Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 99 (2008) 5270–5295
BIORESOURCE TECHNOLOGY

Review

Trends in biotechnological production of fuel ethanol from different feedstocks

Óscar J. Sánchez [a,b], Carlos A. Cardona [a,*]

[a] Department of Chemical Engineering, National University of Colombia at Manizales, Cra. 27 No. 64–60 Of. F-505, Manizales, Caldas, Colombia
[b] Department of Engineering, University of Caldas, Calle 65 No. 26–10, Manizales, Caldas, Colombia

Received 12 July 2006; received in revised form 4 November 2007; accepted 6 November 2007
Available online 26 December 2007

Abstract

Present work deals with the biotechnological production of fuel ethanol from different raw materials. The different technologies for producing fuel ethanol from sucrose-containing feedstocks (mainly sugar cane), starchy materials and lignocellulosic biomass are described along with the major research trends for improving them. The complexity of the biomass processing is recognized through the analysis of the different stages involved in the conversion of lignocellulosic complex into fermentable sugars. The features of fermentation processes for the three groups of studied feedstocks are discussed. Comparative indexes for the three major types of feedstocks for fuel ethanol production are presented. Finally, some concluding considerations on current research and future tendencies in the production of fuel ethanol regarding the pretreatment and biological conversion of the feedstocks are presented.

Keywords: Bioethanol; Sugars; Starch; Lignocellulosic biomass; Ethanolic fermentation

1. Introduction

World faces the progressive depletion of its energetic resources mainly based on non-renewable fuels. At the same time, energy consumption grows at rising rates. The USA is the first oil consumer, but China's spectacular economic growth has imposed serious pressure on the oil market. Global panorama in that market is dark. Permanent crises in the Middle East and the speculation in the stock exchange, among other factors, have caused the oil price to reach such elevated values of 100 dollars per barrel. World economy could experience stagnation if the oil maintains these high prices. In addition, the intensive utilization of fossil fuels has led to the increase in the generation of polluting gases released into the atmosphere, which have caused changes in the global climate. The solution to this problematic depends on how the development and implementation of technologies based on alternative sources of energy will be undertaken. Through the use of renewable energetic resources, humankind can find part of the solution to their energy requirements in an environmentally friendly way.

One renewable solution is the use of solar energy in form of biomass (bioenergy). Global potential of bioenergy is represented in energy crops and lignocellulosic residues. Conversion of these feedstocks into biofuels is an important choice for the exploitation of alternative energy sources and reduction of polluting gases. In addition, the utilization of biofuels has important economic and social effects. For instance, Sheehan and Himmel (1999) point out that the diversification of fuel portfolio would bring money and jobs back into the USA economy. Moreover, the development of energy crops dedicated to the biofuels production would imply a boost to agricultural sector. This analysis is also valid for developing countries, especially in Latin America, considering the perspective of drastic reduction of proven oil reserves in the mid term. In addition, agricultural products from developing countries have

* Corresponding author. Tel.: +57 6 8810000x50417; fax: +57 6 88 10000x50193.
E-mail address: ccardonaal@unal.edu.co (C.A. Cardona).

0960-8524/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.biortech.2007.11.013

to face a fierce competition from rich countries that grant huge subsidies for their agricultural production.

Ethanol (ethyl alcohol, bioethanol) is the most employed liquid biofuel either as a fuel or as a gasoline enhancer. Ethanol has some advantages when it is used as an oxygenate. Firstly, it has a higher oxygen content that implies a less amount of required additive. The increased percentage of oxygen allows a better oxidation of the gasoline hydrocarbons with the consequent reduction in the emission of CO and aromatic compounds. Related to MTBE, ethanol has greater octane booster properties, it is not toxic, and does not contaminate water sources. Nevertheless, ethanol production costs are higher than those of MTBE, gasoline mixed with alcohol conduces the electricity, and Reid vapor pressure is higher that entails a greater volatilization, which can contribute to ozone and smog formation (Thomas and Kwong, 2001). Many countries have implemented or are implementing programs for addition of ethanol to gasoline (see Table 1). Fuel ethanol production has increased remarkably because many countries look for reducing oil imports, boosting rural economies and improving air quality. The world ethyl alcohol production has reached about 51,000 mill liters (Renewable Fuels Association, 2007), being the USA and Brazil the first producers (see Table 2). In average, 73% of produced ethanol worldwide corresponds to fuel ethanol, 17% to beverage ethanol and 10% to industrial ethanol.

The fuel ethanol can be obtained from energy crops and lignocellulosic biomass. The complexity of the production process depends on the feedstock. In this way, the spectrum of designed and implemented technologies goes from the simple conversion of sugars by fermentation, to the multi-stage conversion of lignocellulosic biomass into ethanol. The big diversity of technological alternatives requires the analysis of the global process along with the design and development of each one of the involved operations. Among the new research trends in this field, process integration has the key for reducing costs in ethanol industry and increasing bioethanol competitiveness related to gasoline. This issue is the main topic analyzed in a previous paper (Cardona and Sánchez, 2007). Several reviews have been published on the theme of fuel ethanol production especially from lignocellulosic biomass (Chandrakant and Bisaria, 1998; Lee, 1997; Lin and Tanaka, 2006; Lynd, 1996). The amount of reviews covering ethanol production form other types of feedstocks like sucrose-based or starchy materials is more reduced (e.g., Kosaric and Velikonja, 1995; Bothast and Schlicher, 2005). Nevertheless, an analysis of this process from the viewpoint of the three major types of feedstock has not been the main objective of those works. In addition, some issues concerning the feedstocks features on a comparative basis have not always been sufficiently emphasized. This paper attempts to achieve this aim considering the literature reviewed in the last one decade. Therefore, the purpose of this work is to analyze the different trends in fuel ethanol production taking into account both mature and developing technologies and making emphasis on the different types of raw materials

Table 2
World production of ethyl alcohol (mill liters)

Country	2006	2005
1. USA	18,376	16,139
2. Brazil	16,998	15,999
3. China	3,849	3,800
4. India	1,900	1,699
5. France	950	908
6. Germany	765	431
7. Russia	647	749
8. Canada	579	231
9. Spain	462	352
10. South Africa	386	390
11. Thailand	352	299
12. United Kingdom	280	348
13. Ukraine	269	246
14. Colombia[a]	269	27
15. Poland	250	220
Total	51,056	45,988

[a] These data correspond to the fuel ethanol produced in new distilleries whose construction started in 2005 (Londoño, 2007); industrial and beverage alcohol are not included, although their share is significantly lower. Modified from Renewable Fuels Association, 2007.

Table 1
Fuel ethanol programs in some countries

Country	Feedstock	Percentage of ethanol in gasoline blends, % (v/v)	Remarks
Brazil	Sugar cane	24	ProAlcool program; hydrous ethanol is also used as fuel instead of gasoline
USA	Corn	10	Oxygenation of gasoline is mandatory in dirtiest cities; tax incentives; some states have banned MTBE; 85% blends are also available
Canada	Corn, wheat, barley	7.5–10	Tax incentives; provincial programs aimed to meet Kyoto Protocol
Colombia	Sugar cane	10	Began in November 2005; total tax exemption
Spain	Wheat, barley	–	Ethanol is used for ETBE production; direct gasoline blending is possible
France	Sugar beet, wheat, corn	–	Ethanol is used for ETBE production; direct gasoline blending is possible
Sweden	Wheat	5	85% blends are also available; there is no ETBE production
China	Corn, wheat		Trial use of fuel ethanol in central and north-eastern regions
India	Sugar cane	5	Ethanol blends are mandatory in 9 states
Thailand	Cassava, sugar cane, rice	10	All gasoline stations in Bangkok must sell ethanol blends; ethanol blends will be mandatory from 2007

Adapted from Murray (2005) and Berg (2004).

from which fuel ethanol is obtained, and the possibilities for using alternative feedstocks leading to an improvement of the global process.

2. Ethanol from sugars

Main feedstock for ethanol production is sugar cane in form of either cane juice or molasses (by-product of sugar mills). About 79% of ethanol in Brazil is produced from fresh sugar cane juice and the remaining percentage from cane molasses (Wilkie et al., 2000). Sugar cane molasses is the main feedstock for ethanol production in India; cane juice is not presently used with this purpose (Ghosh and Ghose, 2003). Beet molasses are other source of fermentable sugars for ethanologenic fermentation. The most employed microorganism is *Saccharomyces cerevisiae* due to its capability to hydrolyze cane sucrose into glucose and fructose, two easily assimilable hexoses. Aeration is an important factor for growth and ethanol production by *S. cerevisiae*. Although this microorganism has the ability to grow under anaerobic conditions, small amounts of oxygen are needed for the synthesis of substances like fatty acids and sterols. The oxygen may be supplied through the addition to the medium of some chemicals like urea hydrogen peroxide (carbamide peroxide), which also contributes to the reduction of bacterial contaminants as claimed in the patent of Narendranath et al. (2000). Other yeasts, as *Schizosaccharomyces pombe*, present the additional advantage of tolerating high osmotic pressures (high amounts of salts) and high solids content (Bullock, 2002; Goyes and Bolaños, 2005). In fact, a fermentation process using a wild strain of this yeast has been patented (Carrascosa, 2006). Among bacteria, the most promising microorganism is *Zymomonas mobilis*, which has a low energy efficiency resulting in a higher ethanol yield (up to 97% of theoretical maximum). However, its range of fermentable substrates is too narrow (glucose, fructose and sucrose) (Claassen et al., 1999). Other disadvantage of the use of this bacterium during the fermentation of sugar cane syrup and other sucrose-based media is the formation of the polysaccharide levan (made up of fructose units), which increases the viscosity of fermentation broth, and of sorbitol, a product of fructose reduction that decreases the efficiency of the conversion of sucrose into ethanol (Lee and Huang, 2000).

The high osmolality of the media based on cane molasses is negative for ethanolic fermentation. This osmolality is related to the concentration of sugars and salts in the medium. Different studies have been carried out in order to obtain *S. cerevisiae* strains with greater salt and temperature tolerance. For example, Morimura et al. (1997) developed by protoplast fusion and manipulating culture conditions, flocculating strains capable of growing at 35 °C and at molasses concentration of 22% (w/v). Under these conditions and using repeated-batch cultures at laboratory scale, ethanol concentration of 91 g/L and productivities of 2.7 g/(L h) were obtained. However, the principal approach for avoiding the negative influence of salts and other compounds on the fermentation is through the conditioning of molasses by the addition of different compounds neutralizing the inhibitory effects of the medium components. In addition, molasses should be supplemented with nutritional factors promoting the yeast growth.

Agents used for cane molasses have demonstrated their usefulness for conditioning beet molasses such as EDTA, ferrocyanide and zeolites (Ergun et al., 1997). According to Castellar et al. (1998), zeolites could also act as a pH regulator. In the case of fermentations with high glucose concentration and using *Saccharomyces bayanus*, the zeolite use allows the maintenance of the medium pH around 3.7 leading to the consumption of all the initial glucose and, therefore, to a higher ethanol concentration. Other substances have demonstrated their usefulness during the preparation of media based on sugar cane. The addition of a commercial enzymatic complex of amylases, cellulases and amylopectinases allows the conversion of non-fermentable substances into assimilable compounds improving the alcoholic fermentation (Acevedo et al., 2003). On the other hand, adding a minimum inhibitory concentration of hop acids to molasses will stop bacteria growth, increase ethanol yields and avoid the need for antibiotics as described in the patent of Maye (2006).

2.1. Batch and semicontinuous processes

The *Melle-Boinot* process is the typical process for fuel ethanol production by batch fermentation. This process comprises the weight and sterilization of feedstock, followed by the adjustment of pH with H_2SO_4 and of the degrees Brix to values of 14–22. Obtained wort is fermented by yeasts. The produced wine is decanted, centrifuged and sent to ethanol separation stage, whereas the yeasts are recycled to the fermentation in order to reach high cell concentration during cultivation (Kosaric and Velikonja, 1995).

Fed-batch culture implies low levels of substrate concentration during the course of fermentation, while ethanol is accumulating in the medium. This type of cultivation regime, along with the cell recycling, is the most employed technology in Brazil for bioethanol production due to the possibility of achieving higher volumetric productivities. Control of the flow rate of medium feed is quite advantageous because the inhibitory effect caused by high substrate or product concentrations in the fermentation broth can be neutralized. It was observed that the addition of sucrose in linear or exponentially decreasing way leads to 10–14% increase in ethanol productivity (Echegaray et al., 2000). The optimization of feeding policy plays a crucial role for increasing both productivity and ethanol yield of fed-batch fermentations. This issue was analyzed in a previous review (Cardona and Sánchez, 2007). For fed-batch cultures, Alfenore et al. (2004) showed that higher ethanol concentrations (147 g/L) can be obtained in cultures without

oxygen limitation (0.2 vvm) during only 45 h in comparison to microaerobic conditions.

In the case of multiple or repeated batch fermentation, the use of flocculating yeast strains plays an important role. In this process, after starting a conventional batch, the yeasts are decanted in the same vessel where they were cultivated by removing the clarified culture broth. Then, an equal amount of fresh culture medium is added for the following batch. In this way, high cell concentrations are reached and inhibition effect by ethanol is reduced without the need of adding flocculation aids or using separation or recirculation devices. These repeated batches can be carried out until the moment when the activity and viability of culture is lost as a consequence of a high exposition to the fermentation environment. When this occurs, the system should be re-inoculated. Some examples of typical batch, fed-batch and repeated batch fermentations for bioethanol production from sugar cane molasses can be observed in Table 3.

2.2. Continuous processes

The design and development of continuous fermentation systems have allowed the implementation of more cost-effective processes. Continuous processes have several advantages compared to conventional batch processes mainly due to the reduced construction costs of the bioreactors, lower maintenance and operation requirements, better process control, and higher productivities (see Table 3). For those very reasons, 30% of ethanol production facilities in Brazil employ continuous fermentation processes (Monte Alegre et al., 2003). Most of these advantages are due to the high cell concentration found in these processes. Such high densities can be reached by immobilization techniques, recovery and recycling of cell biomass, or control of microbial growth. The major drawback is that yeasts cultivated under anaerobic conditions during long time diminish their ability to synthesize ethanol. In addition, at high dilution rates enabling elevated productivities, the substrate is not completely consumed and yields are reduced. Aeration also plays an important role during continuous cultivation. Cell concentration, cell yield from glucose, and yeast viability may be enhanced by increasing air supply whereas ethanol concentration decreases under both microaerobic and aerobic conditions. Cell growth inhibition by ethanol is reduced at microaerobic conditions compared to fully anaerobic cultivation and specific ethanol productivity is stimulated with the increase of oxygen percentage in the feed (Alfenore et al., 2004).

Continuous processes permit the decrease of product inhibition effect. Through cascade of continuous reactors, ethanol obtained in first reactors is easily transported to the following reducing its inhibitory effect. Other configurations employing one fermentor can also reduce the product inhibition as in the case of the Biostill technology (Kosaric and Velikonja, 1995). Other variants of continuous fermentation have been proposed, but many of them still have not reached the commercial level. Some of them require the use of highly flocculating yeast strains like in tower and fluidized-bed reactors. These types of reactors allow much higher cell concentrations (70–100 g/L) and ethanol productivities, and have a long-term stability due to the self replenishing of fresh yeasts. Moreover, these reactors do not require stirring devices or centrifugation (Gong et al., 1999). One approach to increase process productivity is the continuous ethanol removal from culture broth during fermentation by means of vacuum (see Table 3) or membranes, but the capital costs are increased. These configurations involving the application of reaction-separation integration were analyzed in a previous review (Cardona and Sánchez, 2007).

The stability of the culture is other important issue regarding continuous fermentation. If the system is operated near an unstable steady state, any small perturbation in input parameters (like dilution rate, temperature or substrate concentration of the feed) could not be offset by the culture and the system may start to operate under lower productivity conditions or oscillate with the time.

Table 3
Some fermentation processes for ethanol production from sugar cane molasses using *S. cerevisiae*

Regime	Configuration	Ethanol conc. in broth, g/L	Productivity, g/(L h)	Yield, % of theor. max.	References
Batch	Reuse of yeast from previous batches; yeast separation by centrifugation	80–100	1–3	85–90	Claassen et al. (1999)
Fed-batch	Stirred tank with variable feeding rate (exponent. depend. with time)	53.7–98.1	9–31	73.2–89	Echegaray et al. (2000)
Repeated batch	Stirred tank; flocculating yeast; up to 47 stable batches	89.3–92	2.7–5.25	79.5–81.7	Morimura et al. (1997)
Continuous	CSTR; cell recycling using a settler; flocculating yeast; aeration 0.05 vvm	70–80	7–8		Hojo et al. (1999)
	Biostill; residence time 3–6 h; cell recycling by centrifugation; recycled stream from distillation column to fermentor	30–70	5–20	94.5	Kosaric and Velikonja (1995)
Continuous removal of EtOH	Removal by vacuum; cell recycling	50	23–26.7		Costa et al. (2001); da Silva et al. (1999)

Laluce et al. (2002) constructed a special five-stage continuous fermentation system with cell recycling and with different temperatures in each stage. With this system, they experimentally assessed the effect of temperature fluctuations on fermentation performance. These fluctuations produced variations in both cell concentration and cell viability. Hojo et al. (1999) showed that microaeration plays an important role in the stabilization of ethanol, substrate and cell concentrations during the continuous cultivation of sugar cane syrup with cell recirculation of *S. cerevisiae*. Without air addition at low rates (0.05 vvm), these concentrations had significant fluctuations. One source of fluctuations leading to oscillatory behavior of continuous ethanolic fermentation using either *S. cerevisiae* or *Z. mobilis* is the high ethanol content in the broth as in the case of very high gravity fermentations (see below). Wide variations in ethanol, cell and substrate concentrations are observed under these conditions. Bai et al. (2004) showed that the utilization of packed-bed reactors attenuates these oscillations and quasi-steady states were attained, but the causes and mechanism of such attenuation require further research. Alternatively, an oscillatory regime of fermentation can be employed for ethanol production as patented by Elnashaie and Garhyan (2005). In this case, the required equipment comprises a fermentor, a process control system capable of operating the fermentor under chaotic conditions, and a membrane selective for ethanol. The development of proper models describing this type of processes allows the deep analysis of the stability of this cultivation regime. Tools like dynamic simulation and, especially, bifurcation analysis (Daugulis et al., 1997; Zhang and Henson, 2001) can provide valuable information for the design of more effective continuous fermentation processes. This issue is highlighted in the paper of Cardona and Sánchez (2007).

One of the strategies used for improving the ethanolic fermentation is the utilization of immobilized cells that allow the implementation of continuous processes with higher yields and productivities (see Table 4), and with increased cell concentrations (Claassen et al., 1999). Nevertheless, ethanol concentrations in the effluent tend to be lower that in other variants of continuous processes (see Table 3). Microbial cells are immobilized by entrapping them within porous solid supports like calcium alginate, carrageenan or polyacrylamide. In addition, they can be adsorbed on the surface of materials like wood chips, bricks, synthetic polymers, or other materials with a large surface area (Gong et al., 1999). Nowadays, most of the configurations using immobilized cells are far of commercial operation. Certain support particles have influence on cell metabolism as it has been shown in the case of solid-state fermentation, biofilm reactors, and immobilized cell reactors. Prakasham et al. (1999) claim that the simple addition of a small fraction of solids facilitates the cell anchorage in submerged cultures. This kind of adhesion on materials like river sand, delignified sawdust, chitin and chitosan, enhances the metabolic activity and is a easier and more economical method than the cell immobilization. Hence, the application of these techniques of "passive immobilization" to continuous cultures should be experimentally tested.

3. Ethanol from starch

3.1. Hydrolysis of starch

Starch is a high yield feedstock for ethanol production, but its hydrolysis is required to produce ethanol by fermentation. Starch was traditionally hydrolyzed by acids, but the specificity of the enzymes, their inherent mild reaction conditions and the absence of secondary reactions have made the amylases to be the catalysts generally used for this process. α-amylase obtained from thermoresistant bacteria like *Bacillus licheniformis* or from engineered strains of *Escherichia coli* or *Bacillus subtilis* is used during the first step of hydrolysis of starch suspensions.. For amylases to attack starch, these suspensions should be brought to high temperatures (90–110 °C) for the breakdown of starch kernels. Apar and Özbek (2004) provides information about the effects of operating conditions on the enzymatic hydrolysis of corn starch using commercial α-amylase. In last years, the possibility of hydrolyzing starch at low temperatures for achieving energy savings is being investigated (Robertson et al., 2006). The product of this first step, called liquefaction, is a starch solution containing dextrines and small amounts of glucose. The liquefied starch is subject to saccharification at lower temperatures (60–70 °C)

Table 4
Some continuous processes for bioethanol production from sugar cane and related media using immobilized cells of *S. cerevisiae*

Carrier	Medium	Ethanol conc. in effluent, g/L	Productivity, g/(L h)	Yield, % of theor. max.	References
Calcium alginate and zeolitic base	Cane molasses	54.48	1.835	88.2	Caicedo et al. (2003)
Chrysotile	Cane syrup	25–75	16–25	80.4–97.3	Wendhausen et al. (2001)
	Cane molasses		3.5–10		Monte Alegre et al. (2003)
Calcium alginate	Sucrose	50.6–60.0	10.2–12.1	66–79	Sheoran et al. (1998)
	Molasses[a]	47.4–55.3	7.3–10.4	62–74	
	Glucose	30.6–41.0	2.98[b]	83.1	Gilson and Thomas (1995)

[a] Higher values correspond to acid-treated and clarified molasses.
[b] Measured in g EtOH/(10^{11} cells h).

through glucoamylase obtained generally from *Aspergillus niger* or *Rhizopus* species (Pandey et al., 2000; Shigechi et al., 2004).

3.2. Ethanol production from corn

Ethanol is produced almost exclusively from corn in the USA. Corn is milled for extracting starch, which is enzymatically treated for obtaining glucose syrup. Then, this syrup is fermented into ethanol. There are two types of corn milling in the industry: wet and dry. During wet-milling process, corn grain is separated into its components. Starch is converted into ethanol and the remaining components are sold as co-products. During dry-milling, grains are not fractionated and all their nutrients enter the process and are concentrated into a distillation co-product utilized for animal feed called Dried Distiller's Grains with solubles (DDGS). In general, the liquefaction, saccharification and fermentation steps are the same for both technologies. Fermentation is performed using *S. cerevisiae* and is carried out at 30–32 °C with the addition of ammonium sulfate or urea as nitrogen sources. Proteases can be added to the mash to provide an additional nitrogen source for the yeast resulting from the hydrolysis of corn proteins (Bothast and Schlicher, 2005). Burmaster (2007) has patented a method for improving the fermentation of corn mashes and other feedstocks through the monitoring and control of oxidation reduction potential. This method allows achieving higher yields, shorter cultivation times, and decreased by-product formation. Configurations involving a higher degree of integration as the simultaneous saccharification and fermentation (SSF) have been successfully implemented, especially in the dry-milling process (see Cardona and Sánchez, 2007). The SSF performed at a temperature above 34 °C employing a thermotolerant yeast enables the reduction of cooling requirements and the improvement of the conversion process as claimed in the patent of Otto and Escovar-Kousen (2004). In the wet-milling process, the cascade process is also employed. This process involves the separate realization of saccharification and fermentation where hydrolysis of dextrines by glucoamylase, yeast propagation, pre-fermentation, and fermentation are carried out through a cascade system.

Z. mobilis also has been researched for ethanol production from dry-milled corn starch. In particular, a system consisting of a continuous packed bed column with immobilized glucoamylase followed by a continuous fluidized bed reactor with immobilized *Z. mobilis* showed an ethanol concentration of 70 g/L and a productivity of 23 g/(L h). In comparison, the continuous SSF carried out in a fluidized bed reactor with co-immobilized glucoamylase and *Z. mobilis* cells showed lower productivity and ethanol concentrations (Krishnan et al., 1999).

New tendencies in corn-to-ethanol industry are aimed at dry-milling processes. The increase in ethanol production capacity in the USA is mainly represented by corn dry-mill ethanol plants (Tiffany and Eidman, 2003). Other research efforts are oriented to the development of corn hybrids with higher extractable starch or higher fermentable starch content. Genetic engineering can be applied to direct the accumulation of amylases in the endosperm of transgenic corn kernels making possible the utilization of "self-processing" grains (Bothast and Schlicher, 2005).

3.3. Ethanol production from wheat

Although in France ethanol is mostly produced from beet molasses, it is also produced from wheat by a process similar to that of corn. Some efforts have been done for optimizing fermentation conditions. For example, Wang et al. (1999c) have determined the optimal fermentation temperature and specific gravity of the wheat mash. Soni et al. (2003) have optimized the conditions for starch hydrolysis using α-amylase and glucoamylase obtained by solid-state fermentation of wheat bran. To enhance fermentation performance, high gravity fermentations have been proposed, particularly for the case of wheat mashes. In this kind of process, the initial dissolved solids concentration exceeds 200 g/L implying a higher substrate load. Therefore, higher ethanol concentrations are obtained, lower amounts of process water are required, and energy costs are decreased. The drawbacks of this technology include longer fermentation times, and sometimes incomplete fermentations probably caused by product inhibition, high osmotic pressures and inadequate nutrition (Barber et al., 2002). To accelerate high gravity fermentations, the controlled addition of small amounts of acetaldehyde during the fermentation has allowed the reduction in cultivation time from 790 h to 585 h without effect on the ethanol yield. It is believed that this positive effect may be caused by the ability of acetaldehyde to replenish the intracellular acetaldehyde pool and restore the cellular redox balance (Barber et al., 2002).

Fermentation of wheat mashes of very high gravity (VHG) has been proposed as well. These mashes consist of wheat starch hydrolyzates containing 300 g or more of dissolved solids per liter of mash. VHG fermentation technology implies that high ethanol concentrations are obtained from very concentrated sugar solutions. Thomas et al. (1996) emphasize that considerable amounts of water can be saved by applying this technology to ethanol production. Additionally, the implementation of VHG fermentation increases the throughput rate of an ethanol plant without the need of increasing the plant capacity. Bayrock and Ingledew (2001) designed and tested a system that combines the multistage continuous culture fermentation and the VHG cultivation for a feed stream containing 150–320 g/L glucose using *S. cerevisiae*. The maximum ethanol concentration obtained in the process was of 132.1 g/L indicating the feasibility of implementing this technology in the industry, particularly in the continuous production of ethanol from wheat starch.

3.4. Ethanol production from cassava

Cassava represents an important alternative source of starch not only for ethanol production, but also for production of glucose syrups. In fact, cassava is the tuber that has gained most interest due to its availability in tropical countries being one of the top ten more important tropical crops. Ethanol production from cassava can be accomplished using either the whole cassava tuber or the starch extracted from it. Starch extraction can be carried out through a high-yield large-volume industrialized process as the Alfa Laval extraction method (FAO, 2004), or by a traditional process for small- and mid-scale plants. This process can be considered as the equivalent of the wet-milling process for ethanol production from corn. The production of cassava with high starch content (85–90% dry matter) and less protein and minerals content is relatively simple. Cassava starch has a lower gelatinization temperature and offers a higher solubility for amylases in comparison to corn starch. The hydrolysis of cassava flour has been proposed for the production of glucose in an enzymatic hollow-fiber reactor with 97.3% conversion (López-Ulibarri and Hall, 1997) considering that cassava flour production is more simple and economic than cassava starch production. However, it is considered that cassava ethanol would have better economic indicators if the whole tuber is used as feedstock, especially when small producers are involved. Fuel ethanol production from whole cassava is equivalent to ethanol production from corn by dry-milling technology. For this, cassava should be transported as soon as possible from cropping areas considering its rapid deterioration due to its higher moisture content (about 70%). Hence, this feedstock should be processed within 3–4 days after its harvest. One of the solutions to this problem consists in the use of sun-dried cassava chips (Sriroth et al., 2007). The farmers send the cassava roots to small chipping factories where they are peeled and chopped into small pieces. The chips are sun-dried during 2–3 days. The final moisture content is about 14% and the starch content reaches 65%.

The first step of the process in the distillery is the grinding of the dried cassava chips or fresh roots (if a permanent supply is ensured). Milled cassava is mixed with water and undergoes cooking followed by the liquefaction process. Liquefied slurry is saccharified to obtain the glucose, which will be assimilated by the yeast during the next fermentation step. The process can be intensified through the SSF as in the corn case. If fresh roots are employed, a fibrous material is obtained in the stillage after distillation. This material can be used as an animal feed similarly to the DDGS produced in the corn-based process. The wastewater can be treated by anaerobic digestion to produce biogas, which can be used to produce steam and power for the process. Nevertheless, the amount of steam generated is not enough to cover the needs of the process. Hence, natural gas or other fossil fuel is required (Dai et al., 2006).

3.5. Ethanol production from other starchy materials

Besides corn and wheat, ethanol can be produced from rye, barley, triticale (Wang et al., 1997), and sorghum (Zhan et al., 2003). For these cereals, some pretreatments have proven to be useful. Wang et al. (1997) have employed the pearling of wheat, barley, rye and triticale grains for increasing starch content of the feedstock in an average of 12% obtaining a 6.5–22.5% enhance in ethanol yield during fermentation. In addition, VHG technology has been tested with successful results for oats, barley, rye and triticale (Wang et al., 1999b). It has been reported the ethanol production from other plant sources with high starch concentration. Abd-Aziz (2002) suggested the utilization of sago palm for ethanol production in the case of Malaysia. The ethanol production from bananas and banana wastes using commercial α-amylase and glucoamylase has been studied by Hammond et al. (1996). In their work, an ethanol yield of 0.5 L EtOH/kg dry matter of ripe bananas was obtained. The processing of starch-containing food wastes by adding malt to the pulverized feedstock has been patented (Chung and Nam, 2002). One of the most promising crops for fuel ethanol production is the sweet sorghum, which produces grains with high starch content, stalks with high sucrose content and leaves and bagasse with high lignocellulosic content. In addition, this crop can be cultivated in both temperate and tropical countries requiring only 1/3 of the water needed for cane cropping and half of the water required by corn. Moreover, it is tolerant to the drought, flooding and saline alcalinity (Winner Network, 2002). Grassi (1999) reports that from some varieties of sweet sorghum, the following productivities can be obtained: 5 ton/Ha grains, 8 ton/Ha sugar and 17 ton dry matter/Ha lignocellulosics. The estimated price for fuel ethanol production from this feedstock is US$200–300/$m^3$, whereas the corresponding one for sugar cane ethanol is 260, for corn ethanol is 300–420 and for lignocellulosic ethanol is 450.

4. Ethanol from lignocellulosic biomass

It is evident the importance of lignocellulosic biomass as a feedstock for ethanol production. Lignocellulosic complex is the most abundant biopolymer in the Earth. It is considered that lignocellulosic biomass comprises about 50% of world biomass and its annual production was estimated in 10–50 billion ton (Claassen et al., 1999). Many lignocellulosic materials have been tested for bioethanol production as observed in Table 5. In general, prospective lignocellulosic materials for fuel ethanol production can be divided into six main groups: crop residues (cane bagasse, corn stover, wheat straw, rice straw, rice hulls, barley straw, sweet sorghum bagasse, olive stones and pulp), hardwood (aspen, poplar), softwood (pine, spruce), cellulose wastes (newsprint, waste office paper, recycled paper sludge), herbaceous biomass (alfalfa hay, switchgrass, reed canary grass, coastal Bermudagrass, thimothy grass), and

Table 5
Pretreatment methods of lignocellulosic biomass for fuel ethanol production

Methods	Procedure/agents	Remarks	Examples of pretreated materials	References
Physical methods:				
Mechanical comminution	Chipping, grinding, milling	Milling: vibratory ball mill (final size: 0.2–2 mm), knife or hammer mill (final size: 3–6 mm)	Wood and forestry wastes (hardwood, straw) Corn stover, cane bagasse Timothy, alfalfa	Alvo and Belkacemi (1997); Papatheofanous et al. (1998); Sun and Cheng (2002)
Pyrolysis	$T > 300$ °C, then cooling and condensing	Formation of volatile products and char Residues can undergo mild dilute-acid hydrolysis (1 N H2SO4, 2,5 h, $T = 97$ °C) to produce 80–85% reducing sugars (>50% glucose) It can be carried out under vacuum (400 °C, $p = 1$ mm Hg, 20 min)	Wood Waste cotton, corn stover	Khiyami et al. (2005); Sun and Cheng (2002); Yu and Zhang (2003)
Physical–chemical methods:				
Steam explosion	Saturated steam at 160–290 °C, $p = 0{,}69$–$4{,}85$ MPa for several sec or min, then decompression until atm. pressure	It can handle high solids loads Size reduction with lower energy input compared to comminution 80–100% hemicellulose hydrolysis, destruction of a portion of xylan fraction, 45–65% xylose recovery Inhibitors formation Addition of H_2SO_4, SO_2, or CO_2 improves effic. of further enzym. hydrolysis Cellulose depolymerization occurs at certain degree Lignin is not solubilized, but it is redistributed	Poplar, aspen, eucalyptus Softwood (Douglas fir) Bagasse, corn stalk, wheat straw, rice straw, barley straw, sweet sorghum bagasse, *Brassica carinata* residue, olive stones Timothy grass, alfalfa, reed canary grass	Ballesteros et al. (2001, 2002b, 2004); Belkacemi et al. (1997, 2002); De Bari et al. (2002); Hamelinck et al. (2005); Lynd et al. (2002); Nakamura et al. (2001); Negro et al. (2003); Shevchenko et al. (1999); Söderström et al. (2003); Sun and Cheng (2002)
Liquid hot water (LHW)	Pressurized hot water, $p > 5$ MPa, $T = 170$–230 °C, 1–46 min; solids load <20%	80–100% hemicellulose hydrolysis, 88–98% xylose recovery, >50% olygomers Low or no formation of inhibitors Cellulose depolymerization occurs at certain degree Further cellulose conversion >90% Partial solubilization of lignin (20–50%)	Bagasse, corn stover, olive pulp Alfalfa fiber	Ballesteros et al. (2002b); Koegel et al. (1999); Laser et al. (2002); Lynd et al. (2002); Negro et al. (2003); Ogier et al. (1999); Sreenath et al. (2001)
Ammonia fiber explosion (AFEX)	1–2 kg ammonia/kg dry biomass, 90 °C, 30 min, $p = 1.12$–1.36 MPa	Ammonia recovery is required 0–60% hemicellulose hydrolysis in dependence on moisture, >90% olygomers No inhibitors formation Cellulose depolymerization occurs at certain degree Further cellulose conversion can be >90%, for high-lignin biomass (<50%) ~10–20% lignin solubilization	Aspen wood chips Bagasse, wheat straw, barley straw, rice hulls, corn stover Switchgrass, coastal Bermudagrass, alfalfa Newsprint MSW	Dale et al. (1996); Lynd et al. (2002); Sun and Cheng (2002)
CO_2 explosion	4 kg CO_2/kg fiber, $p = 5{,}62$ MPa	No inhibitors formation Further cellulose conversion can be >75%	Bagasse Alfalfa Recycled paper	Sun and Cheng (2002)

(*continued on next page*)

Table 5 (*continued*)

Methods	Procedure/agents	Remarks	Examples of pretreated materials	References
Chemical methods:				
Ozonolysis	Ozone, room temperature and pressure	No inhibitors formation Further cellulose conversion can be >57% Lignin degradation	Poplar sawdust Pine Bagasse, wheat straw, cotton straw, green hay, peanut	Sun and Cheng (2002)
Dilute-acid hydrolysis	0,75–5% H_2SO_4, HCl, or HNO_3, $p \sim 1$ MPa; continuous process for low solids loads (5–10 wt% dry substrate/mixture): $T = 160$–200 °C; batch process for high solids loads (10–40 wt% dry substrate/mixture): $T = 120$–160 °C	pH neutralization is required that generates gypsum as a residue 80–100% hemicellulose hydrolysis, 75–90% xylose recovery Cellulose depolymerization occurs at certain degree High temperature favors further cellulose hydrolysis Lignin is not solubilized, but it is redistributed	Poplar wood Bagasse, corn stover, wheat straw, rye straw, rice hulls Switchgrass, Bermudagrass	Hamelinck et al. (2005); Lynd et al. (2002); Martinez et al. (2000); Rodríguez-Chong et al. (2004); Saha et al. (2005a,b); Schell et al. (2003); Sun and Cheng (2002); Wooley et al. (1999b)
Concentrated-acid hydrolysis	10–30% H_2SO_4, 170–190 °C, 1:1,6 solid–liquid ratio 21–60% peracetic acid, silo-type system	Acid recovery is required Residence time greater compared to dilute-acid hydrolysis Peracetic acid provokes lignin oxidation	Poplar sawdust Bagasse	Cuzens and Miller (1997); Teixeira et al. (1999a,b)
Alkaline hydrolysis	Dilute NaOH, 24 h, 60 °C; $Ca(OH)_2$, 4 h, 120 °C; it can be complemented by adding H_2O_2 (0.5–2.15 vol.%) at lower temperature (35 °C)	Reactor costs are lower compared to acid pretreatment >50% hemicellulose hydrolysis, 60–75% xylose recovery Low inhibitors formation Cellulose swelling Further cellulose conversion can be >65% 24–55% lignin removal for hardwood, lower for softwood	Hardwood Bagasse, corn stover, straws with low lignin content (10–18%), cane leaves	Hamelinck et al. (2005); Hari Krishna et al. (1998); Kaar and Holtzapple (2000); Lynd et al. (2002); Saha and Cotta (2006); Sun and Cheng (2002); Teixeira et al. (1999a)
Oxidative delignification	Peroxidase and 2% H_2O_2, 20 °C, 8 h	Almost total solubilization of hemicellulose Further cellulose conversion can be 95% 50% lignin solubilization	Bagasse	Sun and Cheng (2002)
Wet oxidation	1.2 MPa oxygen pressure, 195 °C, 15 min; addition of water and small amounts of Na_2CO_3 or H_2SO_4	Solubilization of major part of hemicellulose Inhibitors formation Lignin degradation	Corn stover, wheat straw	Bjerre et al. (1996); Varga et al. (2004)
Organosolv process	Organic solvents (methanol, ethanol, acetone, ethylene glycol, triethylene glycol) or their mixture with 1% of H_2SO_4 or HCl; 185–198 °C, 30–60 min, pH = 2.0–3.4	Solvent recovery required Almost total hydrolysis of hemicellulose, high yield of xylose Almost total lignin solubilization and breakdown of internal lignin and hemicellulose bonds	Poplar wood Mixed softwood (spruce, pine, Douglas fir)	Lynd et al. (2002); Pan et al. (2005); Rezzoug and Capart (1996); Sun and Cheng (2002)
Biological methods:				
Fungal pretreatment	Brown-, white- and soft-rot fungi	Fungi produces cellulases, hemicellulases, and lignin-degrading enzymes: ligninases, lignin.peroxidases, polyphenoloxidases, laccase and quinone-reducing enzymes	Corn stover, wheat straw	Sun and Cheng (2002); Tengerdy and Szakacs (2003)

	Cellulase and hemicellulase production by solid-state fermentation of biomass	Very slow process: Pleurotus ostreatus converts 35% of wheat straw into reducing sugars in 5 weeks Brown-rot fungi degrades cellulose White- and soft-rot fungi degrade cellulose and lignin		Itoh et al. (2003)
Bioorganosolv pretreatment	*Ceriporiopsis subvermispora* for 2-8 weeks followed by ethanolysis at 140–200 °C for 2 h	Fungi decompose the lignin network Ethanol action allows hemicellulose hydrolysis Biological pretreatment can save 15% of the electricity needed for ethanolysis Ethanol can be reused; environmentally-friendly process	Beech wood	

Modified from Sánchez and Cardona (2005).

municipal solid wastes (MSW). The composition of most of these materials can be found elsewhere (e.g. Sun and Cheng, 2002).

Numerous studies for developing large-scale production of ethanol from lignocellulosics have been carried out in the world. However, the main limiting factor is the higher degree of complexity inherent to the processing of this feedstock. This is related to the nature and composition of lignocellulosic biomass. Two of the main polymers of the biomass should be broken down into fermentable sugars in order to be converted into ethanol or other valuable products. But this degradation process is complicated, energy-consuming and non-completely developed.

4.1. Pretreatment of lignocellulosic biomass

The main processing challenge in the ethanol production from lignocellulosic biomass is the feedstock pretreatment. The lignocellulosic complex is made up of a matrix of cellulose and lignin bound by hemicellulose chains. During the pretreatment, this matrix should be broken in order to reduce the crystallinity degree of the cellulose and increase the fraction of amorphous cellulose, the most suitable form for enzymatic attack. Additionally, main part of hemicellulose should be hydrolyzed and lignin should be released or even degraded. The fact that the cellulose hydrolysis is affected by the porosity (accessible surface area) of lignocellulosic materials should be also considered. The yield of cellulose hydrolysis is less than 20% of the theoretical when pretreatment is not carried out, whereas the yield after pretreatment often exceeds 90% of theoretical (Lynd, 1996). Therefore, the aim of the pretreatment is the removal of lignin and hemicellulose, the reduction of crystalline cellulose and the increase in the porosity of the materials. Additionally, the pretreatment should improve the formation of sugars or the ability to form them during the succeeding enzymatic hydrolysis, and avoid the formation of inhibitors for subsequent hydrolysis and fermentation processes. For the pretreatment of lignocellulosics, several physical, physical–chemical, chemical and biological processes have been proposed and developed (Sun and Cheng, 2002). The main pretreatment methods reported in the literature are shown in Table 5.

4.1.1. Physical methods

Waste materials can be comminuted by a combination of chipping, grinding and milling to reduce cellulose crystallinity. This reduction facilitates the access of cellulases to the biomass surface increasing the conversion of cellulose. The energy requirements of mechanical comminution of lignocellulosic materials depend on the final particle size and biomass characteristics. Although mechanical pretreatment methods increase cellulose reactivity towards enzymatic hydrolysis, they are unattractive due to their high energy and capital costs (Ghosh and Ghose, 2003). Pyrolysis has also been tested as a physical method for pretreatment of lignocellulosic biomass since

cellulose rapidly decomposes when is treated at high temperatures.

4.1.2. Physical–chemical methods

Physical–chemical pretreatment methods are considerably more effective than physical. The steam explosion is the most studied method of this type. During this process, the use of saturated steam at high pressure causes autohydrolysis reactions in which part of the hemicellulose and lignin are converted into soluble olygomers. The factors affecting steam explosion pretreatment are residence time, temperature, chip size and moisture content. To consider the combined action of both temperature and time over the performance of steam explosion pretreatment, the so-called severity index has been defined including a correction term when this process is carried out under acidic conditions (Shahbazi et al., 2005; Söderström et al., 2003). In some cases (e.g. herbaceous waste), the use of very small particles is not desirable considering the economy of the process (Ballesteros et al., 2002a).This method is recognized as one of the most cost-effective for hardwood (poplar, oak, birch, maple) and agricultural residues, but is less efficient for softwood (pine, cedar). Shahbazi et al. (2005) proposed a fractionation procedure for softwood based on steam explosion and alkaline delignification in order to produce ethanol and related co-products. Söderström et al. (2003) propose a two-step steam pretreatment of softwood by dilute-acid impregnation that includes a partial hydrolysis of cellulose during the second step. According to these authors, this variant of pretreatment is a promising method for increasing the overall yield during ethanol production.

One of the most promising methods is the pretreatment with Liquid Hot Water (LHW) or thermohydrolysis. Laser et al. (2002) mention that under optimal conditions, this method is comparable to dilute acid pretreatment but without addition of acids or production of neutralization wastes. In addition, this technology presents elevated recovery rates of pentoses and does not generate inhibitors (Ogier et al., 1999). Nevertheless, solid load is much less than for steam explosion, which is usually greater than 50%. Negro et al. (2003) compared steam explosion and LHW pretreatments for poplar biomass and showed best results for the latter at 210 °C during 4 min. Other physical–chemical method is the Ammonia Fiber Explosion (AFEX) process whose fundament is similar to steam explosion. As AFEX method and steam explosion, CO_2 explosion uses the same principle but the yields are relatively low (Sun and Cheng, 2002).

4.1.3. Chemical methods

Chemical pretreatments employ different chemical agents as ozone, acids, alkalis, peroxide and organic solvents. Inorganic acids as H_2SO_4 and HCl have been preferably used for biomass pretreatment. Hydrolysis with dilute sulfuric acid has been successfully developed given that high reaction rates can be achieved improving significantly the subsequent process of cellulose hydrolysis. In contrast, the costs of dilute acid pretreatment are higher than the corresponding ones of steam explosion or AFEX process (Sun and Cheng, 2002). Schell et al. (2003) studied the dilute-acid pretreatment of corn stover at pilot plant level using high solid loads obtaining a xylose yield of 77% at 190 °C. This pretreatment method was evaluated through a kinetic model that allowed the prediction of process conditions in order to maximize the yield. Similar kinetic studies were carried out for cane bagasse pretreated with nitric acid (Rodríguez-Chong et al., 2004) or without acid addition (Jacobsen and Wyman, 2002). Dilute acid pretreatment also can be accomplished in a two-stage way. For this, a first depolymerization stage of hemicellulose at 140 °C during 15 min is carried out in order to avoid the formation of furan compounds and carboxylic acids, followed by a second stage at 190 °C during 10 min to make cellulose more accessible to enzymatic hydrolysis (Saha et al., 2005a,b). These authors point out that the realization of dilute-acid pretreatment at low temperatures (121 °C) allows avoiding the degradation of sugars to furfural and hydroxymethylfurfural (HMF), but the sugars yields are lower.

Dilute acid pretreatment along with steam explosion are the most widely studied methods. The National Renewable Energy Laboratory (NREL) of the US Department of Energy, which currently is developing ethanol production technologies from biomass, has preferred the dilute acid pretreatment for the design of its process alternatives (Aden et al., 2002; Wooley et al., 1999b). Lynd (1996) points out that the main advantage of this process related to steam explosion is the higher recovery of sugars derived from hemicellulose. For hardwood, this recovery is about 80% for dilute acid pretreatment, and does not exceed 65% for steam explosion. Ogier et al. (1999) state that the methods appearing as the most efficient are dilute-acid pretreatment, steam explosion with catalyst addition and LHW. These methods are also chosen by Hamelinck et al. (2005) as the more perspective in short-, mid- and long-term evaluations.

Concentrated acids also have been used for pretreatment. A fuel ethanol production process from cane bagasse involving the pretreatment with concentrated sulfuric acid has been patented (Farone and Cuzens, 1996). This technology implies the retrofitting of sugar mills in order to produce ethanol and improve energetic indexes of this kind of processes (Cuzens and Miller, 1997). An alternative approach was tested by Teixeira et al. (1999a,b), which employ a silo type system by introducing the feedstock (bagasse or hybrid poplar) in plastic bags to which a peracetic acid solution was added. Cellulose conversion of pretreated material reached 93.1% during 120 h using 21 wt% acid concentration or during 24 h using 60 wt% acid concentration. This system requires low energy since the process is carried out at room temperature.

Alkaline pretreatment is based on the effects of the addition of dilute bases on the biomass: increase of

internal surface by swelling, decrease of polymerization degree and crystallinity, destruction of links between lignin and other polymers, and breakdown of lignin. The effectiveness of this method depends on the lignin content of the biomass (Sun and Cheng, 2002). In general, the utilization of bases as sodium hydroxide or solvents such as ethanol or methanol (organosolv process) allows the dissolution of lignin, but their costs are so high that these methods are not competitive for large scale plants (Lynd et al., 1999).

4.1.4. Biological methods

Biological pretreatment has low energy requirements and mild environmental conditions. However, most of these processes are too slow limiting its application at industrial level. Many white-rot fungi degrade the lignin and, for this reason, they have been utilized for ligninases production and lignocellulose degradation. Lee (1997) reports the main microorganisms producing lignin-degrading enzymes and indicates the fermentation processes for producing them by both submerged culture and solid-state fermentation. In fact, the fungus *Phanerochaete chrysosporium* has been proposed in the patent of Zhang (2006) for degrading the lignin in a biomass-to-ethanol process scheme involving the separate fermentation of pentoses and hexoses. Tengerdy and Szakacs (2003) and Kang et al. (2004) highlight the viability of producing cellulases and hemicellulases by solid-state fermentation. According to preliminary evaluations of the NREL, the cost of cellulases produced *in situ* by submerged culture is US$0.38/100,000 FPU (Filter Paper Units, a way for measuring cellulase activity). Thus, cellulase costs comprise 20% of ethanol production costs assuming them in US$1.5/gallon. On the other hand, commercial cellulase cost (US$16/100,000 FPU) is prohibitive for this process. In contrast, these authors indicate that the cost of producing cellulases by solid-state fermentation of corn stover could reach US$0.15/100,000 FPU that would correspond to US$0.118/gal EtOH, i.e. near 8% of total costs.

One of the main problems during the pretreatment and hydrolysis of biomass is the variability in the content of lignin and hemicellulose. This variability depends on factors as the type of plant from which the biomass is obtained, crop age, method of harvesting, etc. This makes that no one of the pretreatment methods could be applied in a generic way for many different feedstocks (Claassen et al., 1999). The future trends for improving the pretreatment of lignocellulosic feedstocks also include the production of genetically modified plant materials with higher carbohydrate content or modified plant structure to facilitate pretreatment in milder conditions or using hemicellulases. It is estimated that the use of these new materials along with improved conversion technologies, could reduce the ethanol cost from lignocellulosic biomass in US$0.11/L in the next ten years (Wooley et al., 1999a).

4.2. Detoxification of lignocellulosic hydrolyzates

During pretreatment and hydrolysis of lignocellulosic biomass, a great amount of compounds that can seriously inhibit the subsequent fermentation are formed in addition to fermentable sugars. Inhibitory substances are generated as a result of the hydrolysis of the extractive components, organic and sugar acids esterified to hemicellulose (acetic, formic, glucuronic, galacturonic), and solubilized phenolic derivatives. In the same way, inhibitors are produced from the degradation products of soluble sugars (furfural, HMF) and lignin (cinnamaldehyde, *p*-hydroxybenzaldehyde, syringaldehyde), and as a consequence of corrosion (metal ions) (Lynd, 1996; Palmqvist and Hahn-Hägerdal, 2000b). For this reason and depending on the type of employed pretreatment and hydrolysis, detoxification of the streams that will undergo fermentation is required. Detoxification methods can be physical, chemical or biological. As pointed out by Palmqvist and Hahn-Hägerdal (2000a), these methods cannot be directly compared because they vary in the neutralization degree of the inhibitors. In addition, the fermenting microorganisms have different tolerances to the inhibitors. The main features of the detoxification methods employed for ethanol production from biomass and some examples are summarized in Table 6.

In the model processes developed for NREL, ionic exchange (Wooley et al., 1999b) and overliming (Aden et al., 2002) have been proposed as detoxification methods. Alkali treatment is considered one of the best detoxification methods. By this method, furaldehydes and phenolic compounds are mainly removed leading to great improvement in fermentability, especially in the case of dilute-acid hydrolyzates (Persson et al., 2002a). Treatment with calcium hydroxide (overliming) or ammonia has shown better results than treatment with sodium or potassium hydroxide, but this difference has not been understood. Martinez et al. (2001) performed the experimental optimization of the amount of added lime, which depends on the content of acids in each hydrolyzate. These authors developed a method for predicting the optimal addition dosage based on the titration of hydrolyzate with 2 N NaOH. Persson et al. (2002a) indicate that the positive effects of alkali treatment cannot be completely explained by the removal of inhibitors and that this method could have possible stimulatory effects on fermenting microorganisms.

Other very diverse detoxification methods have been proposed as: neutralization with lime followed by the addition of activated carbon and filtration for acetic acid removal; partial removal of acetic acid, furfural and soluble lignin by molecular sieves; vapor stripping for removal of volatile inhibitors (Olsson and Hahn-Hägerdal, 1996); and adsorption using activated carbon, diatomite, bentonite and zeolite after neutralization or overliming (Yu and Zhang, 2003). An alternative biological method for detoxification of dilute solutions resulting from biomass pretreated by pyrolysis has been proposed (Khiyami et al., 2005). It is based on a biofilm reactor that uses a mixed

Table 6
Detoxification methods of streams resulting of pretreatment and hydrolysis of lignocellulosic biomass for bioethanol production

Methods	Procedure/agents	Examples	Microorganism	Remarks	References
Physical methods:					
Evaporation	Evaporation, separation of volatile and non-volatile fractions and dilution of non-volatile fraction	Willow hz.	*S. cerevisiae*	Reduction of acetic acid and phenolic compounds in non-volatile fraction; roto-evaporation	Palmqvist and Hahn-Hägerdal (2000a)
		Aspen hz.	*P. stipitis*	93% yield of ref. fermn.; removal: 54% acetic acid, 100% furfural, 29% vanillin; roto-evaporation;	Palmqvist and Hahn-Hägerdal (2000a)
Extraction	Organic solvents, 3:1 org. phase: aqueous phase volumetric ratio	Spruce hz.	*S. cerevisiae*	Solv: diethyl ether (solv.); yield comparable to ref. fermn.; removal of acetic, formic and levulinic acids, furfural, HMF	Palmqvist and Hahn-Hägerdal (2000a)
		Aspen hz.	*P. stipitis*	Solv.: ethyl acetate; 93% yield of ref. fermn.; removal: 56% acetic acid, 100% furfural, 100% vanillin, 100% hydroxybenzoic acid	Palmqvist and Hahn-Hägerdal (2000a)
		Pine hz.	*S. cerevisiae*	Solv.: ethyl acetate; removal of low molecular phenolic compounds	Palmqvist and Hahn-Hägerdal (2000a)
		Steam-exploded poplar	*S. cerevisiae*	Solv.: ethyl acetate; EtOH yield (SSF): detoxified hz. 0.51 g/g, undetox. hz. 0 g/g; high degree of phenolic removal	Cantarella et al. (2004)
	Supercritical solvent in countercurrent with the hydrolyzate, 20 MPa, 40 °C; then, depressurization	Dilute-acid spruce hz.	*S. cerevisiae*	Solv.: supercritical CO_2; 98% yield of ref. fermn.; removal: 93% furfural, 10% HMF	Persson et al. (2002b)
Adsorption	Activated carbon, 0.05–0.20 g/g glucose	Steam-exploded concentr. oak hz.	*S. cerevisiae*	Detoxified hz. with 140–170 g/L initial glucose was utilized; undetox. hz. with 100 g/L initial glucose could not be completely utilized	Lee et al. (1999)
	Amberlite hydrophobic polymeric adsorbent XAD-4, 8% (w/v), 1.5 h, 25 °C; regeneration with EtOH; then, neutraliz. with lime	LHW-pretreated corn fiber	Recombinant *E. coli*	Reduction of furfural conc. from 1–5 to <0.01 g/L; 90% yield of theoretical; sugars are not adsorbed	Weil et al. (2002)
Chemical methods:					
Neutralization	$Ca(OH)_2$ or CaO, pH = 6, then membrane filtration or adsorption	Acid hz. of cotton waste pyrolysate	*S. cerevisiae*, *Pichia* sp.	Precipitation or removal of toxic compounds; 10% lower yield for *Pichia* sp.	Yu and Zhang (2003)
		Steam-exploded poplar	*S. cerevisiae*	EtOH yield (SSF): detoxified hz. 0.86 g/g, undetox. hz. 0 g/g	Cantarella et al. (2004)
Alkaline detoxification (overliming)	$Ca(OH)_2$, pH = 9–10,5, then pH adjustment to 5,5–6,5 with H_2SO_4 or HCl	Dilute-acid hz. of spruce		Yield comparable to ref. fermn.; 20% removal of furfural and HMF	Palmqvist and Hahn-Hägerdal (2000a)
		Steam-exploded bagasse	Recombinant *S. cerevisiae*	Removal of acid acetic, furfural and part of phenolic compounds	Martín et al. (2002)
		Acid hz. of cotton waste pyrolysate	*S. cerevisiae*, *Pichia* sp.	7.5% lower yield for *Pichia* sp.	Yu and Zhang (2003)
		Rice hulls hz.	Recombinant *E. coli*	39% reduction in fermentation time	Saha et al. (2005a)
		Wheat straw hz.	Recombinant *E. coli*	Reduction in fermn. time: SSF -18%, SHF - 67%	Saha et al. (2005b)
		Dilute-acid bagasse hz.	Recombinant *E.coli*	Removal: 51% furfural, 51% HMF, 41% phenolic compounds, 0% acetic acid; overliming at 60 °C or 25 °C, at high temperature, the required amounts of lime and acid are reduced	Martinez et al. (2000, 2001)

Combined alkaline detoxification	KOH, pH = 10, then pH adjustment to 6,5 with HCl and addition of 1% sodium sulfite	Bagasse hz.	*P. stipitis*	Reduction of ketones and aldehydes, removal of volatile compounds when hydrolyzate is heated at 90 °C	Palmqvist and Hahn-Hägerdal (2000a)
		Dilute-acid hz. of spruce	*S. cerevisiae*		Palmqvist and Hahn-Hägerdal (2000a)
		Willow hz.	Recombinant *E. coli*		Palmqvist and Hahn-Hägerdal (2000a)
Ionic exchange	Weak base resins Amberlyst A20, regenerated with ammonia	Dilute-acid poplar	Recombinant *Z. mobilis*	Removal: 88% acetic acid, 100% H_2SO_4; 100% sugars recovery	Wooley et al. (1999b)
		Dilute-acid hz. of spruce	*S. cerevisiae*	Removal: >80% phenolic compounds, ~100% levulinic, acetic and formic acids, 70% furfural; considerable lost of fermentable sugars	Palmqvist and Hahn-Hägerdal (2000a)
	Poly(4-vinyl pyridine) (Xie et al. (2005))	Corn-stover hz.	Recombinant *S. cerevisiae*	Sugars eluted earlier than all tested inhibitors; ferment. results were similar to that using pure sugars	Xie et al. (2005)
Biological methods:					
Enzymatic detoxification	Laccase (phenol oxidase) and lignin peroxidase from *Trametes versicolor*: 30 °C, 12 h	Willow hz.	*S. cerevisiae*	2–3-fold increase of EtOH productivity compared to undetox. hz.; laccase selectively removes phenolic low molecular weight compounds and phenolic acids	Palmqvist and Hahn-Hägerdal (2000a); Jönsson et al. (1998)
		Steam-exploded bagasse	Recombinant *S. cerevisiae*	80% removal of phenolic compounds	Martín et al. (2002)
Microbial detoxification	*Trichoderma reesei*	Steam-exploded willow	*S. cerevisiae*	3-fold increase of EtOH productivity compared to undetox. hz.; 4-fold increase of yield; removal of acetic acid, furfural and benzoic acid derivatives	Palmqvist and Hahn-Hägerdal (2000a)
				Aerobic bacteria oxidize aromatic compounds	
	Immobilized to PCS mixed culture of *Pseudomonas putida* and *Streptomyces setonii* cells (biofilm reactor: PCS tubes attached to CSTR agitator shaft)	Diluted pyrolysate of corn stover		Detoxification of 10 and 25 vol.% of pyrolysate medium, and partially detoxification of 50 vol.% of pyrolysate medium	Khiyami et al. (2005)

Observations: Reference fermentation (ref. fermn.) refers to fermentation carried out in a glucose-based medium without inhibitors; hz – hydrolyzate; undetox. hz. – undetoxified hydrolyzate; PCS – plastic composite support. Modified from Sánchez and Cardona (2005).

culture of aerobic bacteria cells (see Table 6) naturally immobilized on a plastic support. In this way, the biofilm-associated cells are more resistant to the toxic substances released during the biomass pretreatment. The use of extraction with supercritical fluids has also been proposed for detoxification of wood hydrolyzates (Persson et al., 2002b).

The presence of inhibitors directly influences on the course of ethanolic fermentation. In continuous or fed-batch fermentations, feed of the bioreactors is carried out with not very high flow rates allowing a low concentration of inhibitors in the broth. In continuous systems, inhibitors diminish the growth rate and, therefore, the process productivity that is directly linked to the dilution rate. In systems with cell retention (e.g., by cell recirculation using filtration, sedimentation or centrifugation), the increase of accumulables, including the inhibitors, makes the productivity to fall down imposing the need of implementing purge streams. Taherzadeh (1999) developed a simple strategy for on-line feedback control of fed-batch cultivation for *in situ* detoxification of spruce and birch hydrolyzates. Through this strategy, the same yeast cells converted the inhibitors and maintained their concentration at low levels without the need of any detoxification treatment. Thus, the maximal specific productivity of ethanol increased in more than 10 times (Nilsson et al., 2001). Purwadi et al. (2007) employed a continuous cultivation system using a flocculating strain of *S. cerevisiae* to ferment a non-detoxified spruce hydrolyzate. Results obtained demonstrated that high-cell system with recycling of cells allow the *in situ* detoxification of the pretreated biomass at high dilution rates without the need of any detoxification method. It has shown the possibility of converting the hydrolyzates into ethanol in two hours (at dilution rates of $0.5\ h^{-1}$), which represents an important outcome in the cultivation of toxic pretreated lignocellulosic biomass.

Most of the studies on the effect of toxic compounds on growth and ethanol production have been performed for *S. cerevisiae* and xylose-fermenting yeast. Palmqvist et al. (1999) carried out extensive experiments for assessing the effect of acetic acid, furfural and *p*-hydroxybenzoic acid on growth and ethanol productivity of *S. cerevisiae* and *C. shehatae* through full factorial design. Oliva et al. (2003, 2004) performed the study of the effect of compounds released during the pretreatment of poplar biomass by steam explosion for the thermotolerant yeast *Kluyveromyces marxianus*, showing that growth is more affected than ethanol production, and that this microorganism exhibits an important resistance to aromatic compounds. Recombinant microorganisms also have been investigated regarding their capacity for fermenting lignocellulosic hydrolyzates containing inhibitors. Zaldivar et al. (1999, 2000) analyzed the effect of several aldehydes and alcohols formed during the dilute-acid pretreatment on the growth and fermentation of genetically-modified *E. coli* capable of assimilating xylose. In the case of aldehydes, these authors showed that only furfural strongly inhibited ethanol production. One new approach to tackle the presence of inhibitors in biomass hydrolyzates is the development of inhibitor-tolerant strains of microorganisms by means of genetic modification and metabolic engineering. However, Belkacemi et al. (2002) point out that due to the synergistic interactions among inhibitors and lack of information about the mechanisms of these interactions, it is not clear against what inhibitor resistance is desired. In this way, intense efforts are being carried out for the identification of inhibitory substances, as well as the determination of their inhibition mechanisms. Palmqvist and Hahn-Hägerdal (2000b) have reviewed the main works carried out in this field applied to wood hydrolyzates. These authors emphasize that these studies will allow the minimization of inhibitors formation during pretreatment and hydrolysis, the prediction of hydrolyzates fermentability and the development of more efficient detoxification methods.

4.3. Hydrolysis of cellulose

For fermentation of lignocellulosic materials, cellulose should be degraded into glucose (saccharification) using acids or enzymes. In the former case, concentrated or dilute acids can be used. If dilute acids (H_2SO_4 and HCl) are employed, temperatures of 200–240 °C at 1.5% acid concentrations are required to hydrolyze the crystalline cellulose, but the degradation of glucose into HMF and other non-desired products is unavoidable under these conditions. Similarly, xylose is degraded into furfural and other compounds. During two-stage regime, a first stage under mild conditions (190 °C, 0.7% acid, 3 min) is carried out to recover pentoses, while in the second stage, the remaining solids undergo harsher conditions (215 °C, 0.4% acid, 3 min) to recover hexoses. In this way, 50% glucose yield is obtained (Hamelinck et al., 2005). One variant of the acid hydrolysis is the employ of extremely low acid and high temperature conditions during batch processes (auto-hydrolysis approach) that has been applied to sawdust (Ojumu and Ogunkunle, 2005). Concentrated acid process using 30–70% H_2SO_4 has higher glucose yield (90%) and is relatively rapid (10–12 h) but the amount of used acid is a critical economic factor. By continuous ion-exchange, over 97% acid recovery is possible (Hamelinck et al., 2005).

However, cellulose hydrolysis is currently carried out using microbial cellulolytic enzymes. Enzymatic hydrolysis has demonstrated better results for the subsequent fermentation because no degradation components of glucose are formed although the process is slower. Most of the commercial cellulases are obtained aerobically from *Trichoderma reesei*, though a small portion is obtained from *A. niger*. *T. reesei* releases a mixture of cellulases, among which at least two cellobiohydrolases, five endoglucanases, β-glucosidases and hemicellulases can be found (Zhang and Lynd, 2004). The action of cellobiohydrolases causes a gradual decrease in the polymerization degree while

endoglucanases cause the rupture of cellulose in smaller chains reducing rapidly the polymerization degree. Endoglucanases especially act on amorphous cellulose, whereas cellobiohydrolases can act on crystalline cellulose as well (Lynd et al., 2002). Although *T. reesei* produces some β-glucosidases, which are responsible of hydrolyzing formed cellobiose into two molecules of glucose, their activities are not very high. Unfortunately, cellobiohydrolases are inhibited by the cellobiose. For this reason, β-glucosidase from other source needs to be added in order to complement the action of the cellulases of this fungus. Factorial optimization techniques have been applied for the design of cellulases mixtures from different sources including β-glucosidase in order to maximize the yield of produced glucose (Kim et al., 1998). The development of a multicellulase plasmid in which the different cellulase genes could be expressed to produce cellulases with an optimum ratio from a single cultivation has been suggested.

Cellulases should be adsorbed on the surface of substrate particles before hydrolysis of insoluble cellulose takes place. The three-dimensional structure of these particles in combination with their size and shape determines whether β-glucosidic linkages are or are not accessible to enzymatic attack (Zhang and Lynd, 2004). This makes cellulose hydrolysis to be slower compared to the enzymatic degradation of other biopolymers. For instance, the hydrolysis rate of starch by amylases is 100 times faster than hydrolysis rate of cellulose by cellulases under industrial processing conditions.

4.4. Fermentation of biomass hydrolyzates

The classic configuration employed for fermenting biomass hydrolyzates involves a sequential process where the hydrolysis of cellulose and the fermentation are carried out in different units. This configuration is known as separate hydrolysis and fermentation (SHF). In the alternative variant, the simultaneous saccharification and fermentation (SSF), the hydrolysis and fermentation are performed in a single unit. The most employed microorganism for fermenting lignocellulosic hydrolyzates is *S. cerevisiae*, which ferments the hexoses contained in the hydrolyzate but not the pentoses.

4.4.1. Separate hydrolysis and fermentation

When sequential process is utilized, solid fraction of pretreated lignocellulosic material undergoes hydrolysis (saccharification). This fraction contains the cellulose in an accessible to acids or enzymes form. Once hydrolysis is completed, the resulting cellulose hydrolyzate is fermented and converted into ethanol. One of the main features of the SHF process is that each step can be performed at its optimal operating conditions. The most important factors to be taken into account for saccharification step are reaction time, temperature, pH, enzyme dosage and substrate load.

By testing lignocellulosic material from sugar cane leaves, Hari Krishna et al. (1998) have found the best values of all these parameters varying in each experimental series the value of one of the factors fixing the other ones. 65–70% cellulose conversion was achieved at 50 °C and pH of 4.5. Although enzyme doses of 100 FPU/g cellulose caused almost a 100% hydrolysis, this amount of cellulases is not economically justifiable. Hence, 40 FPU/g cellulose dosage was proposed obtaining only 13% reduction in conversion. Regarding the substrate concentration, solids loads of 10% was defined as the most adequate considering arising mixing difficulties and accumulation of inhibitors in the reactioning medium. Though these conditions were determined for a specific material pretreated by overliming and the extrapolations to other lignocellulosic feedstocks are risky, found values are within the reported ones in the literature for a wide range of materials. Hydrolysis tests for steam-pretreated spruce also indicate the need of high enzyme loadings of both cellulases and β-glucosidase to achieve cellulose conversions greater than 70% due to the less degradability of the softwood (Tengborg et al., 2001). The composition of lignocellulosic material has an important influence on the enzyme dosage as claimed in the patent of Foody et al. (2000). In particular, the ratio of arabinan plus xylan to total nonstarch polysaccharides determines its relative cellulase requirement to convert the cellulose to glucose. Thus, the higher this ratio, the less enzyme is required after the pretreatment, and hence the more economical the production of ethanol. Feedstocks with values of this ratio over about 0.39 are particularly well suited for a cellulose-to-ethanol process as certain varieties of oat hulls and corn cobs. Saha and Cotta (2006) obtained 96.7% yield of monomeric sugars using an enzymatic cocktail of cellulase, β-glucosidase and xylanase for saccharification of wheat straw pretreated by alkaline peroxide method. An ethanol concentration of 18.9 g/L and a yield of 0.46 g/g of available sugars were achieved in the subsequent fermentation using a recombinant *E. coli* strain capable of assimilating both hexoses and pentoses. Park et al. (2001) have studied the hydrolysis of waste paper contained in MSW obtaining significant sugars yield and evaluating the viscosity as operating parameter. Bioethanol production from the cellulosic portion of MSW has been already patented (Titmas, 1999) and some strategies for improving the fermentability of acid hydrolyzates of MSW have been defined. Nguyen et al. (1999) employed a mixed solids waste (construction lumber waste, almond tree prunings, wheat straw, office waste paper, and newsprint) for producing ethanol by SHF using yeasts. In this process, a recycling of enzymes was implemented through microfiltration and ultrafiltration achieving 90% cellulose hydrolysis at a net enzyme loading of 10 FPU/g cellulose.

S. cerevisiae has demonstrated its elevated resistance to the presence of inhibitors in the lignocellulosic hydrolyzate. In the case of the more productive continuous regime, one way to enhance this resistance is the increase in the cell retention to prevent wash-out and maintain high yeast cell density. Brandberg et al. (2005) employed a microfiltration unit to recirculate the cells under microaerobic conditions

achieving sugar conversion up to 99% for undetoxified dilute-acid pretreated hydrolyzates of softwood (spruce) supplemented with mineral nutrients, although the operation time was not very high (96 h) and the productivity was low. Cantarella et al. (2001) utilized a non-conventional approach for the saccharification process using bi-phasic media, which allow reaching higher glucose concentrations at the end of the hydrolysis (about 150 g/L evaluated in aqueous phase). In this case, greater substrate loads can be used because the added organic phase (acetate esters) ensures the required rheological properties for the process.

4.4.2. Simultaneous saccharification and fermentation

The SSF process shows more attractive indexes than the SHF as higher ethanol yields and less energetic consumption. In this case, the cellulases and microorganisms are added to the same process unit allowing that the glucose formed during the enzymatic hydrolysis of cellulose be immediately consumed by the microbial cells converting it into ethanol. Thus, the inhibition effect caused by the sugars over the cellulases is neutralized. However, the need of employing more dilute media to reach suitable rheological properties makes that final product concentration be low. In addition, this process operates at non-optimal conditions for hydrolysis and requires higher enzyme dosage, which positively influences on substrate conversion, but negatively on process costs. Considering that enzymes account for an important part of production costs, it is necessary to find methods reducing the cellulases doses to be utilized. With this aim, addition of surfactants has been proposed. Alkasrawi et al. (2003) showed that the addition of the non-ionic surfactant Tween-20 to the steam exploded wood during a batch SSF using *S. cerevisiae* has some effects: 8% increase in ethanol yield, 50% reduction in cellulases dosage (from 44 FPU/g cellulose to 22 FPU/g cellulose), increase of enzyme activity at the end of the process, and decrease in the time required for reaching the highest ethanol concentration. It is postulated that the surfactant avoids or diminishes the non-useful adsorption of cellulases to the lignin. However, Saha et al. (2005a) obtained marginal increases (3.5%) in saccharification of rice hulls using 2.5 g/L of Tween 20.

Hari Krishna et al. (1998) evaluated the optimal conditions of the SSF of sugar cane leaves, as they did for the SHF. These authors defined a temperature of 40 °C and pH of 5.1 as the best conditions for 3-d cultivation, achieving 31 g/L of ethanol from an initial substrate load as high as 15%. Nevertheless, the enzyme dosage was quite high (100 FPU/g cellulose). Softwood is more difficult to degrade by SSF than hardwood. Stenberg et al. (2000) used the resulting slurry of the steam pretreatment of SO_2-impregnated spruce in SSF tests using yeasts and determined that the best initial load of substrate was 5% (w/w) reaching an 82% yield based on the cellulose and soluble hexoses present at the start of the process. The productivity was doubled related to SHF. The cellulases load was in the range of 5–32 FPU/g cellulose.

Varga et al. (2004) proposed a non-isothermal regime for batch SSF process in the case of wet oxidized corn stover: In the first step of the SSF, small amounts of cellulases were added at 50 °C to obtain better mixing conditions. In the second step, more cellulases were added along with the yeast *S. cerevisiae* at 30 °C. In this way, the final solid concentration in the hydrolyzate could be increased up to 17% dry matter concentration achieving 78% ethanol yield. In general, increased cultivation temperature accelerates metabolic processes and lowers the refrigeration requirements. Yeasts as *K. marxianus* have been tested as potential ethanol producer at temperatures higher than 40 °C. Kádár et al. (2004) compared the performance of thermotolerant *K. marxianus* and *S. cerevisiae* during batch SSF of waste cardboard and paper sludge not finding great differences between both microorganisms at 40 °C, although cellulose conversions (55–60%) and ethanol yields (0.30–0.34 g/g cellulose) were relatively low. Ballesteros et al. (2001) carried out several fed-batch SSF tests at 42 °C during 72 h using *K. marxianus* in the case of by-products of olive oil extraction. Their results showed ethanol yields of 76% of theoretical for olive pulp, and of 59% for acid-catalyzed steam-exploded olive stones. With the aim of increasing ethanol yields from olive pulp, Ballesteros et al. (2002b) employed LHW pretreatment reaching a yield of 80% of theoretical and recovering potentially valuable phenolic compounds.

4.4.3. Fermentation of pentoses

One of the main problems in bioethanol production from lignocellulosics is that *S. cerevisiae* can ferment only certain mono- and disaccharides like glucose, fructose, maltose and sucrose. This microorganism is not able to assimilate cellulose and hemicellulose directly. In addition, pentoses obtained during hemicellulose hydrolysis (mainly xylose) cannot be assimilated by this yeast. A way to overcome this obstacle is through recombinant DNA technology (genetic engineering) that will be analyzed in a future review. Other approach to this problem is the use of pentose fermenting microorganisms like some species of yeasts and bacteria. In this case, configurations involving the separate fermentation of pentoses and hexoses have been proposed. Yeasts as *Pichia stipitis*, *Candida shehatae* and *Pachysolen tannophilus* can assimilate pentoses but their ethanol production rate from glucose is at least five times less than that observed for *S. cerevisiae*. Moreover, their culture requires oxygen and ethanol tolerance is 2-4 times lower (Claassen et al., 1999). Olsson and Hahn-Hägerdal (1996) extensively reviewed the microorganisms employed for pentose fermentation noting that most of them present inhibition by substrate, besides product inhibition. For example, the productivity of the yeast *C. shehatae* can diminish from 0.47–0.68 g/(L h) for xylose concentrations of 30–90 g/L to 0.42 for xylose concentrations of 200 g/L. Pentose fermenting yeasts require a careful control for maintaining low oxygen levels in the culture medium

needed for their oxidative metabolism. Additionally, these yeasts successfully ferment pure xylose but not the aqueous hemicellulose streams generated during the biomass pretreatment, probably due to the presence of different inhibitors (Chandrakant and Bisaria, 1998).

For pentose utilizing microorganisms, the hexoses are definitely the easier and faster assimilable substrate for the conversion to ethanol. This derives in a diauxic growth. If fermentation time is not sufficiently long, pentoses remain in the medium decreasing the utilization rates of the lignocellulosic complex. As a rule, microorganisms prefer glucose over galactose followed by xylose and arabinose (Gong et al., 1999). This is explained by the catabolic repression that glucose exerts on the uptake rates of xylose and other pentoses as in the case of *C. shehatae*. To offset this effect, sequential fermentations are employed in which *S. cerevisiae* utilizes the hexoses during the first days of cultivation and later xylose-utilizing yeast is added in order to complete the conversion to ethanol (Chandrakant and Bisaria, 1998), but achieved ethanol yields are not very high. In addition, the productivities of pentose utilizing microorganisms are less than those of hexose-fermenting ones. Comparisons are conclusive: ethanol productivity for *S. cerevisiae* can attain values of 170 g/(L h) in the case of continuous systems with cell recycling, whereas the productivity for *C. shehatae* at high cell concentrations reaches only values of 4.4 g/(L h) (Olsson and Hahn-Hägerdal, 1996). On the other hand, there are a few cases where the immobilization of these yeasts increases the ethanol productivity (Chandrakant and Bisaria, 1998), unlike the case of hexose-fermenting yeasts or *Z. mobilis*. In spite of these drawbacks, pentose utilizing microorganisms are important for the design of processes involving separate fermentation of hexoses and pentoses during the processing of resulting streams from biomass pretreatment. As occurs with *S. cerevisiae* or *Z. mobilis*, most pentose-fermenting yeasts are mesophiles.

Thermophilic bacteria have also been considered for fermentation of pentose-containing lignocellulosic hydrolyzates. Thermophilic and saccharolytic clostridia are an important group of ethanol-producing microorganisms and include species as *Clostridium thermohydrosulfuricum*, *Thermoanaerobacter* (formerly *Clostridium*) *thermosaccharolyticum* and *C. thermocellum*. These bacteria may transform pentoses and aminoacids into ethanol and can synthesize up to 2 mol EtOH/mol hexose. Having saccharolytic properties, these microorganisms have the ability to grow on a wide variety of non-treated wastes. *C. thermocellum* can even directly convert lignocellulosic materials into ethanol (McMillan, 1997). Main drawback consists in their very low ethanol tolerance. Consequently the maximal reached concentrations of ethanol are less than 30 g/L. Nevertheless, the fact that the culture can be carried out at high temperatures offers the possibility for a more effective alcohol removal by distillation or pervaporation (Claassen et al., 1999). Lynd et al. (2002) report low ethanol concentrations (in the order of 25 g/L) for *T. thermosaccharolyticum* cultivated in xylose-based media during batch and continuous cultures. These authors studied the influence of different factors limiting the substrate utilization for continuous cultures at progressively higher feed xylose concentrations. Their results indicate that the salt accumulation due to the utilization of bases for controlling the pH during the fermentation limits the growth of this bacterium at elevated values of xylose content in the feed. Xylose-fermenting termophilic bacteria are prospective organisms to be co-cultured with cellulose hydrolyzing bacteria such *C. thermocellum* in order to directly convert pretreated lignocellulosic biomass into ethanol, process named consolidated bioprocessing (CBP). Main trends on CBP for ethanol production are analyzed in a previous work (Cardona and Sánchez, 2007).

Other microorganism capable of fermenting a great variety of sugars including hexoses and pentoses is the fungus *Mucor indicus* reaching ethanol yields of 0.46 g/g glucose when is cultivated under anaerobic conditions. In addition, this fungus assimilates the inhibitors present in dilute-acid hydrolyzates (Sues et al., 2005). The employ of the fungus *Chalara parvispora* for ethanol production from pentose-containing materials has been patented (Holmgren and Sellstedt, 2006). Ogier et al. (1999) have compiled information about the main fermentative indexes for the pentose-assimilating yeasts *C. shehatae*, *P. stipitis* and *Pachysolen tannophilus*, and for the xylose-assimilating thermophilic bacteria *T. thermosaccharolyticum*, *T. ethanolicus* and *B. stearothermophilus*.

5. Comparison of the main types of feedstock

The selection of the most appropriate feedstock for ethanol production strongly depends on the local conditions. Evidently, North American and European countries have based their ethanol industry on the starchy materials due to their agro-ecological conditions. These conditions are not appropriate for cultivation of sugar cane, the highest yielding feedstock. The employ of starchy crops, specifically corn, for bioethanol production has provoked a hot debate on the suitability of these raw materials considering the energy input required for their production (Patzek et al., 2005; Pimentel, 2003; Shapouri et al., 2003; Wang et al., 1999a). Nevertheless, the competitiveness of Brazilian cane ethanol has been widely demonstrated, especially if its output/input energy ratio is considered (see Table 7). Besides energy considerations, crops yield should be analyzed. Note from Table 7 that the calculated ethanol yield from corn is greater than that from sugar cane because of the higher amount of fermentable sugars (glucose) that may be released from the original starchy material. However, the annual ethanol yield from each hectare of cultivated corn is lower than that for sugar cane. For the case of beet molasses, the yield per ton of feedstock is lower compared to corn, but as the beet productivity per cultivated hectare is considerably higher, the annual ethanol yield expressed in L/(Ha year) is higher related to star-

Table 7
Comparative indexes for the three main types of feedstocks for fuel ethanol production

Item	Sucrose-containing materials	Starchy materials	Lignocellulosic biomass	Remarks	References
Feedstock yield, ton/Ha	70–122.9			Sugar cane; Brazil, Colombia	Agrocadenas (2006), Asocaña (2006)
		20		Cassava	Agrocadenas (2006)
		35		Sweet sorghum	Agrocadenas (2006)
		1.5–3.0		Wheat	Agrocadenas (2006)
		6–10.07		Corn; USA	Agrocadenas (2006), FAO (2007)
			19.6–34.40	Cane bagasse	Moreira (2000)
			6.59–11.06	Corn stover	Kim and Dale (2004)
			1.93–3.86	Wheat straw	Kim and Dale (2004)
Feedstock cost, US$/kg	0.0100			Sugar cane; Brazil	Macedo and Nogueira (2005)
		0.0760		Corn; USA	McAloon et al. (2000)
	0.0124	0.1300		Cane/corn; Colombia	Quintero et al. (2007)
			0.0295	Dry corn stover, USA	Aden et al. (2002)
EtOH yield, L/ton	70			Sugar cane juice	Moreira and Goldemberg (1999)
	100			Sugar beet	Berg (2001)
		180		Cassava	Agrocadenas (2006)
		86		Sweet sorghum	Agrocadenas (2006)
		340–350		Wheat	Agrocadenas (2006)
		370		Corn	Agrocadenas (2006)
		403.1		Corn, wet milling	Gulati et al. (1996)
		419.4–460.6		Corn, dry milling	Gulati et al. (1996)
			140	Cane bagasse	Moreira (2000)
			261.3	Wheat straw	Kim and Dale (2004)
			227.7	Corn stover	Kim and Dale (2004)
			330	Dry corn stover; USA	Kadam and McMillan (2003)
Annual EtOH yield, L/(Ha year)	5,345–9,381			Sugar cane; Brazil, Colombia	Agrocadenas (2006), Asocaña (2006)
	6,600			Sugar beet; France	Poitrat (1999)
		3,600		Cassava	Agrocadenas (2006)
		9,030		Sweet sorghum	Agrocadenas (2006)
		1,020–3,214		Wheat	Agrocadenas (2006), Poitrat (1999)
		6,600		Corn	Agrocadenas (2006)
Production costs, US$/L anhydrous EtOH	0.1980			Sugar cane; Brazil	Xavier (2007)
	0.2153	0.3381		Sugar cane/corn; Colombia	Quintero et al. (2007)
		0.2325	0.3963	Corn/corn stover; USA	McAloon et al. (2000)
Output/input energy ratio	8.0			Sugar cane	Berg (2001)
	1.9			Sugar beet	Berg (2001)
		1.34–1.53		Corn; USA	Berg (2001), Shapouri et al. (2003)
			6.0	Biomass	Berg (2001)
Possibility of co-generation	Yes	No	Yes		
Co-products	Concentrated stillage for fertilization	DDGS (dry milling)	Lignin as feedstock for chemicals		

chy materials (beet: 6,600, dry-milled wheat: 3,214, wet-milled wheat: 2,555) (Poitrat, 1999). On the other hand, the high moisture content of the cassava implies the use of a greater amount of feedstock to reach the same starch content related to corn. However, the crop yields of cassava are higher than that of corn. Moreover, the corn yield in some tropical countries is significantly lower than the corn yield in the USA favoring the use of cassava instead of corn in such countries. For instance, the yield of technified corn in Colombia reaches only 3.9 ton/Ha (Agrocadenas, 2006) whereas the cassava yield can reach 30 ton/Ha (Espinal et al., 2005). This yield leads to an ethanol yield of 5,400 L/(Ha year), greater than the expected yield from corn under Colombian conditions that reaches 4,329 L/(Ha year).

Lignocellulosic materials represent a promising option as a feedstock for ethanol production considering their output/input energy ratio, their great availability both in tropical and temperate countries, their low cost (primarily related to their transport), and their ethanol yields (see Table 7). One of the advantages of the use of lignocellulosic biomass is that this feedstock is not directly

related to food production. This implies the production of bioethanol without the need of employing vast extensions of fertile cultivable land for cropping cane or corn exclusively dedicated to the bioenergy production. In addition, lignocellulosics is a resource that can be processed in different ways for production of many other products like synthesis gas, methanol, hydrogen and electricity (Chum and Overend, 2001).

The selection of the lignocellulosic feedstock is in concordance with the interests of each country for transferring value to the produced wastes, especially for those wastes that do not have value as a food. For the case of the USA, corn stover is considered one of the most promising feedstocks due to its wide availability. The total availability of this material in the United States in such a way that its recollection and use be environmentally sustainable, has been estimated in about 80–100 mill dry ton per year becoming the most abundant agricultural residue in that country. Kadam and McMillan (2003), citing non-published data from the NREL, indicate that the theoretical yield of this material is 480 L EtOH/dry ton, assuming that both hexoses and pentoses can be fermented into ethanol. These authors point out that 33 mill ton per year of corn stover would be necessary to ensure the total ethanol production of 11,000 mill liters per year considering a more realistic yield of 330 L EtOH/dry ton. The above-mentioned demonstrates the vast possibilities of biomass taking into account that, in this case, there will be no competition for cultivable land with crops dedicated to food production. Other studies have been oriented to the use of rice straw for ethanol production (Kadam et al., 2000). Kim and Dale (2004) provide an interesting panorama on the size of the bioethanol feedstock resource at global and regional levels considering wasted crops (crops lost in distribution) and lignocellulosic biomass (crop residues and sugar cane bagasse). These authors estimate that the global potential ethanol production from these feedstocks accounts 491 GL/year that is 16 times higher than current ethanol production. This amount of bioethanol could replace 32% of global gasoline consumption. For them, rice straw is the feedstock that potentially could produce the largest amounts of ethanol, followed by wheat straw.

However, the great-scale ethanol production from lignocellulosic biomass could entail serious economic and environmental consequences, like noted by Berndes et al. (2001). These authors estimate that labor requirements for bioenergy production on a great scale in whatever country should not exceed 1% of total manpower. Grassi (1999) points out that the development of bioenergy production technologies would represent the creation of 200,000 direct and indirect jobs and the reduction of 255 mill ton per year of CO_2 in 2010. Nevertheless, the main limitation in the use of this resource is the conversion process of biomass into ethanol as analyzed in Section 4. Once these technological limitations are overcome, lignocellulosic biomass will be the main feedstock for ethanol production.

Certainly, a detailed economic and environmental evaluation of the different feedstocks is required in order to make decisions on the most appropriate raw materials for fuel ethanol production in each case. A useful approach for performing such evaluations is to employ simulation tools based on realistic data obtained from existing ethanol production facilities, pilot plants or mathematical models. In addition, this approach allows the analysis of how different technological configurations (e.g., SHF or SSF) have influence on the indicators of the overall process. Examples of these comparative studies can be found in the works of McAloon et al. (2000) and Cardona et al. (2005) for corn and lignocellulosic ethanol, Quintero et al. (2007) for sugar cane and corn ethanol, and Aden et al. (2002), Wooley et al. (1999a,b) and Sánchez et al. (2006) for lignocellulosic biomass.

6. Conclusions

The massive utilization of fuel ethanol in the world requires that its production technology be cost-effective and environmentally sustainable. In particular, ethanol production costs should be lowered. For current technologies employed at commercial level, the main share in the cost structure corresponds to the feedstocks (above 60%) followed by the processing expenditures. In general, the use of sucrose-containing materials as cane molasses allows producing ethanol with the lowest costs compared to the starchy materials (mostly grains). Particularly, although the ethanol yield from corn is higher than that from sugar cane, the lower annual yield of corn per cultivated hectare makes it necessary to use larger cropping areas. On the other hand, the lignocellulosic biomass represents the most prospective feedstock for ethanol production. The availability and low cost of a wide range of lignocellulosic materials offer many possibilities for the development of bioindustries that could support the growth of the international biofuel market and contribute to the reduction of greenhouse gas emissions worldwide.

The current research tendencies for improving fuel ethanol production are linked to the nature of employed raw materials, processing steps, and related process engineering issues. The trends regarding the latter aspect were discussed in a previous work (Cardona and Sánchez, 2007). In the present paper, main trends in the conversion of different feedstock into ethanol were discussed. Some of them are summarized in Table 8, which complements the Table 4 of the above-mentioned previous work concerning the research priorities for improving ethanol production by means of process engineering. For the three main types of feedstocks, the development of effective continuous fermentation technologies with near to 100% yields and elevated volumetric productivities is one of the main research subjects in the ethanol industry. To this end, many of newly proposed technologies for reducing the product

Table 8
Research trends and priorities for improving fuel ethanol production from different feedstocks

Issue	All feedstocks[a]	Sucrose-containing materials	Starchy materials	Lignocellulosic biomass
Feedstock	Reduction in costs of feedstocks by improving crop yields, pest resistance and cropping systems	Increase in crop productivity	Utilization of native starchy material other than cereal grains (cassava, indigenous roots, etc.)	Evaluation of the use of dedicated energy crops
			Development of corn hybrids with higher extractable starch or with higher fermentable starch content	Genetic modification of herbaceous plants for changing their carbohydrate content
			Genetic improvement of corn (e.g. "self-processing grains")	Economic utilization of different and alternative wastes like MSW
Pretreatment		Removal of impurities and toxic substances from molasses	Reduction of energy costs of liquefaction	Reduction of milling power
				Optimization of steam explosion and dilute-acid pretreatment
				Development of LHW, AFEX and alkaline hydrolysis
				Reduced formation of inhibitors
				Recycling of concentrated acids
Hydrolysis			Low temperature digestion of starch	Increase in specific activity, thermal stability and cellulose-specific binding of cellulases (e.g. by protein engineering)
				Reduction of costs of cellulases production (10-fold reduction)
				Cellulases production by solid-state fermentation
				Recycling of cellulases
				Improvement of acid hydrolysis of MSW
Fermentation	Continuous fermentation with high cell density and increased yields and productivity	Reduction of inhibition by ethanol	Recombinant strains of yeasts with increased productivity and ethanol tolerance	Increase in conversion of glucose and pentoses to ethanol
		Microorganisms with increased osmotolerance or flocculating properties	High cell-density fermentation (e.g. immobilized cells, flocculating yeasts, membrane reactors, etc.)	Recombinant strains with increased stability and efficiency for assimilating hexoses and pentoses, and for working at higher temperatures
			Very high gravity fermentations	Development of strains more tolerant to the inhibitors
				Increase of ethanol tolerance in pentose-fermenting microorg.

[a] Refers to the three analyzed groups of feedstock: sucrose-containing materials, starchy materials and lignocellulosic biomass.

inhibition effect on the cell growth rate should be scaled-up at industrial level. This progress should complement the intense efforts oriented to the selection and development of microbial strains with particular traits like specific flocculating properties or increased tolerance to ethanol, inhibitors and salts.

The three kinds of feedstock analyzed in this paper correspond to resources that are present in almost all the countries. In particular, all populated regions in the world account for vast amounts of lignocellulosic waste materials that eventually can be converted into ethanol. Tropical countries like Colombia exhibit comparative advantages in the availability of feedstocks for ethanol production in comparison to European or North American countries. In fact, the dynamics of the global ethanol market could require these countries to supply the growing demand of those countries that have implemented or will implement ambitious programs for the partial substitution of fossil fuels with renewable liquid fuels. These programs may have dissimilar motivations different to environmental concerns, but humankind and global climate will be benefited in any case.

Acknowledgements

The authors express their acknowledgments to the Colombian Institute for Development of Science and Technology (Colciencias), National University of Colombia at Manizales, and University of Caldas.

References

Abd-Aziz, S., 2002. Sago starch and its utilization. Journal of Bioscience and Bioengineering 94 (6), 526–529.

Acevedo, A., Godoy, R., Bolaños, G., 2003. Increase in ethanol production during fermentation of molasses using the enzymatic complex Rhyzozyme. In: XXII Congreso Colombiano de Ingeniería Química, Bucaramanga, Colombia (in Spanish).

Aden, A., Ruth, M., Ibsen, K., Jechura, J., Neeves, K., Sheehan, J., Wallace, B., Montague, L., Slayton, A., Lukas, J., 2002. Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis for corn stover. Technical Report NREL/TP-510-32438. Golden, CO (USA), National Renewable Energy Laboratory. 143 p.

Agrocadenas, 2006. Segundo informe de coyuntura maíz 2006. Observatorio Agrocadenas Colombia, Ministry of Agricultural and Rural

Development. <http://www.agrocadenas.gov.co/home.htm> (February 2007).

Alfenore, S., Cameleyre, X., Benbadis, L., Bideaux, C., Uribelarrea, J.-L., Goma, G., Molina-Jouve, C., Guillouet, S.E., 2004. Aeration strategy: a need for very high ethanol performance in *Saccharomyces cerevisiae* fed-batch process. Applied Microbiology and Biotechnology 63, 537–542.

Alkasrawi, M., Eriksson, T., Börjesson, J., Wingren, A., Galbe, M., Tjerneld, F., Zacchi, G., 2003. The effect of Tween-20 on simultaneous saccharification and fermentation of softwood to ethanol. Enzyme and Microbial Technology 33, 71–78.

Alvo, P., Belkacemi, K., 1997. Enzymatic saccharification of milled timothy (*Phleum pretense* L.) and alfalfa (*Medicago sativa* L.). Bioresource Technology 61, 185–198.

Apar, D.K., Özbek, B., 2004. σ-Amylase inactivation during corn starch hydrolysis process. Process Biochemistry 39 (12), 1877–1892.

Asocaña, 2006. General issues of sugar sector. Annual report 2005-2006. Asociación de Cultivadores de Caña de Azúcar de Colombia (Asocaña). 67 p. (in Spanish).

Bai, F.W., Chen, L.J., Anderson, W.A., Moo-Young, M., 2004. Parameter oscillations in a very high gravity medium continuous ethanol fermentation and their attenuation on a multistage packed column bioreactor system. Biotechnology and Bioengineering 88 (5), 558–566.

Ballesteros, I., Oliva, J.M., Sáez, F., Ballesteros, M., 2001. Ethanol production from lignocellulosic byproducts of olive oil extraction. Applied Biochemistry and Biotechnology 91–93, 237–252.

Ballesteros, I., Oliva, J.M., Negro, M.J., Manzanares, P., Ballesteros, M., 2002a. Enzymic hydrolysis of steam exploded herbaceous agricultural waste (*Brassica carinata*) at different particule sizes. Process Biochemistry 38, 187–192.

Ballesteros, I., Oliva, J.M., Negro, M.J., Manzanares, P., Ballesteros, M., 2002b. Ethanol production from olive oil extraction residue pretreated with hot water. Applied Biochemistry and Biotechnology, 717–732.

Ballesteros, M., Oliva, J.M., Negro, M.J., Manzanares, P., Ballesteros, I., 2004. Ethanol from lignocellulosic materials by a simultaneous saccharification and fermentation process (SFS) with Kluyveromyces marxianus CECT 10875. Process Biochemistry 39, 1843–1848.

Barber, A.R., Henningsson, M., Pamment, N.B., 2002. Acceleration of high gravity yeast fermentations by acetaldehyde addition. Biotechnology Letters 24, 891–895.

Bayrock, D.P., Ingledew, W.M., 2001. Application of multistage continuous fermentation for production of fuel alcohol by very-high-gravity fermentation technology. Journal of Industrial Microbiology and Biotechnology 27, 87–93.

Belkacemi, K., Turcotte, G., Savoie, P., Chornet, E., 1997. Ethanol production from enzymatic hydrolyzates of cellulosic fines and hemicellulose-rich liquors derived from aqueous/steam fractionation of forages. Industrial & Engineering Chemistry Research 36, 4572–4580.

Belkacemi, K., Turcotte, G., Savoie, P., 2002. Aqueous/steam-fractionated agricultural residues as substrates for ethanol production. Industrial & Engineering Chemistry Research 41, 173–179.

Berg, C., 2001. World Fuel Ethanol. Analysis and Outlook. F.O. Licht. <http://www.agra-europe.co.uk/FOLstudies/FOL-Spec04.html>/ (March 2004).

Berg, C., 2004. World Fuel Ethanol. Analysis and Outlook. <http://www.distill.com/World-Fuel-Ethanol-A&O-2004.html> (November 2006).

Berndes, G., Azar, C., Kåberger, T., Abrahamson, D., 2001. The feasibility of large-scale lignocellulose-based bioenergy production. Biomass and Bioenergy 20, 371–383.

Bjerre, A.B., Olesen, A.B., Fernqvist, T., Plöger, A., Schmidt, A.S., 1996. Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose. Biotechnology and Bioengineering 49, 568–577.

Bothast, R.J., Schlicher, M.A., 2005. Biotechnological processes for conversion of corn into ethanol. Applied Microbiology and Biotechnology 67, 19–25.

Brandberg, T., Sanandaji, N., Gustafsson, L., Franzén, C.J., 2005. Continuous fermentation of undetoxified dilute acid lignocellulose hydrolysate by Saccharomyces cerevisiae ATCC 96581 using cell recirculation. Biotechnology Progress 21, 1093–1101.

Bullock, G.E., 2002. Ethanol from Sugarcane. Sugar Research Institute, Australia, 192 p.

Burmaster, B., 2007. Improved ethanol fermentation using oxidation reduction potential. Patent WO2007064545.

Caicedo, L.A., Cuenca, M.M., Díaz, M. 2003. Scale-up of ethanol production at pilot scale level using a reactor with immobilized cells. In: XXII Congreso Colombiano de Ingeniería Química, Bucaramanga, Colombia (in Spanish).

Cantarella, M., Alfani, F., Cantarella, L., Gallifuoco, A., Saporosi, A., 2001. Biosaccharification of cellulosic biomass in inmiscible solvent–water mixtures. Journal of Molecular Catalysis B: Enzymatic 11, 867–875.

Cantarella, M., Cantarella, L., Gallifuoco, A., Spera, A., Alfani, F., 2004. Comparison of different detoxification methods for steam-exploded poplar wood as a substrate for the bioproduction of ethanol in SHF and SSF. Process Biochemistry 39, 1533–1542.

Cardona, C.A., Sánchez, Ó.J., 2007. Fuel ethanol production: process design trends and integration opportunities. Bioresource Technology 98, 2415–2457.

Cardona, C.A., Sánchez, O.J., Montoya, M.I., Quintero, J.A., 2005. Analysis of fuel ethanol production processes using lignocellulosic biomass and starch as feedstocks. In: Seventh World Congress of Chemical Engineering, Glasgow, Scotland, UK.

Carrascosa, A.V., 2006. Production of ethanol under high osmotic pressure conditions comprises a microorganism for fermentation of molasses must. Patent ES2257206.

Castellar, M.R., Aires-Barros, M.R., Cabral, J.M.S., Iborra, J.L., 1998. Effect of zeolite addition on ethanol production from glucose by *Saccharomyces bayanus*. Journal of Chemical Technology and Biotechnology 73, 377–384.

Chandrakant, P., Bisaria, V.S., 1998. Simultaneous bioconversion of cellulose and hemicellulose to ethanol. Critical Reviews in Biotechnology 18 (4), 295–331.

Chum, H.L., Overend, R.P., 2001. Biomass and renewable fuels. Fuel Processing Technology 71, 187–195.

Chung, B.H., Nam, J.G., 2002. Process for producing high concentration of ethanol using food wastes by fermentation. Patent KR20020072326.

Claassen, P.A.M., van Lier, J.B., López Contreras, A.M., van Niel, E.W.J., Sijtsma, L., Stams, A.J.M., de Vries, S.S., Weusthuis, R.A., 1999. Utilisation of biomass for the supply of energy carriers. Applied Microbiology and Biotechnology 52, 741–755.

Costa, A.C., Atala, D.I.P., Maugeri, F., Maciel, R., 2001. Factorial design and simulation for the optimization and determination of control structures for an extractive alcoholic fermentation. Process Biochemistry 37, 125–137.

Cuzens, J.C., Miller, J.R., 1997. Acid hydrolysis of bagasse for ethanol production. Renewable Energy 10 (2/3), 285–290.

Dai, D., Hu, Z., Pu, G., Li, H., Wang, C., 2006. Energy efficiency and potentials of cassava fuel ethanol in Guangxi region of China. Energy Conversion and Management 47, 1686–1699.

Dale, B.E., Leong, C.K., Pham, T.K., Esquivel, V.M., Rios, I., Latimer, V.M., 1996. Hydrolysis of lignocellulosics at low enzyme levels: application of the AFEX process. Bioresource Technology 56, 111–116.

da Silva, F.L.H., Rodrigues, M.A., Maugeri, F., 1999. Dynamic modelling, simulation and optimization of an extractive continuous alcoholic fermentation process. Journal of Chemical Technology and Biotechnology 74, 176–182.

Daugulis, A.J., McLellan, P.J., Jinghong, L., 1997. Experimental investigation and modeling of oscillatory behavior in the continuous culture of *Zymomonas mobilis*. Biotechnology and Bioengineering 56 (1), 99–105.

De Bari, I., Viola, E., Barisano, D., Cardinale, M., Nanna, F., Zimbardi, F., Cardinale, G., Braccio, G., 2002. Ethanol production at flask and

pilot scale from concentrated slurries of steam-exploded aspen. Industrial & Engineering Chemistry Research 41, 1745–1753.

Echegaray, O., Carvalho, J., Fernandes, A., Sato, S., Aquarone, E., Vitolo, M., 2000. Fed-batch culture of *Saccharomyces cerevisiae* in sugarcane blackstrap molasses: invertase activity of intact cells in ethanol fermentation. Biomass and Bioenergy 19, 39–50.

Elnashaie, S.S.E.H, Garhyan, P., 2005. Chaotic fermentation of ethanol. Patent US2005170483.

Ergun, M., Mutlu, S.F., Gürel, Ö., 1997. Improved ethanol production by *Saccharomyces cerevisiae* with EDTA, ferrocyanide and zeolite X addition to sugar beet molasses. Journal of Chemical Technology and Biotechnology 68, 147–150.

Espinal, C.F., Martínez, H.J., Acevedo, X., 2005. The chain of cereals, balanced feed, aviculture, and pig farming in Colombia. An overview of its structure and dynamics 1991–2005. Working report No. 87. Observatorio Agrocadenas Colombia, Ministry of Agriculture and Rural Development. 94 p. (in Spanish).

FAO, 2004. Global cassava market study. Business opportunities for the use of cassava. Proceedings of the Validation Forum on the Global Cassava Development Strategy, vol. 6. Food and Agriculture Organization of the United Nations (FAO), Rome.

FAO, 2007. FAOSTAT. Food and Agriculture Organization of the United Nations (FAO). <http://faostat.fao.org> (February 2007).

Farone, W.A., Cuzens, J.E., 1996. Method of strong acid hydrolysis. Patent WO9640970.

Foody, B., Tolan, J.S., Bernstein, J.D., Foody Sr., P., 2000. Pretreatment process for conversion of cellulose to fuel ethanol. Patent US6090595.

Ghosh, P., Ghose, T.K., 2003. Bioethanol in India: recent past and emerging future. Advances in Biochemical Engineering/Biotechnology 85, 1–27.

Gilson, C.D., Thomas, A., 1995. Ethanol production by alginate immobilised yeast in a fluidised bed bioreactor. Journal of Chemical Technology and Biotechnology 62, 38–45.

Gong, C.S., Cao, N.J., Du, J., Tsao, G.T., 1999. Ethanol production from renewable resources. Advances in Biochemical Engineering/Biotechnology 65, 207–241.

Goyes, A., Bolaños, G., 2005. Un estudio preliminar sobre el tratamiento de vinazas en agua supercrítica. In: XXIII Congreso Colombiano de Ingeniería Química, Manizales, Colombia.

Grassi, G., 1999. Modern bioenergy in the European Union. Renewable Energy 16, 985–990.

Gulati, M., Kohlman, K., Ladish, M.R., Hespell, R., Bothast, R.J., 1996. Assessment of ethanol production options for corn products. Bioresource Technology 5, 253–264.

Hamelinck, C.N., van Hooijdonk, G., Faaij, A.P.C., 2005. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. Biomass and Bioenergy 28, 384–410.

Hammond, J.B., Egg, R., Diggins, D., Coble, C.G., 1996. Alcohol from bananas. Bioresource Technology 56, 125–130.

Hari Krishna, S., Prasanthi, K., Chowdary, G., Ayyanna, C., 1998. Simultaneous saccharification and fermentation of pretreated sugar cane leaves to ethanol. Process Biochemistry 33 (8), 825–830.

Hojo, O., Hokka, C.O., Souto Maior, A.M., 1999. Ethanol production by a flocculant yeast strain in a CSTR type fermentor with cell recycling. Applied Biochemistry and Biotechnology 77–79, 535–545.

Holmgren, M., Sellstedt, A., 2006. Producing ethanol through fermentation of organic starting materials, involves using fungus e.g., Chalara parvispora, capable of metabolizing pentose compounds. Patent SE527184.

Itoh, H., Wada, M., Honda, Y., Kuwahara, M., Watanabe, T., 2003. Bioorganosolve pretreatments for simultaneous saccharification and fermentation of beech wood by ethanolysis and white rot fungi. Journal of Biotechnology 103, 273–280.

Jacobsen, S.E., Wyman, C.E., 2002. Xylose monomer and oligomer yields for uncatalyzed hydrolysis of sugarcane bagasse hemicellulose at varying solids concentration. Industrial & Engineering Chemistry Research 41, 1454–1461.

Jönsson, L.J., Palmqvist, E., Nilvebrant, N.-O., Hahn-Hägerdal, B., 1998. Detoxification of wood hydrolysates with laccase and peroxidase from the white-rot fungus Trametes versicolor. Applied Microbiology and Biotechnology 49, 691–697.

Kaar, W.E., Holtzapple, M.T., 2000. Using lime pretreatment to facilitate the enzymic hydrolysis of corn stover. Biomass and Bioenergy 18, 189–199.

Kadam, K.L., McMillan, J.D., 2003. Availability of corn stover as a sustainable feedstock for bioethanol production. Bioresource Technology 88, 17–25.

Kadam, K.L., Forrest, L.H., Jacobson, W.A., 2000. Rice straw as a lignocellulosic resource: collection, processing, transportation, and environmental aspects. Biomass and Bioenergy 18, 369–389.

Kádár, Zs., Szengyel, Zs., Réczey, K., 2004. Simultaneous saccharification and fermentation (SSF) of industrial wastes for the production of ethanol. Industrial Crops and Products 20, 103–110.

Kang, S.W., Park, Y.S., Lee, J.S., Hong, S.I., Kim, S.W., 2004. Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. Bioresource Technology 91, 153–156.

Khiyami, M.A., Pometto III, A.L., Brown, R.C., 2005. Detoxification of corn stover and corn starch pyrolysis liquors by *Pseudomonas putida* and *Streptomyces setonii* suspended cells and plastic compost support biofilms. Journal of Agricultural and Food Chemistry 53, 2978–2987.

Kim, S., Dale, B.E., 2004. Global potential bioethanol production from wasted crops and crop residues. Biomass and Bioenergy 26, 361–375.

Kim, E., Irwin, D.C., Walker, L.P., Wilson, D.B., 1998. Factorial optimization of a six-cellulase mixture. Biotechnology and Bioengineering 58 (5), 494–501.

Koegel, R.G., Sreenath, H.K., Straub, R.J., 1999. Alfalfa fiber as a feedstock for ethanol and organic acids. Applied Biochemistry and Biotechnology, 105–115.

Kosaric, N., Velikonja, J., 1995. Liquid and gaseous fuels from biotechnology: challenge and opportunities. FEMS Microbiology Reviews 16, 111–142.

Krishnan, M.S., Nghiem, N.P., Davison, B.H., 1999. Ethanol production from corn starch in a fluidized-bed bioreactor. Applied Biochemistry and Biotechnology 77–79, 359–372.

Laluce, C., Souza, C.S., Abud, C.L., Gattas, E.A.L., Walker, G.M., 2002. Continuous ethanol production in a nonconventional five-stage system operating with yeast cell recycling at elevated temperatures. Journal of Industrial Microbiology & Biotechnology 29, 140–144.

Laser, M., Schulman, D., Allen, S.G., Lichwa, J., Antal Jr., M.J., Lynd, L.R., 2002. A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol. Bioresource Technology 81, 33–44.

Lee, J., 1997. Biological conversion of lignocellulosic biomass to ethanol. Journal of Biotechnology 56, 1–24.

Lee, W.-C., Huang, C.-T., 2000. Modeling of ethanol fermentation using *Zymomonas mobilis* ATCC 10988 grown on the media containing glucose and fructose. Biochemical Engineering Journal 4, 217–227.

Lee, W.G., Lee, J.S., Shin, C.S., Park, S.C., Chang, H.N., Chang, Y.K., 1999. Ethanol production using concentrated oak wood hydrolysates and methods to detoxify. Applied Biochemistry and Biotechnology 77–79, 547–559.

Lin, Y., Tanaka, S., 2006. Ethanol fermentation from biomass resources: current state and prospects. Applied Microbiology and Biotechnology 69, 627–642.

Londoño, L.F. 2007. Informe anual. Sector azucarero colombiano. <http://www.asocana.com.co/informes/Default.aspx> (September 2007).

López-Ulibarri, R., Hall, G.M., 1997. Saccharification of cassava flour starch in a hollow-fiber membrane reactor. Enzyme and Microbial Technology 21, 398–404.

Lynd, L.R., 1996. Overview and evaluation of fuel ethanol from cellulosic biomass: technology, Economics, the Environment, and Policy. Annual Review of Energy and the Environment 21, 403–465.

Lynd, L.R., Wyman, C.E., Gerngross, T.U., 1999. Biocommodity engineering. Biotechnology Progress 15, 777–793.

Lynd, L.R., Weimer, P.J., van Zyl, W.H., Pretorious, I.S., 2002. Microbial cellulose utilization: fundamentals and biotechnology. Microbiology and Molecular Biology Reviews 66 (3), 506–577.

Macedo, I.C., Nogueira, L.A.H., 2005. Evaluation of ethanol production expansion in Brazil. In: Cadernos NAE/Núcleo de Assuntos Estratégicos da Presidência da República. Biofuels, Section 2. Brasília (Brazil), Núcleo de Assuntos Estratégicos da Presidência da República, pp. 113–220 (in Portuguese).

Martín, C., Galbe, M., Wahlbom, C.F., Hahn-Hägerdal, B., Jönsson, L.J., 2002. Ethanol production from enzymatic hydrolysates of sugarcane bagasse using recombinant xylose-utilising *Saccharomyces cerevisiae*. Enzyme and Microbial Technology 31, 274–282.

Martinez, A., Rodriguez, M.E., York, S.W., Preston, J.F., Ingram, L.O., 2000. Effects of $Ca(OH)_2$ treatments ("Overliming") on the composition and toxicity of bagasse hemicellulose hydrolysates. Biotechnology and Bioengineering 69 (5), 526–536.

Martinez, A., Rodriguez, M.E., Wells, M.L., York, S.W., Preston, J.F., Ingram, L.O., 2001. Detoxification of dilute acid hydrolysates of lignocellulose with lime. Biotechnology Progress 17, 287–293.

Maye, J.P., 2006. Use of hop acids in fuel ethanol production. Patent US2006263484.

McAloon, A., Taylor, F., Yee, W., Ibsen, K., Wooley, R., 2000. Determining the cost of producing ethanol from corn starch and lignocellulosic feedstocks. Technical Report NREL/TP-580-28893. Golden, CO (USA), National Renewable Energy Laboratory. 35 p.

McMillan, J.D., 1997. Bioethanol production: status and prospects. Renewable Energy 10, 295–302.

Monte Alegre, R., Rigo, M., Joekes, I., 2003. Ethanol fermentation of a diluted molasses medium by *Saccharomyces cerevisiae* immobilized on chrysotile. Brazilian Archives of Biology and Technology 46 (4), 751–757.

Moreira, J.S., 2000. Sugarcane for energy – recent results and progress in Brazil. Energy for Sustainable Development 4 (3), 43–54.

Moreira, J., Goldemberg, J., 1999. The alcohol program. Energy Policy 27, 229–245.

Morimura, S., Zhong, Y.L., Kida, K., 1997. Ethanol production by repeated-batch fermentation at high temperature in a molasses medium containing a high concentration of total sugar by a thermotolerant flocculating yeast with improved salt-tolerance. Journal of Fermentation and Bioengineering 83 (3), 271–274.

Murray, D., 2005. Ethanol's potential: Looking beyond corn. Eco-Economy Updates. Earth Policy Institute. <http://earth-policy.org/Updates/2005/Update49.htm> (July 2005).

Nakamura, Y., Sawada, T., Inoue, E., 2001. Enhanced ethanol production from enzymatically treated steam-exploded rice straw using extractive fermentation. Journal of Chemical Technology and Biotechnology 76, 879–884.

Narendranath, N.V., Thomas, K.C., Ingledew, W.M., 2000. Use of urea hydrogen peroxide in fuel alcohol production. Patent CA2300807.

Negro, M.J., Manzanares, P., Ballesteros, I., Oliva, J.M., Cabañas, A., Ballesteros, M., 2003. Hydrothermal pretreatment conditions to enhance ethanol production from poplas biomass. Applied Biochemistry and Biotechnology 105–108, 87–100.

Nguyen, Q.A., Keller, F.A., Tucker, M.P., Lombard, C.K., Jenkins, B.M., Yomogida, D.E., Tiangco, V.M., 1999. Bioconversion of mixed solids waste to ethanol. Applied Biochemistry and Biotechnology 77–79, 455–472.

Nilsson, A., Taherzadeh, M.J., Lidén, G., 2001. Use of dynamic step response for control of fed-batch conversion of lignocellulosic hydrolyzates to ethanol. Journal of Biotechnology 89, 41–53.

Ogier, J.-C., Ballerini, D., Leygue, J.-P., Rigal, L., Pourquié, J., 1999. Production d'éthanol à partir de biomasse lignocellulosique. Oil & Gas Science and Technology – Revue de l'IFP 54 (1), 67–94 (in French).

Ojumu, T.V., Ogunkunle, O.A., 2005. Production of glucose from lignocellulosic under extremely low acid and high temperature in batch process, auto-hydrolysis approach. Journal of Applied Sciences 5 (1), 15–17.

Oliva, J.M., Sáez, F., Ballesteros, I., González, A., Negro, M.J., Manzanares, P., Ballesteros, M., 2003. Effect of lignocellulosic degradation compounds from steam explosion pretreatment on ethanol fermentation by thermotolerant yeast *Kluyveromyces marxianus*. Applied Biochemistry and Biotechnology, 141–153.

Oliva, J.M., Ballesteros, I., Negro, M.J., Manzanares, P., Cabañas, A., Ballesteros, M., 2004. Effect of binary combinations of selected toxic compounds on growth and fermentation of Kluyveromyces marxianus. Biotechnology Progress 20, 715–720.

Olsson, L., Hahn-Hägerdal, B., 1996. Fermentation of lignocellulosic hydrolysates for ethanol production. Enzyme and Microbial Technology 18, 312–331.

Otto, E., Escovar-Kousen, J. 2004. Ethanol production by simultaneous saccharification and fermentation (SSF). Patent WO2004046333.

Palmqvist, E., Hahn-Hägerdal, B., 2000a. Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification. Bioresource Technology 74, 17–24.

Palmqvist, E., Hahn-Hägerdal, B., 2000b. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Bioresource Technology 74, 25–33.

Palmqvist, E., Grage, H., Meinander, N.Q., Hahn-Hägerdal, B., 1999. Main and interaction effects of acetic acid, furfural, and *p*-hydroxybenzoic acid on growth and ethanol productivity of yeasts. Biotechnology and Bioengineering 63 (1), 46–55.

Pandey, A., Nigam, P., Soccol, C.R., Soccol, V.T., Singh, D., Mohan, R., 2000. Advances in microbial amylases. Biotechnology and Applied Biochemistry 31, 135–152.

Pan, X., Arato, C., Gilkes, N., Gregg, D., Mabee, W., Pye, K., Xiao, Z., Zhang, X., Saddler, J., 2005. Biorefining of softwoods using ethanol organosolv pulping: preliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-products. Biotechnology and Bioengineering 90 (4), 473–481.

Papatheofanous, M.G., Billa, E., Koullas, D.P., Monties, B., Koukios, E.G., 1998. Optimizing multisteps mechanical–chemical fractionation of wheat straw components. Industrial Crops and Products 7, 249–256.

Park, E.Y., Michinaka, A., Okuda, N., 2001. Enzymatic hydrolysis of waste office paper using viscosity as operating parameter. Biotechnology Progress 17, 379–382.

Patzek, T.W., Anti, S.-M., Campos, R., Ha, K.W., Lee, J., Li, B., Padnick, J., Yee, S.-A., 2005. Ethanol from corn: Clean renewable fuel for the future, or drain on our resources and pockets? Environment, Development and Sustainability 7, 319–336.

Persson, P., Andersson, J., Gorton, L., Larsson, S., Nilvebrant, N.-O., Jönsson, L.F., 2002a. Effect of different forms of alkali treatment on specific fermentation inhibitors and on the fermentability of lignocellulose hydrolysates for production of fuel ethanol. Journal of Agricultural and Food Chemistry 50, 5318–5325.

Persson, P., Larsson, S., Jönsson, L.J., Nilvebrant, N.-O., Sivik, B., Munteanu, F., Thörneby, L., Gorton, Lo., 2002b. Supercritical fluid extraction of a lignocellulosic hydrolysate of spruce for detoxification and to facilitate analysis of inhibitors. Biotechnology and Bioengineering 79 (6), 694–700.

Pimentel, D., 2003. Ethanol fuels: energy balance, economics, and environmental impacts are negative. Natural Resources Research 12 (2), 127–134.

Poitrat, E., 1999. The potential of liquid biofuels in France. Renewable Energy 16, 1084–1089.

Prakasham, R.S., Kuriakose, B., Ramakrishna, S.V., 1999. The influence of inert solids on ethanol production by *Saccharomyces cerevisiae*. Applied Biochemistry and Biotechnology 82, 127–134.

Purwadi, R., Brandberg, T., Taherzadeh, M.J., 2007. A possible industrial solution to ferment lignocellulosic hydrolyzate to ethanol: continuous cultivation with flocculating yeast. International Journal of Molecular Sciences 8, 920–932.

Quintero, J.A., Montoya, M.I., Sánchez, O.J., Giraldo, O.H., Cardona, C.A., 2007. Fuel ethanol production from sugarcane and corn: Comparative analysis for a Colombian case. Energy, doi:10.1016/j.energy.2007.10.001.

Renewable Fuels Association. 2007. Industry statistics. <http://www.ethanolrfa.org/industry/statistics>/ (September 2007).

Rezzoug, S.-A., Capart, R., 1996. Solvolysis and hydrotreatment of wood to provide fuel. Biomass and Bioenergy 11 (4), 343–352.

Robertson, G.H., Wong, D.W.S., Lee, C.C., Wagschal, K., Smith, M.R., Orts, W.J., 2006. Native or raw starch digestion: a key step in energy efficient biorefining of grain. Journal of Agricultural and Food Chemistry 54, 353–365.

Rodríguez-Chong, A., Ramírez, J.A., Garrote, G., Vázquez, M., 2004. Hydrolysis of sugar cane bagasse using nitric acid: a kinetic assessment. Journal of Food Engineering 61 (2), 143–152.

Saha, B.C., Cotta, M.A., 2006. Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw. Biotechnology Progress 22, 449–453.

Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V., 2005a. Dilute acid pretreatment, enzymatic saccharification, and fermentation of rice hulls to ethanol. Biotechnology Progress 21, 816–822.

Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V., 2005b. Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. Process Biochemistry 40, 3693–3700.

Sánchez, O.J., Cardona, C.A., 2005. Biotechnological production of fuel alcohol I: production from different feedstocks. Interciencia 30 (11), 671–678, in Spanish.

Sánchez, O.J., Fraga, E.S., Cardona, C.A., 2006. Process synthesis for fuel ethanol production from lignocellulosic biomass using an optimization-based strategy. In: World Renewable Energy Congress IX and Exhibition, Florence, Italy.

Schell, D.J., Farmer, J., Newman, M., McMillan, J.D., 2003. Dilute-sulfuric acid pre-treatment of corn stover in pilot-scale reactor. Investigation of yields, kinetics, and enzymatic digestibilities of solids. Applied Biochemistry and Biotechnology 105 (1–3), 69–85.

Shahbazi, A., Li, Y., Mims, M.R., 2005. Application of sequential aqueous steam treatments to the fractionation of softwood. Applied Biochemistry and Biotechnology, 973–987.

Shapouri, H., Duffield, J.A., Wang, M., 2003. The energy balance of corn ethanol revisited. Transactions of the ASAE 46 (4), 959–968.

Sheehan, J., Himmel, M., 1999. Enzymes, energy, and the environment: A strategic perspective on the U.S. Department of Energy's research and development activities for bioethanol. Biotechnology Progress 15, 817–827.

Sheoran, A., Yadav, B.S., Nigam, P., Singh, D., 1998. Continuous ethanol production from sugarcane molasses using a column reactor of immobilized *Saccharomyces cerevisiae* HAU-1. Journal of Basic Microbiology 38 (2), 123–128.

Shevchenko, S.M., Beatson, R.P., Saddler, J.N., 1999. The nature of lignin from steam explosion/enzymatic hydrolysis of softwood. Applied Biochemistry and Biotechnology 77–79, 867–876.

Shigechi, H., Fujita, Y., Koh, J., Ueda, M., Fukuda, H., Kondo, A., 2004. Energy-saving direct ethanol production from low-temperature-cooked corn starch using a cell-surface engineered yeast strain co-displaying glucoamylase and α-amylase. Biochemical Engineering Journal 18, 149–153.

Söderström, J., Pilcher, L., Galbe, M., Zacchi, G., 2003. Two-step steam pretreatment of softwood by dilute H_2SO_4 impregnation for ethanol production. Biomass and Bioenergy 24, 475–486.

Soni, S.K., Kaur, A., Gupta, J.K., 2003. A solid state fermentation based bacterial α-amylase and fungal glucoamylase system and its suitability for the hydrolysis of wheat starch. Process Biochemistry 39, 185–192.

Sreenath, H.K., Koegel, R.G., Moldes, A.B., Jeffries, T.W., Straub, R.J., 2001. Ethanol production from alfalfa fiber fractions by saccharification and fermentation. Process Biochemistry 36, 1199–1204.

Sriroth, K., Lamchaiyaphum, B., Piyachomkwan, K., 2007. Present situation and future potential of cassava in Thailand. <http://www.cassava.org/doc/presentsituation2.pdf> (Februrary 2007).

Stenberg, K., Bollók, M., Réczey, K., Galbe, M., Zacchi, G., 2000. Effect of substrate and cellulase concentration on simultaneous saccharification and fermentation of steam-pretreated softwood for ethanol production. Biotechnology and Bioengineering 68 (2), 204–210.

Sues, A., Millati, R., Edebo, L., Taherzadeh, M.J., 2005. Ethanol production from hexoses, pentoses, and dilute-acid hydrolyzate by *Mucor indicus*. FEMS Yeast Research 5, 669–676.

Sun, Y., Cheng, J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource Technology 83, 1–11.

Taherzadeh, M.J., 1999. Ethanol from Lignocellulose: Physiological Effects of Inhibitors and Fermentation Strategies. Ph.D. thesis, Chalmers, University of Technology Göteborg, Sweden. 56 p.

Teixeira, L.C., Linden, J.C., Schroeder, H.A., 1999a. Alkaline and peracetic acid pretreatments of biomass for ethanol production. Applied Biochemistry and Biotechnology 77–79, 19–34.

Teixeira, L.C., Linden, J.C., Schroeder, H.A., 1999b. Optimizing peracetic acid pretreatment conditions for improved simultaneous saccharification and co-fermentation (SSCF) of sugar cane bagasse to ethanol fuel. Renewable Energy 16, 1070–1073.

Tengborg, C., Galbe, M., Zacchi, G., 2001. Influence of enzyme loading and physical parameters on the enzymatic hydrolysis of steam-pretreated softwood. Biotechnology Progress 17, 110–117.

Tengerdy, R.P., Szakacs, G., 2003. Bioconversion of lignocellulose in solid substrate fermentation. Biochemical Engineering Journal 13, 169–179.

Thomas, V., Kwong, A., 2001. Ethanol as a lead replacement: phasing out leaded gasoline in Africa. Energy Policy 29, 1133–1143.

Thomas, K.C., Hynes, S.H., Ingledew, W.M., 1996. Practical and theoretical considerations in the production of high concentrations of alcohol by fermentation. Process Biochemistry 31 (4), 321–331.

Tiffany, D.G., Eidman, V.R., 2003. Factors associated with success of fuel ethanol producers. Staff Paper Series P03-7. University of Minnesota, USA. 54 p.

Titmas, J.A., 1999. Apparatus for hydrolyzing cellulosic material. Patent US5879637.

Varga, E., Klinkle, H.B., Réczey, K., Thomsen, A.B., 2004. High solid simultaneous saccharification and fermentation of wet oxidized corn stover to ethanol. Biotechnology and Bioengineering 88 (5), 567–574.

Wang, S., Sosulski, K., Sosulski, F., Ingledew, M., 1997. Effect of sequential abrasion on starch composition of five cereals for ethanol fermentation. Food Research International 30 (8), 603–609.

Wang, M, Saricks, C, Santini D., 1999a. Effects of fuel ethanol use on fuel-cycle energy and greenhouse gas emissions, report no. ANL/ESD-38. Argonne, IL: Argonne National Laboratory, Center for Transportation Research, 1999. See also: <www.transportation.anl.gov/pdfs/TA/58.pdf>.

Wang, S., Thomas, K.C., Sosulski, K., Ingledew, W.M., Sosulski, F.W., 1999b. Grain pearling and very high gravity (VHG) fermentation technologies for fuel alcohol production from rye and triticale. Process Biochemistry 34, 421–428.

Wang, S., Ingledew, W., Thomas, K., Sosulski, K., Sosulski, F., 1999c. Optimization of fermentation temperature and mash specific gravity for fuel alcohol production. Cereal Chemistry 76 (1), 82–86.

Weil, J.R., Dien, B., Bothast, R., Hendrickson, R., Mosier, N.S., Ladisch, M.R., 2002. Removal of fermentation inhibitors formed during pretreatment of biomass by polymeric adsorbents. Industrial & Engineering Chemistry Research 41, 6132–6138.

Wendhausen, R., Fregonesi, A., Moran, P., Joekes, I., Augusto, J., Rodrigues, R., Tonella, E., Althoff, K., 2001. Continuous fermentation of sugar cane syrup using immobilized yeast cells. Journal of Bioscience and Bioengineering 91 (1), 48–52.

Wilkie, A.C., Riedesel, K.J., Owens, J.M., 2000. Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. Biomass and Bioenergy 19, 63–102.

Winner Network., 2002. Village Level Bioenergy System Based on Sweet Sorghum. <http://www.w3c.org/TR/1999/REC-html401-19991224/loose.dtd>/ (October 2004).

Wooley, R., Ruth, M., Glassner, D., Sheejan, J., 1999a. Process design and costing of bioethanol technology: a tool for determining the status and direction of research and development. Biotechnology Progress 15, 794–803.

Wooley, R., Ruth, M., Sheehan, J., Ibsen, K., Majdeski, H., Gálvez, A., 1999b. Lignocellulosic biomass to ethanol process design and eco-

nomics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis. Current and futuristic scenarios. Technical Report NREL/TP-580-26157. Golden, CO (USA), National Renewable Energy Laboratory. 123 p.

Xavier, M.R., 2007. The Brazilian sugarcane ethanol experience. Issue Analysis, no. 3, Washington, USA, Competitive Enterprise Institute. 11 p.

Xie, Y., Phelps, D., Lee, C.-H., Sedlak, M., Ho, N., Wang, N.-H.L., 2005. Comparison of two adsorbents for sugar recovery from biomass hydrolyzate. Industrial & Engineering Chemistry Research 44, 6816–6823.

Yu, Z., Zhang, H., 2003. Pretreatments of cellulose pyrolysate for ethanol production by *Saccharomyces cerevisiae*, Pichia sp. YZ-1 and *Zymomonas mobilis*. Biomass and Bioenergy 24, 257–262.

Zaldivar, J., Martinez, A., Ingram, L.O., 1999. Effect of selected aldehydes on the growth and fermentation of ethanologenic *Escherichia coli*. Biotechnology and Bioengineering 65 (1), 24–33.

Zaldivar, J., Martinez, A., Ingram, L.O., 2000. Effect of alcohol compounds found in hemicellulose hydrolysate on the growth and fermentation of ethanologenic *Escherichia coli*. Biotechnology and Bioengineering 68 (5), 524–530.

Zhan, X., Wang, D., Tuinstra, M.R., Bean, S., Seib, P.A., Sun, X.S., 2003. Ethanol and lactic acid production as affected by sorghum genotype and location. Industrial Crops and Products 18, 245–255.

Zhang, B.S., 2006. Process for preparing fuel ethanol by using straw fiber materials. Patent CN1880416.

Zhang, Y., Henson, M.A., 2001. Bifurcation analysis of continuous biochemical reactor models. Biotechnology Progress 17, 647–660.

Zhang, Y.-H.P., Lynd, L.R., 2004. Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulose systems. Biotechnology and Bioengineering 8 (7), 797–824.