Increased Degradability of Cellulose by Dissolution in Cold Alkali

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To enhance the degradability of cellulosic materials for further industrial purposes, different qualities of cellulose were dissolved in cold sodium hydroxide solution and precipitated by lowering the pH with sulfuric acid. The precipitated cellulose was subjected to acidic and enzymatic hydrolysis. The results showed that the precipitated cellulose degraded considerably faster both with enzymes and acid relative to the untreated controls. Important pretreatment parameters that can influence the degradability of the pretreated cellulosic materials were found to include temperature and concentration of the cellulose in NaOH solution. Increasing amounts of cellulose were hydrolysed with decreasing pretreatment temperature; the degradability of the pretreated cellulose increased with decreasing cellulose concentration. The degree of polymerization (DP) also can influence the pretreatment efficiency. Diluted sulfuric acid was able to decrease the DP and enhance the effect of dissolution and precipitation. The results showed that the lower DP of cellulosic materials caused an increase of degradability for the NaOH pretreated samples compared to untreated samples. The NaOH pretreatment was more effective for shorter chain cellulose.

Keywords: Cellulose degradation; Cold alkali pretreatment; DP; Crystallinity; Acidic hydrolysis; Enzymatic hydrolysis

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INTRODUCTION

Along with the environmental problems associated with fossil fuels, searching for new sustainable energy has become increasingly important. Therefore, environmentally friendly biofuel is now produced (Demirbas 2009). First-generation biofuel production resulted from the conversion of sugar, starch, or oil crops such as oil palm, rapeseed, and soybean (Naik *et al.* 2010), but there are some drawbacks. For example, the costs are quite high, and arable lands raising such crops can lead to the less availability for food production. Moreover, glucose, lipids, and starch are present in minor amounts in most plants, while lignocellulosic materials can be produced in large amounts from plants cultivated in areas not suitable for food production and as byproducts of traditional agriculture (Lucia 2008). Thus, cellulose-ethanol research of so called second-generation bioethanol, in which cellulose is broken down to glucose and fermented to ethanol, has become a hot point of research worldwide. This approach can utilize the most abundant raw materials such as wood, pulp, grass, agricultural residues, *etc.* (Tan *et al.* 2008). However, a problem with this concept is that cellulose is very resistant to degradation. One method is acid hydrolysis by strong acids (*i.e.*, sulfuric acid), which is an inexpensive way to degrade cellulose to sugars under relatively harsh conditions; however, this method produces undesirable byproducts toxic to ethanol fermenting microorganisms (Badger

2002). Moreover, concentrated acids are corrosive, requiring corrosion-resistant reactors, which makes this process very expensive. Thus, hydrolysis with cellulose-degrading enzymes is typically preferred, although enzymes are costly and the degradation rate is rather slow. These problems with sugar production from cellulose have so far prevented large-scale commercialisation of ethanol from cellulose.

Cellulose is a linear, unbranched polysaccharide consisting of several hundred to several thousand D-glucose units joined by β-1,4-glucosidic linkages, as shown in Fig. 1 (O'Sullivan 1997). Each chain is stabilised by intra-chain hydrogen bonds formed between the C6 hydroxyl and the C2 hydroxyl and between the C5 oxygen and C3 hydroxyl, which provide rigidity to the structure. There are also hydrogen bonds between cellulose chains stabilising sheets of parallel cellulose chains. The individual chains are arranged in crystalline structures, *i.e.*, fibrils.

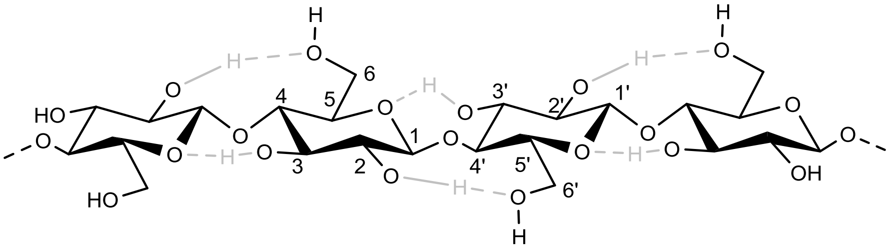

Fig. 1. Molecular structure of cellulose

Not all cellulose forms perfect crystals; there is unordered cellulose, probably located on the fibre surface and between fibrils, and also as segments in the fibrils. Crystalline and unordered regions are distributed in the cellulose component of the biomass. The intra- and intermolecular hydrogen bonds lead to the limited accessibility of reactive agents to cellulose substrates. The poor chemical reactivity of cellulose is, to a large degree, due to the crystalline structure of cellulose, which can exclude water almost completely (Zhao *et al*. 2006).

Since less molecular surface is exposed in the crystalline region, cellulose reactions that require penetration of the reactive agents into the cellulose matrix proceed more slowly than in regions that are more disordered. Therefore, it is widely accepted that the unordered regions are much easier to degrade in comparison to the crystalline regions. Some cellulosic materials often contain a small quantity of hemicelluloses and lignin, which can also inhibit degradation (Rahikainen *et al*. 2013). To increase the chemical reactivity of cellulose, pretreatments decreasing the crystallinity of cellulose are required (Sun and Cheng 2002).

One way to increase the degradability of cellulose may be to convert crystalline cellulose to less ordered cellulose structures. This can be accomplished by dissolving cellulose and thereafter precipitating it quickly. The cellulose structure will be highly unordered and easily hydrolysed. Many efficient cellulose dissolving agents are relatively expensive (*i.e.*, ionic solvents) or represent potential environmental problems (*i.e.*, phosphoric acid), and are less attractive for industrial applications. Cold sodium hydroxide solution represents a possible solvent, and alkaline pretreatment has indeed been reported to improve the degradability of cellulose (Zhao *et al*. 2008). In this work, we present a simple and industrially feasible method to convert cellulose to unordered forms using cold sodium hydroxide that are more easily hydrolysed than crystalline structures.

EXPERIMENTAL

Materials

The primary cellulose sample used was Avicel PH-101 microcrystalline cellulose (~50 μm particle size), purchased from FMC Corporation (USA). Avicel, a purified, partially depolymerised α-cellulose derived from special grades of wood, has a high crystallinity but relatively low degree of polymerization. The remaining cellulose samples, cotton linter, birch kraft pulp, sulfite pulp, unbleached softwood pulp, and bleached softwood pulp, were supplied by the Department of Fibre and Polymer, KTH Royal Institute of Technology, Sweden.

The enzyme Novozym 342 (Novozym, Denmark) is a cellulolytic culture filtrate containing mainly endoglucanase, cellobiohydrolase, and β-glucosidase (Liu *et al*. 2009; Žnidaršič-Plazl *et al*. 2009). Disodium hydrogen phosphate (Na_2HPO_4) was supplied by Merck KGaA (Germany), and sodium dihydrogen phosphate (NaH_2PO_4) by KEBO AB (Stockholm, Sweden). Phosphate buffer used in enzymatic hydrolysis was prepared by mixing 9.0 mM of Na_2HPO_4 and 11.0 mM of NaH_2PO_4, with a final pH of 7.00±0.05.

Sodium hydroxide (NaOH) was supplied by Fisher Scientific Company (Sweden). Sodium hydroxide solutions were prepared by mixing NaOH solid with deionized water. All concentrations are expressed in weight percent. Sulfuric acid was provided by Merck KGaA. Varying concentrations of sulfuric acid was prepared by mixing the concentrated sulfuric acid (95 to 97%) with quantitative deionized water.

Methods

Pretreatment procedure

One gram cellulosic materials were mixed with 50 mL NaOH solution at different temperatures (4 ºC, 10 ºC, RT, 40 ºC) with magnetic stirring. Thereafter, cellulose was precipitated using any of three different methods for degradation experiments and characterisation: (1) the pH was adjusted to approximately 7 with sulfuric acid at room temperature to precipitate the cellulose in the liquid; (2) the pH was adjusted to approximately 12 by flushing the alkaline solution with carbon dioxide at room temperature; (3) increasing the cellulose solution temperature to 100 ºC. In each case the precipitate was collected *via* a glass filter and washed with deionised water.

Some samples were subjected to an acid pre-treatment prior to the above alkaline treatment to lower the degree of polymerization. After pre-thermosetting to 90 ºC, the cellulosic materials were mixed with 2 M sulfuric acid and incubated at 90 ºC on a magnetic stirrer for 5 min or 40 min. The concentration was 4 g (dry weight) of cellulose/pulp in 100 mL of 2 M sulfuric acid. The reaction was terminated by washing with approximately 1 L of ice water and 20 mM phosphate buffer, pH 7 in a glass filter. A portion of the residue solid was used to determine viscosity and the yield of the samples; the remaining samples were used for NaOH pretreatment (as described above) followed by enzymatic hydrolysis.

Hydrolytic degradation procedure

The cellulosic materials were subjected to three different degradation experiments, *i.e.*, hydrolysis by dilute sulfuric acid (0.1 M), hydrolysis by concentrated sulfuric acid (4 M), and hydrolysis by cellulolytic enzymes. The cellulose samples were placed in water overnight to swell before the hydrolysis. The acid hydrolysis was performed with the swelled original cellulose and pretreated cellulose samples in a 90 ºC water bath at varying times (20 min, 40 min, 1 h, 2 h, and 5 h). When the hydrolysis process was complete, the samples were cooled with ice water and neutralised with NaOH to about pH 7. Finally, the solution was centrifuged at 2,451 G for 5 min, washed with water 3 times, and dried overnight in an oven at 105 °C for residue weight evaluation.

The enzymatic hydrolysis was carried out with Novozym 342 at a dose of 90 ECU/g in a 40 °C water bath at varying times. One g (oven dry weight) sample was suspended in 30 mL of sodium phosphate buffer (pH 7.0 ± 0.05). The reaction was terminated by placing the reaction system in a 90 °C water bath for 15 min; the residue solid was filtered with glass filter and dried in an oven overnight at 105 °C for dry weight evaluation.

Viscosity

Viscosity was determined according to the international standard ISO 5351 (2004) and is proportional to the average degree of polymerization of cellulose.

RESULTS AND DISCUSSION

To test the dissolution and precipitation of cellulose prior to degradation, Avicel was selected as a model system. Avicel is microcrystalline cellulose, which is resistant to depolymerization and thus a suitable model compound to study cellulose degradability. Seven different concentrations of Avicel (*i.e.*, 0.1%, 0.2%, 0.5%, 1%, 2%, 4%, and 10%) were chosen and dissolved in four different concentrations of NaOH solution (*i.e.*, 1%, 5%, 8%, and 10%) at 4 °C with magnetic stirring (Fig. 2). After 1 h, a different appearance of the solutions was obtained. With an increase in concentration of sodium hydroxide, the dissolution of cellulose also increased. In summary, it appears that NaOH concentrations of 8% and 10% can dissolve cellulose at concentrations up to 2%. Although higher cellulose concentrations may be soluble at 10% NaOH concentrations, the solutions become viscous and gel-like. These results are generally in line with data in the literature, with dissolution of cellulose fibres in NaOH solutions in water (Moigne and Navard 2010). This result indicates that cold alkali can produce a semi-transparent liquid without any visible precipitation when left for several hours without stirring. This solution is most likely not a "solution" in strict chemical terms, but rather a mixture of a solution and a colloidal suspension of cellulose. From a technical perspective, it can be regarded as a liquid, and referred to as a "solution"; therefore, 10% NaOH with 2% Avicel was chosen as the standard.

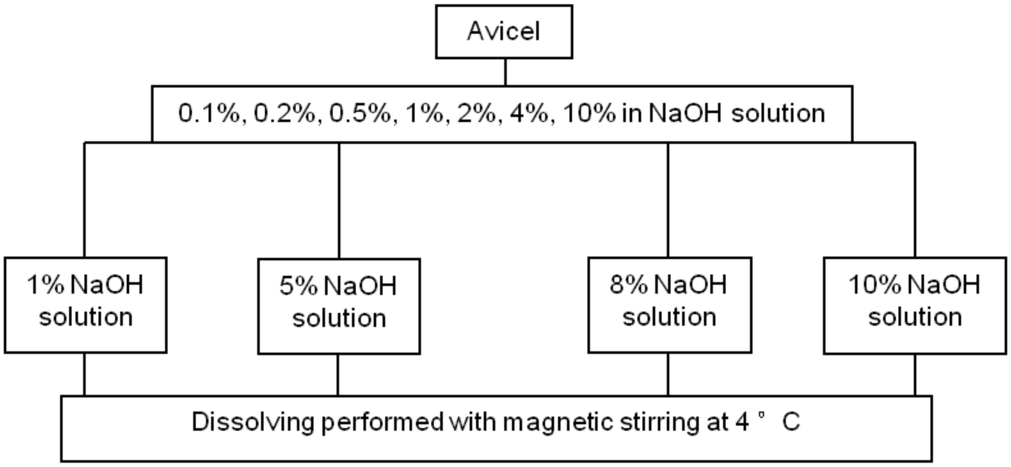

Fig. 2. Experimental design for dissolving Avicel in NaOH

Three different methods to precipitate the cellulose were investigated. The evaluation measured the yield of each precipitate (Table 1). The data clearly indicates that neutralization with H_2SO_4 led to a higher yield of cellulose than lowering of the pH with CO_2. An increase of the temperature was a much less effective method for precipitation. For future studies, precipitation should be performed by adjusting the pH to 7 with sulfuric acid at room temperature.

Table 1. Methods for Precipitation of Cellulosic Materials

Precipitation Method	Weight of Precipitate (g)	Yield (%)
Neutralise to pH 7 with H_2SO_4	1.008	100.0
Lowing pH with CO_2	0.861	86.1
Increasing temperature to almost 100 °C	0.355	35.5 Precipitate turned yellow

The original and cold NaOH-pretreated Avicel (1 g) were subjected to hydrolysis with dilute (0.1 M) and concentrated (40%) sulfuric acid, or with the commercial cellulolytic culture filtrate Novozym 342. The results of acid hydrolysis, shown in Fig. 3, strongly indicate that pretreated samples were degraded considerably faster by both strong acid and dilute acid than the untreated controls. The effects were greater with the strong sulfuric acid hydrolysis than the diluted acid. After 5 h of 40% sulfuric acid hydrolysis, about 96% of pretreated samples were degraded, whereas 36% of solid residue remained in the untreated control.

Cellulolytic enzymes (Novozym 342) also degraded the pretreated sample much faster than the untreated control, as shown in Fig. 3. The degradability differences between pretreated and untreated samples appeared to be larger than acid hydrolysis, but the overall degradation was much slower than strong acid. After 48 h incubation, more than 85% of the cellulose was degraded in the pretreated cellulose, whereas less than 40% was degraded in the untreated control. The hydrolysis of cellulose is based on the hydrolysis of glycosidic bonds in the polysaccharide chains.

From the cellulose structure (Fig. 1) described in the Introduction, the polysaccharide chains are closely connected by the hydrogen bonds, intra-molecular hydrogen bonds within a single chain, and intermolecular hydrogen bonds between the adjacent chains. The hydrogen bonds hold the cellulose chains together to form the rigid fibrous structure, and the state of hydrogen bonds can also determine other physical properties of cellulose such as crystallinity. Therefore, the state of hydrogen bonds was considered the primary factor dominating the resistance of cellulose hydrolysis, by providing a crystalline and resistant structure to the cellulose.

During the cold NaOH treatment, the hydroxyl ions take part in swelling and at least partial dissolution of the cellulose. Furthermore, alkali pretreatment may lead to the enlargement of the pore ratio and inner surface areas of the cellulose (He *et al*. 2008). This enlargement helps to explain why the degradability can be increased by the pretreatment at 4 °C in the hydrolysis process.

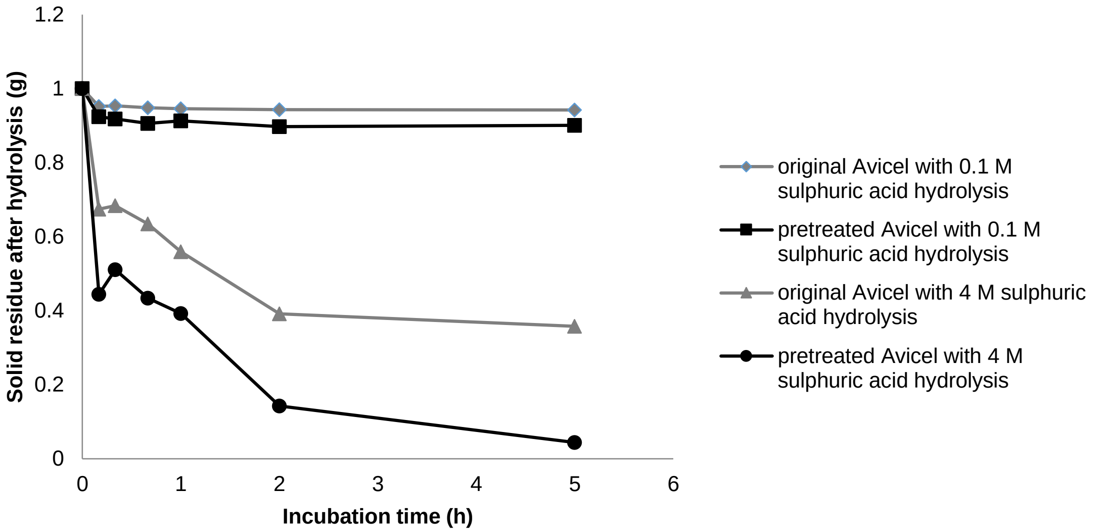

Fig. 3. Acidic hydrolysis of Avicel and pretreated Avicel

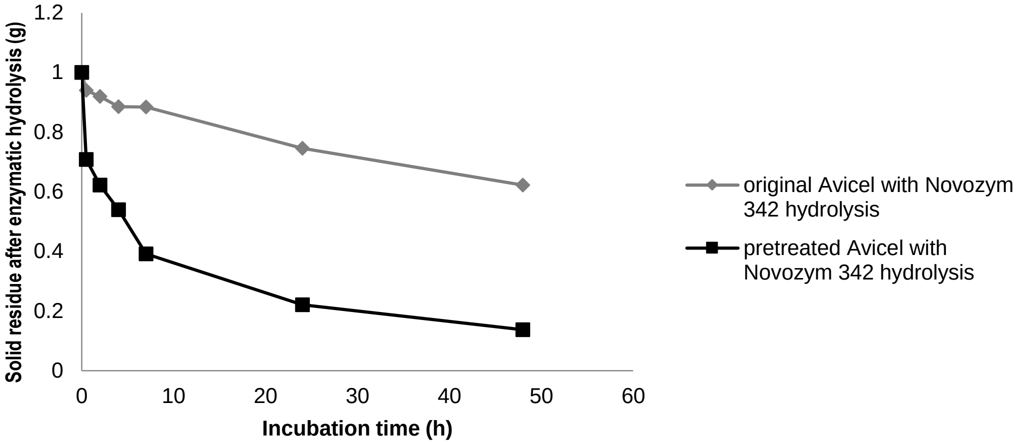

Fig. 4. Enzymatic hydrolysis of Avicel and pretreated Avicel

The pretreatment with NaOH at 4 °C can increase the degradability of cellulose samples. To study other pretreatment temperatures, Avicel samples were added in 10% NaOH solution at other temperatures, *i.e.*, 10 ºC, RT (23 to 25 ºC), and 40 ºC. After approximately 1 h, the samples were evaluated for clarity (Fig. 5). All the celluloses dissolved in 4 °C, 10 °C, and RT (23 to 25 °C), but precipitant was present at 40 °C. The effect of the pretreatment at varying temperatures was also tested. Original and reprecipitated Avicel underwent hydrolysis for 5 h and 20 h with Novozym 342, and the weight of the solid residue determined (Fig. 6). The results on the enzymatic degradation showed that the effect was clearly better if the dissolution was carried out at a lower temperature. At 40 ºC, no dissolution occurred, but there was an improvement of the degradation by the enzymatic hydrolysis.

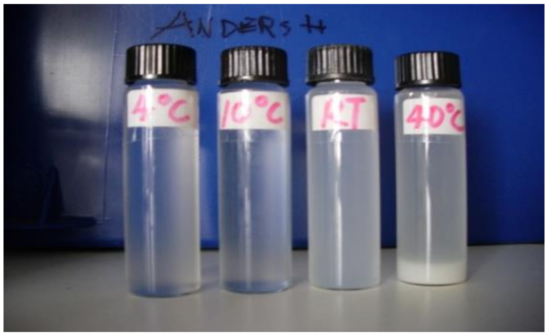

Fig. 5. The appearance of 1% Avicel treated with 10% NaOH solution at different temperatures

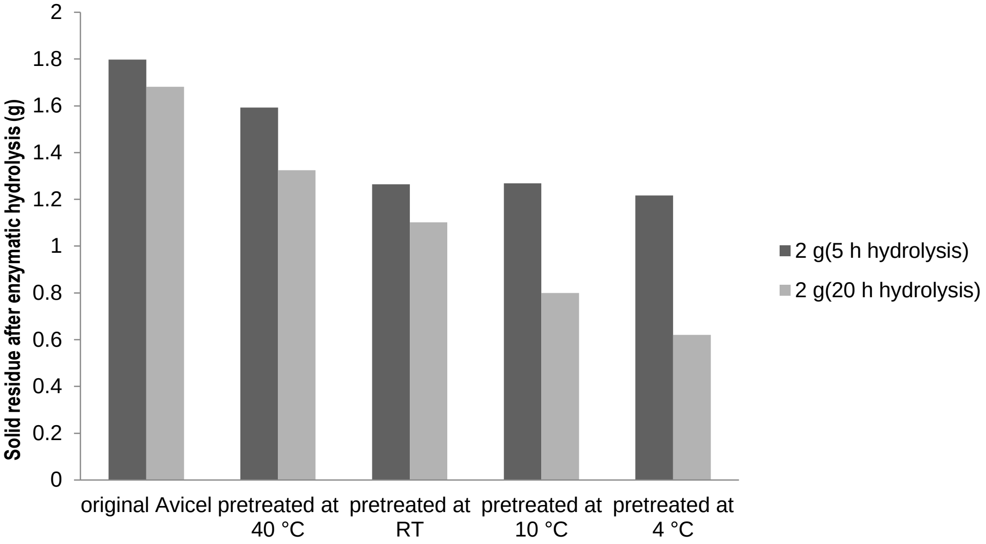

Fig. 6. Enzymatic hydrolysis of Avicel with varying pretreatment temperatures

Thus, for Avicel, a short-chain cellulose with high crystallinity, the lower temperature pretreatment with NaOH was very effective for the degradation efficiency both in acidic and enzymatic hydrolysis (Figs. 3 and 4). What about other cellulose qualities? From the supra-molecular view, the degree of polymerization (DP = number of anhydroglucose units) can also influence the degradability of cellulose; a higher DP is probably associated with lower solubility and thereby less activation effect of cold NaOH pretreatment. Therefore, cotton linter, bleached birch kraft pulp, and bleached sulfite pulp widely used in the pulp and paper industry and processing varying DP (as measured by viscosity according to ISO 5351 (2004) were dissolved in 10% NaOH at 4 ºC at the same concentration as Avicel, 2% cellulosic materials in 10% NaOH solution (Fig. 7).

Table 2. The Viscosity of Cellulosic Materials

Sample	Viscosity (mL/g)
Avicel	120
Bleached sulfite pulp	550
Bleached birch kraft pulp	710
Cotton linter	900

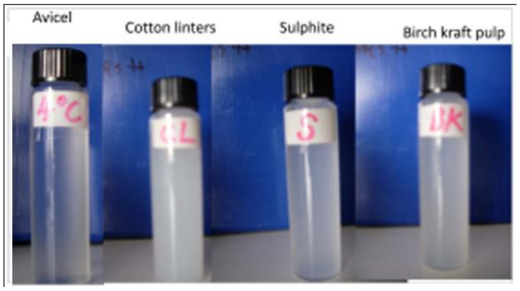

Fig. 7. The appearance of different cellulose solution

As shown in Fig. 7 and Table 2, the solutions were more turbid as the viscosity increased. Thus, colloid suspension is greater when the DP is higher. After the pretreatment and precipitation, Novozym 342 hydrolysis was performed with both the non-treated and pretreated materials (including Avicel) for 5 h and 20 h. The results of the hydrolysis are shown in Fig. 8.

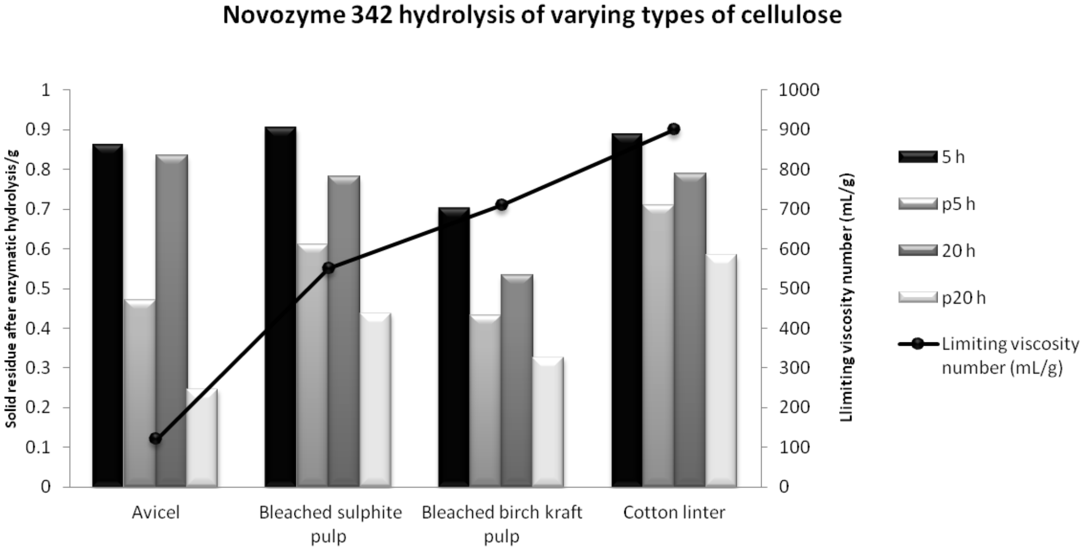

Fig. 8. The effects of the pretreatments of cellulose with different degree of polymerizations (expressed as limiting viscosity number). Oven-dried residuals in gram (starting material was 1 g) is shown after enzymatic hydrolysis. *5 h, untreated control incubated 5 h with Novozym 342; p5 h, sample dissolved and reprecipitated cellulose incubated 5 h with Novozym 342; 20 h, untreated control treated 20 h with Novozym 342; and p20 h, dissolved and reprecipitated cellulose incubated with Novozym 342 for 20 h.

Avicel (microcrystalline cellulose) has a viscosity of 120 mL/g, has a low degree of polymerization, but has a very high degree of crystallinity. Fully bleached mixed spruce and pine sulfite pulp have a viscosity of 550 mL/g, and a relative low degree of polymerization. They were pulped in acidic conditions and have a rather high crystallinity. Fully bleached birch kraft pulp, viscosity 710 mL/g, has a rather high degree of polymerization. It was pulped in an alkaline condition and most likely contains a portion of unordered swelled cellulose. Cotton linter, viscosity 900 mL/g, was not pulped and has a very high degree of polymerization. Most of the cellulose has a high degree of crystallinity. The results indicate that for the untreated samples, the birch kraft pulp was the easiest to degrade, and Avicel the most difficult to degrade. This indicates that the degree of crystallinity is important for the degradation rate and that the enzymatic hydrolysis had little relationship with the DP of the untreated samples. The pretreatment worked well for the different cellulose qualities; the largest improvement effects based on the viscosity values were on Avicel followed by sulfite pulp, and the smallest effects were on cotton linter. This illustrated that the DP was important for the efficiency of the pretreatment technique to increase the cellulose reactivity, and it worked better on shorter chain cellulose materials than longer materials. In support of this, the longer chain cellulose also produced less transparent suspensions, as shown in Fig. 7.

Thus, cellulose qualities with lower DP seem to react better with this pretreatment, and thereby the degradability increased more after pretreatment compared with the original materials. Therefore, shortening of long chained cellulose material with, for instance, a short time of mild acid hydrolysis, would increase the effectiveness of a cold alkali pretreatment. To test this hypothesis, an unbleached softwood kraft pulp with high viscosity was treated with 2M sulfuric acid for 5 min and 40 min, and the yield and viscosity of the pretreated samples were determined. From the literature, it is known that dilute sulfuric acid was principally involved with the hydrolysis of hemicellulose and the subsequent removal of its component sugars and their degradation products (Taherzadeh and Karimi 2007). Another effect was the hydrolysis of some amorphous regions when acid incubation was performed on the cellulose samples. This can lead to an increase in the crystallinity of the samples. More amorphous regions were hydrolysed, and the degree of crystallinity increased with increasing incubation time. Therefore, after sulfuric acid incubation for 5 min and 40 min the yield was not 100%. The result can be seen in Table 3 and Fig. 9. The dilute sulfuric acid incubation also caused shortening of the cellulose chains, as illustrated by the viscosity data shown in Table 3 and Fig. 9; the viscosity decreased after acid incubation, which means that the degree of polymerization decreased, and the longer incubation time the more DP decreasing. Both the original unbleached softwood pulp and dilute sulfuric acid incubated pulp were exposed to the cold alkali pretreatment. Afterwards, cold alkali pretreatment was carried out on unbleached softwood pulp with or without sulfuric acid incubation. Finally, enzymatic hydrolysis was carried out on these six samples (samples with sulfuric acid incubation, with or without cold alkali pretreatment) with Novozym 342. The evaluation was also done by weighing the solid residue and the results were shown in Fig. 9. The results proved that cold alkaline pretreatment was more effective for the degradation of the shorter chain cellulose, *i.e.*, lower DP than the degree of crystallinity, but for the non-cold alkali pretreated sample, the degree of crystallinity is the main factor to influence the enzymatic hydrolysis efficiency. This experiment was performed on unbleached kraft softwood pulp that contained some residual lignin, but this seems not to have affected the reaction to a large degree.

Table 3. Yield and Viscosity of Unbleached Softwood Pulp Treatments

Sample	Yield (%)	Viscosity (mL/g)
Unbleached softwood pulp	100	1300
5 min 2 M sulfuric acid incubated unbleached softwood pulp	88.25	520
40 min 2 M sulfuric acid incubated unbleached softwood pulp	76.33	300

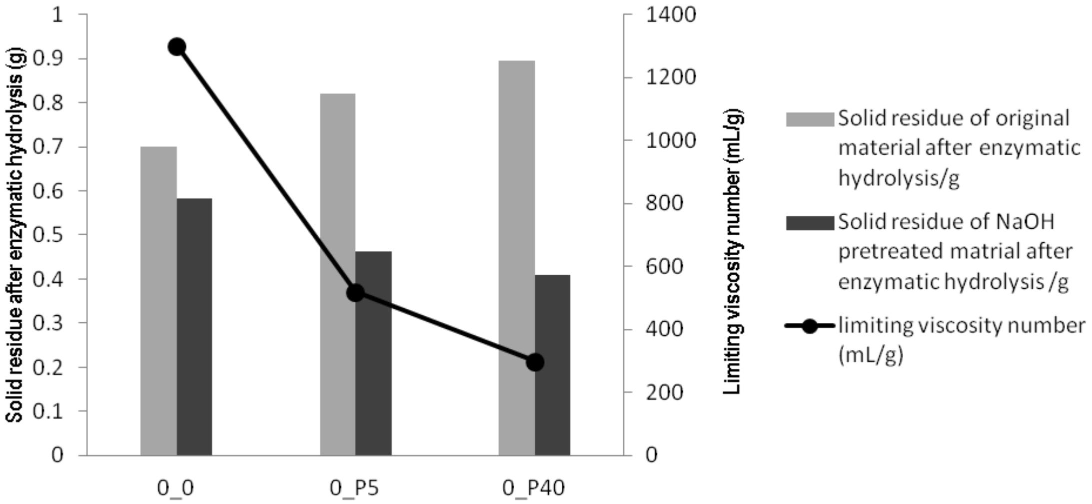

Fig. 9. Efficiency of acidic and alkali pretreatment. *0_0, Unbleached softwood pulp, 0_P5, 5 min 2 M sulfuric acid incubated unbleached softwood pulp, 0_P40, 40 min 2 M sulfuric acid incubated unbleached softwood pulp

Technical significance

The present method increases the degradability of cellulose both with acid hydrolysis and with enzymes, and may have the potential to solve technical problems with both processes. Acid hydrolysis generates different kinds of by-products that may work as inhibitors for fermenting organisms and therefore disturb production of ethanol from the hydrolysates. In the present experiments, the liquid of the acid hydrolysate was dark, indicating that by-products were formed. A faster hydrolysis may therefore allow acidic hydrolysis conditions that are milder, and therefore less by-products may be formed which are beneficial for the possibility to perform fermentations. Also for enzymatic degradation, the pretreatment method may be beneficial, since the speed of the process is increased and may increase the yield and production of industrial hydrolysis units.

Is the idea of dissolution and re-precipitation of cellulose in NaOH feasible in an industrial setting? Since the ethanol from cellulose concept will be a large-scale industrial project producing a bulk product, the production costs must be low, and direct use of NaOH and sulfuric acid as in the present experiments is most likely too expensive. However, it is also possible to perform the precipitation by flushing with carbon dioxide. The solution left following cellulose precipitation consists of a mixture of NaOH and Na_2CO_3, and this can be regenerated to a NaOH solution by reactions similar to the lime cycle in a kraft mill.

The process was shown here to work successfully with relatively pure celluloses and chemical pulps containing also residual lignin and hemicelluloses, but an experiment using more native biomass (milled straw) was unsuccessful. This is probably because lignified tissues consist of large covalent networks of lignin and hemicelluloses (Lawoko *et al*. 2006) that cannot be dissolved by the cold alkali. Thus, some rudimentary chemical pulping or similar process may be required prior to dissolution in alkali. Such pulping can also shorten the cellulose chains to a suitable length and produce fuel in the form of black liquor lignin. Figure 10 shows a flow chart for an ethanol production plant using cellulose as the raw material.

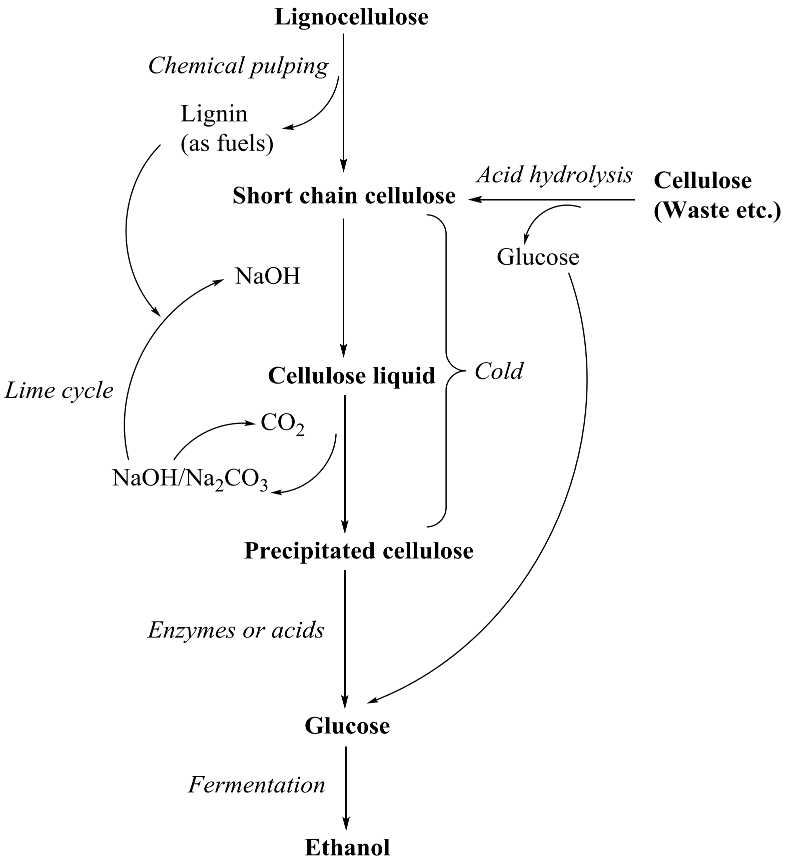

Fig. 10. Flow chart of a hypothetical mill for ethanol production from lignocelluloses and waste products

CONCLUSIONS

1. Short chain cellulose can be dissolved into a liquid that most likely is a mixture of a solution and a colloidal suspension, by 10% NaOH at 4 °C. After precipitation this cellulose can be considerably faster degraded by both acid and enzyme hydrolysis, producing sugars.
2. The efficiency of the method decreases with increasing degree of polymerization (DP). The DP can however easily be shortened on long chains cellulose by a mild acid hydrolysis, and thereafter the cold alkali pretreatment works better.
3. Some important pretreatment parameters that could influence the degradability of the pretreated cellulose samples were found; one parameter was the temperature of pretreatment. The lower the pretreatment temperature, the more cellulose was hydrolysed. Another parameter was the concentration of the cellulose in NaOH solution. The lower the cellulose concentration, the higher the degradability of the pretreated cellulose, which meant that the more volume NaOH solution was used to dissolve the cellulose, the better the pretreatment effect. Moreover, DP and the crystallinity of the cellulose also could influence the pretreatment efficiency. For the original cellulosic materials without cold alkali pretreatment, crystallinity was the main factor influencing the degradability. The higher the crystallinity, the lower was the degradability. But after NaOH pretreatment, DP becomes the main parameter. The lower the DP, the greater

was the extent of degradation regardless of whether the degree of crystallinity was high or low.

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