'Cradle-to-grave' assessment of existing lignocellulose pretreatment technologies

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“Cradle-to-grave” assessment of existing lignocellulose pretreatment technologies

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Summary

Pretreatment is considered to be a central unit process in a biorefinery to convert lignocellulosic biomass into fuels and chemicals, affecting all other operations in the process. A variety of technologies to pretreat lignocellulosic biomass are available today, which encompass a wide range of physical, chemical, and biological based processes. Among these, chemical based pretreatments are considered to be the most promising for future biorefineries. However, several
key criteria regarding technical, economical and environmental considerations should be critically analyzed when adapting these technologies for the nascent biorefinery industry. This review will discuss the most important pretreatment methods available today and will highlight key criteria for the development of a future ideal pretreatment.

Introduction

The increasing interest in renewable sources of energy is a result of several factors, which include an increase in energy consumption by rapidly industrializing nations, disruption of oil supply as a consequence of exclusive monopoly by politically volatile countries, petroleum price instability, as well as environmental concerns due to the increasing greenhouse gas (GHG) emissions from fossil fuels and its detrimental effects on climate change [1]. Considering the fact that petroleum is the most important source of energy available today and that the largest fraction of its production is used in transportation (http://www.iea.org), development of economically viable liquid fuels has become a priority for many nations across the globe. For this purpose, biomass has been seen as the only sustainable resource, capable of delivering liquid fuels and chemical products on a large scale [2,3]. The US alone has the potential to produce more than 1.3 billion tons of biomass without major changes in land use or agricultural and forestry practices, being capable of sustainably supplying more than 30% of the US current petroleum consumption [3,4].

Governments and private enterprises have been adopting measures to promote renewable energies, either by enforcing policies to stimulate its use or by funding research and the
development of environmentally friendly technologies, making them competitive with existing ones. In one of these initiatives, the United States government approved the Energy Independence and Security Act of 2007 (EISA) [5] which mandates the production of 21 billion gallons of advanced biofuels by 2022, of which 16 billion gallons must derive from lignocellulosic feedstocks. Sustainable production of economically viable biofuels, such as ethanol, from lignocellulosic biomass is considered to be one of the most suitable alternatives. Lignocellulosic materials like agricultural and forest residues, municipal solid waste (MSW) and dedicated energy crops are present in great abundance, providing simultaneously the required attributes for reducing greenhouse gas emissions.

The term “lignocellulosic biomass” is often used to describe the material that composes the plant cell wall, which includes primarily cellulose (30-50%), hemicellulose (15-35%) and lignin (10-30%). As a result of the organization and interaction between these polymeric structures, the plant cell wall is naturally recalcitrant to biological degradation [6]. In the context of biological conversion of lignocellulosic biomass to ethanol (Figure 1), a thermochemical pretreatment step prior to enzymatic hydrolysis is required to facilitate the access of the enzymes to its substrates, enhancing the rate of hydrolysis by 3-10 fold, depending on the type of pretreatment and nature of the substrate. Several economic models acknowledge that pretreatment is a major unit operation in a lignocellulosic biorefinery, accounting for 16-19% of its total capital investment [7] and being the second largest expense after the power plant generator [8]. For this reason it is important to mature existing technologies and develop new methodologies to decrease the economical impact of pretreatment on the overall biorefinery system. In this review, the importance of pretreatment in a biorefinery, leading pretreatment technologies available and key criteria for choosing the ideal pretreatment will be discussed.
Importance of pretreatment in the biorefinery context

In plants, the cell wall acts naturally as a physical protection against pathogens [9]. Some of these pathogens carry hydrolytic enzymes that target different plant cell wall components promoting the disruption of the fibers [9]. From the evolutionary perspective, the plant cell wall structure developed naturally to avoid pathogen penetration. Substrate-related factors affecting enzymatic digestibility are normally associated in a macro-scale with cell wall porosity [10] and biomass particle size [11]. In a micro-scale, these factors are associated with cellulose crystallinity and degree of polymerization [12], hemicellulose side branching [13], lignin composition [14] and coumarate and ferulate cross-linking [14,15]. For an efficient biological conversion of cellulose into fermentable sugars, it is important to modify the physical and chemical properties of the plant cell wall, which can be obtained through a pretreatment step.

Different pretreatments have varying chemistries and ways of interacting with the plant cell wall components. Also, there are an extensive variety of lignocellulosic materials, ranging from grasses, softwoods and hardwoods, which also have different physical and chemical properties. For this reason there is interdependence between pretreatment, the type of substrate and the way it is eventually processed.

Moreover, it is evident that the choice of pretreatment will also impact the physicochemical properties of the pretreated biomass. These properties will also profoundly affect downstream processing of the biomass such as enzyme and microbial selection, pre-conditioning, by-product utilization, waste residue handling and ethanol recovery (Figure 2). Consequently, the choice of pretreatment will have an economic impact on each one of the biorefinery stages. These aspects
make pretreatment the central unit operation in a biologically based sugar-platform for processing lignocellulosic biomass into fuels and chemicals.

Important Pretreatment Technologies Available Today

Several pretreatment technologies are available today, which can be classified, based on the nature of the cell wall disruption, into four categories: a) physical, b) solvent fractionation, c) chemical and d) biological pretreatment.

Physical Pretreatment

Physical pretreatments operate based on the principle of particle size reduction by mechanical stress. This can be obtained by dry, wet vibratory and compression based ball milling procedures [16-18], increasing the enzyme performance by improving the surface area to volume ratio and in some cases by reduction in degree of polymerization and crystallinity of cellulose [19]. Although physical pretreatments are not sufficient to dramatically increase sugar conversions, most pretreatments require a minimal particle size reduction in order to be effective, especially to overcome mass and heat transport problems. It is also important to notice that beyond a certain particle size this type of pretreatment becomes economically unfeasible.

Solvent fractionation
This category of pretreatment technologies applies the principle of differential solubilization and partitioning of various components of the plant cell wall, including cellulose by disruption of the hydrogen bonding between microfibrils [20]. Several fractionation methodologies are available today, however the most attractive ones are the organosolv process, phosphoric acid fractionation, and ionic liquids based fractionation.

**Organosolv process** uses organic solvents, usually alcohols in the presence of an acidic catalyst to extract lignin from lignocellulosic biomass [21]. In this pretreatment, operating temperatures range between 90-120°C for grasses and 155-220°C for woods, with a residence time ranging from 25-100 minutes. Depending on the substrate, catalyst concentration can vary from 0.83 to 1.67% and alcohol concentration from 25 up to 74% (v/v). Lignol Inovation Corporation, Vancouver, Canada, is investing in the organosolv process using ethanol as a preferred solvent. In their process, the insoluble cellulose fraction is used to produce fermentable sugars via enzymatic hydrolysis, while the liquid phase containing lignin, furfural, xylose, acetic acid and lipophylic extracts is processed to recover these compounds [22]. Also, oligosaccharides are subjected to a mild acid hydrolysis to convert them into fermentable sugars to produce more ethanol. The solvent is recovered by distillation and reused while the lignin is used as an additive binder for other applications.

In **Phosphoric acid fractionation**, a series of different solvent extractions using phosphoric acid, acetone and water [23] at temperatures around 50°C are used to fractionate the plant cell wall into amorphous cellulose, hemicellulose, lignin and acetic acid. The fractionation is possible due to the preferential solubility of various plant cell wall components in different solvents. Using this methodology, it is possible to decrystallize cellulose fibers, removing at the same time most
of the hemicelluloses and lignin [23]. This pretreatment has several advantages, since it can operate at a low temperature and pressure (atmospheric pressure) giving at the same time high yields of glucose and xylose [24]. However, the challenges facing this method have to do with finding economical ways of recovering and purifying phosphoric acid (and other solvents) for recycle at the end of each pretreatment batch.

Only recently Ionic liquids were first applied as a pretreatment technology to convert lignocellulosic biomass into fermentable sugars [25]. Ionic liquids have the capability to form hydrogen bonds with cellulose at high temperatures, due to the presence of anions like chloride, formate, acetate or alkyl phosphonate. This pretreatment uses ionic liquids in a ratio of 1:10 (Biomass: Ionic Liquid, w/w) and temperatures ranging from 100-150ºC. The soluble biomass is regenerated using anti-solvents such as water, methanol and ethanol, and then subjected to enzymatic hydrolysis to produce fermentable sugars. One important factor is the fact that residual ionic liquids remaining in the biomass after regeneration could interfere with hydrolytic enzyme activities (and downstream fermentation steps), affecting the final sugar and biofuel yields [26]. After regeneration, ionic liquids may be recovered from anti-solvents by flash distillation and reused in the process [27]. Ionic liquids have tremendous potential for pretreating lignocellulosic biomass, producing a substrate that can achieve more than 90% cellulose digestibility [28], however the cost of these chemicals must be significantly reduced or their recovery made highly efficient, in order to make this technology a serious candidate for an industrially based biorefinery process.

Chemical Pretreatment
Most of the leading pretreatment technologies are represented in this category, which comprises *acidic*, *alkaline* and *oxidative* based pretreatments. Optimal operational conditions for some leading chemical pretreatments to maximize enzymatic digestibility of corn stover are summarized in Table 1. In this category, most pretreatments differ in the types of chemistries and mechanisms that are responsible for cell wall ultra-structural and chemical modifications (Table 2) that result in improved enzyme accessibility and higher sugar yields.

**Acidic based pretreatments**

Several acidic based pretreatment technologies are available, such as concentrated and dilute acid, steam explosion and liquid hot water pretreatment. Although the chemistry governing these pretreatments is quite similar, they operate with different methodologies. While dilute acid uses an externally added acid catalyst, steam explosion and liquid hot water pretreatments operate in acidic conditions due to the fact that water behaves like an acid at high temperatures and also due to liberation of acetic acid from hemicellulose under these conditions [29]. The acid is responsible for chemical hydrolysis, targeting mainly hemicelluloses and lignin, solubilizing a great part of these components from the plant cell wall structure and improving the enzyme accessibility to cellulose. However, during this process several degradation products are formed, mainly furfural, 5-hydroxymethylfurfural, phenolic acids and aldehydes, levulinic acid and other aliphatic acids which can inhibit both enzymatic hydrolysis and fermentation [30,31].

*Dilute-acid* pretreatments use typically sulfuric acid as catalyst for hemicellulose and lignin solubilization at low acid concentrations (0.05-5%) and temperatures ranging from 160-220°C.
[3,32], minimizing the formation of degradation products. Naturally, the sugar degradation will also be minimized, which contributes to higher sugar yields at the end of the process. However, this methodology requires extensive washing of the cellulose rich slurry fraction after pretreatment and/or detoxification of the hydrolyzates before fermentation [31,33]. One advantage of this pretreatment is that hemicellulose hydrolytic enzymes (e.g. xylanases) need not be added during enzymatic hydrolysis, which can be considered a definite cost saving feature. However, most microbes have been unable to ferment xylose from the pretreatment liquor without substantial detoxification [33].

Steam explosion has been one of the most widely implemented pretreatment technologies, being successfully applied to several types of lignocellulosic biomass (e.g. softwoods, hardwoods, agricultural residues). This pretreatment has been demonstrated in several variants, with and without the presence of several chemical catalysts (e.g. sulfuric acid, sulfur dioxide, sodium hydroxide, ammonia) prior to steam explosion [34-38]. It operates at high temperature (160-290ºC) and pressure for a certain duration of time (ranging from a few seconds to several minutes) before the pressure is explosively released [34-37].

Liquid hot water pretreatment uses water at high temperatures (160-230ºC) and pressures (>5 MPa), in order to maintain water in the liquid state [29]. Several methodologies have been developed to promote an effective contact between the biomass and the liquid water, varying from co-current, counter-current and flow-through [39]. Recent variants of this pretreatment allow better pH control between 4 to 7, minimizing the non-specific degradation of polysaccharides [40].
In addition, several other mineral and organic acids (nitric, carbonic, succinic, fumaric, maleic, citric acid) have been used successfully as pretreatment catalysts [41-43].

Alkaline Pretreatments

This kind of pretreatment uses alkaline catalysts, such as calcium oxide (lime), ammonia and sodium hydroxide. Most of these chemicals specifically target hemicellulose acetyl groups and lignin-carbohydrate complex based ester linkages [13,15]. These reactions help solubilize and extract lignin from the biomass, reducing non-specific binding during enzymatic hydrolysis [44]. However, cell wall chemical and ultra-structural modifications still need to be understood for most alkaline pretreatments in order to develop proper enzyme mixtures that can effectively hydrolyze both cellulose and hemicellulose.

Ammonia Fiber Expansion (AFEX) is a low temperature process (60-140°C) where concentrated ammonia is used as a catalyst (0.3-2 kg ammonia per kg of dry weight biomass). Ammonia is added to pre-wetted biomass (0.6-2 kg moisture per kg of dry weight biomass) in a high pressure reactor and is cooked for 5-45 minutes before the pressure is rapidly released. The volatility of ammonia allows it to be recovered and reused, leaving the dried biomass ready for enzymatic hydrolysis [45]. Since ammonolysis reactions do not produce major inhibitors (e.g. organic acids) for enzymes and microbes, it is possible to ferment and hydrolyze this substrate without any sort of detoxification [46,47]. However, AFEX treated biomass requires the utilization of hemicellulose hydrolytic enzymes in addition to cellulases to produce fermentable sugars. X-ray
diffraction studies also revealed that ammonia de-crystallizes cellulose [48], facilitating enzyme activity on this substrate.

Other ammonia based methodologies (e.g. Ammonia Recycle Percolation (ARP), Soaking in Aqueous Ammonia (SAA), supercritical ammonia and ammonia-hydrogen peroxide pretreatments [49,50]) are available, differing mostly in the thermodynamic state of ammonia-water mixtures, and ammonia concentrations. For example, ARP is carried out in a flow-through, recycle mode by percolating ammoniacal solutions (5-15% concentration) through a packed bed reactor with the biomass under pressure and high temperatures. During this process, hemicellulose and lignin are removed from the biomass in the liquid phase.

**Oxidative pretreatments**

Oxidizing agents can also be used to remove lignin and hemicellulose from biomass, in order to increase enzymatic digestibility of cellulose [51]. These agents can react selectively with lignin aromatics and alkyl/aryl ether linkages or also target hemicellulose and cellulose, which will contribute negatively to the final sugar hydrolysis yields. Several kinds of degradation products may be formed (primarily aliphatic aldehydes and aliphatic organic acids) that have been found to inhibit enzymatic hydrolysis of cellulose.

Alkaline wet oxidation is a form of oxidative pretreatment which operates under alkaline conditions and high temperatures (170-220°C) using pressurized air/oxygen or hydrogen peroxide as oxidant [52,53]. The alkali in this pretreatment is sodium carbonate, which helps
solubilize hemicellulose [52] and also minimizes the formation of furan based degradation products which could inhibit enzymes.

Biological Pretreatment

Biological pretreatments, as opposed to chemical based pretreatments, are not energy intensive processes [54], normally performed using fungi (e.g. white rot basidiomycetes) and certain actinomycetes. During pretreatment, these organisms secrete extracellular enzymes like lignin peroxidases and laccases that help remove a considerable amount of lignin from the biomass [55]. However, biological processes require longer residence time (several hours to few days) [56], which constitutes a serious limitation for its application in large scale biorefinery related operations. Also, most microbes typically consume some part of the carbohydrates available in the biomass during growth, which will negatively impact the sugar yield at the end of the process. Important advantages of this pretreatment may be more evident when less recalcitrant genetically modified plant materials are available with higher carbohydrate content, requiring milder pretreatments.

Considerations for an ideal pretreatment technology

The evaluation of a pretreatment should comprise not only technological factors, but also economical and environmental criteria. Feedstock cost represents the highest variable cost in a biorefinery [7]. For this reason, sugar yield, concentration and effectiveness of pretreatment contribute to a large extent to the overall economical feasibility of the process (e.g. biomass
utilization, volume of reactors). At the same time environmental impacts due to land use management factors [57] and waste residue production would also affect process economics. Energy requirements are associated with chemical and water utilization, temperature, pressure, mixing and chemical recovery during pretreatment. These parameters have economical and environmental impacts, however, it is important not to place boundaries around the pretreatment step only, since this will impact all other unit operations in a biorefinery [58**] (Figure 2). One of these is the fermentation based unit operation. Some pretreatments require pre-conditioning of hydrolyzates due to inhibitory compounds produced during pretreatment [31], contributing to additional costs and solid-waste management concerns. The type of chemicals utilized is also a criterion to take into consideration. Besides the reactor volume, the construction material is also going to affect the total capital investment for pretreatment. A less corrosive chemical with low toxicity would contribute to reducing this cost as well as increasing safety and environmental benefits.

Lignin recovery is also an important criterion; since this component of the plant cell wall can be used as a precursor to manufacture valuable chemicals [22]. This methodology is evolving and could be considered a valuable option in the future, contributing to the economical feasibility of a biorefinery. Moreover, some pretreatments have the potential to produce dry pretreated substrates, which could benefit distributed processing facilities due to lower transportation costs. Currently, biomass transportation and storage is considered a technical, economical and environmental concern [59**] and should be addressed before choosing a certain pretreatment technology for large-scale implementation. Some of these pretreatment technologies (e.g. AFEX) also have the potential to process biomass for animal feed [60], increasing its digestibility and
reducing the feedstock requirements for animal production [59**]. This application can create conditions for sustainable land use management, both for bioenergy and feed production, promoting at the same time the desired environmental benefits of lignocellulosic biofuels.

**Conclusion**

For biological conversion of lignocellulosic biomass, pretreatment plays a central role affecting all unit operations in the process and is also an important cost deterrent to the commercial viability of the process. Currently, not all pretreatments are capable of producing biomass that can be converted to sugars in high enough yield and concentration, while being economically viable. However, one cannot decouple enzyme related factors from hydrolysis performance. These comprise specific activity of enzymes, enzyme synergy, enzyme inactivation due to non-specific binding to lignin or other compounds, product inhibition and inhibition by enzyme interactions with pretreatment degradation products [61-63]. In this perspective, it is important to develop this industry integrating the pretreatment with enzymatic hydrolysis and fermentation as inter-connected modular units, aiming to reduce pretreatment severity, total enzyme loading and increase microbial robustness to achieve economically feasible and environmentally sustainable yields of desired end-products (e.g. biofuels and biochemicals).
Acknowledgements

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Conflict of interest

The authors declare that there is no conflict of interest.
References

- of special interest
- of outstanding interest


This paper provides a comprehensive summary of the current status and future projections for the energy portfolio, with special reference to renewable resources, of the world and how that would influence political, economic and environmental policies.


Farrell and co-authors explored the existing loopholes in the fierce net-energy debate for corn and cellulosic ethanol due to lack of suitable metrics in previous studies to show the positive effect of ethanol on GHG emissions compared to conventional fossil fuels.


This paper shows that the optimal dilute acid pretreatment conditions that maximize glucose and xylose yields may not be always the same.

Billion-Ton Annual Supply. Edited by. Oak Ridge: Oak Ridge National Laboratory, United States Department of Energy; 2005:1-78. [Agriculture USDo, Energy USDo (Series Editor): This DOE-USDA sponsored report looks at the total available forest and agricultural land that can be used to sustainably produce a billion-tons of biomass in the US, with relatively few changes to land use and agricultural practices, to produce biofuels and biochemicals.


Himmel and colleagues have provided the current state-of-the-art understanding of the nature of lignocellulosic cell wall recalcitrance to cellulolytic enzymes and pretreatment chemicals.


With tremendous advances being made in the field of pretreating lignocellulosic biomass with ionic liquids, this paper provides a useful overview to several types of solvents that have been used in the past to solubilize cellulose.


The current article explores a novel low-temperature pretreatment utilizing phosphoric acid, acetone and water to fractionate lignocellulosic biomass into its constituent components that results in highly digestible amorphous cellulose and potentially high-value by-products such as acetic acid, hemicellulose and lignin.


This paper examines the nutrient value of ammonia (AFEX) pretreated corn stover hydrolyzate that allowed fermentation at high ethanol yields with no detoxification or external nutrient supplementation.

This paper demonstrates the feasibility of producing ethanol from ammonia (AFEX) pretreated lignocellulosic biomass with no detoxification and external nutrient supplementation of the hydrolyzate offering the potential to significantly improve the economics of cellulosic ethanol production.


   This is a comprehensive review article exploring the interactions between pretreatments and other up/down-stream unit-operations in a lignocellulose biorefinery.


   Carolan and colleagues explore the economic feasibility of regional biomass processing centers, using ammonia based pretreatment (AFEX), to co-produce animal feed and biofuel feedstock for the biorefinery.


Figure 1 – Process flow diagram representing the unit operations for biological conversion of lignocellulosic biomass into ethanol.
Figure 2 – Pretreatment choice affects all biorefinery related operations. This fact gives pretreatment a central role affecting the design and economic viability of a lignocellulosic biorefinery.
Table 1

Table 1 – Optimum operating conditions of some of the leading pretreatment technologies that maximize enzymatic digestibility of pretreated corn stover [2,106-108].

<table>
<thead>
<tr>
<th>Pretreatment Type</th>
<th>Temperature (°C)</th>
<th>Reaction time (min.)</th>
<th>Chemical used</th>
<th>Chemical Loading (g/g dry biomass)</th>
<th>Water Loading (g/g dry biomass)</th>
<th>Specific Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilute acid</td>
<td>160</td>
<td>20</td>
<td>Sulfuric acid</td>
<td>0.015</td>
<td>3</td>
<td>Batch process</td>
</tr>
<tr>
<td>Sulfur dioxide</td>
<td>190</td>
<td>5</td>
<td>Sulfur dioxide</td>
<td>0.03</td>
<td>4</td>
<td>Soaked overnight in 3% acid solution before pretreatment</td>
</tr>
<tr>
<td>Controlled pH</td>
<td>190</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>5.25</td>
<td>Flowthrough mode</td>
</tr>
<tr>
<td>liquid hot water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFEX</td>
<td>90</td>
<td>5</td>
<td>Ammonia</td>
<td>1</td>
<td>0.6</td>
<td>Liquid ammonia added to moist biomass prior to heating reactor</td>
</tr>
<tr>
<td>ARP</td>
<td>170</td>
<td>10</td>
<td>Ammonia</td>
<td>0.5</td>
<td>2.8</td>
<td>Flowthrough mode using 5ml/min of ammoniacal solution, 15% w/w</td>
</tr>
<tr>
<td>Lime</td>
<td>55</td>
<td>4 weeks</td>
<td>Calcium hydroxide</td>
<td>0.5</td>
<td>10</td>
<td>Purged with air</td>
</tr>
</tbody>
</table>
Table 2 – Effect of leading thermochemical pretreatments on the physicochemical properties of corn stover and respective glucan and xylan conversion after enzymatic hydrolysis. Enzymatic hydrolysis was conducted using 15 FPU/g glucan cellulase loading (Spezyme CP, Genencor). Glucan and xylan conversions were calculated relative to the initial composition of corn stover, including both monomeric and oligomeric sugars. The cellulose crystallinity index for untreated corn stover is 50 units and the degree of polymerization is 7000 [7,67,71,106,108-111].

<table>
<thead>
<tr>
<th>Pretreatment Type</th>
<th>Cellulose crystallinity index</th>
<th>% Cellulose removed</th>
<th>% Hemicellulose removed</th>
<th>% Lignin removed</th>
<th>Cellulose degree of polymerization</th>
<th>% Acetyl groups removed</th>
<th>% Glucan Conversion</th>
<th>% Xylan Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilute Acid</td>
<td>53</td>
<td>5-10</td>
<td>70-75</td>
<td>18</td>
<td>2700</td>
<td>55</td>
<td>92</td>
<td>93</td>
</tr>
<tr>
<td>Sulfur dioxide</td>
<td>ND</td>
<td>3-5</td>
<td>40</td>
<td>40-45</td>
<td>3000</td>
<td>55</td>
<td>87 a)</td>
<td>78 a)</td>
</tr>
<tr>
<td>Steam Explosion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controlled pH</td>
<td>45</td>
<td>5-10</td>
<td>40</td>
<td>ND</td>
<td>5600</td>
<td>55</td>
<td>91</td>
<td>81</td>
</tr>
<tr>
<td>liquid hot water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFEX</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6600</td>
<td>30-35</td>
<td>96</td>
<td>91</td>
</tr>
<tr>
<td>ARP</td>
<td>26</td>
<td>1-5</td>
<td>50-60</td>
<td>75-85</td>
<td>4600</td>
<td>85-90</td>
<td>90</td>
<td>88</td>
</tr>
<tr>
<td>Lime</td>
<td>56</td>
<td>1-3</td>
<td>30-35</td>
<td>55-60</td>
<td>3200</td>
<td>90-95</td>
<td>94</td>
<td>76</td>
</tr>
</tbody>
</table>

a) Enzymatic hydrolysis was performed using Celluclast 1.5L at 65 FPU/g mixture and 17 β-glucosidase IU/g mixture.
ND – Not determined.