Monatshefte für Chemie 116, 961—971 (1985)

Monatshefte für Chemie
Chemical Monthly

The Alkaline Degradation of Cellobiose to Glucose and Fructose

G. Bonn[a], H. Binder[a], H. Leonhard[b], and O. Bobleter[a,*]

[a] Institute of Radiochemistry, University of Innsbruck, A-6020 Innsbruck, Austria

[b] Institute of Physical Chemistry, University of Innsbruck, A-6020 Innsbruck, Austria

(Received 19 September 1984. Accepted 21 January 1985)

The alkaline degradation of cellobiose by 0.01 to 0.1 *N* NaOH at temperatures between 60 and 85 °C was studied. At the same time the formation of glucose and fructose was analyzed. At lower alkali concentrations, the production of acidic compounds lowered the *pH* to such an extent that the hydrolyzing reaction ceased within a relatively short time. At higher alkali concentrations the *pH* change is much smaller and first order reaction rates of the cellobiose degradation were obtained. Through the application of a simplified reaction mechanism the consecutive reactions of the glucose and fructose formation were evaluated mathematically.

(Keywords: Alkaline degradation; Carbohydrates; Kinetic studies)

Alkalischer Abbau von Cellobiose zu Glucose und Fructose

Der alkalische Abbau von Cellobiose wurde mit 0.01 *N* bis 0.1 *N* NaOH im Temperaturbereich von 60 bis 85 °C untersucht. Gleichzeitig wurden die gebildete Glucose und Fructose analysiert. Bei niedrigen Alkalikonzentrationen wird der *pH* Wert stark herabgesetzt, sodaß es innerhalb kurzer Reaktionszeiten zu einer Beendigung der Reaktion kommt. Bei höheren Alkalikonzentrationen ist die *pH*-Abnahme wesentlich geringer und der alkalische Abbau von Cellobiose gehorcht einer Reaktion 1. Ordnung. Durch die Anwendung eines vereinfachten Reaktionsschemas konnten auch die Folgereaktionen der Glucose- und Fructosebildung mathematisch erfaßt werden.

* Dedicated to Prof. Dr. *H. Tuppy* on the occasion of his 60th birthday.

Introduction

Interest in alkaline degradation of cellulose and hemicellulose is about as old as the manufacture of paper and the mercerisation of cotton. More recently, alkaline biomass treatment is gaining in importance for the production of animal fodder[1,2]. A precise understanding of the behaviour of cellulose in alkaline solutions is also relevant for examinations comparing this process with hydrothermolysis[3–6], acid hydrolysis[7] and the enzymatic decomposition of plant biomass[8].

A comprehensive survey of the reactions of cellulose with aqueous alkali is given by *P. M. Molton* and *T. F. Demmitt*[9]. When regenerated cellulose is treated with 1% NaOH, trioses, tetroses, pentoses, low molecular neutral compounds, acids and lactones are found to be present[10]. The use of solid alkali and higher temperatures (5% NaOH, 180 °C) favour the production of acids, e.g. isosaccharinic acids, lactic acid and formic acid[11]. Aqueous alkaline degradation of low molecular carbohydrates has been described in the temperature range between 25–40 °C[12–14].

The reaction of biomass with alkali at high temperatures (300 °C) yields up to 78 by-products and can be used for the production of oil-type substances as energy-carriers[15–17].

In the present work cellobiose, 4-(β-*D*-glucopyranosyl)-*D*-glucopyranose, was selected as the starting compound to obtain information on its reaction behaviour and to compare these results with alkaline hydrolysis data of other carbohydrates and with different hydrolysis processes.

The degradation of cellobiose and the formation of glucose and fructose were measured in the range 60–85 °C applying low alkaline conditions. Furthermore, the change in *pH* values and the kinetic assessment of the reaction were important criteria for a thorough understanding of the transformation mechanism.

Experimental

Materials and Methods

The degradation of cellobiose was carried out in stainless steel autoclaves (316 SS) with a volume of 8 cm^3. The autoclaves were filled with 4 cm^3 of the standard solution, which contained 1 g/100 cm^3 cellobiose (Merck, Darmstadt, FRG) and were 0.1 *N*, 0.05 *N*, 0.01 *N* respectively in NaOH. The autoclaves were immersed in an oil-bath and, after the chosen reaction time, cooled in an ice-bath. The temperature was measured with a thermocouple which had been placed inside one of the autoclaves for each series of tests. The heating-up time was up to 90 seconds, which was deducted from the total time to obtain the actual reaction time. The alkaline hydrolysis was carried out with 0.01 *N* NaOH at 66 °C, 70 °C and 85 °C, with 0.05 *N* NaOH at 60 °C and 70 °C and with 0.1 *N* NaOH at 60 °C, 70 °C and 80 °C.

Analytical Methods

For cellobiose, glucose and fructose, as well as certain by-products to be analyzed, high performance liquid chromatography (HPLC) and thin layer chromatography (TLC) were used.

HPLC: All samples were analyzed directly without any pretreatment. For the sugar analyses a column was packed with silica gel containing chemically bound amino groups. As mobile phase a mixture of acetonitrile and water (75 : 25) was chosen, the flow rate being 2 ml/min. A refractive index detector (R. I.) was used [18] (Knauer 6100 UV/RI). The analysis of the by-products hydroxymethylfurfural (*HMF*) and furfural was accomplished with an RP-C 18 column. A mixture of methanol and water (70 : 30) as mobile phase and a UV detector at 254 nm (Spectra Physics SF 770) served for this purpose.

TLC: TLC plates (Polygram SIL G/UV 254, Macherey Nagel) were employed and 10 μl of the reaction solution was analyzed. The mobile phase was a mixture of isopropanol, ethyl acetate and water (5 : 10 : 7). The quantitative photometric analysis was effected with a TLC—chromatogram scanner Shimadzu, Kyoto, Japan (CS 920) [19].

The pH values were taken before and after the degradation reaction.

Results and Discussion

Degradation of Cellobiose with 0.01 N NaOH

As Fig. 1 *a* shows, in the first few minutes substantial degradation of cellobiose as well as the formation of glucose and fructose take place. Then the reaction ceases.

At temperatures between 66 and 85 °C, a consumption of 40 to 57% of the original cellobiose occurs after a reaction time of 24 minutes. At the same time only 1 to 2% of fructose and approximately 10% of glucose are formed. The 70 °C curves were also measured, but not plotted for the sake of clarity.

Hydroxymethylfurfural (*HMF*) and furfural, which appear as by-products in hydrothermolysis and acid hydrolysis, could, in alkaline hydrolysis, only be found as traces at a higher alkali concentration (0.1 *N* NaOH, 70 °C). The formation of other by-products such as methylglyoxal (2-oxo-propanal), glyceraldehyde and acids, e.g. formic acid, acetic acid, lactic acid, glycolic acids, saccharinic acids is, however, very marked.

The acidic products are therefore responsible for the decrease in *pH*. By measurement of the *pH* it is shown that the alkaline hydrolysis of cellobiose comes to a standstill when, depending on the temperature, a particular *pH* level is reached (Fig. 1 *b*).

Degradation of Cellobiose with 0.05 N NaOH

As shown in Fig. 2 *a*, under these reaction and temperature conditions the conversion of cellobiose and the formation of glucose and fructose

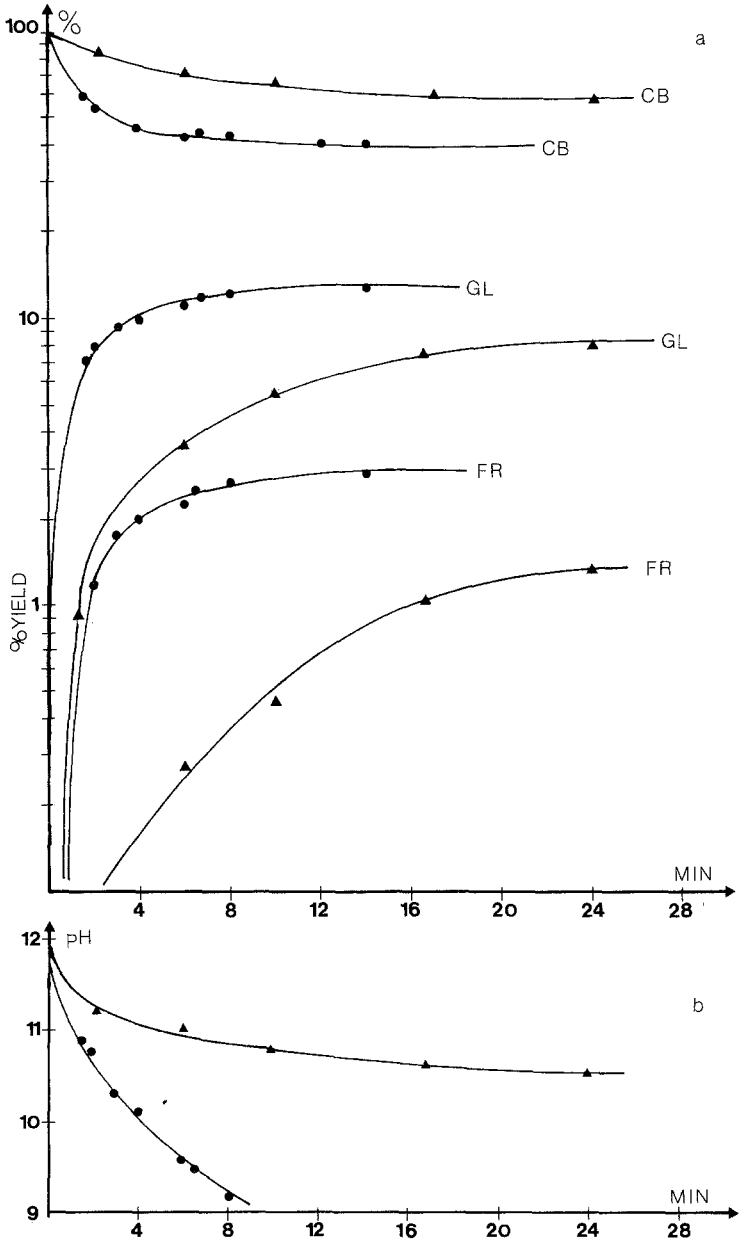

Fig. 1 *a*. Degradation of cellobiose (*CB*) with 0.01 *N* NaOH at 66 °C (▲), 85 °C (●) and formed glucose (*GL*) and fructose (*FR*) at 66 °C (▲), and 85 °C (●). The degradation rate in percent is plotted versus the reaction time in minutes

Fig. 1 *b*. *pH*-values of the degradation of cellobiose with 0.01 *N* NaOH at 66 °C (▲), 85 °C (●). The *pH*-value is plotted versus the reaction time in minutes

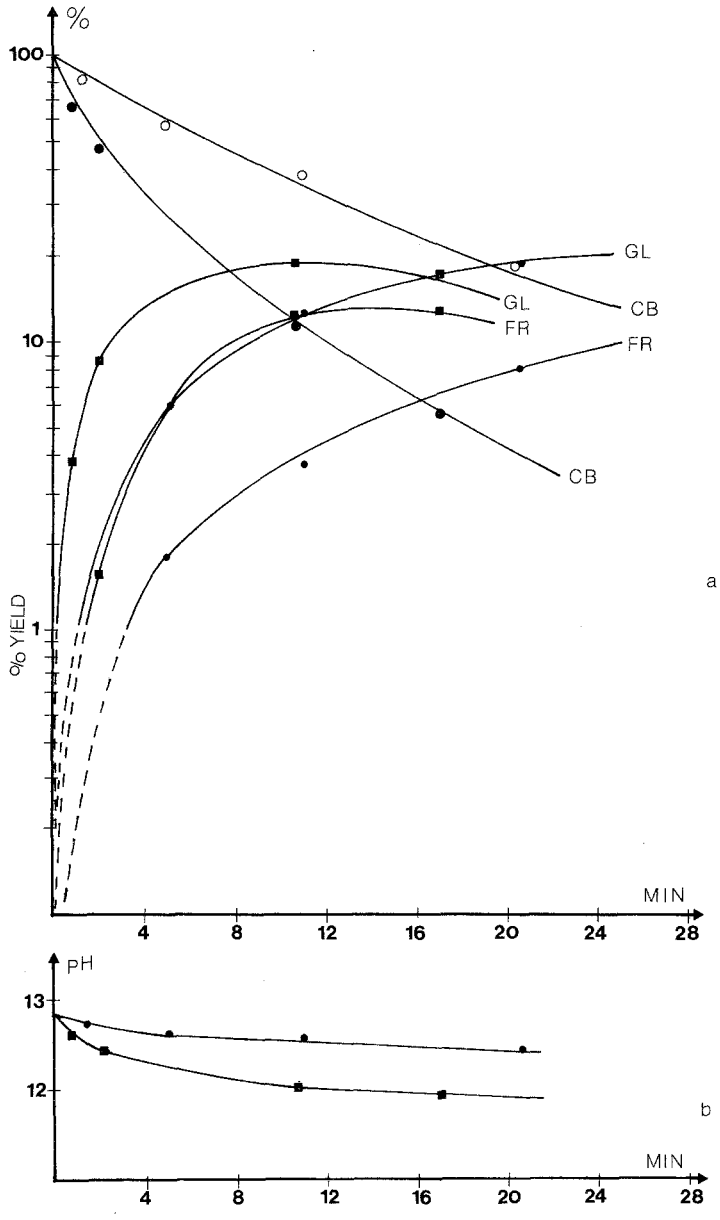

Fig. 2 *a*. Degradation of cellobiose (*CB*) with 0.05 *N* NaOH at 60 °C (○) and 70 °C (●) and formed glucose (*GL*) and fructose (*FR*) at 60 °C (●) and 70 °C (■). The degradation rate in percent is plotted versus the reaction time in minutes

Fig. 2 *b*. *pH*-values of the degradation of cellobiose with 0.05 *N* NaOH at 60 °C (●) and 70 °C (■). The *pH*-value is plotted versus the reaction time in minutes

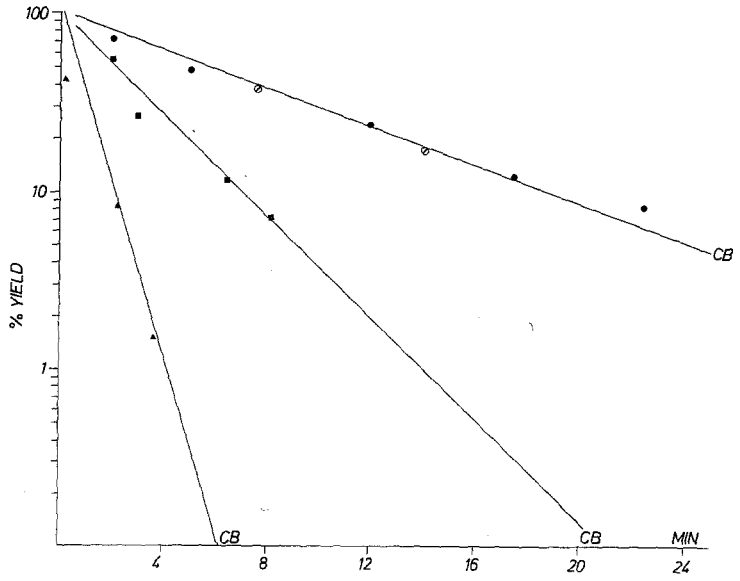

Fig. 3 a. Alkaline degradation of cellobiose (*CB*) with 0.1 *N* NaOH (first order reaction) at 60 °C (●), 70 °C (■) and 80 °C (▲). 60 °C after elimination of oxygen (He, H_2) cellobiose (CB) (⌀). The degradation (yield in percent) is plotted versus the reaction time in minutes; plotted curves obtained by iteration

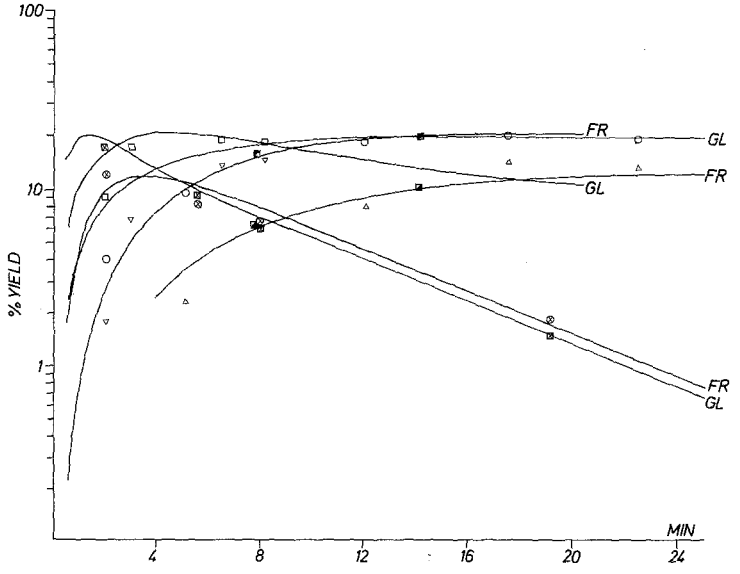

Fig. 3 b. Formation of *D*-glucose (*GL*) and *D*-fructose (*FR*) during alkaline treatment of cellobiose (*CB*) at 60 °C, glucose (○), fructose (△), 60 °C after elimination of oxygen (He, H_2) glucose (◩), fructose (◪), 70 °C glucose (□), fructose (▽), 80 °C glucose (⊠), fructose (⊗). The formation, yield in percent of the initial cellobiose, is plotted versus the reaction time in minutes. The calculated results are given in the solid curves

persist throughout the whole measuring period. After 10 minutes and at 70 °C, maxima of glucose and fructose (with approximately 30% yield) are observed (Fig. 2 *b*).

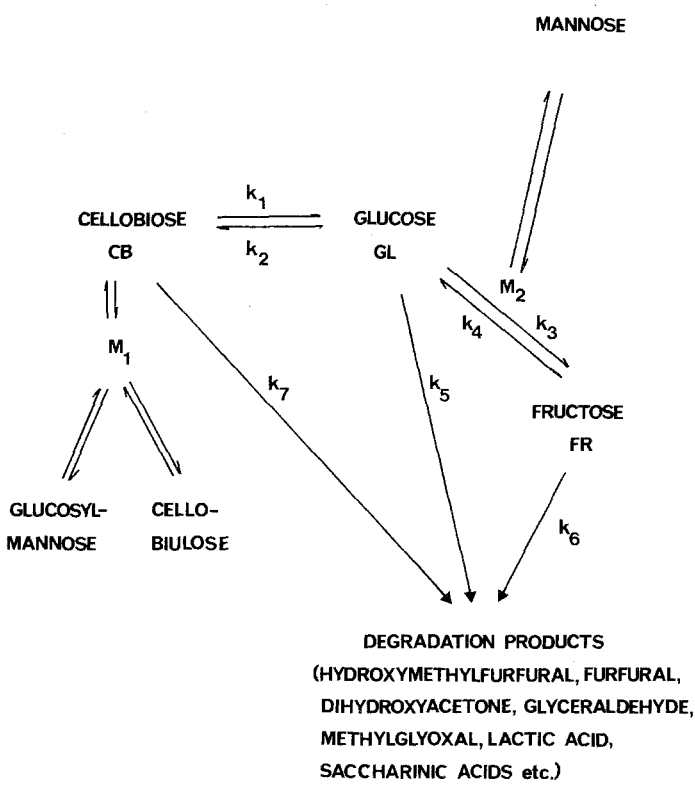

Fig. 4. Reaction scheme of the alkaline degradation of cellobiose

Degradation of Cellobiose with 0.1 N NaOH

In the experiments with 0.1 *N* NaOH the *pH* was lowered only from 13 to 12.75 at all three temperatures investigated until 90% of the cellobiose was consumed. An average alkali concentration could be assumed for these experiments corresponding to a *pH* of approximately 12.9. This relative *pH* stability is the necessary condition for obtaining clear first order reaction mechanisms. In Fig. 3 *a* the consumption of cellobiose is given. Fig. 3 *b* shows the yield of the reaction products, *D*-glucose and *D*-fructose.

To ensure that the oxygen present in the reaction vessel does not

influence the reaction, a further series of experiments was carried out at 60 °C (0.1 *N* NaOH), the reaction vessels having been rinsed with nitrogen and helium before use. The corresponding values are shown in Fig. 3 *a* and *b*; there was no deviation, either in sugar concentrations or in *pH*, from the experiments performed without oxygen exclusion.

Table 1. *Rate constants k (min^{-1}) obtained by iteration*

T°C	60	70	80
k(min^{-1})			
k_1	0.05	0.13	0.45
k_2	0.01	0.01	0.01
k_3	0.10	0.15	0.45
k_4	0.15	0.07	0.35
k_5	0.0001	0.005	0.07
k_6	0.0002	0.01	0.18
k_7	0.085	0.21	0.8
$k_1 + k_7$	0.135	0.34	1.25
k_{exp}	0.128	0.34	1.36

A simplified reaction model (Fig. 4) is proposed, in which the intermediate states of cellobiose-cellobiulose-glucosylmannose (M_1) are neglected, and the glucose(GL)-fructose(FR)-mannose rearrangement over the intermediate state (M_2) (*Lobry de Bruyn-van Ekenstein*) is characterized by only 2 reaction steps (k_3 and k_4). By mathematical iteration[20] the first order reaction constants k_1 to k_7 were obtained (see Table 1). From these the yield curves were calculated for cellobiose, *D*-glucose and *D*-fructose and plotted as solid lines in Fig. 3 *a* and *b*. There is a good agreement with the experimental data. Cellobiose (CB) is disintegrated to lower-molecular products following the reaction:

$$\frac{d[CB]}{dt} = -k_1[CB] - k_7[CB] + k_2[GL] \quad (1)$$

Due to the low k_2 value, equation 1 can be approached by neglecting the reverse reaction $k_2[GL]$, which is responsible for the recombination of glucose into cellobiose,

$$\frac{d[CB]}{dt} \approx -(k_1 + k_7)[CB] \quad (2)$$

The experimentally obtained reaction constant for the cellobiose degradation (k_{exp}) should therefore be approx. equal to the mathematically evaluated constants ($k_1 + k_7$). In Table 1 these values are also given and

the good agreement proves that the simplifications in the reaction scheme are acceptable.

The evaluation of the *pH* curves is of special interest. In the case of the 0.01 *N* NaOH solutions (Fig. 1), most of the alkali is consumed during the 24 minutes reaction (Table 2). At the same time only a small amount of the cellobiose (initially 10 g/l) is transformed into glucose and fructose. On the assumption that lactic acid is the characteristic acidic compound of the degradation pathway only approximately 0.9 g/l is produced. Obviously

Table 2. NaOH *consumption and product formation after 20 minutes reaction time*

T °C	consumption NaOH (*N*)	consumption Cellobiose g/l	glucose + fructose g/l
0.01 *N* initial NaOH concentration			
66	0.0092	3.6	0.97
70	0.0094	4.9	1.29
85	0.0100	5.9	1.57
0.05 *N* initial NaOH concentration			
60	0.025	8.3	2.68
70	0.042	9.6	2.40
0.1 *N* initial NaOH concentration			
60	0.050	9.2	3.1
70	0.050	10.0	3.2
80	0.075	10.0	0.32

ca. 60% of the consumed cellobiose is transformed into other neutral compounds.

Using 0.05 *N* NaOH, the NaOH consumption was 0.025 to 0.042 *N* at the end of the reaction time (Table 2) and therefore the acid formation is markedly increased.

In the experiments with 0.1 *N* NaOH after 20 minutes reaction time 0.05 and 0.075 *N* of the alkali is neutralized. If the values at 80 °C are transformed into the assumed lactic acid production, 67.5% of the hexoses are converted into organic acids.

Two tendencies can be observed: with increasing temperatures hydrolysis to C-6 sugars increases and with increasing alkali concentration the yield of acids formed from carbohydrates grows. This is in agreement with *De Bruijn*[21] and coworkers who found a 100% acid formation at *pH* 14, with 50% lactic and 30% C-6 saccharinic acids.

Comparison with Hydrothermal and Acid Hydrolysis

The analysis of the by-products showed that in alkaline hydrolysis no, or only very little, hydroxymethylfurfural (*HMF*) and furfural are formed. These, however, are decisive by-products in hydrothermal and mineral acid degradation of cellobiose[22], which are usually carried out at higher temperatures, thus facilitating the elimination of water which yields *HMF* and furfural.

The isomerization rate (*Lobry de Bruyn-van Ekenstein* rearrangement) occurring during the build-up of the hexoses under alkaline conditions is significant. With 0.1 *N* NaOH solutions nearly equal glucose and fructose concentrations are obtained. Mannose was below our detection limit. The same isomerization also takes place with cellobiose where cellobiulose and glucosyl-mannose reach an equilibrium. These isomerizations are much less marked under hydrothermal, and negligible under acidic, conditions.

In the case of alkaline treatment of cellobiose no more than approx. 30% can be obtained as glucose and fructose. The remaining amount is directly converted into C-3 compounds, acids (e.g. saccharinic, formic, acetic, and lactic acid) and condensation products[23]. In hydrothermolysis an approximate 60% hexose conversion rate can be achieved[6]. The initial hexose production in acid hydrolysis is close to 100%, but due to the high sugar degradation rate, the maximum obtainable hexose yields usually remain below those of hydrothermolysis in technical installations.

Alkaline pretreatment renders plant matter (e.g. straw) more digestible to ruminants. Obviously, the accessibility of the enzymes can be increased by this process and inhibitory effects are minimized. Both effects can be explained by the extraction of the hemicellulose and a relatively large part of the lignin. In addition, *the Lobry de Bruyn-van Ekenstein* rearrangement may reduce the crystallinity of high molecular carbohydrates.

Comparing on this basis alkaline pretreatment with hydrothermolysis at approx. 200 °C, the results are in favour of the latter: hemicellulose and part of the lignin are extracted without the application of chemicals and are therefore available for further use in aqueous solution. The remaining cellulose fraction is at least as well enzymatically hydrolyzable as plant matter pretreated with alkali.

These experiments gave further evidence that alkaline, hydrothermal and acidic hydrolysis are three individual processes with characteristic differences.

Acknowledgement

The authors thank the Fonds zur Förderung der wissenschaftlichen Forschung (Vienna) for supporting this work.

References

[1] *Rexen F., v. Thomsen,* Anim. Feed Sci. Technol. **1**, 73 (1976).
[2] *Jackson M. G.,* Anim. Feed Sci. Technol. **2**, 105 (1977).
[3] *Bobleter O., Pape G.,* Austrian Pat. 263666 (1968).
[4] *Bobleter O., Binder H.,* Holzforschung **34**, 48 (1980).
[5] *Bonn G., Concin R., Bobleter O.,* Wood Sci. Technol. **17**, 195 (1983).
[6] *Bobleter O., Bonn G., Concin R.,* in: Alternative Energy Sources III (*Veziroglu,* ed.), Vol. 3, p. 323. Washington: Hemisphere Corp. 1983.
[7] *Seaman J. F.,* Symposium on Biomass as an non-fossil Fuel Source, Honululu, April 1–6, 1979.
[8] *Erikson K. E.,* Biotechnol. and Bioeng. **20**, 317 (1978).
[9] *Molton P. M., Demmitt T. F.,* Polym. Plast. Technol. Eng. **11**, 127 (1978).
[10] *Mayat N. S., Golova O. P., Nikolaeva I. I.,* Vysokomol. Soedin **5**, 873 (1963).
[11] *Machell G., Richards G. N.,* J. Chem. Soc. **1960**, 1924.
[12] *Russell J. A., Molton P. M., Landsman S. D.,* in: Alternative Energy Sources III (*Veziroglu T. N.,* ed.), Vol. 3. Washington: Hemisphere Corp. 1983.
[13] *Miller R. K., Molton P. M., Russell J. A.,* see Ref.[12].
[14] *Molton P. M., Miller R. K., Donovan J. M., Demmitt T. F.,* Carbohydr. Res. **75**, 199 (1979).
[15] *MacLaurin D. J., Green J. W.,* Can. J. Chem. **47**, 3957 (1969).
[16] *MacLaurin D. J., Green J. W.,* Can. J. Chem. **47**, 3947 (1969).
[17] *De Wit G., Kieboom A. P. G., van Bekkum H.,* Carbohydr. Res. **74**, 157 (1979).
[18] *Binder H.,* J. Chromatogr. **189**, 414 (1980).
[19] *Bobleter O., Pape G.,* Monatsh. Chem. **99**, 1560 (1968).
[20] *Frost A., Pearson R.,* Kinetic and Mechanism. New York: J. Wiley. 1953.
[21] *De Bruijn J. M., Kieboom A. P. G., van Bekkum H., van der Poel P.,* XIIth Int. Carbohydr. Symp., Utrecht, July 1–7, 1984, Abstracts p. 189, VONK Publishers.
[22] *Bobleter O., Bonn G.,* Carbohydr. Res. **124**, 185 (1983).
[23] *Bonn G. K., Pfeifer P. A., Hoermeyer H., Bobleter O.,* Fresenius Z. Anal. Chem. **318**, 30 (1984).