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# THE HYDROTHERMOLYSIS OF CELLOBIOSE AND ITS REACTION-PRODUCT D-GLUCOSE

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## ABSTRACT

The hydrothermolysis of cellobiose in the range 180–249° has been studied. Kinetic analysis of the reaction showed that 60% of the cellobiose is converted into D-glucose, and 40% into other products. The rate ($k_1$) of cellobiose disintegration is approximately eight times that ($k_2$) of D-glucose. Thus, hydrothermolysis differs from acidic hydrolysis. Hydrothermolysis is not dependent on pH, at least in the range 3–7.

## INTRODUCTION

The treatment of carbohydrates with pure water at elevated temperatures has attracted only sporadic interest[1–3]. Most of the experiments involved autoclaves, and there was significant formation of reactive degradation-products (*e.g.*, hydroxymethylfurfural, furfural, and methylglyoxal) after a relatively short reaction-time. As soon as it was shown that the yield of monomeric sugars from cellulose could be substantially increased by the use of a dynamic process[4–8], research on the hydrolysis of polysaccharides with pure water was intensified, including investigation of pretreatment of the biomass with steam[9,10].

In our dynamic process (hydrothermolysis), continuous elution with water rapidly transports to the cooling units that part of the biomass which has been solubilised, thus reducing the extent of further degradation. By this method, >90% of the plant matter (*e.g.*, wood, straw, and paper) can be solubilised. At ~200°, hemicellulose is decomposed into soluble oligomers (together with a fraction of easily dissolvable lignin); at 270°, cellulose is largely converted into D-glucose, and, at >300°, the remaining lignin is also degraded[11–13].

Biomass research has been aided by the development of improved analytical methods[14–16]. However, a comparison of hydrothermolysis with acidic hydrolysis and alkaline degradation of carbohydrates has not yet been fully investigated[17–21]. We now report on the hydrothermolysis of cellobiose (4-*O*-β-D-glucopyranosyl-D-glucopyranose) involving static experiments in stainless-steel autoclaves.

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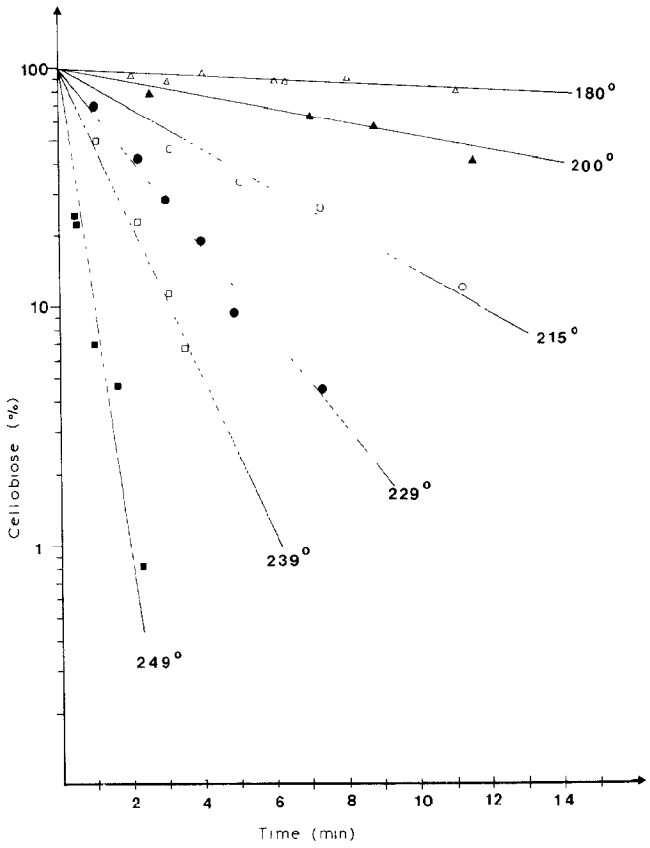



Fig. 1. Hydrothermolysis of cellobiose in the range 180–249°.

## RESULTS AND DISCUSSION

The hydrothermal treatment of cellobiose, carried out in the range 180–249°, involved a first-order reaction (Fig. 1). At 180°, there was only a slight reaction of cellobiose, but, at 249°, >90% of cellobiose had reacted after 1 min. Fig. 2 shows the formation of D-glucose from cellobiose. In the range 200–215°, the final yield of D-glucose was 50%; at 229°, 239°, and 249°, maxima of 40–51% were obtained after 1–5 min. However, D-glucose also decomposes quickly under these conditions.

The reaction rate-constants for cellobiose, calculated from Fig. 1, are plotted logarithmetically against the inverse absolute temperature in Fig. 3. According to the Arrhenius equation, an activation energy of 136.0 $kJ.mol^{-1}$ (32.5 $kcal.mol^{-1}$) was computed for the hydrothermolysis of cellobiose.

Figs. 1 and 2 show that cellobiose (Cel) and D-glucose (Glc) disintegrate according to a first-order mechanism. The maximum yield of D-glucose of ~50% in-

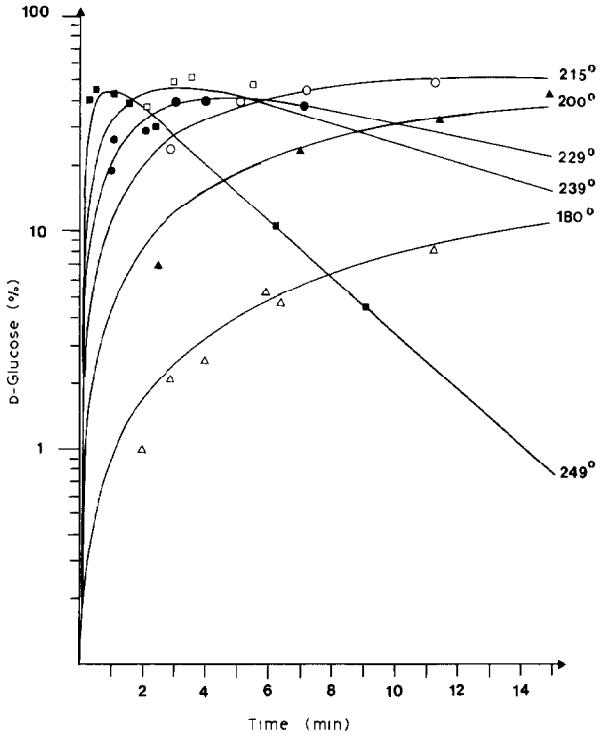



Fig. 2. Formation of D-glucose during the hydrothermal treatment of cellobiose in the range 180–249°. The curves were calculated according to equation *6*.

dicated that, in addition to D-glucose formation, the cellobiose is involved in at least one other reaction. The following, simplified reaction scheme can be assumed:

$$\text{Cel} \xrightarrow{k_1} \begin{cases} \xrightarrow{k_3} \text{Glc} \xrightarrow{k_2} \text{degradation products} \\ \xrightarrow{k_4} \text{degradation products} \end{cases} \qquad (1)$$

The overall rate of reaction of cellobiose ($k_1$) is therefore

$$k_1 = k_3 + k_4. \qquad (2)$$

According to equation *1*, the D-glucose concentration is given by

$$\frac{d[\text{Glc}]}{dt} + k_3[\text{Cel}] - k_2[\text{Glc}], \qquad (3)$$

which integrates to

$$[\text{Glc}]_t = \frac{k_3}{k_2 - (k_3 + k_4)} [\text{Cel}]_o (e^{-(k_3+k_4)t} - e^{-k_2 t}) + [\text{Glc}]_o e^{-k_2 t}. \qquad (4)$$

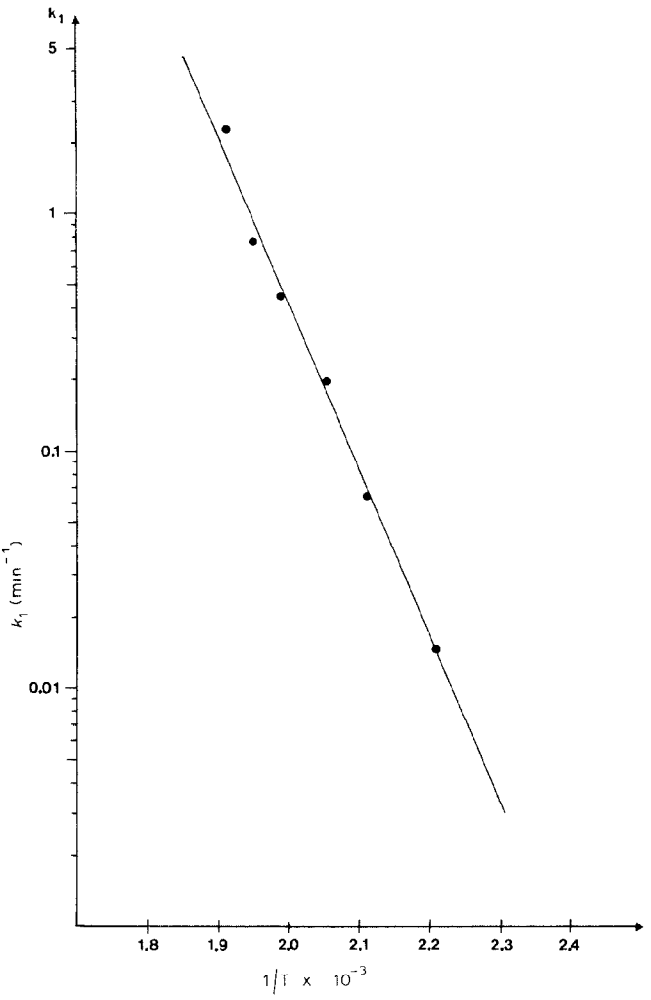



Fig. 3. Arrhenius plot for the hydrothermal reaction of cellobiose.

It is assumed that $k_4/k_3$ = constant = $k_a$, so that equation *2* can therefore be written as

$$k_1 = k_3(1 + k_a) = \frac{1}{H} k_3. \tag{5}$$

By inserting this expression and equation *2* into equation *4*, and at the same time cancelling the last term because at the beginning of the reaction $[Glc]_o = 0$, equation *4* becomes

$$[Glc]_t = \frac{k_1 \cdot H}{k_2 - k_1} [Cel]_o (e^{-k_1 t} - e^{-k_2 t}). \tag{6}$$

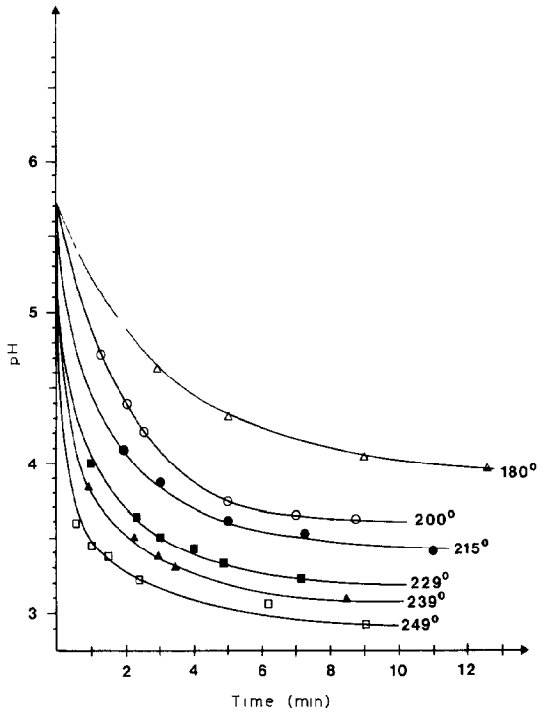


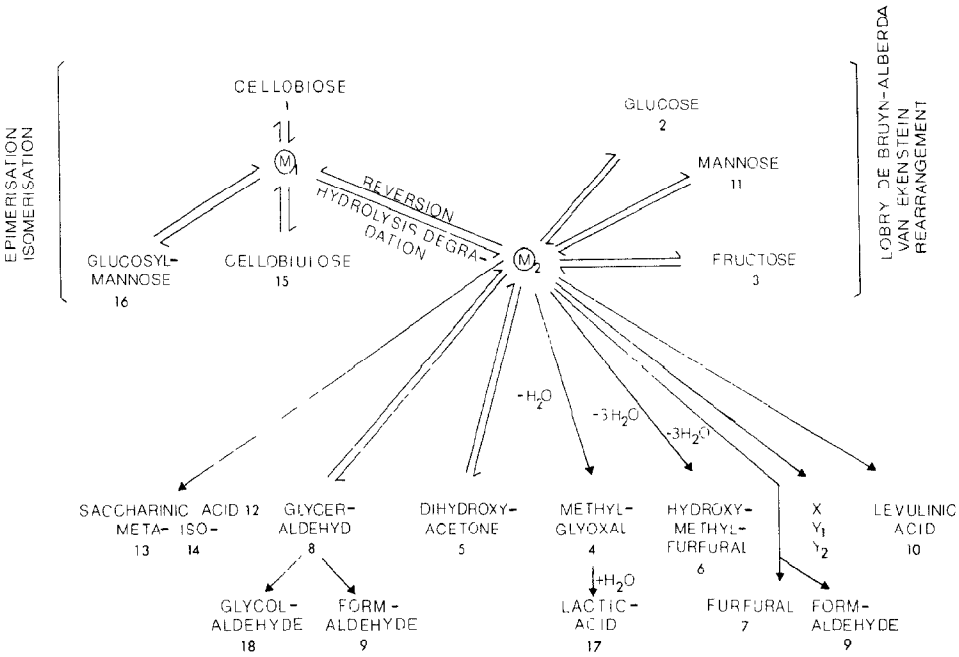

Fig. 4. Values of pH for cellobiose solutions treated hydrothermally in the range 180–249°.

The experimental values of $k_1$ are given in Fig. 1. The values of $k_2$ were obtained from the data shown in Fig. 2. The 249° curve, for example, gives a relation for $k_1/k_2$ (2.3/0.3) of 7.7. Similar experiments involving acid hydrolysis of cellobiose gave [29] $k_1/k_2$ ratios of 50–80.

By an iteration method, a value for H of 0.60 was obtained which corresponded well with the experimental results at all temperatures. From this H value and equation 5, a $k_3/k_4$ ratio of 1.50 is obtained. Therefore, the probability that cellobiose hydrolyses to give two D-glucose molecules is 50% higher than the simultaneous reaction path of cellobiose to degradation products.

*The pH-dependency of hydrothermolysis in the degradation of cellobiose.* — The pH values of the reaction solution were measured after each experiment, and Fig. 4 shows them as a function of time at degradation temperatures in the range 180–249°. The starting pH was 5.7. At 180°, the pH decreased to 4 within 12 min, whereas, at 249°, it fell to 2.92 after 9 min. The difference in pH units (1.7 and 2.78, respectively) means that a 50–600-fold increase of $[H^+]$ has little effect on the reaction rate (see Fig. 1 ). Even if the marked decrease in pH in the first minute is neglected, there are still changes in $[H^+]$ between 3.6 and 16.4. These changes would be associated with marked changes in reaction rate if hydrothermal degradation followed the same $H^+$-dependency as acid hydrolysis.

*Comparison of hydrothermal degradation with acidic and alkaline hydrolysis.* — In order to obtain further insight into the relevant reaction steps, the known facts about the decomposition of cellobiose and D-glucose were compiled in Scheme 1. Cellobiose (**1**) is in equilibrium with cellobiulose (**15**) and D-glucosyl-mannose (**16**), presumably through an intermediate ($M_1$). Alkaline conditions usually promote rapid isomerisation and epimerisation[22,23]. Through hydrolysis and degradation, an assumed second intermediate ($M_2$) is formed, and subsequently D-glucose (**2**), D-mannose (**11**), and D-fructose (**3**). At the same time, an equilibrium between these three sugars occurs by the Lobry de Bruyn–Alberda van Ekenstein rearrangement. A certain amount of dimeric sugars can be attributed to reversion.




Scheme 1. Products associated with the hydrothermal, alkaline, and acidic degradation of cellobiose.

A series of degradation products[24,25] is formed from $M_2$, namely, saccharinic acids (**12–14**), glyceraldehyde (**8**), dihydroxyacetone (**5**), methylglyoxal (**4**), lactic acid (**17**), hydroxymethylfurfural (**6**), furfural (**7**), formaldehyde (**9**), unknown products (X, $Y_{1,2}$), and levulinic acid (**10**).

Fig. 5 shows an h.p.l.c. fractionation of the products formed on treatment of D-glucose with alkali. The formation of acids is strong enough to neutralise the base (0.01M NaOH) after a few minutes and terminate the reaction. At this stage, there is a strong peak for fructose (**3**), mannose is below the detection limit (0.6 g/L), methylglyoxal (**4**) and dihydroxyacetone (**5**) are present, but not glyceraldehyde, furfural, or the saccharinic acids (see Scheme 1). Thus, the reaction of glyceraldehyde and dihydroxyacetone cannot occur, and the possible reaction of dihy-

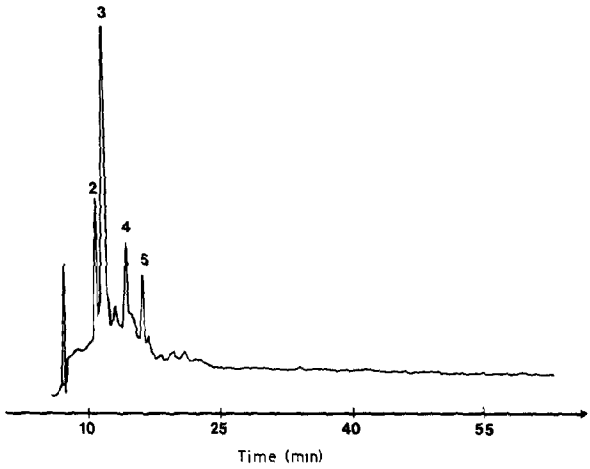



Fig. 5. H.p.l.c. after degradation of D-glucose with 10mM NaOH at 80° for 10.45 min: 2, glucose; 3, fructose; 4, methylglyoxal; and 5, dihydroxyacetone.

droxyacetone and methylglyoxal is still to be proved. Lactic acid can be formed[26] from methylglyoxal or even from saccharinic acids.

The situation changes markedly if D-glucose is degraded hydrothermally (Fig. 6). Hydroxymethylfurfural (**6**) and furfural (**7**) are the main products. As expected, the isomerisation to D-fructose (**3**) is less than under alkaline conditions.

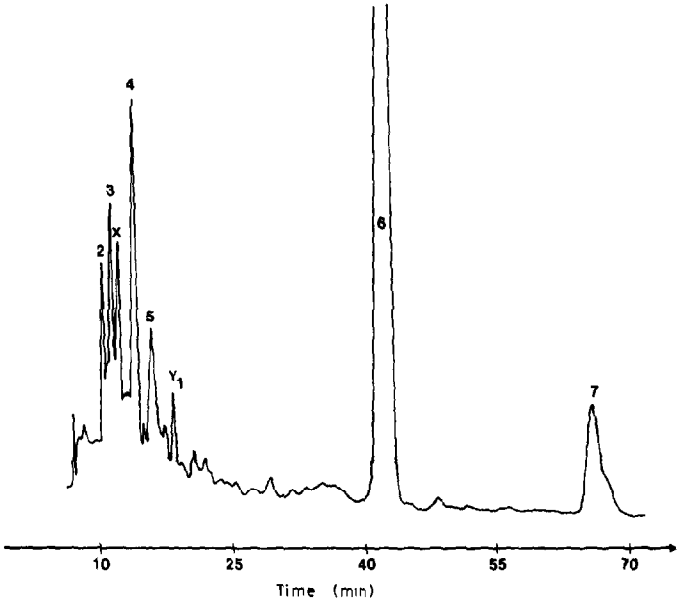



Fig. 6. H.p.l.c. after degradation of D-glucose hydrothermally at 230° for 1.40 min: 2, glucose; 3, fructose; 4, methylglyoxal; 5, dihydroxyacetone; 6, hydroxymethylfurfural; and 7, furfural.

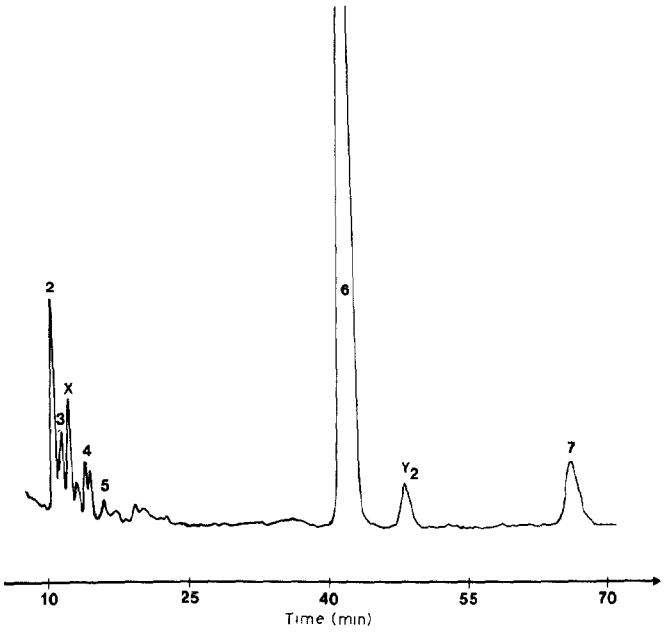



Fig. 7. H.p.l.c. after degradation of D-glucose with 5mM $H_2SO_4$ at 230° for 1 min: 2, glucose; 3, fructose; 4, methylglyoxal; 5, dihydroxyacetone; 6, hydroxymethylfurfural; and 7, furfural.

A relatively high concentration of methylglyoxal (**4**) develops, as well as two unknown substances (X and $Y_1$) (see Scheme 1). The formation of acid is much lower than in the alkaline treatment and, even at the highest temperature, a $[H^+]$ of only 1.2mM is reached; in the alkaline solution, 10mM NaOH is neutralised within a few minutes.

In the acidic degradation of D-glucose (Fig. 7), little isomerisation to D-fructose (**3**) occurs, little methylglyoxal (**4**) and very little dihydroxyacetone (**5**) are formed (see Scheme 1). The greater part of the D-glucose is transformed into hydroxymethylfurfural (**6**) and furfural (**7**). In addition to $Y_1$, another unknown compound ($Y_2$) is detected. According to the literature[27], levulinic acid appears only as a reaction product at acid concentrations >M.

## EXPERIMENTAL

*Materials and methods.* — Stainless-steel autoclaves (volume 8 mL) containing solutions (4 mL, 1 g/100 mL) of cellobiose were heated in an oil bath. The heating-up period, which was deducted from the total reaction time, reached 105 s at 260°. The reaction was quenched in an ice–water mixture.

*Analytical methods.* — Cellobiose, D-glucose, and by-products were determined by h.p.l.c. and t.l.c. H.p.l.c. was performed directly on samples using a column packed with silica gel containing chemically bound amino groups and aceto-

nitrile–water (75:25) at 2 mL/min. A refractive index detector was used[14] (Knauer 6100 UV/RI). For hydroxymethylfurfural and furfural, an RP-C 18 column was used together with methanol–water (70:30) and a u.v. detector at 254 nm (Spectra Physics SF 770). Assessment of D-glucose degradation (Figs. 5–8) by h.p.l.c. involved[28] an Aminex HPX 87 H column (BioRAD) at 40° and 5mM $H_2SO_4$ at 0.5 mL/min.

T.l.c. was performed on Polygram SIL G/UV 254 (Macherey Nagel) with 2-propanol–ethyl acetate–water (5:10:7); detection was effected with phenol–sulphuric acid (98%)–ethanol (3 g, 5 mL, 95 mL) at 110° for 10 min and photometric evaluation using a Shimadzu scanner (CS 920).

The pH was determined before and after the degradation reaction.

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