or their enzymes from other organisms to synthesize or transform raw materials into new products, recover/purify such bioproducts and subsequently any needed downstream modifications.

Bioconversions of lignocellulosic materials into useful, higher value products normally require multi-step processes which include:

(i) Pretreatment (mechanical, chemical or biological) (Grethlein, 1984; Grethlein and Converse, 1991).

(ii) Hydrolysis of the polymers to produce readily metabolizable molecules (e.g. hexose or pentose sugars).

(iii) Bio-utilization of these molecules to support microbial growth or to produce chemical products.

(iv) The separation and purification.

Lignocellulose degradation is of great importance for biotechnological conversion of lignocellulosic materials into value added products. There is some excellent and comprehensive literature (Wong and Saddler, 1992a,b; Kuhad *et al.,* 1993, 1997ab; Bhat, 2000; Beg *et al.,* 2001; Sun and Cheng, 2002; Beauchemin *et al.,* 2001, 2003; Subramaniyan and Prema, 2002) available on the various bioproducts from lignocellulose-degrading microbes and their applications. Lignocellulolytic enzymes are used in various industries such as pulp and paper, textile, detergents, fodder, bioconversion, food, environment, chemical and pharmaceutical.

Technologies are currently available for all steps in the bioconversion of lignocelluloses to ethanol and other chemical products. However, these technologies must be improved and new technologies developed to produce renewable biofuel and other bioproducts at prices, which can compete with current production costs.

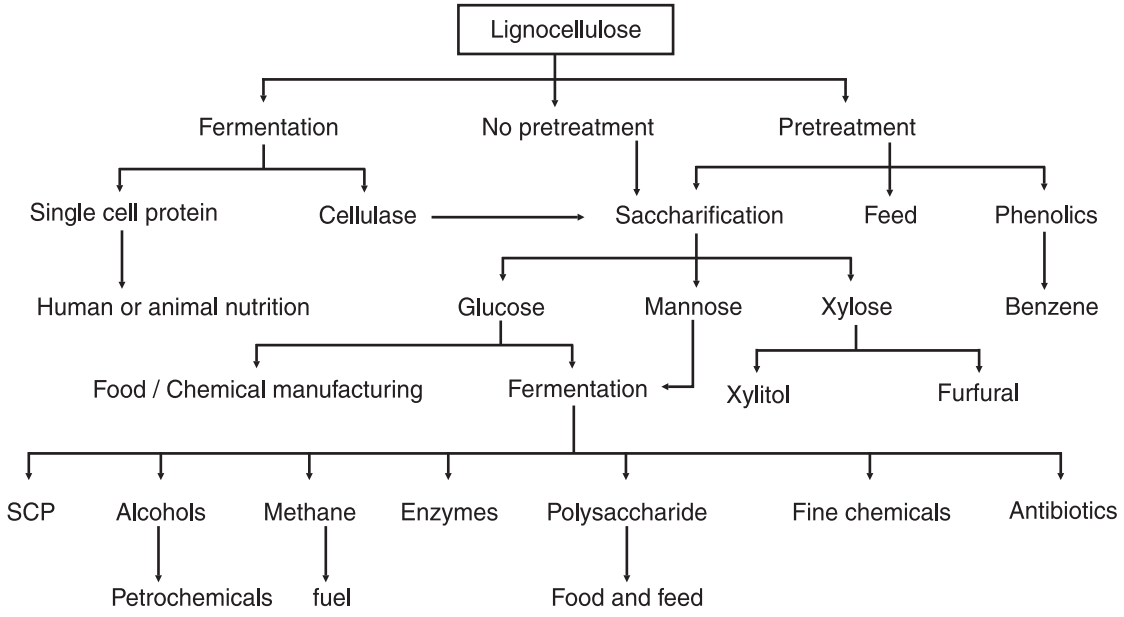
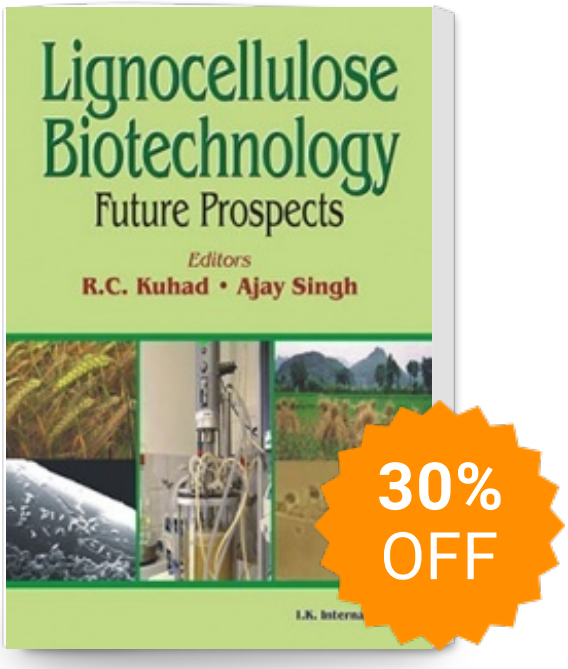



**Fig. 1.** Generalized process stages in lignocellulose bioconversion into value-added bioproducts

## Pulp and Paper Industry

Cellulases alone, or in combination with a few other enzymes such as xylanases, have been found useful for deinking different types of paper waste. Conventional deinking uses surfactants to float the toner followed by high temperatures to aggregate it, and then vigorous dispersion for size reduction. Those processes are expensive and energy-consuming. Cellulases and xylanases release toner particles facilitating flotation and subsequent steps. Endoglucanases and endoxylanases have been demonstrated to effectively deink selected old newsprint waste and to improve optical and strength properties of paper from

# Lignocellulose Biotechnology Future Prospects

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