Chapter 8
Hydrolases from Microorganisms used for Degradation of Plant Cell Wall and Bioenergy

Maria L.T.M. Polizeli, Emanuelle C.P. Corrêa, Aline M. Polizeli, and João A. Jorge

1 Introduction

The first consideration one should be aware of regarding biomass is what it exactly means. The definition of biomass has received different meanings during the course of the years, since it was first used in the 1930s. Currently, it differs according to the purpose of its use. Biologically and etymologically, biomass encompasses everything which is alive on Earth. A broader definition would include the three domains of life – Archaea, Eukarya, and Bacteria – they being alive or dead, along with their wastes. However, some other definitions exclude water, considering biomass as being the dry weight of living beings and their wastes; others claim biomass means the biodegradable fraction of products, waste and residues from agriculture (including vegetal and animal substances), aquaculture, forestry and related industries, the biodegradable fraction of industrial and municipal waste as well as waste water sludge. Lastly, biomass can also be defined as mass provided by living and/or dead plants only. From an energetic approach, biomass is every renewable resource from organic matter which can be used to produce energy. Consequently, all these different ways to define biomass can lead to multi-interpretations of single information. Additionally, we could incur in the mistake of calculating the total biomass of a unit or place without any life in it. To avoid such misunderstanding, we propose and use herein the following terms:

- BIOMASS as being all three domains of life while alive.
- BIODMASS (pronounced bio-d-mass) being biomass plus their wastes and dead organisms.
- BIOEMASS (pronounced bio-e-mass) as the biomass used to produce energy being it any fraction of the original biodmass.

Everything in the universe can be summarized in atoms and energy. Although natural energy takes place daily in our cosmos regardless of any active actions of living beings, life on earth, especially for mankind and as it is known today, depends on

M.L.T.M. Polizeli (✉)
Departament of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto (FFCLRP), São Paulo University, Av. Bandeirantes, 3900 – 14040-901, Ribeirão Preto, SP, Brazil
e-mail: polizeli@ffclrp.usp.br

M.S. Buckeridge and G.H. Goldman (eds.), *Routes to Cellulosic Ethanol*,
DOI 10.1007/978-0-387-92740-4_8,

energy drivingly and promptly produced. The demand for energy by transport and service sectors has increased greatly. Road transport has contributed the most to the increase in overall transport energy consumption (Shafiee and Topal 2009). According to International Energy Agency, between 1990 and 2005, road transport energy use increased by 41%. The possible dark side of this lies in the consequences of this increase, which brought CO_2 emissions up approximately 25%.

Despite theories of lack in relatedness between the amount of CO_2 and climate change, especially global warming (http://www.creationworldview.org/articles _ view.asp?id=67), there seems to be an agreement among the scientific community that less CO_2 released into the atmosphere would strongly favor the environment.

The world's most used energy sources, mainly for transport, are fossil fuels which presumably take millions of years to form (for a different interpretation see http://www.creationworldview.org/articles_view.asp?id=51). If the world continues to consume fossil fuels at 2006 rates, the reserves of oil, coal, and gas will last approximately an additional 40, 200, and 70 years, respectively (Shafiee and Topal 2009), but projections from the EIA show that the consumption tends to increase. Additionally, according to the same department (http://www.eia.doe.gov/emeu/steo/pub/ contents. html#GlobalCrude_Oil And_Liquid_Fuels), despite the stability on the demand for fossil fuels in 2008 and most of 2009, the economic recovery in the United States and elsewhere will lead to a rebound in oil demand growth perhaps yet this year. For some, this can be looked upon as a time gift from "Mother Nature" to humankind so a secure, reliable, and affordable energy source is found to replace the fossil ones.

Exploiting fossil fuel reserves to their full technical potential might bring undesirable, and possibly, irreversible results to the environment (Nel and Copper 2009). Currently, there are alternative energy sources being evaluated and studied. Converting bioemass into energy is one alternative, if not the one which will most achieve its goals. Other possible sources of energy are: geothermal, hidropower, nuclear, ocean, solar, and wind sources, among others.

2 Biomass and Biofuels

Human domination on Earth has changed its landscape greatly. Although when we think of bioemass, we primarily picture plants (wood), the total biodmass on Earth goes much beyond accounting for over two trillion tons of raw matter (Ramage and Scurlock 1996). A great amount of this is suitable to be used to produce energy by being burned and/or transformed into biofuels among others. Biofuel refers to any solid, liquid, or gas fuel that has been derived from bioemass. It can be produced from any carbon source that is easy to replenish when compared with fossil fuels.

There are many advantages to biofuels. The goal of any biofuel, regardless of the type, is for it to be carbon neutral and, therefore, reduce greenhouse gas emissions. Although the energy spent through crop production, transportation, and processing to generate biofuels plus the consumption of carbon through this route result in a balance different from neutrality, biofuels still prove to be substantially more environmentally friendly than their alternatives. Additionally, their production also helps in the development of

rural areas, ensure long-term fuel availability, and improve the economy equilibrium of many countries making them less dependent on imported products.

Biofuels can be obtained directly by extraction from nature, however, raw matter sometimes needs to be processed. The result can be gaseous, liquid, or solid forms of fuels, the latter form being in use ever since man discovered fire. Solid biofuels such as wood, grass cuttings, domestic refuse, charcoal, dried manure, and residues among others can be burned to produce energy. The most common gaseous biofuels are biogas and syngas. Liquid biofuels such as vegetable oil, ethanol, and biodiesel can be easily pumped and handled. This is the main reason why almost all vehicles use liquid form of fuel for combustion purposes.

3 Sugarcane and Bioemass

In this scenario, sugarcane, among many raw matters suitable to be used as bioemass, has increasingly drawn our attention. Brazil was colonized to a large extent to produce the sugarcane plant and extract sugar from it. After the legislation of 1931 requiring the addition of ethanol to gasoline, the production plants in Brazil increased their production (www.iea.org). very efficient and advanced.

1. This increase in ethanol demand associated to many research results in different fields such as genetic engineering, enzymes enhancement, mechanical improvements for the machinery involved, chemical products easing the crops among others, leaded to a more efficient and advanced process which included the growth, harvesting, and processing technology regarding sugarcane and the production of alcohol.
2. Besides the energy involved in the production of sugarcane, ethanol production needs very little external sources of energy due to its bagasse, which supplies almost all necessary energy for the industrial phase. The alcohol from sugarcane has a superior energy balance being able to result in eight times more fuel in the end of the production process than the amount of fossil fuel used to enable it.

Sugarcane crops are very stable when compared with corn and other plants. Furthermore, ethanol is biodegradable, presents a low toxicity to aquatic and soil forms of life, has virtually no sulfur and particulate emissions, reduces CO emissions and other toxic substances, can be blended with other fuels, is a source of hydrogen (fuel cells), has increased the market of flex fuel vehicles and now motorcycles (www.iea.org), it is an alternative fuel for boats and airplanes, and scientists are even willing to make plastic from it.

Brazil's sugarcane plantations are spread across almost every state, but being concentrated mostly in the central south and northeast. The biggest production comes from the São Paulo state with approximately 296 million tons of sugarcane being grown in 2007–2008 (UNICA). Brazil's production of sugarcane was around 493 million tons, and with the increase in the available area to be harvested in 2008–2009, the total sugarcane production had already increased to a half billion tons by January 2008 in only the central south region. Consequently, the production of ethanol has increased from 22.5 to 27 billion liters in 2008–2009 (http://brasilatual.com.br/sistema/index.php?s=etanol+2009). However, there is a high potential for extension

of crops in São Paulo and other states as well. At least 90 million hectares can still be used to plant sugarcane in Brazil (http://www.embrapa.br/imprensa/artigos/2008/A%20expansao%20da%20cana-de-acucar%20e%20a%20sua%20sustentabilidade.pdf). This fact makes Brazil a target place for national and international financial investments in research toward sugarcane improvement and alcohol production.

The discussion regarding the pros and cons of sugarcane crop expansion has become as intense as useless. It is obvious the country has a great potential for expansion without harming the Amazon forest, pantanal, or any other protected place, leaving enough space for cattle and food crops. However, we know sugarcane crops can be expanded only to a certain extent worldwide and that leaves us the only alternative of researching ways to increase productivity without increasing cropping areas.

4 Sugarcane Bagasse and Energy Production

Bagasse sugar and ethanol are mainly derived from sugarcane juice. Bagasse accounts for approximately 1/4 of the total sugarcane produced and it is usually burned to be used to produce energy for the production plant or sold to the animal food industry (http://www.agronline.com.br/agronoticias/noticia.php?id=2860) among other small usages for it. The straw is almost entirely left in the fields to be naturally recycled and part of the nutrients go back into the soil.

The straw corresponds to approximately 1/5 of the sugarcane production (UNICA). The total volume of bagasse will differ from production plant to production plant, as they use different machinery, some produce electricity and some do not, and whether or not the production plant sells energy. Overall, we can say that independently of the differences there might be among plants and amount of bagasse produced, if Brazil produces more than 500 million tons of sugarcane, there will be at least 125 million tons of bagasse and 100 million tons of straw. If we could efficiently use only half of the amount of bagasse (31.25 million tons of dry bagasse), there would be an increase of maybe more than 9 billion liters of ethanol production. Furthermore, an efficient methodology which brought affordability of extracting ethanol from bagasse from sugarcane would also provide insights to general methods of extracting ethanol from any wood.

5 Component of Sugarcane Bagasse

The sugarcane bagasse fibers contain approximately 46% cellulose, 25% hemicelluloses, and 21% lignin as the main components. The conversion of cellulose and hemicellulose fractions into ethanol by microbial enzymes should be considered a viable process. In order to evaluate the participation of enzymatic hydrolysis on sugarcane bagasse, a brief description of cellulose, hemicellulose, and lignin molecules is described below.

Cellulose is the main renewable carbon source in nature. It is a linear polysaccharide constituted by glucose subunits linked by β-1,4 bounds. Each residue is rotated 180° compared with its neighbors. The degree of polymerization (*DP*) of native cellulose is in the range of between 7,000 and 15,000, where:

DP = Molecular weight of cellulose / Molecular weight of one glucose unit.

The cellulose chains are oriented in parallel and form highly organized crystalline domains interspersed by more disorganized, amorphous regions (Fig. 1). Cellulose chains form numerous intra- and intermolecular hydrogen bonds, which account for the formation of rigid, insoluble microfibrils. The native crystalline form has a structure designated as type 1, which can be converted into type II by alkaline treatment. The two types differ in their intrachain hydrogen bonding pattern. Additionally, most native celluloses are composed of two slightly different forms. They are identified by solid state Nuclear Magnetic Ressonance spectrum in type I cellulose, denominated I_{α} and I_{β} which differ in their intermolecular hydrogen bonding and in proportions, depending on the cellulose source (Rahi et al. 2009). The binding of cellulose microfibrils to the hemicellulose xylan results in a noncovalently cross-linked cellulose-hemicellulose network which gives the wall tensile strength.

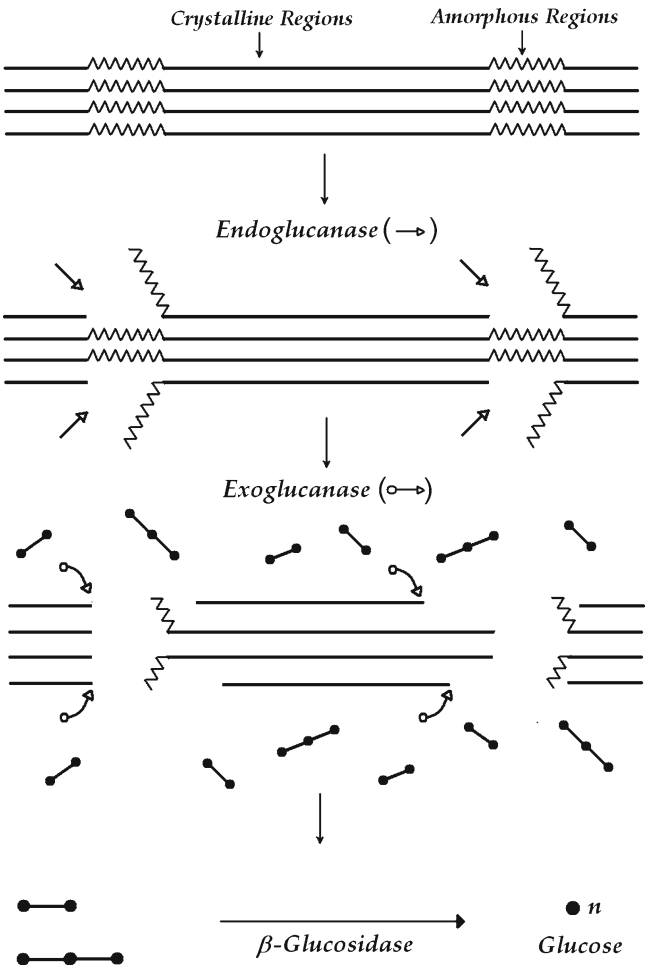

Fig. 1 Schematic representation of the sequential stages of the cellulolytic enzymes action

Hemicellulose is a heteropolysaccharide composed of various proportions of monosaccharide units of D-xylose, D-mannose, D-galactose, and L-arabinose (Polizeli et al. 2005). The hemicellulose molecules can be composed of branched chains with *O*-acetyl groups, D-glucuronic acid, 4-*O*-methyl-α-D-glucuronopyranosyl acid, D-galacturonic acid, ferulic acid, and *p*-coumaric acid. Classes of hemicellulose are usually named according to the main sugar unit. Hydrolyzed polymer which yields only xylose is named xylan; in the same sense, hemicelluloses include mannans, glucans, arabinans, and galactans.

In nature, wood hemicelluloses are amorphous complex structures made of more than one polymer and the most frequent are mannan (galactoglucomannans, glucomannans), glucuronoxylans, arabinogalactans, and arabinoglucuronoxylans. Then, the hydrolysis of β-1,4 linkages from glucomannan yields glucose and mannan. The hydrolysis of β-1,4 linkages from galactoglucomanan produces both manann and glucomanann and α-1,6 galactosyl residues linked. Because the amorphous morphology, hemicelluloses are partially soluble in water. The amount of each component varies from species to species and even among parts of the same plant. Sugarcane cell wall, for instance, is composed of arabinoxylans, but also of polysaccharides β-glucans, mannans, and pectins with neutral branching galactan (Silva 2005).

Xylans are considered the principal class of hemicelluloses and contribute to the total dry weight of angiosperms, as sugarcane, with 15–30%. Xylans are linear polymers of β-D-xylopyranosyl units (xylose) linked by β-(1-4) glycosidic bonds (Fig. 2). Depending on the origin of xylan the chain has substituent groups in the polysaccharide backbone. These include acetyl groups, 4-*O*-methyl-α-D-glucuronopyranosyl units and α-L-arabinofuranosyl groups. Angiosperm xylans have a high rate of

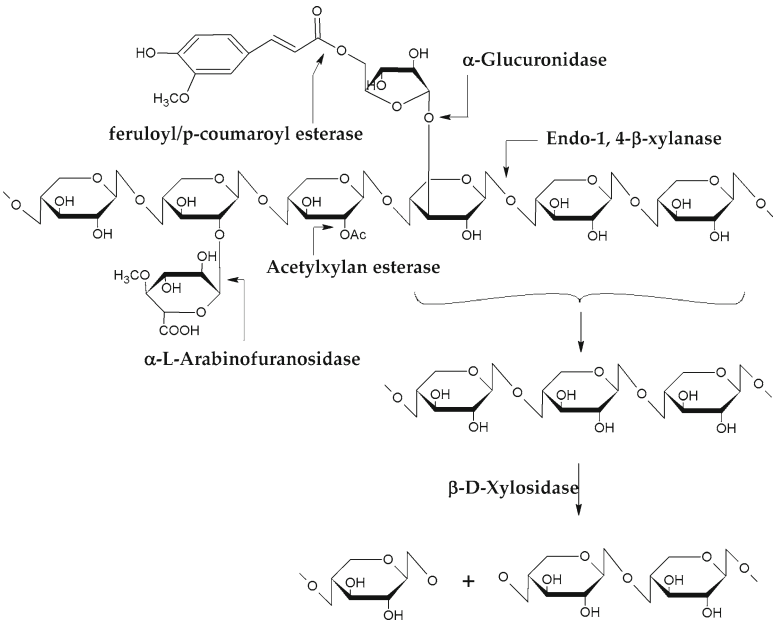

Fig. 2 Schematic representation of a xylan molecule and of the xylanolytic enzymes

substitution (70–80%) by acetyl groups, at position 2 and/or 3 of the β-D-xylopyranosyl, conferring to xylan its partial solubility in water. Subsequently, the lateral chains of xylan determine its solubility, physic conformation and the reactivity of the molecule of xylan with hemicellulosic components and, therefore, greatly influence the way and extension of its enzymatic hydrolysis (Polizeli 2009).

Lignin is a highly crossed-linked and stereochemically complex macromolecule of the cell wall which is formed from polymerization of phenylpropanoid precursors, as shown in Fig. 3. The synthesis of lignin starts with the deamination of L-phenylalanine by phenylalanine ammonia-lyase resulting into cinnamic acid. A series of enzymatic hydroxylations and methylations leads to the formation of *p*-coumaric acid, caffeic acid, ferulic acid, 5'-hydroxyferulic acid and sinapyl acid. Furthermore, reduction provides the monolignols: *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, which differs in the number of methoxyl groups on the aromatic ring. The monolignols are transported

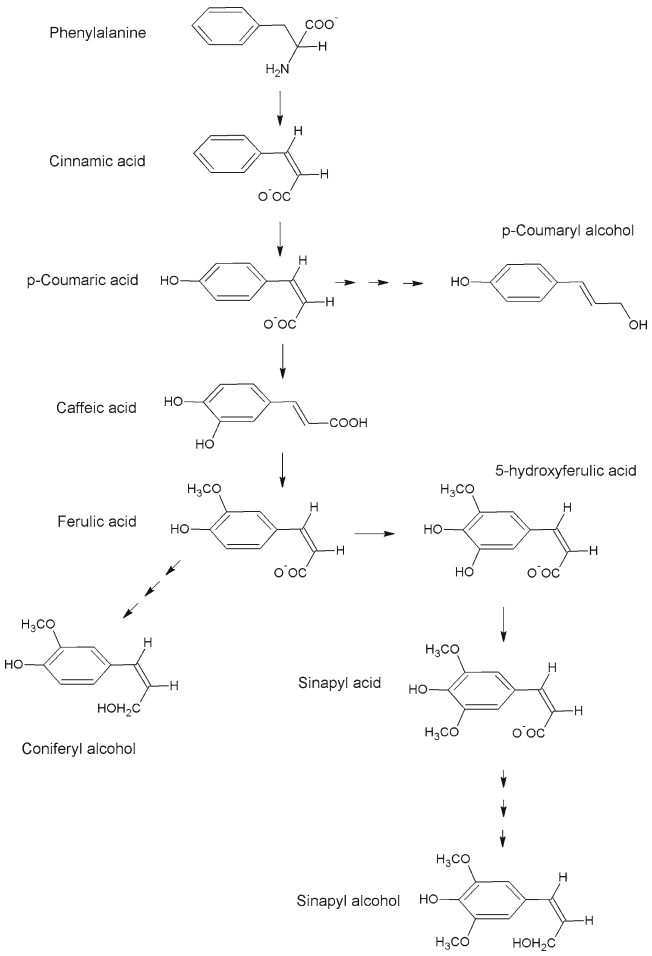

Fig. 3 Schematic representation of the formation of lignin precursors: *p*-coumaryl, coniferyl alcohol and sinapyl alcohol

from the cytosol, where they are synthetized as glucosides, to the cell wall, where the lignin polymerization takes place. The glucose is added to the monolignol to make them water soluble and to reduce their toxicity. After the transporting of glucose is its subsequently removal, the monolignols can couple with each other randomly, polymerizing the lignin to form a complex and irregular crossed-linked network structure.

Thus, lignin is considered one of the hardest organic polymers to degrade due to its insolubility, high molecular weight, and nonlinear and nonuniform structure. Different production plants use different monolignols. The total content of phenolic compounds in the prehydrolysated sugarcane bagasse corresponds to phenylpropanoid derivatives, such as *p*-coumaric and ferulic acids, accounting for more than 50% of the phenols identified by steam explosion) prehydrolysates (Martin et al. 2007).

6 Cell Wall Degrading Enzymes

The diversity and heterogeneity of the polysaccharides of cell wall structure contribute to a large extent to the difficulty found in enzymatic determinations. In this context, according to its chemical structure and the substituent groups of the lateral chain, the xylan and the overlapping sheath of the lignin are covalently bound. Ferulic acid tends to be associated with hemicellulose and *p*-coumaric acid seems to be generally esterified to lignin (Fig. 4). The covalently linked layers of xylans

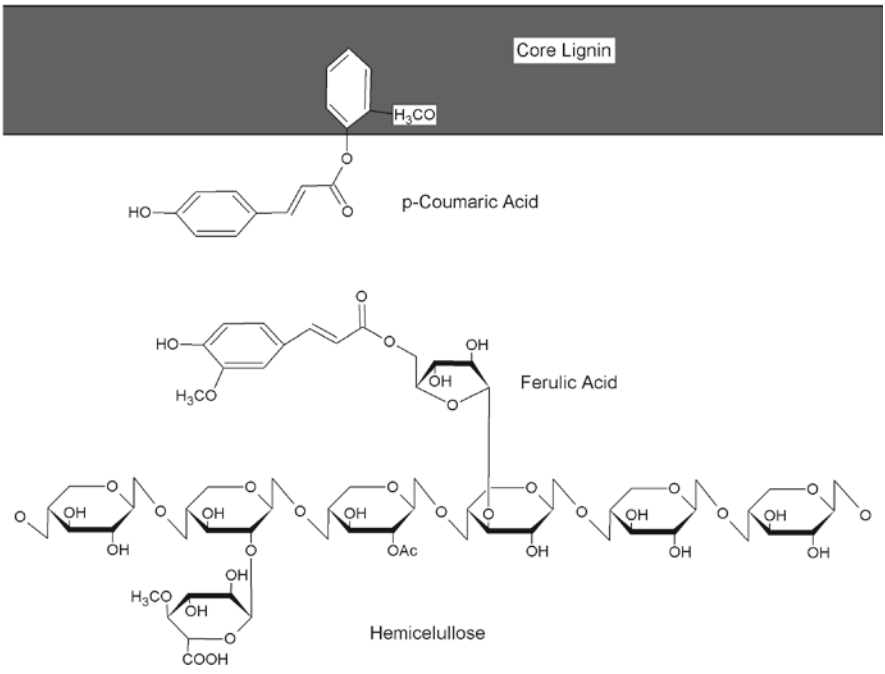

Fig. 4 Interaction of *p*-coumaric and ferulic acids to lignin of cell wall

and lignins and their noncovalent interaction with cellulose are important for the maintenance of the integrity of the cellulose in situ, and consequently, for the protection of the fibers against the action of cellulase. The xylan ramification points have an important function because they may cause steric hindrance. They prevent the formation of the enzyme-substrate complex; alter the xylan structure changing its solubility, and also change the configuration and the linking of phenolic components in the lignin (Polizeli 2009).

Therefore, complete cell wall hydrolysis demands enzymes that hydrolyze the branches (lateral chains), and synergistically contribute to the hydrolysis of the polysaccharide main chains.

7 Microbial Cellulose Degradation

Cellulose is degraded into glucose or low molecular weight soluble saccharides. This is due to the action of a multienzymatic system, which consists of three major components:

(1) Endoglucanase or endo 1,4- β-glucanase (EC 3.2.1.4) acting randomly on 1,4-β-linkages. It prefers to degrade amorphous (noncrystalline) rather than crystalline cellulose.
(2) Exoglucanase or exo-1,4-β-glucanase (EC 3.2.1.91, exo-1,4-β-D-glucan cellobiohydrolase; (CBH); CBH releases both glucose and cellobiose units from the nonreducing ends of cellulose chains.
(3) β-glycosidases or 1,4 – β-glycosidases (EC 3.2.1.21) hydrolyze main cellobiose to glucose, and cellobionic acids to glucose and gluconolactone.

The three enzymes act synergistically in the decomposition of bioemass and in a sequence of events. Endoglucanases randomly disrupt cellulose amorphous regions while exoglucanases act on exposed chain ends by splitting off cellobiose which is subsequently hydrolyzed by β-glucosidase into glucose (Fig. 1). β-glycosidases are the components which regulate the velocity of the process because endo- and exocelulases are inhibited by their product, glucose.

Cellulases are preferentially produced from wood decaying basidiomycetes as *Phanerochaete chrysosporium* (Igarashi et al. 2008), *Trametes versicolor* (Lahjouji et al. 2007), *T. hirsute* (Nozaki et al. 2007), *Ceriporiopsis subvermispora* (Magalhães et al. 2006), *Agaricus bisporus* (De Groot et al. 1998), *Volvariell volvacea* (Ding et al. 2006), and *Schizophyllum commune* (Henrissat et al. 1989). However, as reported by Henrissat et al (1989), the bacteria *Clostridium thermocellum*, *Erwinia chrysanthemi, Bacillus sp., Cellulomonas fimi, Streptomyces sp, Pseudomonas fluorescens*, and *Cryptococcus albidus* also produce cellulases in high levels. Filamentous fungi as *Hypocrea jecorina (Trichoderma reesei)* (Schmoll and Kubicek 2003) and in minor extension *Aspergillus sp* (Dedavid and Silva et al. 2009) and *Penicillium* (Camassola and Dillon 2007) are also reported as cellulase producers.

Phanerochaete chysosporium also produces oxidative enzymes that also play an important function in the cellulose degradation. The flavoprotein cellobiose:quinine oxidoreductase (EC 1.1.5.1) reduces the quinines and phenoxy radicals which were produced during lignin degradation, in the presence of cellobiose, which is oxidized to cellobio-α-lactone. The flavoheme protein, cellobiose oxidase (EC 1.1.3.25) is another enzyme which oxidizes cellobiose and higher cellodextrins into their corresponding onic acids using molecular oxygen. Both enzymes have been shown to bind at microcrystalline cellulose while retaining enzyme activity. Some authors (Wood and Wood 1992) have suggested that cellobiose:quinine oxidoreductase is not an independent enzyme, but a breakdown product of cellulose oxidase instead, which acts as a link within the lignin–cellulose degradation processes.

8 Microbial Hemicellulose Degradation

Due to the heterogeneous structure of hemicelluloses, their complete degradation requires a microbial enzymatic system with several enzymes, acting on the main chain or the lateral chain due to the presence of substituent groups. Important enzymes which act in the main chain are: endo-1,5-α-L-arabinase (arabinan endo-1,5-α-L-arabinosidase EC 3.2.1.99) acting in the endohydrolysis of 1,5-α-arabinofuranosidic linkages in 1,5-arabinans (Sakamoto et al. 2003); β-D-mannanase (mannan endo-1,4-β-mannosidase, EC 3.2.1.78), perform randoming hydrolysis of 1,4- β-D-mannosidic linkages in mannans, galactomannans and glucomannans (Luo et al. 2009); β-D-mannosidase (β-D-mannoside mannohydrolase EC 3.2.1.25) hydrolyzing terminal, nonreducing beta-D-mannose residues in beta-D-mannosides (Athanasopoulos et al. 2005; Tanaka et al. 2009), and enzymes of the xylanolytic system, as described above. Mannanase is reported in microorganism as fungi (Luo et al. 2009), bacteria (Tanaka et al. 2009), and heterologous expression in yeast (Setati et al. 2001). Fungal β-D-mannanase has different characteristics in relation to molecular mass, isoelectric point, pH and temperature.

The main xylanolytic enzymes are endo-1,4-β-xylanase (1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8) and β-D-xylosidase (1,4-β-D-xylan xylohydrolase; EC 3.2.1.37) (Fig. 2). Endo-1,4-β-xylanase cleaves glycosidic bonds in the xylan backbone, producing xylooligosaccharides of variable lengths, resulting in a reduction of the Degree of Polymerization of the substrate. The xylan is not attacked randomly, since the bonds selected for hydrolysis depend on the chain length, the degree of branching and the presence of substituents (Polizeli et al. 2005; Polizeli 2009). Initially, the main products of xylanase action are xylose oligomers, but at a later stage, small molecules such as the mono-, di-, and trisaccharide of β-D-xylopyranosyl may be released. The accumulation of these short oligomers of xylose may inhibit the endoxylanase activity.

The β-D-xylosidase hydrolyses that these short oligomers formed by endo-1,4-β-xylanase action removing the cause of inhibition and increasing the efficiency of xylan hydrolysis (Polizeli et al. 2005; Polizeli 2009). The best substrate for β-xylosidase is xylobiose. Its affinity for xylooligosaccharides is inversely

proportional to their Degree of Polymerization. β-xylosidases are also able to cleave artificial substrates, such as *p*- and *O*-nitrophenyl-β-D-xylopyranoside. A good example of stable β-xylosidase is the enzyme from *Aspergillus phoenicis*, which retains 100% of its activity after 4 h at 60°C or 21 days at room temperature (Rizzatti et al. 2001). Transxylosylation activity has also been detected in fungi, resulting in higher molecular weight products than the original substrates (Kurakabe et al. 1997).

The association of xylanases results in a "xylanosome," a term applied to structures observed as protein aggregates with many subunits. It is an analog structure to cellulosome that is a multienzyme system that may mediate the adhesion of the bacterium to cellulose (Polizeli et al. 2005; Jiang et al. 2004, 2006; Deng et al. 2005). Xylanosomes have very high molecular weights (500–600 kDa) and consist of more than 10 proteins with xylanolytic activity, several of which are endoxylanases. In a few microorganisms, a cellulosome may be associated with a xylanosome, forming large complexes responsible for the hydrolysis of both cellulose and xylan (Sunna and Antranikian 1997).

Other microbial enzymes act on xylan branched groups (Fig. 2):

(1) Acetylxylan esterase (Li et al. 2008) (EC 3.1.1.72), catalyzes the hydrolysis of acetyl groups from polymeric xylan, acetylated xylose, acetylated glucose, α-naphthyl acetate, and *p*-nitrophenyl acetate.
(2) α-D-Galactosidase (Simerska et al. 2007) (α-D-galactoside galactohydrolase, EC 3.2.1.22), hydrolyses of terminal, nonreducing α-D-galactose residues into α-D-galactosides, including galactose oligosaccharides, galactomannans, and galactohydrolase.
(3) α-Glucuronidase (Tenkanen and Siika-aho 2000) (xylan-α-D-1,2-(4-*O*-methyl) glucuronohydrolase, EC 3.2.1.131), that hydrolyses α-D-1,2-(4-*O*-methyl) glucuronosyl links in the main chain of hardwood xylans.
(4) Feruloyl/*p*-coumaroyl esterase (Hermoso et al. 2004) (4-hidroxy-3-methoxycinnamoyl-sugar hydrolase, EC 3.1.1.73) that cleaves ester bonds on xylan between arabinose and ferulic acid and/or arabinose and *p*-coumaric acid side-groups.
(5) Two types of α-arabinofuranosidase, with distinct modes of action: the most common, exo-α-L-arabinofuranosidase (Rahman et al. 2003) (α-L-arabinofuranoside arabinofurano-hydrolase, EC 3.2.1.55), degrades *p*-nitrophenyl-α-L-arabinofuranosides and branches arabinans, whereas endo-1,5-α-L-arabinase (Takao et al. 2002) (arabinan endo-1,5-alpha-L-arabinosidase, EC 3.2.1.99) only hydrolyzes linear arabinans.

The xylanases were originally classified into two families of glycosyl hydrolases, denominated F and G, or 10 and 11 (Henrissat and Bairoch 1993; Sapag et al. 2002), respectively. This classification is based in the structural homology of the hydrophobic regions, deduced from 2D representations of the primary sequences. More recently Collins et al. (2005) reported the existence of enzymes with xylanase activity also belonging to families 5, 8, and 43.

The family F/10 consists of a cellulose-binding domain and a catalytic domain, which is a TIM barrel domain, interconnected by a flexible spacer and a substrate-binding domain. The family G/11 is composed of highly specific endoxylanases of low Mw,

with 40–90% sequence identity, with different pIs, thermostabilities, pH profiles, structures, and catalytic properties. According to Törrönen and Rouvinen (1997), besides these homology-based classes, xylanases could be further divided into those with alkaline and those with acidic pI, but some microorganisms produce both types, e.g., *Aspergillus* and *Trichoderma* sp. This resulted in a further classification of the xylanases in classes such as A, B, C, D, 1, 2, 3, I, II, III (Kulkarni et al. 1999a, b).

Aiming biotechnological applications, the ideal source of xylanase would be one that produces an adequate amount of each of the enzymes composing the xylanolytic system. The synergistic interactions among the xylan-degrading enzymes enhance the susceptibility of the xylan to the attack of endoxylanases. Among microbial sources, filamentous fungi are especially interesting as they secrete the enzymes into the medium, and their xylanase levels are higher than those in yeasts and bacteria.

Cloning, sequencing, and expression of xylanase genes in *Escherichia coli* has been performed for microorganisms of different genera (Kulkarni et al. 1999b; Liu et al. 1999). The xylanases in bacteria are restricted to the intracellular or periplasmic fractions (Schlacher et al. 1996), although extracellular activity has also been reported (Karlsson et al. 1998; Ebanks et al. 2000). Furthermore, enzymes expressed in bacteria are not subjected to posttranslation modifications such as glycosylation.

Besides bacteria, xylanases have also been expressed in the yeasts *Saccharomyces cerevisiae* and *Pichia pastoris. Saccharomyces cerevisiae* is an attractive host for the expression of heterologous xylanases (Romanos et al. 1992). It provides an efficient posttranslational processing such as glycosylation, proper folding of the proteins, proteolysis (Sa-Pereira et al. 2003). Besides being a host that secretes only some proteins, *S. cerevisiae* expressed proteins are easier purified (Das and Shultz 1987). On the other hand, heterologous gene expression by the methylotrophic yeast *Pichia pastoris* is a useful alternative when scaling up to industrial process and has several advantages over *Saccharomyces cerevisiae* because of high secretion efficiency, high cell densities attained in inexpensive culture media, and the relative ease of scaling up the industrial process (Cregg 1999). It is a particularly attractive expression host due to the availability of strong and regulatory promoters that are involved in methanol metabolism (Tsai and Huanga 2008).

Filamentous fungi are attractive hosts for protein expression because of their natural ability to secrete large amounts of proteins into the medium. Fungi dominating the market as expression hosts are *Aspergillus niger* (Levasseur et al. 2005), *Aspergillus nidulans* (Perez-Gonzalez et al. 1998), *Aspergillus oryzae* (Kimura et al. 2002), and *Trichoderma reesei* (Mantyla et al. 2007). Most of the xylanase genes have been expressed in fungi under homologous systems.

Xylanases may be induced either by incubating microorganisms in submerged liquid culture (SbmF) (Rizzatti et al. 2004) or on different solid substrates (SSF) as wheat bran (Betini et al. 2009), rice bran (Dos Santos et al. 2003), sugarcane bagasse (Dos Santos et al. 2003; Sandrim et al. 2005), and others. The induction of the xylanolytic system is supposed to occur through physical contact between some recognition site on the cell surface and the inducer. Most fungal strains are induced by xylan, and carbon catabolite repression by glucose is reported as well. Relatively low levels of constitutive xylanases may be responsible for the initial hydrolysis of

xylan, producing xylotriose, xylobiose, and/or xylose (Polizeli et al. 2005; Polizeli 2009). These oligosaccharides are transported into the cell with the aid of β-xyloside permeases, and then trigger the transcription of the xylanolytic system genes. Permease activity is reduced by the presence of glucose, but it is very efficient in the presence of the xylanolytic inducers.

In filamentous fungi, carbon catabolite repression is mediated by the protein CREA (transcription repressor). Few studies detail the transcriptional regulation of xylanase and the participation of the cAMP in the repression by glucose. Morosoli et al. (Morosoli et al. 1987) report the xylanase from *Cryptococcus albidus* is induced either by xylan or β-methylxyloside, a nonmetabolizable inducer, and that xylose represses the production of the enzyme. In this microorganism, addition of exogenous cAMP elicits a 1.5–2-fold increase in xylanase production in the presence of the inducer, but does not relieve the repression caused by xylose. These authors suggest that a 15-nucleotide sequence located upstream from the xylanase gene could be part of a cAMP regulatory site. Rizzatti et al. (2008) reported that the xylanase of *Aspergillus phoenicis* was induced by xylan, xylose, and β-methylxyloside but repressed by the addition of glucose. The glucose repression was alleviated by addition of cAMP or dibutyryl-cAMP. These physiological observations were supported by a Northern analysis using part of the xylanase gene *ApXLN* as a probe. Gene transcription was shown to be induced by the same inducers, and was repressed by glucose, where the repression was partially relieved by the addition of cAMP or dibutyryl cAMP.

Catabolic repression in *Aspergillus nidulans* and *A. niger* is also described by Ruijter and Visser (Ruijter and Visser 1997). These authors suggest that the CREA protein plays a major role in carbon repression by binding to specific sequences in the promoter of many target genes, and inhibiting their transcription. More recently, it was isolated a transcriptional activator of xylanases (XlnR) from *Aspergillus niger* (van Peij et al. 1998) and a transcriptional activator (AoXlnR) in *Aspergillus oryzae* which is XlnR homolog. Northern blot analysis reveals the controls of the expression of genes encoding xylanolytic enzymes (Marui et al. 2002). Repression of xylanolytic genes by glucose has been assigned to the protein CREA in *A. niger* (de Graaff et al. 1994)) and to its homolog Cre1 in *Trichoderma reesei* (Strauss et al. 1995). CREA protein modulates the expression of XlnR induced by xylose in *A. niger* (de Vries et al. 1999) and *A. nidulans* (Prathumpai et al. 2004).

In general, fungal endoxylanases have optimum activity and stability in the range of 40–75°C and pH 4.0–6.5 although extremophile bacteria produce endoxylanases with an optimal temperature higher than 80°C (Polizeli 2009). Endoxylanases are usually small glycoproteins (6–50 kDa), with multiplicity of forms, being that in some cases three or more enzymatic isoforms from a single culture (Rizzatti et al. 2004). A number of factors may be responsible for this phenomenon, such as differential processing of mRNA, posttranslation modifications (glycosylation and self-aggregation) or proteolysis. Multiple endoxylanases can also be expressed by distinct alleles of one gene, or even by completely separate genes (Polizeli et al. 2005).

9 Microbial Lignin Enzymes

Lignin is degraded by different combinations of extracellular oxidative enzymes found mainly in ligninolytic cultures of white-rot fungi. Here, we comment briefly about Lignin Peroxidase (LiP); Manganese Peroxidase (MnP), and laccase.

Lignin Peroxidase: (1,2-bis [3,4-dimethoxyphenyl] propane-1,3-diol:hydrogen-peroxide oxidoreductase, EC 1.11.1.14) belongs to the family of oxidoreductases specifically those acting on a peroxide as acceptor (peroxidases). It is an extracellular hemeprotein, with an unusual high redox potential and low optimum pH. It reacts with a wide variety of lignin related compounds end even unrelated molecules. The chemical reaction catalyzed is:

$$\begin{aligned}&1,2-\text{bis}(3,4-\text{dimethoxyphenyl})\text{propane}-1,3-\text{diol}+H_2O_2\\&\rightleftharpoons 3,4-\text{dimethoxybenzaldehyde}+1-(3,4-\text{dimethoxyphenyl})\\&\text{ethane}-1,2-\text{diol}+H_2O.\end{aligned}$$

Manganese Peroxidase: (Mn[II]: hydrogen-peroxide oxidoreductase, EC 1.11.1.13) is another type of heme peroxidase. This oxidoreductase shows a strong preference for Mn(II) and acts on a peroxide as acceptor (peroxidase). The product Mn(III) forms a complex with organic acids and diffuses away from the enzyme to oxidize other materials, such as lignin. The chemical reaction is:

$$2\text{Mn(II)}+2H^{+}+H_2O_2 \rightleftharpoons 2\text{Mn(III)}+2H_2O$$

The redox potential of the Mn peroxidase is lower than that of lignin peroxidase and it has only shown capacity to oxidize in vitro phenolic substrates (Rahi et al. 2009), which are oxidized into phenoloxy radicals, further reacting through demethylation, alkyl-phenyl cleavage, Cα-oxidation, or Cα-Cβ cleavage. Manganese peroxidase seems to be more widespread among white-rot fungi than lignin peroxidase.

Laccase (EC 1.10.3.2): is a copper-containing oxidase found in many plants and microorganisms and it does not require peroxidase, H_2O_2. These enzymes oxidize phenol and similar compounds, forming phenoxy radicals. However, in presence of the artificial substrate ABTS (2,2-azinobis(3-ethylbenzothiazoline-5-sulphonate) or some other synthetic mediators, laccase can also oxidize certain nonphenolic compounds, veratryl alcohol and Mn(II). Laccase has the capability to both depolymerize and polymerize lignin model compounds. It is proposed that laccases play a role in the formation of lignin by promoting the oxidative coupling of lignols.

10 Microbial Enzymes and Thermophile

Within an evolutionary context, thermophiles (Cooney and Emerson 1964) probably emerged from a mesophile branch as a consequence of many mutations which allowed their growth in hot environments and limited it in cold temperatures. Glycosylation

itself could not explain the ability for these organisms to survive in such different conditions. Punctual site mutations in the amino acid sequences can also change this characteristic. As for hyperthermophiles, the thermal stability of enzymes and other proteins is also due to saline bridges. Furthermore, there are hydrophobic bounds in the inner side of the proteins which favor the resistance to the unfolding and denaturation of the molecule in aqueous medium (Madigan et al 2000).

Thermophiles and hyperthermophile cells also present changes which help keep their thermal characteristic. These changes are mainly in the biochemical machinery and more heat-stable cell structures. The membranes of thermophiles are rich in saturated fatty acids which turn the medium into a much more hydrophobic environment helping the maintenance of membrane stability. Hyperthermophiles, encompassing many organisms from the domain Archaea, do not present lipids with fatty acids in their membranes but isopropane, a compound constituted by repeated units of five carbons linked to glycerol phosphate.

Another curious fact is that hyperthermophiles increase its concentration of chaperon proteins drastically in each cell when submitted to high temperatures close to the maximum tolerant. Chaperone proteins aid other proteins protecting them and increase cell thermal resistance (Madigan et al 2000). Pyrodictium, an archaeum organism, can survive for 1 h at 121°C achieving a chaperone protein concentration of 80% out of total citoplasmatic proteins. Thermophiles exhibit an increase of heat shock proteins and trehalose in response to temperature increasing. This disaccharide interacts with the solvatation layer of the protein keeping the aggregation and denaturation of the molecule from happening.

Thermophiles are of great interest because they produce considerable quantities of enzymes (Bhat and Maheshari 1987; Latif et al. 1995) and they are more thermal stable when compared with similar ones produced by mesophiles. The high growing temperature also avoids contamination by undesirable microorganisms (Bhat and Maheshari 1987; Latif et al. 1995). *Scytalidium thermophilum* produces a mycelial β-glycosidase, which is activated by glucose or xylose at a concentration varying from 50 to 200 mM (Zanoelo et al 2004a). This kind of enzyme is greatly desirable in scarification processes because a high rate of hydrolysis could be attained without glucose inhibition. This same species of fungus is also found to produce a β-xylosidase tolerant to its product, xylose, in range until 200 mM (Zanoelo et al 2004b) and when grown in Reese minimum medium (Reese and Mandels 1963) added with rice straw as the only carbon source can produce high amounts of xylanases and only traces of cellulases (Gaur et al 2005), which is important to pulp production used in paper manufacturing.

11 Perspectives

The population growth and the increasing demand for fuels have intensified the research and development of utilization of renewable raw material in substitution of fossil sources. The advances in this area signalize that the exploitation of renewable

raw material, including residues, will revert this dependence on crude oil. Until now, corn and sugarcane broth were the major resources in the emerging cellulose-to-ethanol strategy for biofuels with technologies commercially established. The next step is the exploitation of the bagasse and straw of sugarcane for ethanol production.

Cellulose and hemicellulose together, represent approximately 70% of lignin–cellulosic materials. The potential bioconversion of these carbohydrates into biofuels is limited by association with aromatic constituents (lignin). Structural studies over the years have identified the limitation of fiber degradation imposed by aromatic barriers, e.g., coniferyl lignin appears to be the most effective limitation for biodegradation, existing in xylem cells of vascular tissues. Cell walls with syringyl lignin, e.g., leaf sclerenchyma, are often less intractable. On the other hand, ferulic and *p*-coumaric acids esterified into xylan constitute a major limitation for biodegradation of nonlignified cell walls. Nonchemical methods to improve bioconversion of the lignocelluloses include pretreatment of sugarcane bagasse with phenolic acid esterases, plant breeding to modify cell wall aromatics and separation and collection of aromatics to increase availability of carbohydrates for fermentation.

In addition, a better judgment of the regulatory system of the lignocellulosic enzymes is still limited. Great hopes are concentrated on technological advance, but there is also a search for new microorganisms, within the immense biodiversity of this planet, which may possess better adaptive characteristics in relation to temperature, pH of the medium and adaptability to low-cost substrates, hardly exploited up to now. These microorganisms responsible for lignincellulosic biodegradation also present ecological importance because they generate metabolic energy that maintains the flow of carbon, and their importance in the natural recycling of biomass is enormous. The huge potential of thermophiles, thermotolerant, and extremophile microorganisms should also be emphasized, considering their impact in several areas of application. On the other hand, we cannot forget the undeniable contribution of the advent of recombinant DNA technology and phylogenetic studies which can provide a better focus on wanted targets and minimize efforts by seeking closely related organisms sharing desirable characteristics. All of these alternatives should be considered to improve the value added to coproducts of bioconversion from sugarcane into biofuel.

References

Athanasopoulos, V. I., Niranjan, K., and Rastall, R. A. 2005. The production, purification and characterization of two novel α-D-mannosidases from *Aspergillus phoenicis*. Carbohydr. Res. 340(4):609–617.

Betini, J. H. A., Michelin, M., Peixoto-Nogueira, S. C., Jorge, J. A., Terenzi, H. F., and Polizeli, M. L. T. M. 2009. Xylanases from *Aspergillus niger*, *Aspergillus niveus* and *Aspergillus* ochraceus produced under solid-state fermentation and their application in cellulose pulp bleaching. Bioprocess Biosystems Engineering 32:819-824.

Bhat, K. M., and Maheshari, R. 1987. *Sporotrichum thermophile* growth, cellulose degradation, and cellulase activity. Appl. Environ. Microbiol. 53:2175–2182.

Camassola, M., and Dillon, A. J. 2007. Production of cellulases and hemicellulases by *Penicillium echinulatum* grown on pretreated sugar cane bagasse and wheat bran in solid-state fermentation. J. Appl. Microbiol. 103(6):2196–204.

Collins, T., Gerday, C., and Feller, G. 2005. Xylanases, xylanases families and extremophilic xylanases. FEMS Microbiol. Rev. 29:3–23.

Cooney, D. G., and Emerson, R. 1964. In Thermophilic fungi. Freeman, WH, San Francisco, pp.189.

Cregg, J. M. 1999. Expression in methylotrophic yeast *Pichia pastoris*. In Gene Expression Systems, ed. J. M. Fernandez, and J. P. Hoeffler, pp 157–191. Academic Press, New York.

Das, R. C., and Shultz, J. L. 1987. Secretion of heterologous proteins from *Saccharomyces cerevisiae*. Biotechnol. Prog. 3:43–48.

Dedavid e Silva, L. A., Lopes, F. C., Silveira, S. T., and Brandelli, A. 2009. Production of cellulolytic enzymes by *Aspergillus phoenicis* in grape waste using response surface methodology. Appl. Biochem. Biotechnol. 152(2):295–305.

de Graaff, L. H., van den Broeck, H. C., and Ooijen, A. J. J. 1994. Regulation of the xylanase-encoding *xlnA* gene of *Aspergillus tubigensis*. Mol. Microbiol. 12:479–490.

De Groot, P. W., Basten, D. E., Sonnenberg, A., Van Griensven, L. J., Visser, J., and Schaap, P. J. 1998. An endo-1,4-beta-xylanase-encoding gene from *Agaricus bisporus* is regulated by compost-specific factors. J. Mol. Biol. 277(2):273–284.

de Vries, R. P., Visser, J., and de Graaff, L. H. 1999. CreA modulates the XlnR-induced expression on xylose of *Aspergillus niger* genes involved in xylan degradation. Res. Microbiol. 150:281–285.

Deng, W., Deng, W., Jiang, Z. Q., Li, L. T., Wei, Y., Shi, B., and Kusakabe, I. 2005. Variation of xylanosomal subunit composition of *Streptomyces olivaceoviridis* by nitrogen sources. Biotechnol. Lett. 27(6):429–433.

Ding, S., Ge, W., and Buswell, J. A. 2006. Cloning of multiple cellulose cDNAs from *Volvariella volcacea* and their differential expression during substrate colonization and fruiting. FEMS Microbiol. Lett. 263(2):207–213.

Dos Santos, E., Piovan, T., Roberto, I. C., and Milagres, A. M. 2003. Kinetics of the solid state fermentation of sugarcane bagasse by *Thermoascus aurantiacus* for the production of xylanase. Biotechnol. Lett. 25(1):13–16.

Ebanks, R., Dupont, M., Shareck, F., Morosoli, R., Kluepfel, D., and Dupont, C. 2000. Development of an *Escherichia coli* expression system and thermostability screening assay for libraries of mutant xylanase. J. Ind. Microbiol. Biotechnol. 25:310–314.

Gaur, R., Lata, S., and Khare, S. K. 2005. Immobilization of xylan-degrading enzymes from *Scytalidium thermophilum* on Eudragit L-100. World J. Microbiol. Biotechnol. 21:1123–1128.

Henrissat, B., and Bairoch, A. 1993. New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem. J. 293:781–788.

Henrissat, B., Claeyssens, M., Tomme, P., Lemesle, L., and Mornon, J. P. 1989. Cellulase families revealed by hydrophobic cluster analysis. Gene 81(1):83–95.

Hermoso, J. A., Sanz-Aparicio, J., Molina, R., Juge, N., Gonzalez, R., and Faulds, C. B. 2004. The crystal structure of feruloyl esterase A from *Aspergillus niger* suggests evolutive functional convergence in feruloyl esterase family. J. Mol. Biol. 338:495–506.

Igarashi, K., Ishida, T., Hori, C., and Samejima, M. 2008. Characterization of an endoglucanase belonging to a new subfamily of glycoside hydrolase family 45 of the basidiomycete *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 74(18):5628–5634.

Jiang, Z. Q., Deng, W., Li, L. T., Ding, C. H., Kusakabe, I., and Tan, S. S. 2004. A novel, ultra-large xylanolytic complex (xylanosome) secreted by *Streptomyces olivaceoviridis*. Biotechnol. Lett. 26(5):431–436.

Jiang, Z., Dang, W., Yan, Q., Zhai, Q., Li, L., and Kusakabe, I. 2006. Subunit composition of a large xylanolytic complex (xylanosome) from *Streptomyces olivaceoviridis* E-86. J. Biotechnol. 126(3):304–312.

Karlsson, E. N., Dahlberg, L., Torto, N., Gorton, L., and Holst, O. 1998. Enzymatic specificity and hydrolysis pattern of the catalytic domain of the xylanase *xyn*1 from *Rhodothermus marinus*. J. Biotechnol. 60:23–35.

Kimura, T., Suzuki, H., Furuhashi, H., Aburatani, T., Morimoto, K., Sakka, K., and Ohmiya, K. 2002. Molecular cloning, characterization and expression analysis of the *xynF*3 gene from *Aspergillus oryzae*. Biosci. Biotechnol. Biochem. 66:285–292

Kulkarni, N., Lakshmikumaran, M., and Rao, M. 1999a. Xylanase II from an alkaliphilic thermophilic *Bacillus* with distinctly different structure from other xylanases, evolutionary relationship to alkaliphilic xylanases. Biochem. Biophys. Res. Commun. 263:640–645.

Kulkarni, N., Shendye, A., and Rao, M. 1999b. Molecular and biotechnological aspects of xylanases. FEMS Microbiol. Rev. 23:411–456.

Kurakabe, M., Shinjii, O., and Komaki, T. 1997. Transxylosilation of β-xylosidase from *Aspergillus awamori* K4. Biosci. Biotech. Biochem. 6112:2010–2014.

Lahjouji, K., Storms, R., Xiao, Z., Joung, K.B., Zheng, Y., Powlowski, J., Tsang, A., and Varin, L. 2007. Biochemical and molecular characterization of a cellobiohydrolase from *Trametes versicolor*. Appl. Microbiol. Biotechnol. 75(2):337–346.

Latif, F., Rajoka, M. I., and Malik, K. A. 1995. Production of cellulases by thermophilic fungi grown on *Leptochloa fusca* straw. World J. Microbiol. Biotechnol. 11:347–348.

Levasseur, A., Asther, M., and Record, E. 2005. Overproduction and characterization of xylanase B of *Aspergillus niger*. Can. J. Microbiol. 51:177–183.

Li, X. L., Skory, C. D., Cotta, M. A., Puchart, V., and Biely, P. 2008. Novel family of carbohydrate esterases based on identification of the *Hypocrea jecorina* acetyl esterase gene. Appl. Environ. Microbiol. 74(24):7482–7489.

Liu, W., Lu, Y., and Ma, G. 1999. Induction and glucose repression of endo-β-xylanase in the yeast *Trichosporon cutaneum* SL409. Process Biochem 34:67–72.

Luo, H., Wang, Y., Wang, H., Yang, J., Yang, Y., Huang, H., Yang, P., Bai, Y., Shi, P., Fan, Y., and Yao, B. 2009. A novel highly acidid beta-mannanase from the acidophilic fungus *Bispora* sp. MEY-1: gene cloning and overexpression in *Pichia pastoris*. Appl. Microbiol. Biotechnol. 82(3):453–461.

Madigan, M. T., Martinko, J. M., and Parker, J. 2000. Brock Biology Microorganisms. 9th ed., pp. 153–154. Prentice-Hall, New Jersey.

Magalhães, P. O., Ferraz, A., and Milagres, A. F. 2006. Enzymatic Properties of two beta-glucosidases from *Ceriporiopsis subvermispora* produced in biopulping conditions. J. Appl. Microbiol. 101(2):480–486.

Mantyla, A., Paloheimo, M., Hakola, S., Lindberg, E., Leskinen, S., Kallio, J., Vehmaanpera, J., Lantoo, R. and Suominen, P. 2007. Production in *Trichoderma reesei* of three xylanases from *Chaetomium thermophile*: a recombinant thermoxylanase for bleaching of kraft pulp. Appl. Microbiol. Biotechnol. 76:377–386.

Martin, C., Klinke, H. B., Marcet, M., García, L., Hernández, E., and Thomsen, A. B. 2007. Study of the phenolic compounds formed during pretreatment of sugarcane bagasse by wet oxidation and steam explosion. Holzforschung 61(5):483–487.

Marui, J., Tanaka, A., Mimura, S., de Graaff, L. H., Visser, J., Kitamoto, N., Kato, M., Kobayashi, T., and Tsukagoshi, N. 2002. A transcriptional activator, AoXlnR, controls the expression of genes encoding xylanolytic enzymes in *Aspergillus oryzae*. Fungal Genet. Biol. 35:157–169.

Morosoli, R., Durand, S. and Letendre, E. 1987. Induction of xylanase by β-methylxyloside in *Cryptococcus albidus*. FEMS Microbiol. Lett. 48:261–266.

Nel, W.P. and Cooper, C.J. 2009. Implications of fossil fuel constraints on economic growth and global warming. Energy Policy 37:166–180.

Nozaki, K., Seki, T., Matsui, K., Mizuno, M., Kanda, T. and Amano, Y. 2007. Structure and characteristics of an endo-beta-1,4-glucanase, isolated from *Trametes hirsuta* with high degradation to crystalline cellulose. Biosci. Biotechnol. Biochem. 71(10):2375–2382.

Perez-Gonzalez, J. A., van Peij, N. N. M. E., Bezoen, A., MacCabe, A. P., Ramon, D., and Graff, L. H. D. 1998. Molecular cloning and transcriptional regulation of the *Aspergillus nidulans xlnD* gene encoding a β-xylosidase. Appl. Environ. Microb. 64:1412–1419.

Polizeli, M. L. T. M. 2009. Properties and commercial applications of xylanases from fungi. In Advances in Fungal Biotechnology, ed. M. Rai, pp. 82–108. I.K. International, New Delhi.

Polizeli, M. L. T. M., Rizzatti, A. C. S., Monti, R., Terenzi, F. H., Jorge, J. A., and Amorim, D. S. 2005. Xylanases from fungi: properties and industrial applications. Review. Appl. Microbiol. Biotechnol. 67:577–591.

Prathumpai, W., McIntyre, M., and Nielsen, J. 2004. The effect of CreA in glucose and catabolism in *Aspergillus nidulans*. Appl. Microbiol. Biotechnol. 63:748–753.

Rahi, D. K., Rahi, S., Pandey, A. K. and Rajak, R. C. 2009. Enzymes from mushrooms and their industrial applications. In Advances in Fungal Biotechnlogy, ed. M. Rai, pp. 136–184. I.K. International, New Delhi.

Rahman, A. K. M. S., Sugitani, N., Hatsu, M., and Takamizawa, K. 2003. A role of xylanase, alpha-L-arabinofuranosidase, and xylosidase in xylan degradation. Can. J. Microbiol. 49:58–64.

Ramage, J., and Scurlock, J. 1996. Biomass. In Renewable Energy: Power For A Sustainable Future, chapter 4, ed. Boyle, G. pp. 137–182. Oxford University Press, Oxford.

Reese, E. T. and Mandels, M., 1963. Enzymatic hydrolysis of cellulose and its derivatives. Meth. Carb. Chem. 3:139–143.

Rizzatti, A. C. S., Jorge, J. A., Terenzi, H. F., Rechia, C. G. V., and Polizeli, M. L. T. M. 2001. Purification and properties of a termostable extracellular β-xylosidase produced by a thermotolerant *Aspergillus phoenicis*. J. Ind. Microbiol. Biotechnol. 26:156–160.

Rizzatti, A. C. S., Sandrim, V. C., Jorge, J. A., Terenzi, H. F., and Polizeli, M. L. T. M. 2004. Influence of temperature on the properties of the xylanolytic enzymes of the thermotolerant fungus *Aspergillus phoenicis.* J. Ind. Microbiol. Biotechnol. 31:88–93.

Rizzatti, A. C. S., Freitas, F. Z., Bertolini, M. C., Peixoto-Nogueira, S. C., Jorge, J. A., Terenzi, H. F., and Polizeli, M. L. T. M. 2008. Regulation of xylanase in *Aspergillus phoenicis*: a physiological and molecular approach. J. Ind. Microbiol. Biotechnol. 35:237–244.

Romanos, M. A., Scorer, C. A., and Clare, J. J. 1992. Foreign gene expression in yeast: a review. Yeast 8:423–488.

Ruijter, G. J. G., and Visser, J. 1997. Carbon repression in *Aspergilli.* FEMS Microbiol. Lett. 151:103–114.

Sakamoto, T., Ihara, H., Kozaki, S. and Kawasaki, H. 2003. A cold-adapted endo-arabinase from *Penicilium chrysogenum*. Biochim. Biophys. Acta. 1624(1–3):70–75.

Sandrim, V. C., Rizzatti, A. C. S., Terenzi, H. F., Jorge, J. A., Milagres, A. M. F., and Polizeli, M. L. T. M. 2005. Purification and biochemical characterization of two xylanases produced by *Aspergillus caespitosus* and their potential for the bleaching of kraft pulp. Process Biochem. 40 (5):1823–1828.

Sapag, A., Wouters, J., Lambert, C., de Ioannes, P., Eyzaguirre, J., and Depiereux, E. 2002. The endoxylanases from family 11: computer analysis of protein sequences reveals important structural and phylogenetic relationships. J. Biotechnol. 95:109–131.

Sa-Pereira, P., Paveia, H., Costa-Ferreira, M., and Aires-Barros, M. R. 2003. A new look at xylanases: An overview of purification strategies. Mol. Biotechnol. 24:257–281.

Schlacher, A., Holzmann, K., Hayn, M., Steiner, W., and Schwab, H. 1996. Cloning and characterization of the gene for the thermostable xylanase XynA from *Thermomyces lanuginosus.* J. Biotechnol. 49:211–218.

Schmoll, M., and Kubicek, C. P. 2003. Regulation of *Trichoderma* cellulose formation: lessons in molecular biology from an industrial fungus. A Review. Acta Microbiol. Immunol. Hung. 50(2–3):125–145.

Setati, M. E., Ademark, P., van Zyl. W. H., Hahn-Hägerdal, B., and Stålbrand, H. 2001. Expression of the *Aspergillus aculeatus* endo-β-1,4-mannanase encoding gene (man1) in *Saccharomyces cerevisiae* and characterization of the recombinant enzyme. Protein. Expr. Purif. 21(1):105–114.

Shafiee, S., and Topal, E. 2009. When will fossil fuel reserves be diminished? Energy Policy 37:181–189.

Silva, A. M. 2005. Doctor Thesis: Characterization of cell wall of *Saccharum officinarum* L. (sugarcane) and *Brachiaria decumbens* Stapf (braquiaria). UNICAMP, SP, Brazil.

Simerska, P., Monti, D., Cechova, I., Pelantova, H., Mackova, M., Bezouska, K., Riva, S., and Kren, V. 2007. Induction and characterization of an unusual alpha-D-galactosidase from *Talaromyces flavus*. J. Biotechnol. 128:61–71.

Strauss, J., Mach, R. L., Zeilinger, S., Stoffler, G., Wolschek, M., Hartler, G., and Kubicek, C. P. 1995. Cre I the carbon catabolite repressor protein from *Trichoderma reesei*. FEBS Lett. 376:103–107.

Sunna, A., and Antranikian, G. 1997. Xylanolytic enzymes from fungi and bacteria. Crit. Rev. Biotechnol. 17(1):39–67.

Takao, M., Akiyama, K., and Sakai, T. 2002. Purification and characterization of thermostable endo-1,5-alpha-L-arabinase from a strain of *Bacillus thermodenitrificans*. Appl. Environ. Microbiol. 68:1639–1646.

Tanaka, M., Umemoto, Y., Okamura, H., Nakano, D., Tamaru, Y., and Araki, T. 2009. Clonning and characterization of a beta-1,4-mannanase 5C prossessing a family 27 carbohydrate-binding module from a marine bacterium *Vibrio* sp. strain MA-138. Biosci. Biotechnol. Biochem. 73(1):109–116.

Tenkanen, M., and Siika-aho, M. 2000. An alpha-glucuronidase of *Schizophyllum commune* acting on polymeric xylan. J. Biotechnol. 78:149–161.

Törrönen, A., and Rouvinen, J. 1997. Structural and functional properties of low molecular weight endo-1,4-β-xylanases. J. Biotechnol. 57:137–149.

Tsai, C. T. and Huanga, C. T. 2008. Overexpression of the *Neocallimastix frontalis* xylanase gene in the methylotrophic yeasts *Pichia pastoris* and *Pichia methanolica*. Enzyme Microb. Technol. 42:459–465.

van Peij, N. N. M. E., Visser, J. and de Graaff, L. H. 1998. Isolation and analysis of xlnR, encoding transcriptional activator co-ordinating xylanolytic expression in *Aspergillus niger*. Mol. Microbiol. 27:131–142.

Wood, J. D., and Wood, P. M. 1992. Evidence that cellobiose:quinine oxidoreductase from *Phaenerochaete chrysosporium* is a breakdown product of cellobiose oxidase. Biochem. Biophys. Acta. 1119(1):90–96.

Zanoelo, F. F., Polizeli, M. L. T. M., Terenzi, H. F., and Jorge, J. A. 2004a. ß-Glucosidase activity from the thermophilic fungus *Scytalidium thermophilum* is stimulated by glucose and xylose. FEMS Microbiol Lett 240:137–143.

Zanoelo, F. F., Polizeli, M. L. T. M., Terenzi, H. F., and Jorge, J. A. 2004b. Purification and biochemical characterization of a thermostable xylose-tolerant ß-xylosidase from *Scytalidium thermophilum*. J. Ind. Microbiol. Biotechnol. 31:170–176