

Cellulose Hydrolysis Using Zinc Chloride as a Solvent and Catalyst

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ABSTRACT

Cellulose gel with <10% of crystallinity was prepared by treatment of microcrystalline cellulose, Avicel, with zinc chloride solution at a ratio of zinc chloride to cellulose from 1.5 to 18 (w/w). The presence of zinc ions in the cellulose gels enhanced the rate of hydrolysis and glucose yield. The evidence obtained from X-ray diffraction, iodine absorption experiments; and Nuclear Magnetic Resonance spectra analysis suggested the presence of zinc-cellulose complex after Avicel was treated with zinc chloride. Zinc-cellulose complex was more susceptible to hydrolysis than amorphous cellulose. Under the experimental condition, cellulose gels with zinc ions were hydrolyzed to glucose with 95% theoretical yield and a concentration of 14% (w/v) by cellulases within 20 h. The same gel was hydrolyzed by acid to glucose with 91.5% yield and a concentration of 13.4% (w/v).

Index Entries: Cellulose; zinc chloride; cellulases; hydrolysis; zinc-cellulose complex.

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INTRODUCTION

As an orderly structured polymer, crystalline cellulose is more difficult to hydrolyze to its monomeric form (glucose) than other biopolymers. Hydrolysis of cellulose can be achieved by either cellulases or acids. Because of their small molecular size, protons can penetrate the crystalline structure of cellulose to achieve a pseudo-first-order sequential reaction resulting in a higher rate of conversion than enzymatic hydrolysis. The major disadvantage of acid hydrolysis as compared with enzymatic hydrolysis is that a high temperature and pressure are required to achieve a high rate of conversion resulting in high rate of byproduct formation.

Normally, cellulose hydrolysis occurs through the adsorption of catalysts to cellulose followed by the formation of catalyst-cellulose complex. However, cellulose in crystalline state is more resistant to hydrolysis than in amorphous state (1) because of the limited adsorption sites of crystalline cellulose to catalysts. According to Fan and Lee (2), the extent of chemical and enzyme adsorbed to cellulose can be increased almost linearly if the specific surface area is increased. The effectiveness of hydrolysis is also dependent on the initial crystallinity of cellulose, i.e., the lower the initial crystallinity index of cellulose, the higher the extent of conversion to sugars (3). Thus, the key to enhance the rate of cellulose hydrolysis lies in the increasing the surface area and/or decreasing its initial crystallinity.

There are methods to render cellulose more susceptible to hydrolysis, especially to enzymatic hydrolysis (4). These methods either decrease the degree of polymerization of cellulose and/or alter its physical configuration. They include physical treatments, such as milling and steam explosion, and chemical treatments by solvents, acids, alkali, and oxidation agents. Among chemical agents, zinc chloride is known to be an effective swelling agent of cellulose (5). Under the 60–70% concentration range, zinc chloride can also dissolve native cellulose and reduce the crystallinity of cotton. Using zinc chloride under weak acidic condition, low-DP water-soluble and insoluble celluloses could be prepared from native cellulose. The resulting modified cellulose can be hydrolyzed to glucose either by enzymes or acids effectively to produce a high concentration of glucose (6). Moreover, the recycle of zinc chloride is an inexpensive technology used in the vulcanized paper industry. Therefore, we believe that zinc chloride is a better cellulose pretreatment agent than other chemicals.

In the previous work, it was noticed that the presence of zinc ions in the cellulose gel treated by zinc chloride can enhance the rate of cellulose hydrolysis as well as improve the glucose yield (7,8). In this work, we are trying to determine if the formation of zinc-cellulose complex after zinc chloride treatment is responsible for the enhancement of cellulose hydrolysis. Zinc ion was known to form complex compounds with sugars and oligosaccharides (9,10). Our work focused the effect of zinc ions on cellulose hydrolysis by acid and enzymes after zinc chloride pretreatment, and

the existence of zinc-cellulose complex by the aids of X-ray diffraction, NMR analyses, and analysis of iodine adsorption of pretreated cellulose.

MATERIALS AND METHODS

Materials

Microcrystalline cellulose (Avicel, pH 101 from FMC, Newark, DE) was used as the pure cellulose material. Technical-grade zinc chloride was obtained from Ashland Chemical Company. Cellulase derived from *Trichoderma viride* (TAP, 800 U/g) and cellulase derived from *Aspergillus niger* (AP, 400 U/g) were obtained from Enzyme Development Corp. One unit of cellulase activity in this experiment is defined as the release of 1 μ mol of glucose in 1 h at pH 5.0 and 48°C. Cellobiose and D₂O were purchased from Sigma Chemical Company (St. Louis, MO).

Methods

Preparation of Zinc-Cellulose Complex

One hundred milliliters of zinc chloride solution ranging from 64 to 70% (w/w) were added to 10 g of cellulose at 65°C while stirring. After 30 min, the cellulose solution was poured into ethanol, and zinc-cellulose complex was precipitated, recovered, and washed with ethanol and water. Residual water was then removed by a hydraulic press at 100 psi. The water content of cellulose gels was 83–85%. The procedures for the preparation of cellulose gels are summarized in Fig. 1.

Preparation of Amorphous Cellulose

Amorphous cellulose was prepared from zinc-cellulose complex by washing cellulose complex thoroughly with water until the residual zinc ion remaining in cellulose gel was below 0.1% (w/w, based on dry wt). The samples of zinc-cellulose gel were taken after each washing and exhibited different zinc ion contents.

Cellulose Hydrolysis

Enzymatic hydrolysis of cellulose: One gram of cellulase (TAP) and 1 g of cellulase (AP) were first dissolved in 4 mL of acetate buffer solution (0.05N, pH 5.0). The enzyme solution was then added to 100 g of wet cellulose gel in 250-mL flasks. Small glass beads were added to the slurry to provide agitation in the thick slurry. The slurry was then incubated in a shaker at 450 rpm and 48°C. Samples were taken at intervals and centrifuged. The glucose concentration in the supernatant was measured by a glucose analyzer (Beckman Instrument Inc.). The zinc ion concentrations in gel and supernatant were measured by the Atomic Absorption Spectrophotometer (2380, Perkin-Elmer) after the samples were ashed.

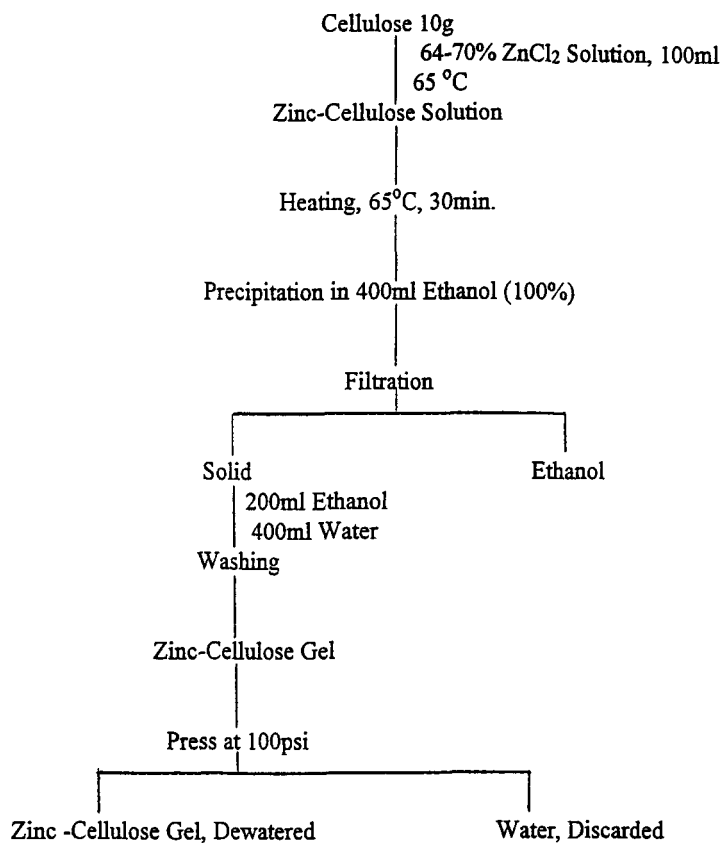


Fig. 1. The procedure of the preparation of cellulose gels.

ACID HYDROLYSIS OF CELLULOSE

One hundred grams of wet cellulose gel were mixed with 100 mL HCl aqueous solution (0.1%, w/v) in a 500-mL flask equipped with mechanical stir. The slurry was incubated in a water bath at 85 °C. Samples were taken at intervals and centrifuged, and the glucose and zinc ion concentrations in the samples were determined.

¹³C-NMR Spectra

Cellobiose was dissolved in zinc chloride solution. One sample was prepared at 25 °C, and another was prepared at 65 °C for 2 h. The samples were then cooled to room temperature. ¹³C-NMR spectra were taken on a Nicolet 50 MHz NT-200 NMR spectrometer equipped with GENT 1180 computer program. Solvent was D₂O solution containing dioxane at 67.4 ppm related to TMS. According to the standard ¹³C-NMR spectrum of Sabtler Research Laboratories, a complete assignment of all the C signals of cellulose has been achieved.

Table 1
Ratio of Zinc Chloride to Cellulose and Yield of Enzymatic Hydrolysis

Ratio ^a	18.0	9.0	5.0	3.0	1.5
Yield ^b	92.6	88.5	88.1	87.6	87.8

^a Ratio: Zinc chloride to cellulose (w/w).

^b Enzymatic hydrolysis at 48°C for 40 h, based on glucose yield (% w/w).

X-ray Powder Diffractogram

Wide-angle X-ray diffraction experiments were conducted using SIEMENS D500. All the samples had been vacuum-dried before the determination in order to eliminate the disturbance by water.

RESULTS AND DISCUSSION

Mole Ratio of Zinc Chloride to Cellulose

Zinc chloride is a solvent of cellulose; the amounts of zinc chloride required can be reduced drastically without decreasing the susceptibility of cellulose gels to enzymatic hydrolyses. Table 1 shows the results of cellulose hydrolysis by enzymes using samples prepared by different concentrations of zinc chloride. There are small differences among samples with different zinc chloride to cellulose ratios.

Effect of Zinc Content in Cellulose Gel on the Hydrolysis Rate

Cellulose gels with different zinc ion contents were subjected to either enzyme or acid hydrolysis, using microcrystalline cellulose (Avicel) as reference. Figure 2 shows the effect of zinc ion on the hydrolysis of cellulose gels by cellulases. When untreated microcrystalline cellulose was used as substrate, only about 10% was hydrolyzed to glucose after 80 h of incubation. In contrast, the pretreated cellulose gels by zinc chloride were more susceptible to enzymatic hydrolysis because of the disruption of crystalline structure in cellulose. There was a direct relationship between amount of residual zinc ions and degree of susceptibility toward cellulases hydrolyses. For example, the initial hydrolysis rate of cellulose gel with 1.2% of residual zinc ions (w/w, based on wet wt) was fourfold faster than the sample with trace amount of residual zinc ions. At 1.2% of zinc ions, cellulose gel was hydrolyzed completely within 20 h, and produced about 14% (w/v) of glucose with 95% of theoretical yield. Since zinc ions had no effect on the cellulase activity at low concentration until zinc ion concentration in aqueous solution was above 1.4% (w/v, Fig. 3),

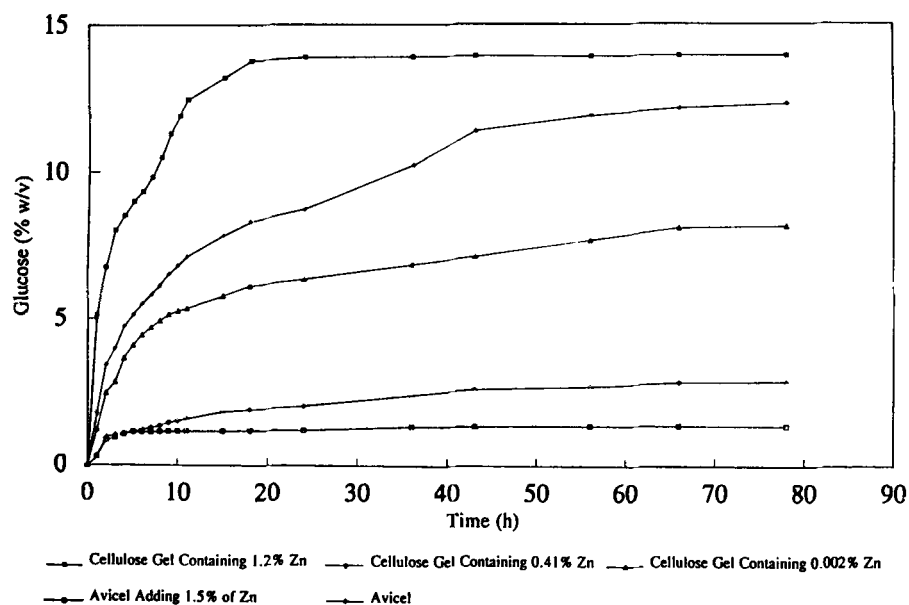


Fig. 2. Effect of zinc content of cellulose gels on enzymatic hydrolysis.

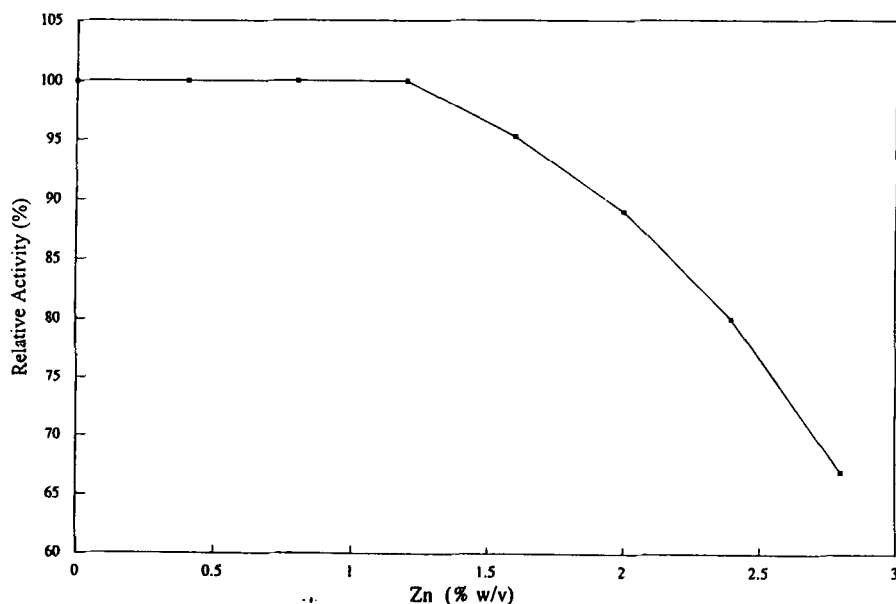


Fig. 3. Effect of zinc concentration on cellulase (TAP) activity.

the increase in rate of hydrolysis of cellulose gel was not the result of the change of enzymatic activity, but the special role of zinc ions on cellulose molecules. Hence, the change of crystalline structure in cellulose was a precondition of increasing hydrolysis rate of cellulose gels. Zinc chloride pretreatment not only rendered the cellulose more susceptible to enzymatic hydrolysis, but also enhanced the hydrolysis rates. Similar results were obtained using acid hydrolysis (Fig. 4).

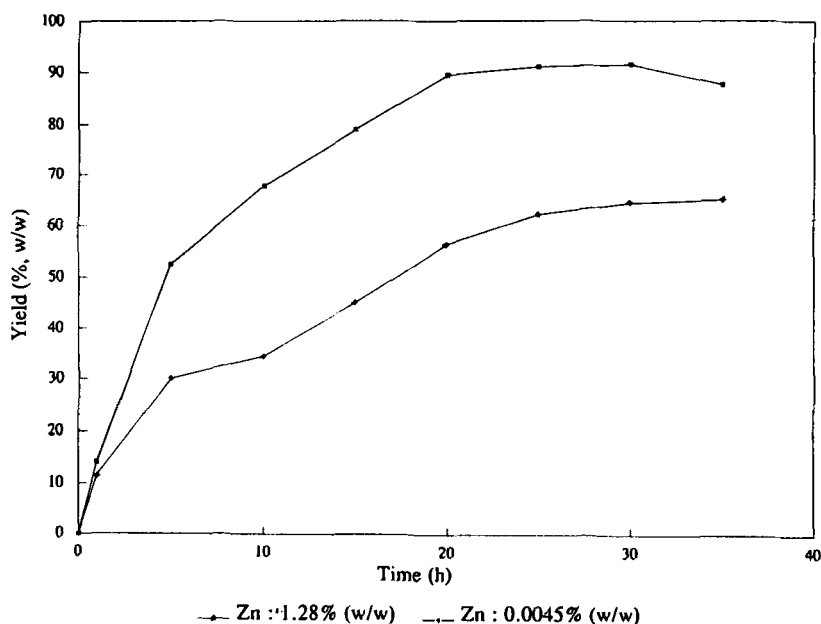


Fig. 4. Effect of zinc content of cellulose gel on acid hydrolysis.

Formation and Function of Zinc-Cellulose Complex

As expected, the disruption of crystalline structure of cellulose molecules after zinc chloride treatment increased the susceptibility of cellulose toward enzyme and acid.

However, the fact that a high level of zinc in cellulose gel rendered the higher hydrolysis rate was unexpected, because the enzymatic activity started to decline if 1.5% of free zinc ion was present in the buffer solution. This unexpected result led to a speculation that zinc-cellulose complex was more susceptible to enzyme and acid than the amorphous cellulose. As shown in Fig. 5, the sample containing 0.08% of zinc ion (w/w, based on dry wt; if based on wet wt, it is 0.0045% of zinc ion) had the same crystallinity as the sample containing 8.6% of zinc ion (w/w, based on dry wt; based on wet wt it is 1.28% of zinc ion). Both samples existed mostly in amorphous state and contained about 10% crystalline region, which was similar to ball-milled cellulose.

All cellulose gel samples, in spite of their difference in residual zinc ion content, adsorbed iodine to a similar degree and produced deep-blue color by iodine staining procedures. The formation of deep-blue color again indicated that the cellulose gels obtained after zinc chloride treatment existed as amorphous cellulose. When samples were subjected to enzymatic digestion, the results again showed the direct relationship between zinc ion content and susceptibility toward hydrolyses (Table 2). Thus, the possibility existed that zinc ions could have in some ways interacted with cellulose to form zinc-cellulose complex after zinc chloride pretreatment, and the complex was more susceptible than the amorphous cellulose.

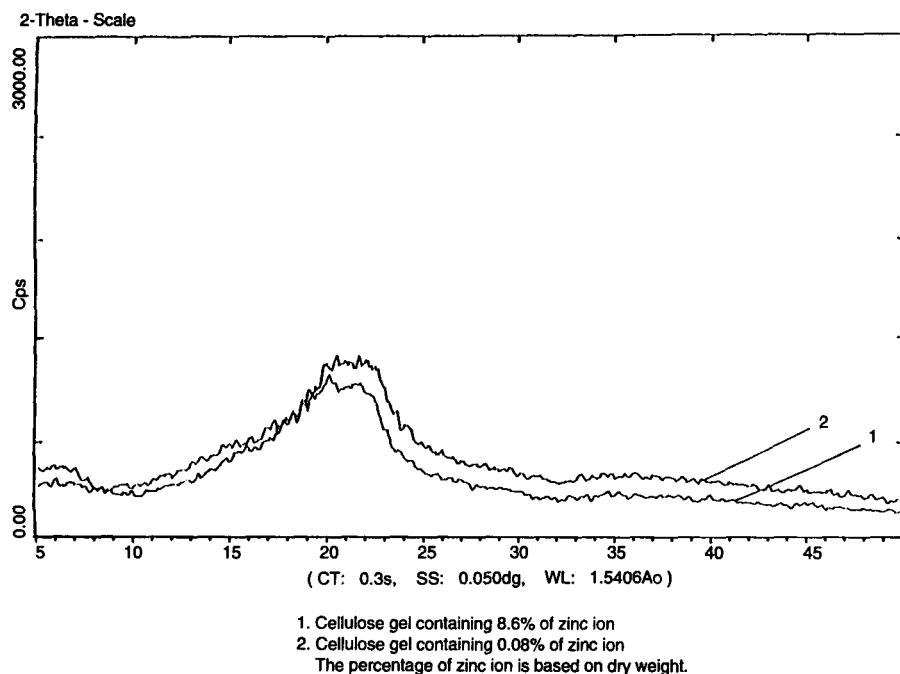


Fig. 5. X-ray powder diffractogram of cellulose gels.

Table 2
 Zinc Content and Iodine Adsorption of Cellulose Gel

Sample	Zn, %, w/w ^a	I ₂ /G, mol/mol ^b	Yield, % w/w ^c
1	1.4	0.075	95.0
2	1.28	0.072	94.3
3	0.41	0.067	76.2
4	0.11	0.065	62.5
5	0.09	0.06	55.7
6	0.0045	0.06	53.6
Avicel	—	0.008	12.6

^aCellulose gel zinc content, based on wet wt.

^bMole iodine absorbed/mol of glucose in cellulose gel.

^cEnzymatic hydrolysis yield, based on glucose at 48°C for 40 h.

The interaction of zinc ion with cellulose can be further elucidated using Nuclear Magnetic Resonance (¹³C-NMR) method. We used cellobiose (a basic cellulose unit) as model compound to show the possible interaction between zinc ion and cellulose. As shown in Fig. 6, ¹³C-NMR spectra of cellobiose after treatment with zinc chloride show the configuration transition and chemical shift. In addition to C₆, the chemical shift changes

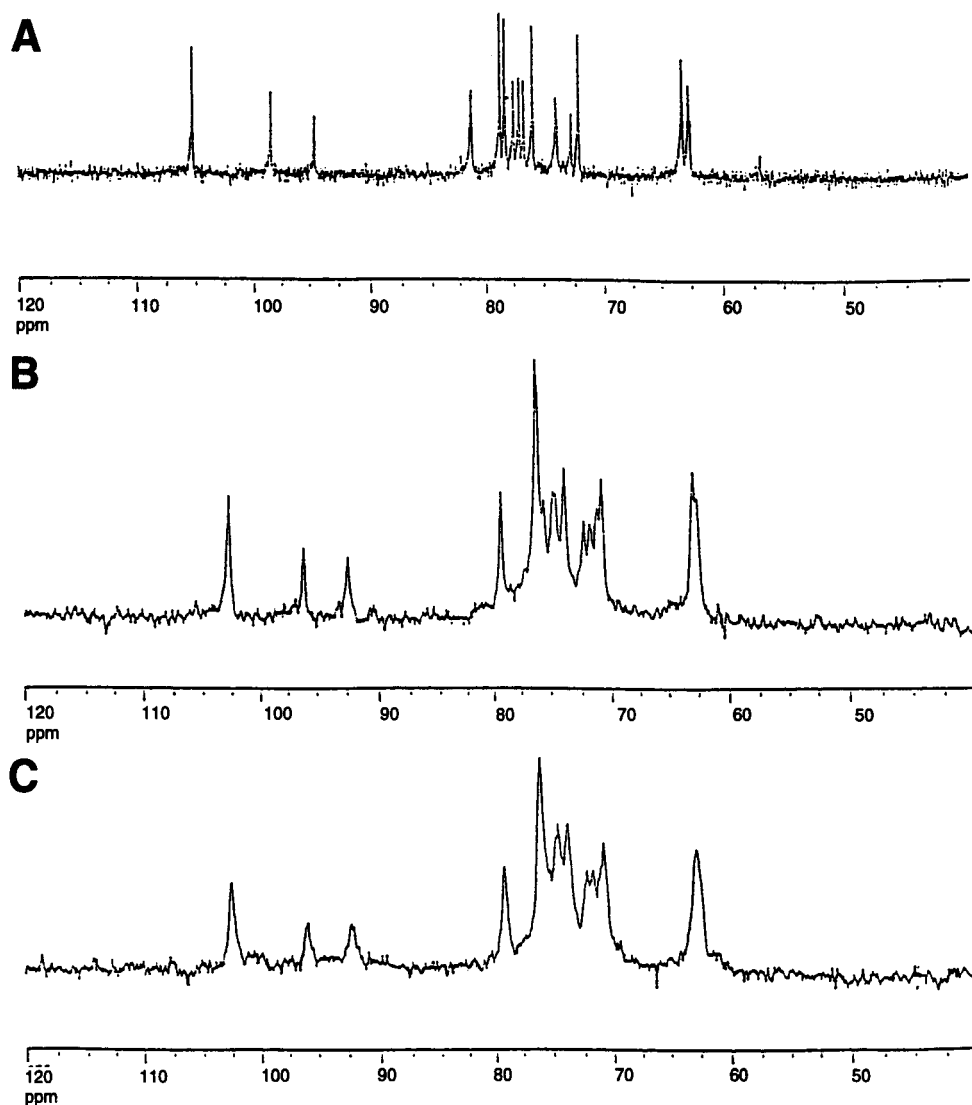


Fig. 6. ^{13}C -NMR of cellobiose (A) and zinc-cellobiose at 25°C (B) and 65°C (C).

of other atoms are between 2 and 4 ppm, particularly C_1 , and C_4 ; the widening of all peaks was observed. The conformational changes could be the result of the interaction between zinc ions and hydroxyl groups to form complex so that it causes the result of the glycoside bonds to be more susceptible to hydrolysis and, in turn, enhances the rates of hydrolysis and glucose yield. Our work also showed the zinc-cellulose complex was stable over a temperature treatment range from ambient to 85°C.

CONCLUSION

Decrease in crystallinity of cellulose can be achieved using a low concentration of zinc chloride treatment. The resulting cellulose can be hydrolyzed readily to produce a high concentration of glucose. Based on the results described, zinc ions can interact with cellulose to form zinc-cellulose complex, which is more susceptible to hydrolysis than amorphous cellulose. The procedure for the preparation of zinc-chloride-treated cellulose is simple and effective. Zinc chloride is stable with low toxicity, and can be recovered and reused economically. Therefore, using zinc chloride to treat cellulose for the production of an inexpensive substrate for fermentation industries is a possibility.

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