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REVIEW

MICROBIAL UTILIZATION OF LIGNOCELLULOSE COMPOSITES: FUNDAMENTALS AND APPLICATIONS

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ABSTRACT

In this review we describes processes by which microorganisms are able to alter structure of lignocellulose. Decay patterns caused by bacteria and fungi are overviewed. In next, applications of these processes in lignocellulolytic enzymes, animal feed and soil fertilizer production are described. Biopulping and biobleaching is illustrated as an environmental friendly alternative to traditional bleaching techniques. Methods of lignocellulose hydrolysis and fermentation of pentose sugars to ethanol are closely overviewed as a most promising technology.

KEY WORDS: lignocellulose, decomposition, ethanol, hydrolysis, pentose fermentation

INTRODUCTION

Wood and other lignocellulosic materials are formed by three main polymeric constituents: cellulose, hemicelluloses and lignin. Cellulose is a linear (often crystalline) polymer of cellobiose (D-glucopyranosyl-β-1,4-D-glucopyranose) that represents approximately 50% of wood dry weight (Tab. 1). The second structural component, hemicelluloses, is made up of different pentoses and hexoses, which are often acetylated and form branched chains. Lignin is a three-dimensional network built up of phenylpropane units. It is highly resistant towards chemical and biological degradation. Lignin and hemicelluloses forms an amorphous matrix in which the cellulose fibrils are sheltered against biological attack by most microorganisms. Many softwoods contain also system of resin and resin canals protecting them in case of injury. Despite of these protective components there are microbes, which are able to invade tree, untreated green lumber, treated wood and finished wooden products. These microorganisms are able to penetrate and disrupt the wood structure, utilizing one or more of wood composites. This process plays an important role in nature. By this way carbon fixed by photosynthesis can be integrated into carbon cycle. From the view of human benefits, biological degradation can be considered as unfavourable in the case of wood in structures, heritage collections etc. In another case specific utilization of lignocellulose composites is used with profit as a technological step in biobleaching and biopulping process, or in

solid-state fermentation (SSF). After saccharification, lignocellulose can serve as carbon and energy source for microorganisms in wide spectrum of technologies. In this paper we illustrate interactions between lignocellulose and microorganisms in native conditions and in some of the most perspective applications. The last but not least of them is lignocellulose to ethanol processing, which has been intensively studied for the last twenty years.

Tab. 1: Lignocellulose components of common agricultural residues

Lignocellulosic material	Cellulose	Hemicellulose	Lignin
Hardwoods	40-55	24-40	18-25
Softwoods	45-50	25-35	25-35
Wheat straw	30	50	15
Rice straw	32	24	18
Corn cob	45	35	15
Bermuda grass	25	36	6
Switch grass	45	31	12
Cotton seed hairs	80-95	5-20	0

Wood Biodeterioration

Biodeterioration is defined as a change in physical, chemical and mechanical characteristics of materials after interference with organisms. Wood biodeterioration can be caused by microorganisms, insect (wood-boring beetles, termites) and by marine borers. In this paper we mentioned only the case of microbial deterioration of wood.

In suitable conditions (e.g. moisture content, see below), if no proper prevention steps are undertaken, bacteria and filamentous fungi can attack the wood structure, utilising specific wood components. Some bacteria can live on the non-structural components in sapwood and may increase the permeability of wood by destroying pit membranes. This can be significant as an initial step of microbial decay. The rate of bacterial degradation is in common conditions very slow. Bacteria, together with actinomycetes, play a significant role in the humification processes associated with soils and composts (Lacey 1997). More significant is the decay process caused by fungi (Vargas-García et al. 2007, Hordová et al. 1998). The woodcolonizing fungi are usually divided into stain fungi, soft-rot fungi, and wood-rotting basidiomycetes. A limited number of ascomycetous fungi (e.g. *Ophiostoma* and *Ceratocystis* spp.) and deuteromycetous fungi (e.g. *Aureobasidium pullulans*, *Phialophora* spp. and *Trichoderma* spp.), called stain fungi, can colonize wood through parenchymatic rays and resin channels. On that occasion stain fungi can also play the role of initial decay microbe. This sort of fungi causes discoloration of softwood tissues but a very limited degradation, which mainly affects extractives and water-soluble materials. The category of soft-rot fungi includes some ascomycetes (*Chaetomium globosum, Ustulina deusta*) and deuteromycetes (*Alternaria alternata*, *Thielavia terrestris*, *Paecilomyces* spp.). Soft-rot fungi can degrade polymers of lignocellulose matrix under extreme environmental conditions (high or low humidity) that inhibit the activity of other fungi. This results in decrease in the mechanical properties of wood. Degradation process is obvious, in wet environment wood goes soft consistency and in dry environment it goes crumbly and brown. Wood-rotting basidiomycetes degrade wood much faster than the soft-rot fungi. They are divided to brown-rot fungi (e.g. *Coniophora puteana, Gloeophyllum trabeum, Laetiporus sulphureus, Piptoporus betulinus, Postia placenta* and

Serpula lacrimans) and white-rot fungi (e.g. *Phanerochaete chrysosporium, Ganoderma australe, Phlebia tremellosa, Ceriporiopsis subvermispora, Pleurotus* spp. and *Phellinus pini*). Brown-rot fungi degrade primarily the cellulose, but they do modify lignin leaving brown, dry and charred like wood of brittle consistency. White-rot fungi, the most frequent wood-rotting organisms, are characterized by their ability to degrade lignin, hemicelluloses, and cellulose selectively, often giving rise to a cellulose-enriched white material (Akhtar et al. 1997, Kerem et al. 1992).

The capability of lignocellulose degradation is connected with wide scale of hydrolytic and oxidative enzymes. The main of ligninolytic enzymes are laccase, lignin peroxidase, manganese peroxidase, versatile peroxidase, H_2O_2 - forming enzymes such as glyoxal oxidase, and aryl alcohol oxidase. Lignin degrading enzymes are non-specific oxidases and hydrolases, well described in literature, e.g., in the review of Vicuña (2000). Among hemicellulose degrading enzymes endo-xylanase, β-xylosidase and accessory enzymes such as xylan esterases, ferulic and *p*-coumaric esterases, α-l-arabinofuranosidases, and α-4-O-methyl glucuronosidases acting synergistically to efficiently hydrolyze wood xylans and mannans. The enzymes and mechanisms involved in cellulose saccharification are described below. For more information about lignocellulolytic enzymes see also review of Pèrez et al. (2002).

Lignocellulose applications

Agriculture and woodworking industries produce large amounts of plant biomass often considered as a waste. It has been estimated that within the agricultural sector in the EU, 1500 million tons of biomass could be processed biologically each year (Amon et al. 2001). Forest residues, sawdust, wood chips and crop residues like wheat straw or corn stover are very cheap and widely accessible, therefore they represent well suited substrate for biotechnological production. Natural facility of microorganisms to utilize or modify lignocellulose and its composites gives us wide application possibilities. The most important of them are described in separate chapters. Lignocellulosic materials can be fermented in its natural state or after foregoing saccharification (Fig. 1). By direct fermentation wide range of lignocellulolytic or further types of enzymes can be produced. Agricultural residues are used for mushroom cultivation, animal feed and soil fertilizers production. Lignocellulose is as well a raw material for the paper industry. Biobleaching and biopulping, solid-state fermentation of wooden material, can be used with benefits in the paper industry. Saccharides (mainly glucose and xylose) obtained by hydrolysis represent two separated solutions in the case of two-step hydrolysis, or a mixture of saccharides in the case of one-step hydrolysis (see next). Hemicellulose hydrolysate is a source of xylose, which can be converted by biological way to xylitol or ethanol (Winkelhausen and Kuzmanova 1998). Cellulose hydrolysate can serve as a substrate in many starch-glucose based technologies, i.e. single cell proteins, enzymes, fine chemicals or antibiotics production. These technologies are not specific for lignocellulose as a raw material, therefore we did not mention it. It is expected that in the near future lignocellulosic biomass will become the main feedstock for fuel ethanol production (see next).

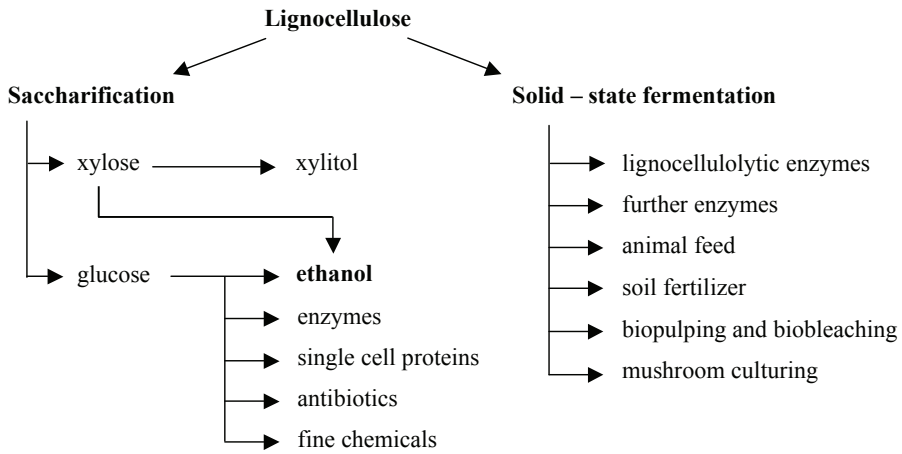

Fig. 1: Biotechnological applications of lignocellulose

Lignocellulolytic enzymes production

Lignocellulolytic enzymes have numerous applications and biotechnological potential for various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture. Lignocellulolytic enzymes (mainly cellulase, laccase and xylanase) are usually produced by submerged fermentation, using hyperproducing mutants. In recent years, an increasing interest can be observed in the solid-state fermentation, since several studies showed superior product yields and simpler downstream processing than submerged fermentation. Szendefy et al. (2003) have demonstrated that SSF can be successfully used for efficient xylanase production by *Thermomyces lanuginosus*. Subsequently the fermented material was applied in biobleaching of the same pulp without a prior downstream processing of the xylanase. Due to the considerably lower production costs, the SSF xylanase proved more cost-efficient when compared to commercial liquid enzyme products. Other advantages of SSF are low capital investment, low energy requirement and less water output. On the other hand, disadvantages of SSF are high specificity for each substrate and microbe pair, and complicated aeration, temperature and pH regulation. Feasibility of different lignocellulosic waste materials for lignocellulolytic enzymes production was demonstrated for fungi (Latifian et al. 2007) and bacteria (Asha Poorna and Prema 2007). Improved enzyme production can be achieved under nitrogen limitation conditions (Buswell et al. 1984), or by mixed fungal cultures (Stoilova et al. 2005). Synergistic interactions between fungi depend on the particular species combination, on the mode of interaction between species, and on the environmental or nutritional conditions in the substrate (Chi et al. 2007). For more information about lignocellulolytic enzymes and its production see the reviews of Howard et al. (2003) and Hölker et al. (2004).

Animal feed production

Microorganisms cultivated on agro-industrial wastes are very attractive feedstuffs, because of production of large amounts of cells rich in proteins. Furthermore, the growth of microbes on lignocellulosic wastes is able to furnish all the hydrolytic enzymes often added in the preparation of feeds, and also makes the minerals more available for absorption by the animal. During microbial processes for conversion of lignocellulosic wastes into feeds, at least one of

three objectives must be reached: an increase in the protein level, an increase in the digestibility of the lignocellulosic material and an improvement in the dry product palatability, although this last factor can be easily improved by ensiling or mixing the substrate with other more palatable foods (Flachowsky et al. 1999).

Digestibility of lignocelluloses is connected with lignin content. Therefore biomass with low lignin content (i.e. straws and grasses) is suggested to be better feedstock. Royse and Sanchez (2007) consider, that the spent shiitake substrate from wheat straw-amended substrate would be excellent for ruminant feed, after potassium and fat content adjusting. Jonathan et al. (2007) achieved improved digestibility by ruminants after fermentation of wood wastes from different economically important Nigerian trees by white rot fungus *Pleurotus tuber-regium*. Although further increase in digestibility is required for widespread feed potential. The same conclusion was made by Okano et al. (2005) after screening the potential of five white-rot fungi to convert Japanese red cedar into feed for ruminants. Peñaloza et al. (1985) came to conclusion that SSF (performed by *A. niger*) represents an alternative way to improve the nutritive value of coffee pulp for monogastric animal feeding. A lowering in the cellulose and hemicellulose content of coffee pulp and a partial utilization of the caffeine, lignin, and tannin components seem to be enough to warrant the inclusion of the material at a 10% level in a growing chicken's diet, ensuring a feed efficiency equal to that of the standard diet.

We have not mentioned the traditional preserving of green feed for ruminants by ensilaging, because lignocellulose is not the subject of fermentation in this process.

Soil fertilizer production

Large-scale alternative for biomass combustion is composting. Compost has proved to be an attractive material for improving soil structure in tilled soils and for increasing dry matter production in grassland soils, owing to its high organic matter content and availability of essential plant nutrients. Direct incorporation of crop residues into soil is limited by the great bulk of residues, slow biodigestion in soil and risk of diseases harbouring. Ex situ composting represents a potential way to recover value and avoid burning or disposal to landfill. From microbiological point of view, composting process is very similar to natural lignocellulose decomposition. The main role is played by actinomycetes and wood-rotting basidiomycetes (Abdulla and El-Shatoury 2007, Lacey 1997). In this sense, also spent mushroom substrate is a beneficial product for the enrichment of soils in fruit, vegetable and flower greenhouses production (Batista et al. 2000). One of the beneficial properties of compost-amended soils is also the microbially induced suppression of soil borne plant pathogens and diseases (Craft and Nelson 1996). In this context the most frequently studied is the fungal genera *Trichoderma* (soil fungi present in plant root ecosystems) and bacterial genera such as *Agrobacterium*, *Pseudomonas*, *Streptomyces* and *Bacillus* (Harman et al. 2004, Hoitink and Boehm 1999, De La Fuente et al. 2007, Joshi et al. 2007, Rezzonico et al. 2003). Using of lignocellulose-based agricultural byproducts as a cheap substrate is one of the proper production possibilities for biocontrol agents production (Suárez-Estrella et al. 2007).

Biopulping and biobleaching

Biopulping is defined as the treatment of wood chips with lignin-degrading fungi prior to pulping. As we have described, fungi are able to degrade or alter the structure of lignin in the wood cell walls, which affects softening of wood. This substantially reduces the electrical energy needed for mechanical pulping, increases mill throughput and leads to improvements in the paper strength properties (Scott et al. 2002). The fungal pre-treatment is a natural

process, therefore no adverse environmental consequences are foreseen. White-rot fungi, e.g. *Ceriporiopsis subvermispora* and *Phanerochaete chrysosporium*, are frequently referred as the most feasible for biopulping, because of their ability of selective lignin degradation (Aguiar and Ferraz, 2007, Vicentim and Ferraz, 2007). Wide array screenings of white-rot fungi are made for better delignification systems to be used in biopulping and other biotechnological applications. Wolfaardt et al. (2004) have tested two hundred and seventy eight strains of wood inhabiting basidiomycetes representing 44 genera for the pre-treatment of softwood chips for kraft pulping. Under the specific pulping conditions of the screening trials, 38 strains of white-rot fungi were more suitable than the reference strains of *P. chrysosporium* and *C. subvermispora*. Berrocal et al. (2000 and 2004) have reported *Streptomyces cyaneus* as an actinomycete capable of degrading lignin with only a limited attack of the cellulose component. In following work, they have concluded that the pre-treatment of wheat straw with *S. cyaneus* has a positive effect on some strength properties of the pulps. The main drawback is a long time period (approximately two weeks) needed to effective wood treatment. For overcoming this disadvantage, process integration is suggested (Akhtar et al. 1998).

Environmental and economical benefits, i.e. lower chemicals inputs, can be achieved also by biobleaching. Biobleaching is defined as a process of decolourization of wood pulp by microorganisms or enzymes. Xylanase can be used in many stages of pulp and paper processing and its beneficial for biobleaching has been well documented. Khandeparkar and Bhosle (2007) have used thermoalkalophilic xylanase from *Arthrobacter sp.* and reached 20 % reduction in kappa number of the kraft pulp without much change in viscosity. This was equivalent to 29 % reduction in chlorine during the bleaching process. Moreover, the enzyme treatment enhanced the brightness of the pulp by 9.6 % compared to the untreated pulp. For biobleaching of kraft pulp, laccase has been widely investigated, second only to xylanase. Treatment of pulp with laccase in combination with a mediator such as violuric acid has been found to be a promising delignification method. The mediator is necessary because laccase itself is unable to penetrate the pulp fibers wall. The large size of laccase limits the action of the enzyme to the fiber surface (Chakar and Ragauskas 2004, Stone et al. 1969).

Biological treatment by lignolytic enzymes is used also for remediation of waste-waters (El Hajjouji et al. 2007) and pulp and paper industry effluents, which is closely described in a review of Thompson et al. (2001).

Lignocellulose hydrolysis

The complex structure of lignocellulose can be broken down not only in biological way, but also by different physical, termophysical and chemical ways, in the process of hydrolysis. The objective of hydrolysis is to gain solution of reducing sugars from polysaccharides contained in raw material. Hydrolysis of lignocellulose by concentrated or diluted acids is a well-known method to obtain fermentable saccharides. There is substantial difference in stability of cellulose, hemicelluloses and lignin. Hydrolysis in two steps can be performed and two different hydrolysates can be obtained. Degradation of hemicelluloses comes into being in relatively moderate conditions. Hydrolysate contains mainly pentoses, xylose and arabinose, but also hexoses, glucose, galactose and mannose. Cellulose is degraded at higher temperature and pressure. Hydrolysate contains mainly glucose. However, the hydrolysates contain not only fermentable sugars but also acetic acid and some furan compounds such as furfural and 5-hydroxymethylfurfural, which are formed by the degradation of sugars, and various phenolic compounds, which are formed by degradation of lignin. Composition of compounds depends on the type of used lignocellulosic material and the chemistry and nature of the pretreatment process.

These degradation products inhibit the growth and fermentative activity of microorganisms. There are different detoxification methods such as extraction with organic solvents, overliming, evaporation, steam stripping, sulfite treatment, ion exchange, ammonium hydroxide, enzyme treatment, zeolite treatment and activated carbon treatment (Larsson et al. 1999, Mussatto and Roberto 2004).

The use of corrosive agents and high temperatures and pressures makes acid hydrolysis method expensive. These disadvantages do not concern the case of enzymatic hydrolysis. Biological process produces fewer by-products in comparison with acid hydrolysis, no aggressive chemicals or high temperature and pressure are needed, but it is more expensive owing to high price and unrepeatable use of enzymes. Typical saccharification schemes involve a pre-treatment step to improve substrate accessibility, followed by enzymatic hydrolysis of the cellulose component to produce glucose. Cellulose hydrolysis and glucose fermentation can be integrated in simultaneous saccharification and fermentation. The main benefit of simultaneous saccharification and fermentation is absence of end product inhibition of cellulose hydrolysis (see next).

The point of lignocellulose pre-treatment is to remove lignin and hemicellulose, reduce cellulose crystallinity, increase the porosity of materials and make by this way cellulose more accessible to the enzymes (Mosier et al. 2005). A variety of physical, physico-chemical, and chemical processes have been used for pretreatment of lignocellulosic materials (Tab. 2).

Tab. 2: Methods for lignocellulose pre-treatment

Type of pre-treatment	**Pre-treatment reagent**	**Reference**
Physical	Liquid hot water	Liu and Wyman, 2005
Physico-chemical	Steam explosion	Fernandez-Bolanos et al., 2001
	CO_2 explosion	Dale and Moreira, 1982
	Ammonia fiber explosion	Dale et al., 1996
Chemical	Concentrated acid	Goldstein and Easter, 1992
	Dilute acid	Sun and Cheng, 2005
	Alkaline	Koullas et al., 1993
	SO_2	Clark and Mackie, 1987
	Lime	Kim and Holtzapple, 2005
	Hydrogen peroxide	Dawson and Boopathy, 2007
	Organic solvent	Chum et al., 1988

Enzymatic hydrolysis of cellulose requires synergistic activities of three types of enzymes. Endo-β-1,4-glucanases hydrolyze accessible regions on cellulose chains to provide new sites for attack by exo-acting cellobiohydrolases which remove successive cellobiose units from newly created chains ends. Finally, β-glucosidase hydrolyzes cellobiose, and smaller amounts of higher cellooligomers, to glucose. The rate-limiting step is the ability of endoglucanases to reach amorphous regions within the crystalline matrix and create new chain ends, which exo-cellobiohydrolases can attack. Cellulolytic enzymes are inhibited by end products cellobiose and to a lesser extent by glucose. Several methods have been developed to reduce the inhibition, including the use of high concentrations of enzymes, the supplementation of β-glucosidases during hydrolysis, and the removal of sugars during hydrolysis by ultrafiltration or simultaneous saccharification and fermentation. In the last named, enzymatic degradation of cellulose is integrated with the fermentation. Glucose in operation is immediately eliminated

by ethanol producing microorganism. Drawback of simultaneous processes is that the optimal temperatures for hydrolysis (40-50°C) and fermentation (30-35°C) are different, which implies a difficult control and optimization of process parameters (Claassen et al. 1999, Olsson and Hahn-Hägerdal 1996). Nevertheless simultaneous saccharification and fermentation can reach higher rates, yields and ethanol concentrations in comparison with separate hydrolysis and fermentation (Wyman et al. 1992). Limiting fact for large scale enzymatic hydrolysis is high enzyme cost. After low cost techniques for enzymes production using SSF, the most promising is the strategy of process integration.

Lignocellulose to ethanol processing

Ethanol is considered as the most important renewable fuel contributing to the reduction of negative environmental impacts generated by the worldwide utilization of fossil fuels. Ethanol can be made synthetically from petroleum or by microbial conversion of sugars through fermentation. About 90 % of ethanol is produced by the fermentation method, the rest by the synthetic method (Taherzadeh 1999). The method of microbial conversion generally uses three steps: (1) the formation of a solution of fermentable sugars, (2) the fermentation of these sugars to ethanol, and (3) the separation and purification of the ethanol, usually by distillation. A dramatic increase in ethanol production using the current cornstarch-based technology seems not to be practical because corn production for ethanol will compete for the limited agricultural land needed for food and feed production. A potential low-cost source of fermentable sugars for ethanol production is lignocellulosic waste material (Sun and Cheng 2002).

The process overview and stumbling blocks in lignocellulose saccharification, the first technological step, were described above. The second technological step is fermentation of sugars concluded in hydrolysates. Hydrolysates of cellulose and hemicellulose are usually produced and fermented separately. Glucose obtained from cellulose is fermented by yeasts *S. cerevisiae* in the same way as starchy glucose. This process is well known and described elsewhere (Rebroš et al. 2005, Purwadi et al. 2007, Tao et al. 2005). But *S. cerevisiae* does not ferment xylose, the second most abundant sugar obtained from lignocellulose (Purwadi and Taherzadeh, 2007). Several authors have illustrated that efficient fermentation of pentoses is important for the overall economy of ethanol production from lignocellulosic materials (Cardona and Sánchez 2007, Zaldivar et al. 2001). First xylose fermenting yeasts were referred in 1981 in parallel by Jeffries (1981) and Schneider et al. (1981). Later, *Candida shehatae*, *Pichia stipitis* and *Pachysolen thannophilus* were selected as the best natural xylose fermenting yeasts in widespread screening works. In following research pentose metabolism and mainly oxygen demand of these producers were investigated. Xylitol production, sensibility to oxygenation and catabolic repression by hexoses were determined as the main problems of xylose fermentation. Low ethanol tolerance and inhibition effect of hydrolysis byproducts are the next bottlenecks of natural xylose fermenting yeasts. In many cases metabolism was redirected toward improved ethanol production by genetic manipulations (Jeffries 2006).

Use of recombinant techniques for ethanol production improvement

Recombinant techniques were used for introducing xylose and arabinose metabolism into *S. cerevisiae* and *Zymomonas mobilis*, both naturally ethanologenic microorganisms. The initial metabolic engineering of *S. cerevisiae* for xylose assimilation attempted the heterologous expression of bacterial xylose isomerase (Walfridsson et al. 1996). Further

recombinant strains with ability of xylose fermentation were constructed by integrating the *P. stipitis* genes XYL1 and XYL2 (encoding the *P. stipitis* xylitol dehydrogenase) and the endogenous XKS1 (encoding the *S. cerevisiae* xylulokinase) (Eliasson et al. 2000). At using of both strategies, difficulties, namely co-production of xylitol, low ethanol production rates, low xylose transport rates, and repression of xylose utilization by glucose have arisen. For *S. cerevisiae* further success was achieved by protein engineering of xylitol dehydrogenase (Watanabe et al. 2007, Jeppsson et al. 2006). Recently, simultaneous co-utilization of xylose and arabinose in recombinant strains of *S. cerevisiae* was demonstrated in the work of Karhumaa et al. (2006).

Into *Z. mobilis* four *E. coli* enzymes (genes) for xylose metabolism were introduced, xylose isomerase (xylA), xylulose kinase (xylB), transketolase (tktA), and transaldolase (talB) (Zhang et al. 1995). Resultant strain *Z. mobilis* CP4 (pZB5) and later constructed strains ferment glucose and xylose in mixtures simultaneously with high yields. The same strategy was successfully used to create arabinose-fermenting strains of *Z. mobilis* (Deanda et al. 1996). Genetic stability of recombinant plasmid-bearing *Z. mobilis* was improved by chromosomal integrating of inserted genes (Mohagheghi et al. 2002). General disadvantage for recombinant *Z. mobilis* strains is high sensitivity to microbial inhibitors commonly associated with hydrolysates, especially acetic acid, and lower ethanol tolerance than in original strains. These obstructions can be avoided by long-term adaptation for given conditions (Lawford et al. 1999, Lawford and Rousseau 1999).

Bacteria have usually a wide spectrum of utilized sugars. This advantage was used in construction of recombinant ethanologenic *Escherichia coli*, *Erwina chrysanthemi* and *Klebsiella oxytoca*. Ingram et al. (1987) have introduced into *E. coli* plasmid expressing alcohol dehydrogenase II (*adh II*) and pyruvate decarboxylase (*pdc*) from *Z. mobilis*. These first ethanol producing *E. coli* strains were genetically unstable, plasmids carrying the *adh* and *pdc* genes were in long-term fermentation lost in the absence of antibiotics. Difficulties with phenotypic stability remain also after chromosomal integrating of the genes (Ohta et al. 1991a, Dumsday et al. 1999). Long-term adaptation and further selection were used for achieving strain *E. coli* LY01 with superior phenotypic stability, tolerance to ethanol and inhibitors found in lignocellulose hydrolysates (Yomano et al. 1998, Zaldivar and Ingram, 1999, Zaldivar et al. 1999 and 2000). Ingram and colleagues have also transformed the related Gram-negative bacteria, *K. oxytoca* and *E. chrysanthemi* with the same *Z. mobilis* genes. Though the resulting strains have lower ethanol yields than *E. coli*, significant progress has since been made in developing improved *K. oxytoca* strains especially well suited for converting cellulose to ethanol (Ohta et al. 1991 b).

Within bacteria genetically improved for ethanol from lignocellulose production is also strain *Thermoanaerobacter* BG1L1, which is a lactate dehydrogenase deficient mutant of the wild-type thermophilic (thermal optimum 70°C) anaerobic bacterial strain *Thermoanaerobacter* BG1 (Georgieva et al. 2007). Georgieva and Ahring (2007) demonstrated that the use of immobilized *Thermoanaerobacter* BG1L1 for continuous ethanol fermentation could be promising in a commercial ethanol process in terms of system stability to process hardiness and reactor contamination.

The global research effort is directed towards developing a stable, ethanol-tolerant, ethanol-producing organism capable of tolerating common fermentation inhibitors generated during lignocellulose saccharification. The larger sizes, thicker cell walls, better growth at low pH, less stringent nutritional requirements, and greater resistance to contamination give yeasts advantages over bacteria. Therefore, despite of rapid progress in

recombinant bacterium strains construction, commercial ethanol producers abide with *S. cerevisiae* yeasts.

Currently, there are only few plants processing biomass to ethanol. Iogen, a Canadian company located in Ottawa, established a pilot plant in 2002, which can process 25 tons of wheat straw per week and correspondingly, produce 320,000 liters of ethanol per year (www.iogen.ca). The second one, BioGasol, a Danish company located in Bornholm Island, established a pilot plant in 2006, which can produce around 10 million liters of ethanol per year (www.biogasol.dk). Abengoa Bioenergy has opened in Nebraska a pilot plant processing 70 tones of wheat straw a day and producing over 5 million liters of fuel grade ethanol per year. The state-of-the-art plant is dedicated to the research and development of biofuel production processes (www.abengoabioenergy.com).

CONCLUSIONS

This literature survey has shown that wood even though its protective composition may be invaded by microorganisms. A chain of events including variety of bacteria and fungi leads to wood decay. Our current knowledge of the enzymology, physiology, biochemistry, and molecular biology of these microbes and involved enzymes is considerable. Consequently, processes that use enzymes and microorganisms are being developed to explore the potential for their biotechnological applications. Biopulping and biobleaching are leading to cleaner and more efficient pulp and paper manufacture. Biomass energy can play an important role in reducing greenhouse gas emissions, since CO_2 that arises from biomass wastes would originally have been absorbed from the air, the use of biomass for energy offsets fossil fuel greenhouse gas emissions. Production of ethanol from lignocellulosic biomass can provide renewable energy source for transportation use independent from fossil fuels resources. Commercialization of the lignocellulosic fuel ethanol will necessitate effective fermentation of both, glucose and xylose fractions of lignocellulose hydrolysates. In spite of some stumbling blocks in xylose utilization, achievements in this field are expected since the advanced progress of recombinant techniques.

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