



## Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review

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### ABSTRACT

Biofuel produced from lignocellulosic materials, so-called second generation bioethanol shows energetic, economic and environmental advantages in comparison to bioethanol from starch or sugar. However, physical and chemical barriers caused by the close association of the main components of lignocellulosic biomass, hinder the hydrolysis of cellulose and hemicellulose to fermentable sugars. The main goal of pretreatment is to increase the enzyme accessibility improving digestibility of cellulose. Each pretreatment has a specific effect on the cellulose, hemicellulose and lignin fraction thus, different pretreatment methods and conditions should be chosen according to the process configuration selected for the subsequent hydrolysis and fermentation steps. This paper reviews the most interesting technologies for ethanol production from lignocellulose and it points out several key properties that should be targeted for low-cost and advanced pretreatment processes.

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### 1. Introduction

The increasing problem of the CO<sub>2</sub> emissions besides some energy security concerns has strengthened the interest in alternative, nonpetroleum-based sources of energy. Biomass is the only suitable and renewable primary energy resource than can provide alternative transportation fuels such as bioethanol or biodiesel in the short-term (Hamelinck et al., 2005; Sun and Cheng, 2002).

Current production of bioethanol relies on ethanol from starch and sugars but there has been considerable debate about its sustainability. In this context, bioethanol produced from lignocellulosic biomass is an interesting alternative since lignocellulosic raw materials do not compete with food crops and they are also less expensive than conventional agricultural feedstocks.

Lignocellulose is the most abundant renewable biomass; its annual production has been estimated in  $1 \times 10^{10}$  MT worldwide (Sánchez and Cardona, 2008). The biological conversion of different lignocellulosic feedstocks such as forest and agricultural residues, or lignocellulosic crops dedicated to ethanol offers numerous benefits but its development is still hampered by economic and technical obstacles (Sánchez and Cardona, 2008). In this context, some of the most important factors to reduce ethanol production cost are: an efficient utilization of the raw material to obtain high ethanol yields, high productivity, high ethanol concentration in the distillation feed, and also process integration in order to reduce

the energy demand (Galbe and Zacchi, 2007; Tomás-Pejó et al., 2008).

Ethanol production from lignocellulosic biomass comprises the following main steps: hydrolysis of cellulose and hemicellulose, sugar fermentation, separation of lignin residue and, finally, recovery and purifying the ethanol to meet fuel specifications. The task of hydrolyzing lignocellulose to fermentable monosaccharides is still technically problematic because the digestibility of cellulose is hindered by many physico-chemical, structural and compositional factors. Owing to these structural characteristics, pretreatment is an essential step for obtaining potentially fermentable sugars in the hydrolysis step. The aim of the pretreatment is to break down the lignin structure and disrupt the crystalline structure of cellulose for enhancing enzymes accessibility to the cellulose during hydrolysis step (Mosier et al., 2005b).

Current pretreatment research is focused on identifying, evaluating, developing and demonstrating promising approaches that primarily support the subsequent enzymatic hydrolysis of the treated biomass with lower enzyme dosages and shorter bioconversion times. A large number of pretreatment approaches have been investigated on a wide variety of feedstocks types and there are several recent review articles which provide a general overview of the field (Carvalho et al., 2008; Hendriks and Zeeman, 2009; Taherzadeh and Karimi, 2008; Yang and Wyman, 2008).

Besides being considered a crucial step in the biological conversion to ethanol, biomass pretreatment represents one of the main economic costs in the process. In fact, it has been described as the second most expensive unit cost in the conversion of lignocellulose

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to ethanol based on enzymatic hydrolysis preceded by feedstocks cost (Mosier et al., 2005b).

Since different lignocellulosic materials have different physico-chemical characteristics, it is necessary to adopt suitable pretreatments technologies based on the lignocellulosic biomass properties of each raw material. Furthermore, the choice of certain pretreatment has a large impact on all subsequent steps in the overall conversion scheme in terms of cellulose digestibility, generation of toxic compounds potentially inhibitory for yeast, stirring power requirements, energy demand in the downstream process and wastewater treatment demands (Galbe and Zacchi, 2007). Other studies describe several parameters (pentose recovery, chip size required, concentration of toxic compounds formed during pretreatment and low energy demand) as deciding factors in an effective pretreatment (Sun and Cheng, 2002).

The purpose of this work is to review the most interesting pretreatment technologies and recent advances for ethanol production from lignocellulose as well as to analyse the interrelated factors between pretreatment, hydrolysis and fermentation.

## 2. Key factors for an effective pretreatment of lignocellulosic biomass

There are several key properties to take into consideration for low-cost and advanced pretreatment process (Yang and Wyman, 2008):

- *High yields for multiple crops, sites ages, harvesting times.*  
Various pretreatments have been shown to be better suited for specific feedstocks. For example, alkaline-based pretreatment methods such as lime, ammonia fiber explosion (AFEX), and ammonia recycling percolation (ARP), can effectively reduce the lignin content of agricultural residues but are less satisfactory for processing recalcitrant substrate as softwoods (Chandra et al., 2007). Acid based pretreatment processes have been shown to be effective on a wide range of lignocellulose substrate, but are relatively expensive (Mosier et al., 2005b).
- *Highly digestible pretreated solid.*  
Cellulose from pretreatment should be highly digestible with yields higher than 90% in less than five and preferably less than 3 days with enzyme loading lower than 10 FPU/g cellulose (Yang and Wyman, 2008).
- *No significant sugars degradation.*  
High yields close to 100% of fermentable cellulosic and hemicellulosic sugars should be achieved through pretreatment step.
- *Minimum amount of toxic compounds.*  
The liquid hydrolyzate from pretreatment must be fermentable following a low-cost, high yield conditioning step. Harsh conditions during pretreatment lead to a partial hemicellulose degradation and generation of toxic compounds derived from sugar decomposition that could affect the proceeding hydrolysis and fermentation steps (Oliva et al., 2003). Toxic compounds generated and their amounts depend on raw material and harshness of pretreatment. Degradation products from pretreatment of lignocellulose materials can be divided into the following classes: carboxylic acids, furan derivatives, and phenolic compounds. Main furan derivatives are furfural and 5-hydroxymethylfurfural (HMF) derived from pentoses and hexoses degradation, respectively; (Palmqvist and Hahn-Hägerdal, 2000). Weak acids are mostly acetic and formic and levulinic acids. Phenolic compounds include alcohols, aldehydes, ketones and acids (Klinke et al., 2002).
- *Biomass size reduction not required.*  
Milling or grinding the raw material to small particle sizes before pretreatment are energy-intensive and costly technologies.

- *Operation in reasonable size and moderate cost reactors.*  
Pretreatment reactors should be low in cost through minimizing their volume, employing appropriate materials of construction for highly corrosive chemical environments, and keeping operating pressures reasonable.
- *Non-production of solid-waste residues.*  
The chemicals formed during hydrolyzate conditioning in preparation for subsequent steps should not present processing or disposal challenges.
- *Effectiveness at low moisture content.*  
The use of raw materials at high dry matter content would reduce energy consumption during pretreatment.
- *Obtaining high sugar concentration.*  
The concentration of sugars from the coupled operation of pretreatment and enzymatic hydrolysis should be above 10% to ensure an adequate ethanol concentration and to keep recovery and other downstream cost manageable.
- *Fermentation compatibility.*  
The distribution of sugar recovery between pretreatment and subsequent enzymatic hydrolysis should be compatible with the choice of an organism able to ferment pentoses (arabinose and xylose) in hemicellulose.
- *Lignin recovery.*  
Lignin and other constituents should be recovered to simplify downstream processing and for conversion into valuable co-products (Yang and Wyman, 2008)
- *Minimum heat and power requirements.*  
Heat and power demands for pretreatment should be low and/or compatible with the thermally integrated process.

## 3. Factors limiting enzymatic hydrolysis

The pretreatment is a necessary step to alter some structural characteristics of lignocellulose, increasing glucan and xylan accessibility to the enzymatic attack. As it has been mentioned, these structural modifications of the lignocellulose are highly dependent on the type of pretreatment employed and have a great effect on the enzymatic hydrolysis (Kumar et al., 2009b) and subsequent steps. The choice of pretreatment technology for a particular raw material depends on several factors, some of them directly related to the enzymatic hydrolysis step such as sugar-release patterns and enzymes employed. Thus, the combination of the composition of the substrate, type of pretreatment, and dosage and efficiency of the enzymes used for the hydrolysis have a great influence on biomass digestibility; although the individual impacts of these factors on the enzymatic hydrolysis are still unclear.

Main factors that influence the enzymatic hydrolysis of cellulose in lignocellulosic feedstocks can be divided in two groups: enzyme-related and substrate-related factors, though many of them are interrelated during the hydrolysis process. Composition of the liquid fraction and solid process streams resulting from different pretreatment approaches can be widely different. These differences will have a great influence on the requirements for effective enzymatic saccharification in subsequent processing steps.

The reduction of pretreatment severity is sometimes required to reduce economic cost. Low severity factor results in less sugar-release and consequently higher amount and different types of enzymes will be required to achieve high sugar yields from both cellulose and hemicellulose fraction. In this context, development of hemicellulases and other accessory enzymes needed for complete degradation of lignocellulose components has become an important issue. Recent studies show the importance of new balanced enzymatic complexes containing optimal combinations to effectively modify the complex structure of lignocellulosic materials (García-Aparicio et al., 2007; Merino and Cherry, 2007).

Substrate-related factors limiting enzymatic hydrolysis are directly connected to the pretreatment employed. These factors are described separately although their effect is normally interrelated.

### 3.1. Cellulose cristallinity (*cristallinity index, CrI*)

Degree of polymerization and cellulose cristallinity have been considered as important factors in determining the hydrolysis rates of relatively refined cellulosic substrates (Chang and Holtzaple, 2000), but data from several independent investigations indicate that these parameters alone do not explain the recalcitrance of lignocellulosic substrates (Puri, 1984). In fact, cellulases complexes capable of hydrolyzing crystalline cellulose have been identified (Mansfield et al., 1999). In some studies wherein cristallinity was suggested to be important, the lignocellulosic materials were mechanically pretreated, therefore any decrease in cristallinity was accompanied by an alteration of other substrate characteristics such as particle size reduction or increase in available surface area.

It has been observed that pretreatment of lignocellulosics improves its hydrolysability but in some cases increases the CrI of the cellulose fraction. This fact has been suggested to be due to the removal or reduction of more easily available amorphous cellulose after pretreatments such steam explosion (Mansfield et al., 1999). In contrast, high pH pretreatments have been shown to have less effect and even reduced biomass cristallinity in some instances (Kumar et al., 2009b).

### 3.2. Cellulose degree of polymerization (*DP, number of glycosyl residues per cellulose chain*)

Degree of polymerization is essentially related to other substrate characteristics, such as cristallinity. Although the role of glucan chain length is not definitively known, it is believed to affect cellulose hydrolysis (Puri, 1984). Depolymerization depends on the nature of cellulosic substrate. In the enzymatic hydrolysis, endoglucanases cut at internal sites of the cellulose chains, preferentially less ordered, being primarily responsible for decreasing degree of polymerization of cellulosic substrates. However, regardless the substrate being attacked there seems to be a “leveling off” of the cellulose DP, correlated with the increased recalcitrance of the residual crystalline cellulose (Mansfield et al., 1999).

The effect of different pretreatments on cellulose chain length has been studied showing reduced degree of polymerization in solids prepared by different pretreatments suggesting that xylan removal had a more severe impact on cellulose chain length than lignin removal (Kumar et al., 2009b).

### 3.3. Substrates available surface area (*pore volume*)

Accessibility of the substrate to the cellulolytic enzymes is one of the major factors influencing hydrolysis process. Thus, one of the main objectives of the pretreatment is to increase the available surface area for the enzymatic attack.

### 3.4. Lignin barrier (*content and distribution*)

The presence of lignin and hemicellulose difficults the access of cellulase enzymes to cellulose difficult (Mansfield et al., 1999), thus reducing the efficiency of the hydrolysis.

Lignin limits the rate of enzymatic hydrolysis by acting as a physical barrier, preventing the digestible parts of the substrate to be hydrolyzed (Chang and Holtzaple, 2000). Besides, lignin appears to reduce cellulose hydrolysis by non-productively binding cellulolytic enzymes (Esteghlalian et al., 2001). Different strategies have been studied recently to overcome the non-productive

adsorption of cellulase to lignin such as alkali extraction and addition of protein (e.g. BSA) or other additives (e.g. poly ethylene glycol, Tween) (Börjesson et al., 2007; Pan et al., 2005). Although the use of additives introduces an additional cost to the ethanol production process, significant benefits could be achieved by improving the enzymatic hydrolysis step.

Irregular binding of enzymes onto lignin is also influenced by the nature of the substrate (Esteghlalian et al., 2001). While various cellulases differ in their inhibition by lignin, the xylanases and glucosidases are less affected (Berlin et al., 2006).

Some pretreatments have been reported to produce different effects such as melting and lignin relocation (steam explosion) or disruption of lignin-carbohydrates linkages (AFEX) (Laureano-Pérez et al., 2005). Lignin chemistry is actually one of main research areas nowadays. It has a great impact on the enzymatic hydrolysis but also, as one of the main components in lignocellulosic materials, different further applications of lignin are under investigation to obtain potential higher value products in a developed biorrefinery process. The effects of different pretreatments on lignin are further detailed.

### 3.5. Hemicellulose content

Removal of hemicellulose increases the mean pore size of the substrate and therefore increases the accessibility and the probability of the cellulose to become hydrolyzed (Chandra et al., 2007). On the other hand, hemicellulosic sugars recovery in the pretreated solids would be interesting to obtain higher total fermentable sugar production. In this case enzymatic requirements for hemicellulosic modification must be taken into account (Kumar and Wyman, 2009a). Degree of acetylation in the hemicellulose is another important factor because lignin and acetyl groups are attached to the hemicellulose matrix and may hinder polysaccharide breakdown (Chang and Holtzaple, 2000).

### 3.6. Feedstock particle size

There is some evidence to support that reduction of particle size increases specific surface area and subsequently the accessibility of cellulose to the enzymes (Sun and Cheng, 2002).

### 3.7. Porosity

Previous studies have concluded that the pore size of the substrate in relation to the size of the enzymes is the main limiting factor in the enzymatic hydrolysis of lignocellulosic biomass (Chandra et al., 2007). Cellulases can get trapped in the pores if the internal area is much larger than the external area which is the case for many lignocellulosic materials (Zhang and Lynd, 2004). An increase of porosity in pretreatment processes can significantly improve the hydrolysis.

### 3.8. Cell wall thickness (*coarseness*)

The waxy barrier comprising grass cuticle and tree bark impedes penetration of enzymes; even milled, plant stems and woody tissues limit liquid penetration by their nature.

### 3.9. Change in accessibility with conversion

The role of glucan accessibility and its change with conversion has been debatable, with a few studies showing that glucan accessibility becomes limiting with conversion (Wang et al., 2006) and a few others showing not significant decrease of accessibility with conversion or even no change at all (Kumar and Wyman, 2009b).

#### 4. Pretreatment technologies for lignocellulosic biomass

Universal pretreatment process is difficult to envision owing to the diverse nature of different biomass feedstocks. A multitude of different pretreatment technologies have been suggested during the last decades. They can be classified into biological, physical, chemical and physico-chemical pretreatments, according to the different forces or energy consumed in the pretreatment process. Combination of these methods has been also studied.

##### 4.1. Biological pretreatments

Fungal pretreatment has been previously explored to upgrade lignocellulosic materials for feed and paper applications. Recently, this environmentally friendly approach has received renewed attention as a pretreatment method for enhancing enzymatic saccharification of lignocellulosic biomass in ethanol production processes. Biological pretreatments employ microorganisms mainly brown, white and soft-rot fungi which degrade lignin and hemicellulose and very little of cellulose, more resistant than the other components (Sánchez, 2009). Lignin degradation by white-rot fungi, the most effective for biological pretreatment of lignocellulosic materials, occurs through the action of lignin-degrading enzymes such as peroxidases and laccases (Kumar et al., 2009a).

Several white-rot fungi such as *Phanerochaete chrysosporium*, *Ceriporia lacerata*, *Cyathus stercoleris*, *Ceriporiopsis subvermispora*, *Pycnoporus cinnabarinus* and *Pleurotus ostreatus* have been examined on different lignocellulosic biomass showing high delignification efficiency (Kumar et al., 2009a; Shi et al., 2008). Biological pretreatment by white-rot fungi has been combined with organosolv pretreatment in an ethanol production process by simultaneous saccharification and fermentation (SSF) from beech wood chips (Itoh et al., 2003). Results from other recent studies have shown that fungal pretreatment of wheat straw for 10 days with a high lignin-degrading and low cellulose-degrading fungus (fungal isolate RCK-1) resulted in a reduction in acid loading for hydrolysis, an increase in the release of fermentable sugars and a reduction in the concentration of fermentation inhibitors. Ethanol yield and volumetric productivity with *Pichia stipitis* were 0.48 g/g and 0.54 g/L h, respectively (Kuhar et al., 2008). An evaluation of biological pretreatment of sugarcane trash using eight different bacteria and fungi was performed on the basis of quantitative changes in the components of the sugarcane trash, the production of the cellulase enzyme complex, total protein and the release of reducing sugars by different bioagents as well as the interaction among different chemical parameters affecting the pretreatment (Singh et al., 2008). In this case, the microbial pretreatment of trash increased accessibility of sugars for enzymatic hydrolysis.

In general, such processes offer advantages such as low-capital cost, low energy, no chemicals requirement, and mild environmental conditions. However, the main drawback to develop biological methods is the low hydrolysis rate obtained in most biological materials compared to other technologies (Sun and Cheng, 2002).

To move forward a cost-competitive biological pretreatment of lignocellulose, and improve the hydrolysis to eventually improve ethanol yields, there is a need to keep on studying and testing more basidiomycetes fungi for their ability to delignify the plant material quickly and efficiently.

##### 4.2. Physical pretreatments

###### 4.2.1. Mechanical comminution

The objective of the mechanical pretreatment is a reduction of particle size and crystallinity of lignocellulosic in order to increase the specific surface and reduce the degree of polymerization. This

can be produced by a combination of chipping, grinding or milling depending on the final particle size of the material (10–30 mm after chipping and 0.2–2 mm after milling or grinding) (Sun and Cheng, 2002). Different milling processes (ball milling, two-roll milling, hammer milling, colloid milling and vibro energy milling) can be used to improve the enzymatic hydrolysis of lignocellulosic materials (Taherzadeh and Karimi, 2008). The power requirement of this pretreatment is relatively high depending on the final particle size and the biomass characteristics. Taking into account the high energy requirements of milling and the continuous rise of energy prices, it is likely that this process is not economically feasible (Hendriks and Zeeman, 2009).

###### 4.2.2. Extrusion

Extrusion process is a novel and promising physical pretreatment method for biomass conversion to ethanol production. In extrusion, the materials are subjected to heating, mixing and shearing, resulting in physical and chemical modifications during the passage through the extruder. Screw speed and barrel temperature are believed to disrupt the lignocellulose structure causing defibrillation, fibrillation and shortening of the fibers, and, in the end, increasing accessibility of carbohydrates to enzymatic attack (Karunanithy et al., 2008). The different bioreactor parameters must be taken into account to achieve the highest efficiency in the process. In recent studies application of enzymes during extrusion process is being considered as a promising technology for ethanol production.

##### 4.3. Chemical pretreatments

###### 4.3.1. Alkali pretreatments

The effect that some bases have on lignocellulosic biomass is the basis of alkaline pretreatments, which are effective depending on the lignin content of the biomass. Alkali pretreatments increase cellulose digestibility and they are more effective for lignin solubilization, exhibiting minor cellulose and hemicellulose solubilization than acid or hydrothermal processes (Carvalho et al., 2008).

Alkali pretreatment can be performed at room temperature and times ranging from seconds to days. It is described to cause less sugar degradation than acid pretreatment and it was shown to be more effective on agricultural residues than on wood materials (Kumar et al., 2009a). Nevertheless, possible loss of fermentable sugars and production of inhibitory compounds must be taken into consideration to optimize the pretreatment conditions.

Sodium, potassium, calcium and ammonium hydroxides are suitable alkaline pretreatments. NaOH causes swelling, increasing the internal surface of cellulose and decreasing the degree of polymerization and crystallinity, which provokes lignin structure disruption (Taherzadeh and Karimi, 2008). NaOH has been reported to increase hardwood digestibility from 14% to 55% by reducing lignin content from 24–55% to 20% (Kumar et al., 2009a).

Ca(OH)<sub>2</sub>, also known as lime, has been widely studied. Lime pretreatment removes amorphous substances such as lignin, which increases the crystallinity index. Lignin removal increases enzyme effectiveness by reducing non-productive adsorption sites for enzymes and by increasing cellulose accessibility (Kim and Holtzapfle, 2006). Lime also removes acetyl groups from hemicellulose reducing steric hindrance of enzymes and enhancing cellulose digestibility (Mosier et al., 2005b). Lime has been proven successfully at temperatures from 85–150 °C and for 3–13 h with corn stover (Kim and Holtzapfle, 2006) or poplar wood (Chang et al., 2001). Pretreatment with lime has lower cost and less safety requirements compared to NaOH or KOH pretreatments and can be easily recovered from hydrolysate by reaction with CO<sub>2</sub> (Mosier et al., 2005b).

Addition of an oxidant agent (oxygen/H<sub>2</sub>O<sub>2</sub>) to alkaline pretreatment (NaOH/Ca(OH)<sub>2</sub>) can improve the performance by favouring lignin removal (Carvalho et al., 2008). Ethanol yields of 0.33 g/g have been obtained in simultaneous saccharification and cofermentation (SSCF) processes with *Escherichia coli* FBR5 from wheat straw pretreated with alkali peroxide (Saha and Cotta, 2006). Furthermore, no furfural or HMF were detected in hydrolysates obtained with alkaline peroxide pretreatment which favours the fermentation step in an ethanol production process (Taherzadeh and Karimi, 2008).

#### 4.3.2. Acid pretreatment

The main objective of the acid pretreatments is to solubilize the hemicellulosic fraction of the biomass and to make the cellulose more accessible to enzymes. This type of pretreatments can be performed with concentrated or diluted acid but utilization of concentrated acid is less attractive for ethanol production due to the formation of inhibiting compounds. Furthermore, equipment corrosion problems and acid recovery are important drawbacks when using concentrated acid pretreatments. The high operational and maintenance costs reduce the interest of applying the concentrated acid pretreatment at commercial scale (Wyman, 1996).

Diluted acid pretreatment appears as more favourable method for industrial applications and have been studied for pretreating wide range of lignocellulosic biomass. Different types of reactors such as percolation, plug flow, shrinking-bed, batch and counter-current reactors have been applied for pretreatment of lignocellulosic materials (Taherzadeh and Karimi, 2008). It can be performed at high temperature (e.g. 180 °C) during a short period of time; or at lower temperature (e.g. 120 °C) for longer retention time (30–90 min). It presents the advantage of solubilizing hemicellulose, mainly xylan, but also converting solubilized hemicellulose to fermentable sugars. Nevertheless, depending on the process temperature, some sugar degradation compounds such as furfural and HMF and aromatic lignin degradation compounds are detected, and affect the microorganism metabolism in the fermentation step (Saha et al., 2005). Anyhow, this pretreatment generates lower degradation products than concentrated acid pretreatments.

High hydrolysis yields have been reported when pretreating lignocellulosic materials with diluted H<sub>2</sub>SO<sub>4</sub> which is the most studied acid. Hydrochloric acid, phosphoric acid and nitric acid have also been tested (Mosier et al., 2005a). Saccharification yield as high as 74% was shown when wheat straw was subjected to 0.75% v/v of H<sub>2</sub>SO<sub>4</sub> at 121 °C for 1 h (Saha et al., 2005). Olive tree biomass was pretreated with 1.4% H<sub>2</sub>SO<sub>4</sub> at 210 °C resulting in 76.5% of hydrolysis yields (Cara et al., 2008). Recently, ethanol yield as high as 0.47 g/g glucose was achieved in fermentation tests with cashew apple bagasse pretreated with diluted H<sub>2</sub>SO<sub>4</sub> at 121 °C for 15 min (Rocha et al., 2009).

Organic acids such as fumaric or maleic acids are appearing as alternatives to enhance cellulose hydrolysis for ethanol production. In this context, both acids were compared with sulfuric acid in terms of hydrolysis yields from wheat straw and formation of sugar degradation compounds during pretreatment. Results showed that organic acids can pretreat wheat straw with high efficiency although fumaric acid was less effective than maleic acid. Furthermore, less amount of furfural was formed in the maleic and fumaric acid pretreatments than with sulfuric acid (Kootstra et al., 2009).

#### 4.3.3. Ozonolysis

Ozone is a powerful oxidant that shows high delignification efficiency (Sun and Cheng, 2002). This lignin removal increases the yield in following enzymatic hydrolysis. The pretreatment is usually performed at room temperature and normal pressure and does not lead to the formation of inhibitory compounds that can affect

the subsequent hydrolysis and fermentation. Ozonolysis has been applied on several agricultural residues such as wheat straw and rye straw increasing in both cases the enzymatic hydrolysis yield after ozonolysis pretreatment (García-Cubero et al., 2009). Despite of some interesting results further research has to be performed regarding ethanol production from lignocellulosic materials pretreated with ozone. An important drawback to consider is the large amounts of ozone needed, which can make the process economically unviable (Sun and Cheng, 2002).

#### 4.3.4. Organosolv

Organosolvation method is a promising pretreatment strategy, since it has demonstrated its potential for lignocellulosic materials (Papatheofanous et al., 1995). Numerous organic or aqueous solvent mixtures can be utilized, including methanol, ethanol, acetone, ethylene glycol and tetrahydrofurfuryl alcohol, in order to solubilize lignin and provide treated cellulose suitable for enzymatic hydrolysis (Zhao et al., 2009a). Comparing to other chemical pretreatments the main advantage of organosolv process is the recovery of relatively pure lignin as a by-product (Zhao et al., 2009a).

In some studies these mixtures are combined with acid catalysts (HCl, H<sub>2</sub>SO<sub>4</sub>, oxalic or salicylic) to break hemicellulose bonds. A high yield of xylose can usually be obtained with the addition of acid. However, this acid addition can be avoided for a satisfactory delignification by increasing process temperature (above 185 °C).

Organosolv process has been suggested to be combined with previous acid hydrolysis to separate hemicellulose and lignin in a two-stage fractionation. High lignin removal (70%) and minimum cellulose loss (less than 2%) are achieved (Papatheofanous et al., 1995).

Removal of solvents from the system is necessary using appropriate extraction and separation techniques, e.g., evaporation and condensation, and they should be recycled to reduce operational costs. Solvents need to be separated because they might be inhibitory to enzymatic hydrolysis and fermentative microorganisms (Sun and Cheng, 2002). The high commercial price of solvents is another important factor to consider for industrial applications. For economic reasons, among all possible solvents, the low-molecular weight alcohols with lower boiling points such as ethanol and methanol are favored.

#### 4.3.5. Ionic liquids (ILs) pretreatment

The use of ILs as solvents for pretreatment of cellulosic biomass has recently received much attention. ILs are salts, typically composed of large organic cations and small inorganic anions, which exist as liquids at relatively low temperatures; often at room temperature. Their solvent properties can be varied by adjusting the anion and the alkyl constituents of the cation. These interesting properties include chemical and thermal stability, non-flammability, low vapour pressures and a tendency to remain liquid in a wide range of temperatures (Hayes, 2009). Since no toxic or explosive gases are formed, ILs are called “green” solvents. Carbohydrates and lignin can be simultaneously dissolved in ILs with anion activity (e.g. the 1-butyl-3-methylimidazolium cation [C<sub>4</sub>mim]<sup>+</sup>) because ILs form hydrogen bonds between the non-hydrated chloride ions of the IL and the sugar hydroxyl protons in a 1:1 stoichiometry. As a result, the intricate network of non-covalent interactions among biomass polymers of cellulose, hemicellulose, and lignin is effectively disrupted while minimizing formation of degradation products. However, most data showing the effectiveness of ILs has been developed using pure crystalline cellulose, and its applicability to a more complex combination of constituents in lignocellulosic biomass requires further studies. Nevertheless, the use of ILs has also been already demonstrated on some lignocellulosic feedstocks such as straw (Li et al., 2009) or wood (Lee et al., 2009).

For the large-scale application of ILs, development of energy-efficient recycling methods for ILs is a prerequisite and should be investigated in detail. Toxicity to enzymes and fermentative microorganisms must be also studied before ILs can be considered a real option for biomass pretreatment (Yang and Wyman, 2008; Zhao et al., 2009b). Depending on the amount of ILs residues remaining, significant negative effect on cellulase activity may be observed. Thus, ILs residues removal would be required to prevent decrease of final sugars concentrations.

In a pretreatment study using 1-ethyl-3-methyl imidazolium diethyl phosphate, the yield of reducing sugars from wheat straw pretreated with this ionic liquid at 130 °C for 30 min was 54.8% after being enzymatically hydrolyzed for 12 h (Li et al., 2009). The fermentability of the hydrolysates obtained after enzymatic saccharification of the regenerated wheat straw was also evaluated. Results obtained using *Saccharomyces cerevisiae* indicated that wheat straw pretreated by this IL did not bring any negative effect on the growth of *S. cerevisiae* (Li et al., 2009).

Further research is needed to improve the economics of ILs pretreatment before they can be applied at industrial scale. Technology is still expensive and commercial IL recovery methods have not been fully developed. In addition, techniques need to be developed to recover hemicellulose and lignin from solutions after extraction of cellulose (Hayes, 2009). Despite of these current limitations, advanced research e.g. as potential synthesis of ILs from carbohydrates, may play a role in reducing their cost. Development of ILs pretreatment offers a great potential for future lignocellulose biorefining processes.

#### 4.4. Physico-chemical pretreatments

##### 4.4.1. Steam explosion: SO<sub>2</sub>-steam explosion

Steam explosion is the most widely employed physico-chemical pretreatment for lignocellulosic biomass. It is a hydrothermal pretreatment in which the biomass is subjected to pressurised steam for a period of time ranging from seconds to several minutes, and then suddenly depressurised. This pretreatment combines mechanical forces and chemical effects due to the hydrolysis (autohydrolysis) of acetyl groups present in hemicellulose. Autohydrolysis takes place when high temperatures promote the formation of acetic acid from acetyl groups; furthermore, water can also act as an acid at high temperatures. The mechanical effects are caused because the pressure is suddenly reduced and fibers are separated owing to the explosive decompression. In combination with the partial hemicellulose hydrolysis and solubilization, the lignin is redistributed and to some extent removed from the material (Pan et al., 2005). Removal of hemicelluloses exposes the cellulose surface and increases enzyme accessibility to the cellulose microfibrils.

The most important factors affecting the effectiveness of steam explosion are particle size, temperature, residence time and the combined effect of both temperature ( $T$ ) and time ( $t$ ), which is described by the severity factor ( $R_0$ ) [ $R_0 = t * e^{[T-100]/14.75}$ ] being the optimal conditions for maximum sugar yield a severity factor between 3.0 and 4.5 (Alfani et al., 2000). Higher temperatures result in an increased removal of hemicelluloses from the solid fraction and an enhanced cellulose digestibility, they also promote higher sugar degradation.

Steam explosion process offers several attractive features when compared to other pretreatment technologies. These include the potential for significantly lower environmental impact, lower capital investment, more potential for energy efficiency, less hazardous process chemicals and conditions and complete sugar recovery (Avellar and Glasser, 1998). Among the main advantages, it is worth to mention the possibility of using high chip size, unnecessary addition of acid catalyst (except for softwoods), high sugar

recovery, good hydrolysis yields in enzymatic hydrolysis and its feasibility at industrial scale development. It is remarkable the fact that energy use for obtaining small chips size before pretreatment can make up one third of the power requirements of the entire process (Hamelinck et al., 2005). Furthermore, although the possibility of avoiding acid catalysts has been previously stated as an advantage, the addition of an acid catalyst has been also described as a manner to increase cellulose digestibility, to improve hemicellulose hydrolysis and, depending on the temperature, to decrease the production of degradation compounds (Sun and Cheng, 2002). Since cost reduction and low energy consumption are required for an effective pretreatment, high particle sizes as well as non-acid addition would be desirable to optimize the effectiveness on the process (Hamelinck et al., 2005).

Although acid utilization in steam explosion has been introduced with some disadvantages, many pretreatment approaches (SO<sub>2</sub>-explosion) have included external acid addition (H<sub>2</sub>SO<sub>4</sub>) to catalyze the solubilization of the hemicellulose, lower the optimal pretreatment temperature and give a partial hydrolysis of cellulose (Brownell et al., 1986; Tengborg et al., 1998). Notwithstanding, the main drawbacks when using acids are related to equipment requirements and higher formation of degradation compounds (Mosier et al., 2005b; Palmqvist and Hahn-Hägerdal, 2000). In general, SO<sub>2</sub>-catalyzed steam explosion is regarded as one of the most effective pretreatment method for softwood material (Tengborg et al., 1998).

Steam explosion technology has been proven for ethanol production from a wide range of raw materials as poplar (Oliva et al., 2003), olive residues (Cara et al., 2006), herbaceous residues as corn stover (Varga et al., 2004), and wheat straw (Ballesteros et al., 2006). It has successfully performed with hardwoods and agricultural residues or herbaceous biomass but it is not very effective for softwoods due to its low content of acetyl groups in the hemicellulosic portion (Sun and Cheng, 2002).

With the aim of maximizing sugar recoveries, some authors have suggested a two-step pretreatment (Tengborg et al., 1998). In the first step, pretreatment is performed at low temperature to solubilize the hemicellulosic fraction, and the cellulose fraction is subjected to a second pretreatment step at temperatures higher than 210 °C. It offers some additional advantages such as higher ethanol yields, better use of the raw material and lower enzyme dosages during enzymatic hydrolysis (Söderström et al., 2002). Nevertheless, an economic evaluation is needed to determine the effectiveness of an additional steam explosion (Galbe and Zacchi, 2007).

The main drawbacks of steam explosion pretreatment are the partially hemicellulose degradation and the generation of some toxic compounds that could affect the following hydrolysis and fermentation steps (Oliva et al., 2003). The toxic compounds generated and their amounts depend on the raw material and the harshness of the pretreatment. Hence, the necessity of using a robust strain in the subsequent fermentation step. The major inhibitors are furan derivatives, weak acids and phenolic compounds. The main furan derivatives are furfural and 5-hydroxymethyl furfural derived from pentoses and hexoses degradation, respectively. Both have been reported as inhibitors by prolongation of the lag phase during batch fermentation (Palmqvist and Hahn-Hägerdal, 2000).

Weak acids generated during steam explosion are mostly acetic acid, formed from the acetic groups present in the hemicellulosic fraction, and formic and levulinic acids derived from further degradation of furfural and HMF. Wide range of phenolic compounds are generated due to the lignin breakdown varying widely between different raw materials. Several detoxification methods have been studied in order to reduce the inhibitory effect caused by these compounds on enzymes and yeasts. However, owing to the

additional cost in the overall process, detoxification should be avoided if possible. Besides detoxification, several approaches such as genetic modification, evolutionary engineering or adaptative strategies are nowadays appearing as a promising alternatives to obtain more tolerant yeasts (Liu et al., 2005).

#### 4.4.2. Liquid hot water

Liquid hot water is another hydrothermal treatment which does not require rapid decompression and does not employ any catalyst or chemicals. Pressure is applied to maintain water in the liquid state at elevated temperatures (160–240 °C) and provoke alterations in the structure of the lignocellulose.

The objective of the liquid hot water is to solubilize mainly the hemicellulose, to make the cellulose more accessible and to avoid the formation of inhibitors. The slurry generated after pretreatment can be filtered to obtain two fractions: one solid cellulose-enriched fraction and a liquid fraction rich in hemicellulose derived sugars. To avoid the formation of inhibitors, the pH should be kept between 4 and 7 during the pretreatment because at this pH hemicellulosic sugars are retained in oligomeric form and monomers formation is minimized. Therefore the formation of degradation products is also lower (Mosier et al., 2005a).

Liquid hot water has been shown to remove up to 80% of the hemicellulose and to enhance the enzymatic digestibility of pretreated material in herbaceous feedstocks, such as corn stover (Mosier et al., 2005a), sugarcane bagasse (Laser et al., 2002) and wheat straw (Pérez et al., 2008). Two-step pretreatment has been studied to optimize hemicellulosic sugars recovery and to enhance enzymatic hydrolysis yields. Lignin is partially depolymerized and solubilized as well during hot water pretreatment but complete delignification is not possible using hot water alone, because of the recondensation of soluble components originating from lignin.

Flow through systems have been reported to remove more hemicellulose and lignin than batch systems from some materials. Addition of external acid during the flow trough process has been also studied but it is discussed if hemicellulose and lignin removal is increased with the acid addition (Wyman et al., 2005a).

In general, liquid hot water pretreatments are attractive from a cost-savings potential: no catalyst requirement and low-cost reactor construction due to low-corrosion potential. It has also the major advantage that the solubilized hemicellulose and lignin products are present in lower concentration, due to higher water input, and subsequently concentration of degradation products is reduced. In comparison to steam explosion, higher pentosan recovery and lower formation of inhibitors are obtained, however, water demanding in the process and energetic requirement are higher and it is not developed at commercial scale.

#### 4.4.3. Ammonia fiber explosion (AFEX)

In the AFEX process, biomass is treated with liquid anhydrous ammonia at temperatures between 60 and 100 °C and high pressure for a variable period of time. The pressure is then released, resulting in a rapid expansion of the ammonia gas that causes swelling and physical disruption of biomass fibers and partial decrystallization of cellulose. While some other pretreatments such as steam explosion produce a slurry that can be separated in a solid and a liquid fractions, AFEX produces only a pretreated solid material.

AFEX has been reported to decrease cellulose crystallinity and disrupt lignin-carbohydrates linkages (Laureano-Pérez et al., 2005). During the pretreatment only a small amount of the solid material is solubilized; little hemicellulose and lignin is removed (Wyman et al., 2005a). Deacetylation of hemicellulose is also observed. AFEX removes the least acetyl groups from certain lignocellulosic materials (Kumar et al., 2009b). Digestibility of biomass is increased after AFEX pretreatment (Galbe and Zacchi, 2007) and

therefore the enzymatic hydrolysis results in greater yields. Both cellulases and hemicellulases will be required in hydrolysis process due to the considerable remaining hemicellulose in the pretreated material.

Ammonia recovery and recycle is feasible despite of its high volatility (Teymouri et al., 2005) but the associated complexity and costs of ammonia recovery may be significant regarding commercial potential of the AFEX pretreatment (Eggeman and Elander 2005; Mosier et al., 2005b).

No formation of inhibitors for the downstream biological processes is one of the main advantages of the ammonia pretreatment, even though some phenolic fragments of lignin and other cell wall extractives may remain on the cellulosic surface.

The AFEX pretreatment is more effective on agricultural residues and herbaceous crops, with limited effectiveness demonstrated on woody biomass and other high lignin feedstocks (Wyman et al., 2005a). There have been reported recent strategies to optimize the conditions in the AFEX pretreatment in studies using different materials (Teymouri et al., 2005). At optimal conditions AFEX can achieve more than 90% conversion of cellulose and hemicellulose to fermentable sugars for a broad variety of lignocellulosic materials. In fact, despite of little removal of lignin or hemicellulose in the AFEX process, enzymatic digestion at low enzyme loadings results very high comparing other pretreatment alternatives (Wyman et al., 2005b). This may suggest that ammonia affects lignin and possibly hemicellulose differently than other chemicals, reducing the ability of lignin to adsorb enzyme and/or to make its access to cellulose more difficult.

A reduction of ammonia requirements and concentration, and a decrease of enzyme loadings while maintaining high conversions of cellulose and hemicellulose can diminish the total cost of ethanol production using AFEX process. Recently, AFEX pretreatment has been successfully used in saccharification and cofermentation processes with recombinant *S. cerevisiae* strains obtaining high ethanol yields.

Furthermore, besides ethanol production, AFEX pretreatment has been showed as a feasible method for pretreating switchgrass for protein extraction (Bals et al., 2007). In this context, the integrated recovery of sugars and protein would be a feasible approach to a cellulosic biorefinery implying a reduction in the ethanol selling price.

Attending industrial requirements a larger and continuous version of AFEX process based on extrusion technology, known as FIBEX, has been developed and tested (Wyman et al., 2005a).

Another type of process utilizing ammonia is ammonia recycle percolation (ARP) in which aqueous ammonia (5–15 wt%) passes through a reactor packed with biomass. Temperature is normally fixed at 140–210 °C, reaction time up to 90 min and percolation rate about 5 mL/min (Sun and Cheng, 2002; Kim et al., 2008a). ARP can solubilize hemicellulose but cellulose remains intact. It leads to a short-chained cellulosic material with high glucan content (Yang and Wyman, 2008). An important challenge for ARP is to reduce liquid loading or process temperature to reduce energy cost. In this context Soaking Aqueous Ammonia (SAA) appears as an interesting alternative since it is performed at lower temperature (30–75 °C) being one of the few pretreatment methods where both glucan and xylan are retained in the solids. Due to that, it results in a pretreated material very interesting for being used with pentose fermenting microorganisms. Furthermore, high xylose recovery at lower temperatures is reflected in lower amount of inhibitory compounds released from sugar degradation. Recently, ethanol yields as high as 89.4% of the theoretical ethanol yield was shown from barley hull pretreated using SAA in a SSCF process using a recombinant *E. coli* KO11 (Kim et al., 2008b).

#### 4.4.4. Wet oxidation

Wet oxidation is an oxidative pretreatment method which employs oxygen or air as catalyst. It allows reactor operation at relatively low temperatures and short reactor times (Palonen et al., 2004). The oxidation is performed for 10–15 min at temperatures from 170 to 200 °C and at pressures from 10 to 12 bar O<sub>2</sub> (Olsson et al., 2005). The addition of oxygen at temperatures above 170 °C makes the process exothermic reducing the total energy demand. The main reactions in wet oxidation are the formation of acids from hydrolytic processes and oxidative reactions.

It has been proven to be an efficient method for solubilization of hemicelluloses and lignin and to increase digestibility of cellulose, specially. It has been widely used for ethanol production followed by SSF (Martín et al., 2008).

Phenolic compounds are not end-products during wet oxidation because they are further degraded to carboxylic acids. However, furfural and HMF production is lower during wet oxidation when comparing to steam explosion or LHW methods. Na<sub>2</sub>CO<sub>3</sub> addition has been shown to decrease formation of inhibitory compounds by maintaining pH in the neutral to alkaline range.

To pretreat wheat straw with Na<sub>2</sub>CO<sub>3</sub>, results in 96% recovery of the cellulose (65% converted to glucose) and 70% of hemicellulose yield (Klinke et al., 2002). High yields have been also obtained after wet oxidation pretreatment of corn stover and spruce (Palonen et al., 2004).

In general, low formation of inhibitors and efficient removal of lignin are achieved with wet oxidation pretreatment. On the other hand, cost of oxygen and catalyst are considered one of the main disadvantages for wet oxidation development technologies.

#### 4.4.5. Microwave pretreatment

Microwave-based pretreatment can be considered a physico-chemical process since both thermal and non-thermal effects are often involved. Pretreatments were carried out by immersing the biomass in dilute chemical reagents and exposing the slurry to microwave radiation for residence times ranging from 5 to 20 min (Keshwani, 2009). Preliminary experiments identified alkalis as suitable chemical reagents for microwave-based pretreatment (Zhu et al., 2006). An evaluation of different alkalis identified sodium hydroxide as the most effective alkali reagent.

#### 4.4.6. Ultrasound pretreatment

The effect of ultrasound on lignocellulosic biomass have been employed for extracting hemicelluloses, cellulose and lignin but less research has been addressed to study the susceptibility of lignocellulosic materials to hydrolysis (Sun and Tomkinson, 2002). In spite of the minor research on ultrasound pretreatment from lignocellulose, some researchers have also shown that saccharification of cellulose is enhanced efficiently by ultrasonic pretreatment (Yachmenev et al., 2009).

Higher enzymatic hydrolysis yields after ultrasound pretreatment could be explained because cavitation effects caused by introduction of ultrasound field into the enzyme processing solution greatly enhance the transport of enzyme macromolecules toward the substrate surface. Furthermore, mechanical impacts, produced by the collapse of cavitation bubbles, provide an important benefit of opening up the surface of solid substrates to the action of enzymes, in addition, the maximum effects of cavitation occur at 50 °C, which is the optimum temperature for many enzymes (Yachmenev et al., 2009).

#### 4.4.7. CO<sub>2</sub> explosion

Carbon dioxide explosion is also used for lignocellulosic biomass pretreatment. The method is based on the utilization of CO<sub>2</sub> as a supercritical fluid, which refers to a fluid that is in a gaseous form but is compressed at temperatures above its critical point to a liquidlike density. Supercritical pretreatment conditions can effectively remove lignin increasing substrate digestibility. Addition of co-solvents such ethanol can improve delignification. Supercritical carbon dioxide (SC-CO<sub>2</sub>) has been mostly used as an extraction solvent but it is being considered for non-extractive purposes due to its many advantages (Schacht et al., 2008). In aqueous solution CO<sub>2</sub> forms carbonic acid, which favours the polymers hydrolysis. CO<sub>2</sub> molecules are comparable in size to water and ammonia and they can penetrate in the same way the small pores of lignocellulose. This mechanism is facilitated by high pressure. After the explosive release of CO<sub>2</sub> pressure, disruption of cellulose and hemicellulose structure is observed and consequently accessible surface area of the substrate to enzymatic attack increases.

Operation at low temperatures compared to other methods prevents monosaccharides degradation, but in comparison to steam

**Table 1**

Summary of the advantages and disadvantages with different methods for pretreating lignocellulosic biomass.

Pretreatment method	Advantages	Disadvantages
Biological	<ul style="list-style-type: none"> <li>– Degrades lignin and hemicellulose</li> <li>– Low energy consumption</li> </ul>	<ul style="list-style-type: none"> <li>– Low rate of hydrolysis</li> </ul>
Milling	<ul style="list-style-type: none"> <li>– Reduces cellulose crystallinity</li> </ul>	<ul style="list-style-type: none"> <li>– High power and energy consumption</li> </ul>
Steam explosion	<ul style="list-style-type: none"> <li>– Causes lignin transformation and hemicellulose solubilization</li> <li>– Cost-effective</li> <li>– Higher yield of glucose and hemicellulose in the two-step method</li> </ul>	<ul style="list-style-type: none"> <li>– Generation of toxic compounds</li> <li>– Partial hemicellulose degradation</li> </ul>
AFEX	<ul style="list-style-type: none"> <li>– Increases accessible surface area</li> <li>– Low formation of inhibitors</li> </ul>	<ul style="list-style-type: none"> <li>– Not efficient for raw materials with high lignin content</li> </ul>
CO <sub>2</sub> explosion	<ul style="list-style-type: none"> <li>– Increases accessible surface area</li> <li>– Cost-effective</li> <li>– Do not imply generation of toxic compounds</li> </ul>	<ul style="list-style-type: none"> <li>– High cost of large amount of ammonia</li> <li>– Does not affect lignin and hemicelluloses</li> <li>– Very high pressure requirements</li> </ul>
Wet oxidation	<ul style="list-style-type: none"> <li>– Efficient removal of lignin</li> <li>– Low formation of inhibitors</li> <li>– Minimizes the energy demand (exothermic)</li> </ul>	<ul style="list-style-type: none"> <li>– High cost of oxygen and alkaline catalyst</li> </ul>
Ozonolysis	<ul style="list-style-type: none"> <li>– Reduces lignin content</li> <li>– Does not imply generation of toxic compounds</li> </ul>	<ul style="list-style-type: none"> <li>– High cost of large amount of ozone needed</li> </ul>
Organosolv	<ul style="list-style-type: none"> <li>– Causes lignin and hemicellulose hydrolysis</li> </ul>	<ul style="list-style-type: none"> <li>– High cost</li> <li>– Solvents need to be drained and recycled</li> <li>– High cost of acid and need to be recovered</li> <li>– Reactor corrosion problems</li> <li>– Formation of inhibitors</li> </ul>
Concentrated acid	<ul style="list-style-type: none"> <li>– High glucose yield</li> <li>– Ambient temperatures</li> </ul>	<ul style="list-style-type: none"> <li>– Generation of degradation products</li> </ul>
Diluted acid	<ul style="list-style-type: none"> <li>– Less corrosion problems than concentrated acid</li> <li>– Less formation of inhibitors</li> </ul>	<ul style="list-style-type: none"> <li>– Low sugar concentration in exit stream</li> </ul>

**Table 2**  
Effect of different pretreatment technologies on the structure of lignocellulose.

	Milling	Steam explosion	LHW	Acid	Alkaline	Oxidative	AFEX	ARP	Lime	CO <sub>2</sub> explosion
Increases accessible surface area	H	H	H	H	H	H	H	H	H	H
Cellulose decrystallization	H	–	n.d.	–	–	n.d.	H	H	n.d.	–
Hemicellulose solubilization	–	H	H	H	L	–	M	M	M	H
Lignin removal	–	M	L	M	M	M	H	H	H	–
Generation of toxic compounds	–	H	L	H	L	L	L	M	M	–
Lignin structure alteration	–	H	M	H	H	H	H	H	H	–

H: high effect; M: moderate effect; L: low effect; n.d. not determined.

**Table 3**  
Capital cost of five pretreatment processes (raw material: corn stover) (Eggeman and Elander, 2005).

	Pretreatment direct fixed capital, \$MM	Pretreatment breakdown %reactor/ other	Total fixed capital, \$MM	Ethanol production, MM gal/year	Total fixed capital, \$/gal annual capacity
Diluted acid	25.0	64/36	208.6	56.1	3.72
Hot water	4.5	100/0	200.6	44.0	4.57
AFEX	25.7	26/74	211.5	56.8	3.72
ARP	28.3	25/75	210.9	46.3	4.56
Lime	22.3	19/18	163.6	48.9	3.35
Non pretreatment	0	–	200.3	9.0	22.26
Ideal pretreatment	0	–	162.5	64.7	2.51

and ammonia explosion sugar yields obtained are lower. Nevertheless a comparison of different pretreatment methods on several substrates showed that CO<sub>2</sub> explosion was more cost-effective than ammonia explosion and formation of inhibitors was lower compared to steam explosion (Zheng et al., 1998). In aspen and southern yellow pine supercritical CO<sub>2</sub> improves enzymatic hydrolysis (Kim and Hong, 2001).

Anyway, current efforts to develop these methods do not guarantee economic viability yet. A very high pressure requirements is specially a concerning issue. On the other hand, carbon dioxide utilization is an attractive alternative to reduce costs because of its co-production during ethanol fermentation. Other advantages are non toxicity, non-flammability and easy recovery after extraction.

## 5. Conclusions and perspectives

Different pretreatment methods for lignocellulosic materials have been described and widely studied to improve ethanol production processes. All these methods should make the lignocellulose accessible to enzymatic reactions, where crystallinity of cellulose, its accessible surface area, and lignin and hemicellulose disposal are the main substrate-related factors affecting the enzymatic hydrolysis. Table 1 shows the main advantages and disadvantages of the most promising pretreatment methods, while Table 2 summarizes the most significant effects of the different technologies on the structure of lignocellulose.

Among the different methods, chemical and thermochemical are currently the most effective and include the most promising technologies for industrial applications. Combination of different pretreatments have been also considered and might be interesting to obtain optimal fractionation of the different components and reach very high yields.

Although huge information about the effects of different pretreatments on biomass composition and sugar yields have been reported in literature, few references exist on the comparison of the pretreatment costs. A quantitative economic analysis of some pretreatment methods was given by Eggeman and Elander (2005).

This study was focused on identifying the process economic impact of the different pretreatment approaches related to capital and operating cost investment and glucose and xylose sugar yields. Table 3 shows the capital cost for each studied case. It could be concluded that low-cost pretreatment reactors are often counterbalanced by higher cost pretreatment catalyst recovery or higher costs for ethanol product recovery. These results serve as a guide but they should not be taken as a basis, because no differentiation for variation in the state of development of each technology was made.

To further decrease the cost of the pretreatment step in the biomass conversion to ethanol it is essential to minimize sugar losses, to increase solids concentration as high as possible and to keep low reactors and associated equipment costs. In order to increase sugar yields, efficient conversion and utilization of hemicellulosic sugars has become an important task and an opportunity to reduce ethanol production cost. In this context current efforts have been addressed to obtain new recombinant xylose-fermenting yeasts as well as specific enzymes for an effective hemicellulose deconstruction. Thus, an efficient integrated process should also include the pentose fraction since the yield of ethanol may be double.

From a basic research point of view, one approach which is receiving more attention is the study of the effects of pretreatment at a more fundamental level. Plant cell wall is very complex and research at cellular, ultrastructural and even molecular level could contribute to understand the diverse catalytic reactions acting on biomass as well as the consequences of thermal or chemical pretreatments. This knowledge should be applied to achieve an integrated and efficient biomass conversion process to ethanol.

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