The structural features of cellulose are known to profoundly influence the kinetics of cellulose hydrolysis. Cellulose in biomass is resistant to hydrolysis due to hydrophobic interactions between cellulose sheets, hydrogen bonding between adjacent cellulose chains, and cellulose's close association with lignin. A useful pretreatment disrupts hydrophobic and hydrogen bonds, as well as the lignin seal, in a manner which minimizes chemical change of the cellulose and formation of undesirable degradation products. The resulting polysaccharide structure must be stabilized against spontaneous recrystallization, once pretreatment conditions are removed. Otherwise the benefit of enhanced hydrolysis is lost. This work reports the intercalating effects and mechanisms of surface activators, and the role of water in altering the physical properties of pretreated cellulose. A mechanism is proposed which leads to a leveling off in particle size (LOPS) during enzyme hydrolysis of lignin-free, microcrystalline cellulose.

Biomass materials consist principally of hemicellulose, cellulose, and lignin. Compositions are typically in the range of 25 to 35% hemicellulose, 30 to 40% cellulose, and 10 to 15% lignin. Other components include inorganic compounds, proteins, and other non-cellulosic constituents.

Autohydrolysis in water, or hydrolysis by dilute acids or xylanase enzymes readily removes the hemicellulose fraction and gives pentose rich hydrolysates. These treatments also remove a significant fraction of the other soluble components. The remaining cellulose is closely associated with lignin and has a crystalline structure which makes it resistant to both acid and enzyme hydrolysis. The recalcitrant nature of cellulose continues to impede economical production of fermentable sugars from biomass materials and cellulosic residues. Many pretreatments have been proposed and tested which disrupt the crystalline structure of cellulose and make the cellulose sufficiently reactive to give 80 to 90% yields of fermentable monomer acid or enzyme hydrolysis. These include: cellulase solvents such as cadoxen or Fe/H2A; strong mineral acids; amines; amine oxides; ammonia; alkali/alcohol solutions; modified pulping processes; and physical treatments (grinding, beating, and radiation) (Navard and Haudin, 1986; Woyriyama and Saida, 1986; Laidisch, 1988; Wood and Saddler, 1988; Holtsapple et al., 1992).

A review on the cost of energy or chemicals used in various pretreatments (Fan et
al., 1987) enabled estimation of
the contribution of pretreatment
to the cost of fermentable
sugars (Ladisch and Sveczkopf,
1991). Substrate, and energy or
chemical costs of pretreatment,
in the best case, range from 5
to 20 cents per pound of sugar.
This calculation does not
include capital or labor
operational costs elsewhere in
the process. Both the glucose
and xylose derived from the
hydrolysis of the biomass, were
assumed to be fermentable to
ethanol at close to theoretical
yield (i.e., about 0.8 lbs
ethanol/lb glucose and xylose).

The overall cost (including
raw material, capital, and
operating costs) of biomass
derived sugars needs to be about
4 cents/pound at current
economic conditions to be
competitive with corn as a
substitute for fuel ethanol
production. Consequently,
cellulose pretreatment costs
need to be reduced to improve
the prospects for converting biomass
to value-added fermentation products. Starch
pretreatment by leaching and
gelatinization using principally
water, enzymes, and heat, serves
as a benchmark against which the
cost of cellulose pretreatments can be compared.

Starch, like cellulose, needs
to be susceptible to enzyme
hydrolysis. Pretreatment of
starch involves adding a
thermostable, a-amylase to
temporarily hydrolyze the starch
and reduce the viscosity of the
starch slurry. The slurry is
gelatinized and liquefied at
temperatures ranging from 90 to
163°C, depending on the process
used (Borline, 1980). The
concentration of the starting
slurry is lower for corn than
for refined starch and ranges
from 20 to 35%.

Our research seeks an
approach which is conceptually
similar to that of starch
pretreatment. Such an approach
would be based on water, and
minimizes the addition of
chemicals which would need to be
recovered or recycled.

Cellulose Structure

Cellulose, a linear polymer of
β-1,4 anhydroglucose monomers,
has a lower proportion of
hydroxyl groups suggesting that
solvation than starch. This
difference arises from the β-1,4
bonds of the anhydroglucose monomers in
cellulose compared to the β-1,4
bonds in linear forms of starch.
The β-1,4 bonds result in a
polymer with both inter and
intra molecular hydrogen bonding
and a repeat unit which is a
dimer. X-ray diffraction studies show
the supramolecular and
crystalline structure of
the cellulose is organized into unit
cells with hydrogen bonding
believed to be confined within
sheets of cellulose chains.
Hydrophobic interactions
perpendicular to the sheets are
thought to help hold the sheets
together. Native cellulose thus
has a high cohesive energy which
makes it water insoluble
relative to starch (Neveil
and Zeronian, 1985; French, 1985).

Studies on cotton cellulose
and lignocellulose suggest that
hydrolysis kinetics will be
affected by the surface area
available for reaction (Rowland and
Bertoniere, 1985; Lin et
al., 1985), as well as cellulose
crystallinity. Surface area can be
increased by adding particulate
to give a higher
surface area per unit volume,
and/or by disrupting the
supramolecular and crystalline
structure to give an internal
porosity within the cellulose
particle which is large enough
to facilitate diffusion of the
enzyme into the particle.
Cellulose with internal "pore"
dimensions of approximately 60
angstroms or larger enables
penetration of fungal cellulases
with molecular weights in the
range of 60 kda (Grethlein,
1985; Stone et al., 1989).
In
either case, a major change in
surface area will correspond to a change in the perceived crystallinity of the cellulose. Unlike starch or cellulose, lignocellulose has an additional barrier to hydrolysis - lignin. Lignin is closely associated with both the macromolecular plant cell wall structure as well as the microscopic cellulose crystalline structure of biomass materials.

Lignin imparts resistance to hydrolysis of cellulose regions with which it is associated even though these would otherwise be accessible to cellulytic enzymes, i.e., susceptibility is reduced. Explosive depressurization of wood (Aspen) impregnated with 500 to 1500 psig steam removes the lignin (a potentially valuable co-product of biomass conversion) without radically altering the crystallinity of the resulting cellulose (Bungay, 1982; Nesse, et al., 1977). Increased susceptibility of the cellulose may help to explain the 85% conversion achieved upon subsequent enzyme hydrolysis. Similar effects were obtained for wheat straw at enzyme levels of 80 to 100 FPU/g substrate (Vallender and Eriksen, 1985).

This paper describes results for a lignin-free, microcrystalline cellulose, Avicel(R). This material has well defined properties, including an average particle size of 30 microns (wet basis) and a leveling off degree of polymerization (LDP) of about 200. Microcrystalline cellulose is a useful model substance for probing the effects of different pretreatments on the physical structure of cellulose.

Hydrolysis of Sulfated Cello-oligosaccharides

Celldextrins and sulfated cello-oligosaccharides are obtained by dissolving 100 grams of Avicel in 50 ml of H2SO4 at 4 C for 7 minutes. Water is added and the slurry is heated to 70 C. This is followed by addition of 100 mL ethanol at 4 C and activated carbon to remove color bodies. The resulting slurry is filtered. Next, 14 L ethanol is added to the filtrate. A white precipitate, containing celldextrins and sulfated cello-oligosaccharides, is formed and recovered by centrifugation (Pereira et al., 1988).

Celldextrins are only sparingly soluble at a degree of polymerization (DP) above 6 (molecular weight of 991 D) (Huebner et al., 1978). However, if the cello-oligosaccharides are sulfated to give 1 sulfate per 3 to 5 glucose units, a solubility in excess of 100 g/L is obtained even if the DP exceeds 6. This illustrates the effectiveness of an intercalating agent, e.g., sulfate, in keeping the structure open to the point that the cellulose dissolves. The derivatized cello-oligosaccharides lose the sulfate groups when heated in water at 120 C for several minutes. The resulting decrease in pH is sufficient to cause rapid formation of water soluble cellobiose which, in turn, is completely converted to glucose by cellulase enzymes at pH 4.8 and 50 C in a subsequent hydrolysis step (Mobedshahi, 1987; Pereira et al., 1988).

Sulfation of Avicel(R) can be carried out in strong acid in order to swell the cellulose and make it accessible. Since the sulfation reaction is strongly inhibited by the presence of water, the cellulose must be at low moisture. A practical process thus requires a drying step for agricultural residues and other biomass feedstocks which typically contain at least 40 % moisture. The sulfuric acid must be recycled for both economic and environmental reasons. This can be readily achieved using ion exclusion (Neuman et al., 1986) or other means.

A low temperature sulfuric acid process has been developed.
and piloted for saccharification of corn residue (stalks) based on the use of sulfuric acid for both pretreatment and hydrolysis with an intermediate drying step (Tsao et al., 1978; Bliekowski et al., 1984; Ladisch, 1989; Barrer, 1991). This approach is also compatible with enzyme hydrolysis, although wet processing techniques which avoid an intermediate drying step and minimize extraneous reagents are more attractive.

**Enzyme Hydrolysis of Microcrystalline (Insoluble) Cellulose**

Microcrystalline cellulose was sieved (dry) to give a material with an initial particle size of greater than 53 microns. A quantity of 2.5 grams was then suspended in 48 mL of 50 mM citrate buffer, pH 4.9. Cellulase enzyme (Cyclase CL, Genencor International, 30 GCU/mL), 2 mL, was added and the mixture incubated under constant agitation at 50°C. A control experiment was also run in which the enzyme solution was replaced with 2 mL of buffer. Wet particle size, particle surface characteristics, and conversion to glucose were monitored as a function of time using a particle size analyzer (Malvern 2600C), scanning electron microscope (in the Materials Engineering Department), and glucose analyzer, respectively. The cellulose samples for scanning electron microscopy were prepared by simply drying them under vacuum.

The average initial particle size of the wet cellulose (69 microns) decreased to 23 microns within 5 minutes after the reaction had been started. Part of this decrease is due to the agitation or other factors as indicated by a separate control study in which the particle size decreased from an initial value of 80 microns to 60 microns after 1 hour. The data clearly show that this enzyme preparation causes a significant decrease in cellulose particle size during the initial part of the hydrolysis reaction. Scanning electron microscopy illustrates a typical particle at the beginning of the reaction. (see Figure 1(a)).

The hydrolysis rapidly proceeds to 25% conversion during the first 180 min while the average wet particle size remains essentially unchanged after its initial drop to the 20 micron range (Figure 2(a)). In a separate run at the same conditions, the hydrolysis was monitored over a period of 6 days (Figure 2(b)) during which 82% conversion was obtained. The average particle size at the end of this run was still 20 microns. Scanning electron micrographs indicated that some of the remaining cellulose particles had a splintered appearance (Figure 2(b)). A third hydrolysis experiment, carried out for 11 days, gave 83% conversion to glucose. A glucose conversion of 80 to 85% appears to represent the upper limit at this concentration of enzyme. The known inhibitory effects of cellulose and glucose are a likely factor in the decreasing rate of the reaction, particularly as the glucose concentration approaches 42 g/L at the end of 6 days.

The leveling off of particle size suggests the hypothesis that enzyme hydrolysis of the microcrystalline cellulose is dominated by a tunneling mechanism. We postulate that the enzyme complex attacks the cellulose by penetrating into the interior of the particle. Once a large extent of hydrolysis occurs, and a significant fraction of the particle's interior is depolymerized, a high internal porosity results. Eventually this leads to a decrease in the particle's structural integrity causing the particle to break apart. The average size would thus appear to remain essentially constant until the
Figure 1. SEM of microcrystalline cellulose at 800x magnification (Scale bar = 10 microns): (a) Control sample at t = 0; (b) hydrolyzed cellulose after 143 hours of incubation with enzyme at pH 4.8 and 50 C.
particle disintegrates. In comparison, if hydrolysis of the solid cellulose were to occur by erosion at the outer surface, a gradual decrease in particle size would be expected. This has not been observed. Further experiments are needed to test our proposed explanation of the leveling off in particle size (abbreviated LOPS) during the enzyme hydrolysis of lignin free cellulose.

Solubility of Microcrystalline Cellulose in Sodium Hydroxide

The solubility of microcrystalline cellulose in aqueous sodium hydroxide is known and was used in the development of a procedure for the gel permeation chromatography of non-derivatized cellulose (Bao et al., 1980). We carried out further experiments on the solution properties of microcrystalline cellulose at 5% concentration in 5, 10, 20, and 30 % NaOH (Yang et al., 1991). Three temperatures were examined: ambient, 6 C and -10 C.

There was little change in the cellulose at ambient temperature, even after 3 days, although the sodium hydroxide solutions developed a yellow color. Gelatinization in NaOH occurs at temperatures below ambient. Swelling and the onset of gelatinization increased with increasing NaOH concentration up to a maximum level of about 20 % and was lower at 30 % NaOH. At 6 C and 10 % NaOH, the cellulose began to resemble a gel after 24 hours and became opaque after 3 days. A similar result was obtained in 20 % NaOH, while at 30% a slight precipitate was observed in the gel. At -10 C, an opaque gel was formed in 5, 10, and 20 % NaOH after 24 hours, although the gel in the 20 % NaOH had a yellow hue indicating that degradative reactions were likely occurring. At 30 % NaOH the gel contained a precipitate after 24 hours and large particles after 3 days.
The results show that sodium hydroxide functions as an intercalating agent. However, it must either be removed or neutralized prior to enzyme hydrolysis. These experiments also indicate that both osmotic effects (proportional to NaOH concentration) and entropic effects (reflected by increased swelling with decreasing temperature) may be parameters which affect cellulose swelling by NaOH.

Water Treatment of Microcrystalline Cellulose

Water is known to be an active agent in the cooking of pulp, and can cause undesirable gelatinization in pulp production (Aronovsky and Gottner, 1930; Michel et al., 1957). Prior research on the role of water in cellulose transformations shows that partial cellulose dissolution may occur above 200°C between pH 7 and 8 (Michel et al., 1957). These results form the starting point of our research. As in other pretreatments, the goal of cellulose swelling and gelatinization using water is to increase both accessibility and susceptibility of the cellulose to enzyme hydrolysis.

The development of conditions which give reproducible swelling of microcrystalline cellulose at temperatures above 200°C was initially carried out in 5.5 mL high pressure, stainless steel reaction tubes filled with an aqueous slurry of cellulose. The tubes were heated by submerging them in a preheated sandbath (Technne). Heating of a 16.8% cellulose slurry in deionized water at 190°C for 4 hours gave a swollen form of cellulose having an apparent volume of up to 4 times that of freshly suspended material. At these conditions, hydrolysis is minimal. This result led to the choice of 190°C for preheating the cellulose sample prior to initiating high temperature pretreatment at 220 to 230°C. Conditions need to be controlled since the acidity of water can become a significant factor in chemically degrading the cellulose at temperatures above 200°C.

This pretreatment has been scaled up to a 150 mL volume in a microprocessor controlled, high pressure, stirred tank batch reactor (Autoclave Engineers). This reactor has enabled preparation of 100 mL volumes of swollen cellulose. This is an important step in our research since we can now prepare sufficiently large quantities of pretreated cellulose to enable further study with respect to its physical and chemical changes, and response to enzyme hydrolysis. These small scale runs confirm that the cellulose swells 5-10 fold over its original volume and that the swollen cellulose is stable for periods of up to an hour. This stability is a necessary condition for further characterizing and characterizing the effect of aqueous cooking of cellulose as a pretreatment. We hope that this will help to foster the development of a practical process for obtaining readily saccharified cellulose in water.

Conclusions

Pretreatments using aqueous based approaches show that complete solubilization of cellulose is attainable when sulfate or sodium hydroxide is used as the intercalating agent. However, added reagents are needed to achieve this effect. Consequently, an alternate approach using water alone is being studied. Water has been
found to cause major swelling when cellulose is cooked for short periods of time at temperatures above 200 C. Hydrolysis of untreated microcrystalline cellulose gives in excess of 80% conversion to glucose when incubated with a fungal cellulase system at pH 4.8 and 50 C for 6 days. The cellulose particle size decreases to 20 microns during the first few minutes of the reaction, and then remains at this size. This phenomenon of leveling off of particle size (LOPS) suggests the hypothesis that the enzyme system penetrates the particle, and disrupts the internal cellulose structure.

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