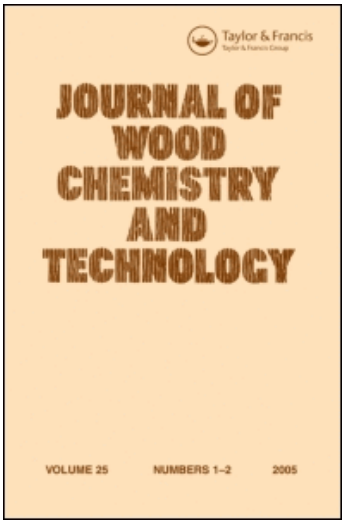

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

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To cite this Article Hörmeyer, H. F. , Bonn, G. , Kim, D. W. and Bobleter, O.(1987) 'Enzymatic Saccharification of Cellulosic Materials after Hydrothermolysis and Organosolv Pretreatments', Journal of Wood Chemistry and Technology, 7: 2, 269 — 283
To link to this Article: DOI: 10.1080/02773818708085267
URL: http://dx.doi.org/10.1080/02773818708085267

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 7(2), 269-283 (1987)

ENZYMATIC SACCHARIFICATION OF CELLULOSIC MATERIALS AFTER HYDROTHERMOLYSIS AND ORGANOSOLV PRETREATMENTS

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ABSTRACT

Different cellulose samples as well as lignocellulosic materials which had been pretreated by either organosolv or hydrothermolysis processes were tested as to their degradability by a crude cellulase preparation from Trichoderma viride. The glucose concentrations in hydrolysate samples were determined by HPLC. End-product inhibition of the cellulase complex by glucose and cellobiose was also studied, as were inhibitory effects caused by lignin- and hemicellulose-derived pretreatment side-products.

INTRODUCTION

Within the scope of research efforts aimed at utilizing cellulose-containing biomass for the produc-

tion of energy and chemicals, enzymatic degradation has, over the years, earned extensive attention (1-3). However, substrate pretreatment steps are very important in order to enhance the accessibility of cellulose to the cellulase complexes. The topochemical restrictions to be overcome include the presence of hemicellulose and encrusting lignin (4,5) as well as unfavorable pore size distribution and, eventually, crystallinity of the cellulose itself (6-8).

The processes designed to render substrates more accessible to the enzyme molecules can be grouped into physical methods - milling, irradiation - (9,10), chemical methods - reprecipitation from solvents; acidic, alkaline and oxidizing agents - (11-16), thermochemical processes - e.g. steam explosion, organosolv and hydrothermolysis - (17-22), and biological pretreatment by rot fungi (23,24).

Hydrothermolysis uses pressurized water instead of steam. In its first stage (at approx. 200 °C and 20-30 bar), the water, which is passed through a vessel containing the biomass material to be disintegrated, rapidly removes all hemicellulose and the soluble lignin from the reaction zone. The equipment available can also be used with mixtures of water and organic solvents, i.e. for both the hydrothermal and the organosolv process. In the present study, the residues

so obtained from several materials - which still contain all of their cellulose - have been tested with a commercial Trichoderma viride cellulase.

EXPERIMENTAL

Cellulase

In all experiments, a technical cellulase preparation of A.B.M. Chemicals Ltd. (Stockport, England), batch no. 3155, was used. This crude lyophilized culture broth of Trichoderma viride, which manifests high β-glucosidase activity, has a pH optimum at 4.0-5.0 and a temperature optimum at 45 °C. However, when cellulosic materials are present, temperatures up to 55 °C are easily tolerated. In the filter paper disintegration assay procedure specified by the supplier, each gram of the enzyme preparation liberates 7.0 mg glucose/min.

Substrates

a) Pure celluloses:

Cellulose, microcrystalline (Avicel(R)), Merck, Darmstadt, West Germany, batch no. 2313920

Cellulose, native, Merck, batch no. 1198536

Cellulose powder, Schleicher & Schüll, Dassel, West Germany

Cotton (from the card)

b) Raw materials for pretreatment:

Poplar wood (Populus deltoides); chemical composition: 41.9% glucan, 14.5% xylan, 21.8% lignin

Wheat straw (Triticum vulgare); chemical composition: 40.5% glucan, 20.9% xylan, 17.2% lignin

Pretreatments

a) Hydrothermolysis:

Percolation of 2-3 g of the raw material with 10 ml/min of deionized water for approx. 30 min (dynamic process); reaction cell volume: 10 ml.

b) Organosolv pretreatment:

Percolation of 2-3 g of the raw material with aqueous methanol (methanol/water ratios given in the respective figures); flow rate: 10 ml/min, time: approx. 30 min, reaction cell volume: 10 ml.

Buffer Solution

0.15 M acetic acid was adjusted to pH 5.0 with caustic soda (chemicals purchased from Merck).

Dry Weight Determinations

In order to relate saccharification yields to the absolute dry mass of each substrate used, weight loss of air-dry and moist substrates was determined after exposing them to 105 °C for 20-24 h.

Enzymatic Hydrolysis

From each substrate, an amount corresponding to ca. 100 mg (abs. dry), together with 25 mg of the cellulase, was placed in a screw-topped 20 ml polyethylene vial and suspended in 8 ml 0.15 M acetate buffer of pH 5.0. For inhibition studies, presumed inhibitors had been dissolved in the buffer in adequate concentrations. The vial was closed and, for the respective period of time, subjected to reciprocal shaking at 120 strokes/min in a water bath thermostated to 50 °C.

After incubation, 6 ml of deionized water was added and the suspension was centrifuged for 5 min at 3300 rpm. The glucose concentration in the supernatant was determined by HPLC. Samples which could not be analyzed immediately were frozen and kept at -17 °C.

Chromatographic Analysis

For glucose quantification, two HPLC systems were available. System A (25) was used for analyses pertaining to wheat straw and poplar wood degradation, system B (26) for those pertaining to the degradation of pure cellulose and inhibition studies.

System A. Column: amino-bonded phase Nucleosil 5-NH_2 (5 µm, Macherey & Nagel, Düren, West Germany), 250 x 4.6 mm I.D. (filled by the slurry-packing

technique; used without pre-column). Eluent: 1.5 ml/min of acetonitrile/water 77:23 (v/v) at 23 °C. Pump: Altex 110 A (Altex Sci., Berkeley/CA, USA).

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System B. Column: Ca-loaded ion-exchange material μ-Spherogel 7.5 (sulfonated polystyrene-divinylbenzene resin, 7.5% cross-linked, Beckman Inc., Berkeley/CA, USA), 300 x 7.5 mm I.D. (pre-packed). Pre-column: ion-exchange (Cation H) Micro-Guard cartridge (Bio-RAD Laboratories, Richmond/CA, USA). Eluent: 0.8 ml/min of water at 85°C (column oven compartment: Shimadzu Corp., Kyoto, Japan). Pump: Beckman 114 M.

In both cases a Waters R 401 refractive index detector was used (Waters Assoc., Milford/MA, USA), and samples were injected through the 20 ul loop of an Altex 210 injection valve.

From the glucose concentrations thus determined, saccharification yields were calculated according to the formula,

$$\% \text{ sacchar.} = \text{mg/ml glucose} \times 14 \text{ ml} \times \frac{100\ \%}{\text{mg substr.}} \times 0.9\ ,$$

where the factor 0.9 represents the molecular weight ratio of glucan monomers ($C_6H_{10}O_5$) to glucose ($C_6H_{12}O_6$). Therefore, throughout this study, extents of saccharification in percent are based on the dry mass of the substrates (pretreatment residues). In addition,

the yields of water insoluble fibers that make up the pretreatment residues are given in the appropriate figure captions. They, too, are on a dry weight basis.

RESULTS AND DISCUSSION

Cellulase Action on Different Types of Cellulose

For proper interpretation of the analytical results, one has to consider that, under the reaction conditions outlined in the preceding section, the crude cellulase preparation also liberates some glucose in absence of substrate. The time course of background glucose concentration *in situ* is given in Fig. 1. Thus, from any of the concentrations determined by HPLC, proportionate values for 14 ml final volume must be subtracted.

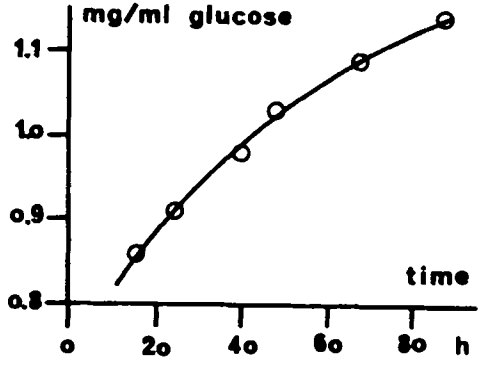

FIG. 1: Glucose formed from the cellulase preparation alone

In Fig.2, saccharification curves of some untreated cellulose samples are compared. The so-called native cellulose is much more accessible to enzymatic hydrolysis than the microcrystalline form, whereas the natural morphologic state prevailing in the cotton sample provides highest stability. After hydrolysis times of 50 hours and more, microcrystalline cellulose is only degraded to 3/4, cotton from the card to 3/10 the extent observed with native cellulose. Hence, native cellulose was used throughout the following inhibition studies.

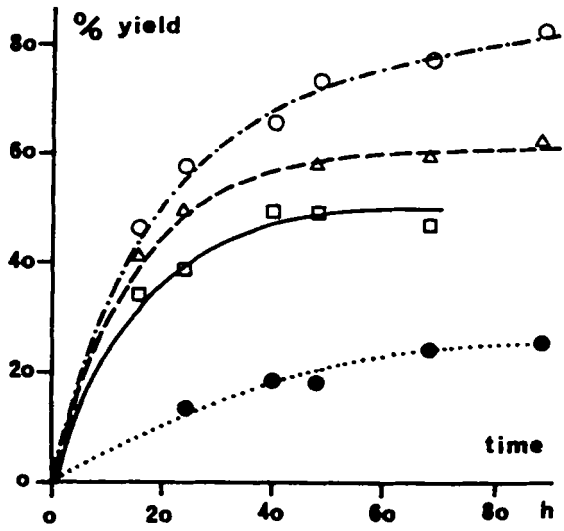

FIG. 2: Enzymatic degradation of cellulose,
·—·—·— native cellulose
— — — — microcrystalline cellulose
——————— cellulose powder
..... cotton (from the card)

Inhibition of the Cellulase Complex

Glucose and cellobiose, i.e. the principal products of the enzyme-catalyzed reaction sequence, are known to play a decisive role in the regulation of cellulase activity. A traditional problem with Trichoderma viride preparations has been that this fungus secretes but little β-1,4-glucosidase (EC 3.2.1.21). Unless cellobiase is supplemented from other organisms, e.g. Aspergillus sp., that deficiency may give rise to cellobiose accumulation (27-30).

The enzyme mixture used in this work exhibited sufficient β-glucosidase activity to ensure quantitative conversion of cellobiose to glucose. Glucose, on the other hand, did inhibit to some extent the degradation of native cellulose (see Fig. 3a).

When a lignocellulosic material is pretreated by hydrothermolysis, compounds such as coniferyl alcohol, syringaldehyde, 4-hydroxybenzoic acid, vanillin etc. will appear in the hydrolysate (31). In part, they will remain adsorbed on the moist cellulose residue, so that their inhibitory capabilities are of interest as well. 4-Hydroxybenzoic acid and vanillin were chosen to represent this class of compounds. When added in concentrations typical for the pretreatment hydrolysates (e.g. 0.03 mg/ml 4-hydroxybenzoic acid, 0.005 mg/ml vanillin), they did not interfere with the

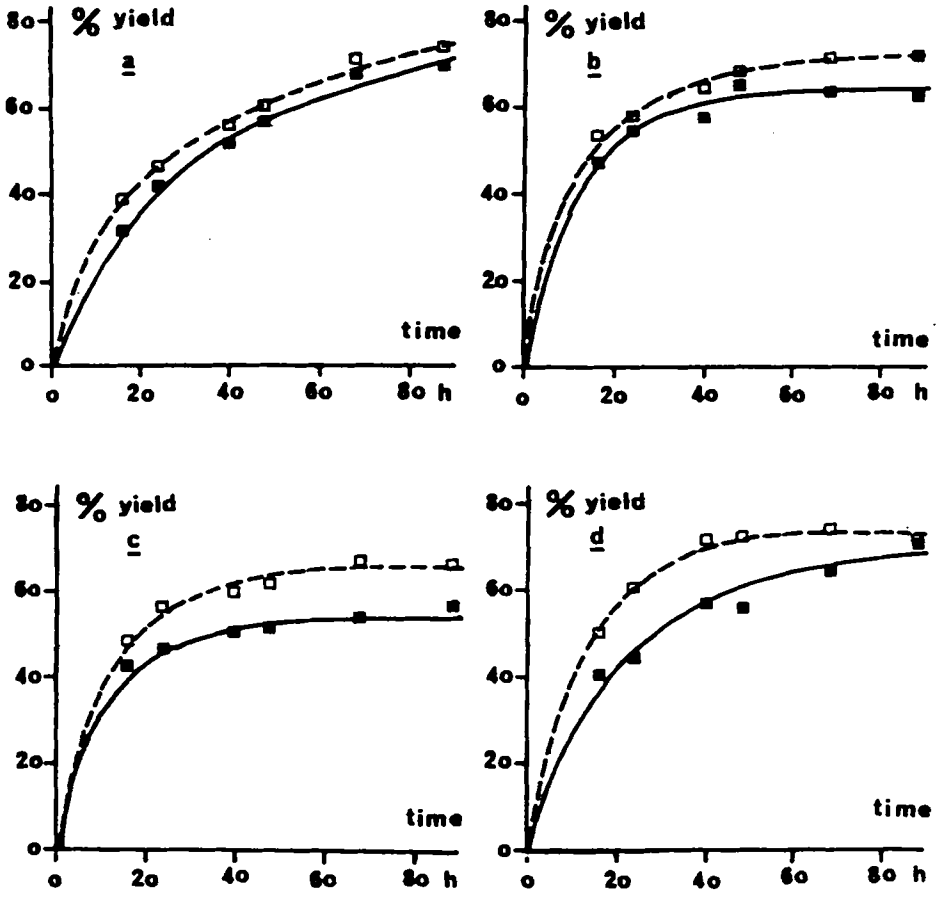

FIG. 3: Inhibition of enzymatic degradation of native cellulose. Full lines: 10 mg/ml, broken lines: 5 mg/ml of a) glucose, b) 4-hydroxybenzoic acid, c) vanillin and d) furfural, respectively. For uninhibited hydrolysis see Fig. 2.

cellulase reaction. Significant inhibition, as shown in Figs. 3b and 3c, only comes about if they are present in higher concentrations (>1 mg/ml). Similar results were obtained for furfural, which is formed from the hemicellulose fraction during hydrothermolysis (see Fig. 3d).

Hydrolysis of Pretreated Composite Substrates

The influence of pretreatment temperature on the enzymatic hydrolysis yield makes itself felt strongly in the saccharification of straw; in fact, a ten degrees' rise can express itself in a yield 1/3 higher (see Fig. 4a). Also at 190 °C, an organosolv treatment ($MeOH/H_2O$ = 1:1 v/v) was compared with a two-step (organosolv-hydrothermolysis) procedure. The result is shown in Fig. 4b: an additional hydrothermolysis step can increase the organosolv saccharification yield by approx. 1/5.

Fig. 5 gives the hydrolysis curves obtained for poplar wood after different kinds of pretreatment at approx. 210 °C. It appears that hydrothermolysis at this temperature has an effect similar to the $MeOH/H_2O$ option if 80% MeOH is used. In both cases, saccharification yields fail to reach the 50% mark. This contrasts with the results of a run with 50% aqueous MeOH at 213 °C, which renders the cellulose

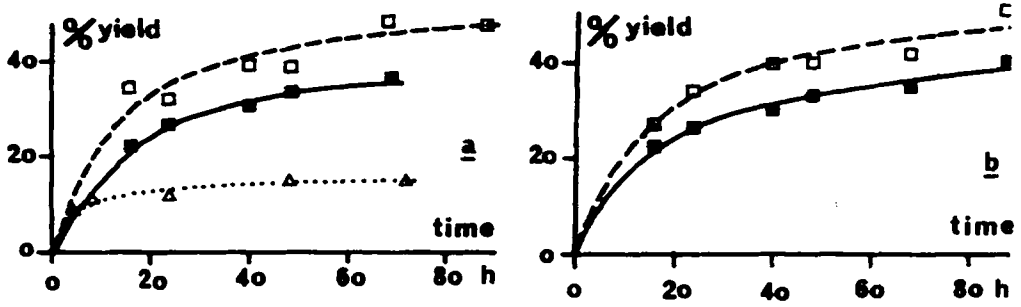

FIG. 4: Enzymatic degradation of straw (water insoluble fiber yields are given in parentheses)
– – – after hydrothermolysis at 189±2 oC (52%)
——— after hydrothermolysis at 180±2 °C (58%)
····· untreated
– – – after MeOH/H_2O - 1:1 treatment followed by hydrothermolysis, both at 191±2 °C (54%)
——— after MeOH/H_2O - 1:1 treatment at 190±2 °C (62 %)

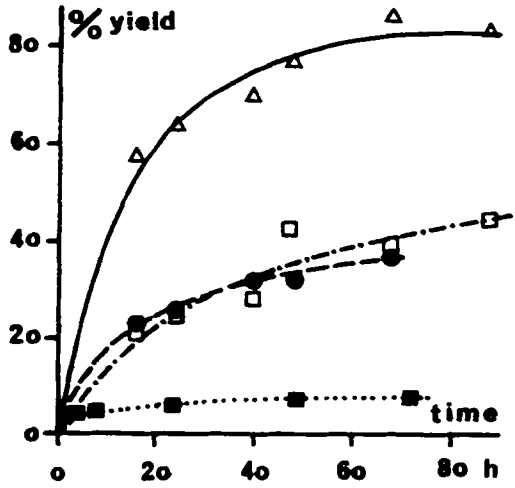

FIG. 5: Enzymatic degradation of poplar wood (water insoluble fiber yields are given in parentheses)
——— after MeOH/H_2O (1:1) treatment at 213±2 °C (43%)
–·–·– after MeOH/H_2O (8:2) treatment at 209±2 °C (70%)
– – – – after hydrothermolysis at 207±2 °C (53%)
····· untreated

about twice as accessible to the enzyme, so that 80-85% saccharification comes about after 3 1/2 days of hydrolysis.

ACKNOWLEDGEMENT

The authors are grateful to the Bundesministerium für Wissenschaft und Forschung (Vienna) for their financial support. One of the authors (H.H.) would like to thank the Austrian Research Center Seibersdorf for granting a bursary.

REFERENCES

1. M.R. Ladisch, K.W. Lin, M. Voloch, and G.T. Tsao, Enzyme Microb. Technol. 5(2), 82 (1983)

2. E.T. Reese and M. Mandels, Annu. Rep. Fermentation Processes 7, 1 (1984)

3. H. Esterbauer, M. Hayn, G. Jungschaffer, E. Taufratzhofer, and J. Schurz, J. Wood Chem. Technol. 3(3), 261 (1983)

4. H.H. Dietrichs and K.I. Zschirnt, Holz als Roh- und Werkstoff 30, 66 (1972)

5. K.-I. Sudo, Y. Matsumura, and K. Shimizu, Mokuzai Gakkaishi 22(12), 670 (1976)

6. H.E. Grethlein, Bio/Technology 3(2), 155 (1985)

7. E.B. Cowling, Biotechnol. Bioeng. Symp. 5, 163 (1975)

8. P.V. Pannir Selvam, T.K. Ghose, and P. Ghose, Proc. Biochem. 18, 3 (1983)

9. M. Mandels, L. Hontz, and J. Nystrom, Biotechnol. Bioeng. 16(11), 1471 (1974)

10. J.F. Saeman, M.A. Millett, and E.J. Lawton, Ind. Eng. Chem. 44, 2848 (1952)

11. B. Philipp, D.C. Dan, K.-J. Linow, E. Polter, and G. Schulz, Acta Biotechnol. 2(3), 275 (1982)

12. K. Shimizu and K. Usami, Mokuzai Gakkaishi 24(9), 632 (1978)

13. D.G. MacDonald, N.N. Bakhshi, J.F. Mathews, A. Roychowdhury, P. Bajpai, and M. Moo-Young, Biotechnol. Bioeng. 25(8), 2067 (1983)

14. M.M. Gharpuray, L.T. Fan, and Y.-H. Lee, Caustic pretreatment study for enzymatic hydrolysis of wheat straw. In: Wood Agric. Residues, J. Soltes, ed., pp. 369-389, New York: Academic Press (1983)

15. M. Gould, Biotechnol. Bioeng. 26(1), 46 (1984)

16. W.C. Neely, Biotechnol. Bioeng. 26(1), 59 (1984)

17. R.F.H. Dekker and A.F.A. Wallis, Biotechnol. Bioeng. 25(12), 3027 (1983)

18. V.P. Puri and H. Mamers, Biotechnol. Bioeng. 25(12), 3149 (1983)

19. K. Shimizu, K. Sudo, S. Nagasawa, and M. Ishihara, Mokuzai Gakkaishi 29(6), 428 (1983)

20. T.N. Kleinert, Tappi 57(8), 99 (1974)

21. E. Edel and J. Feckl, CEC, "Wood as a source of chemicals", presentation at Contractors' Meeting in Friedrichshafen/FRG, 1985/05/08-09 (1985)

22. O. Bobleter and G. Pape, Austrian Pat. 263661 (1968)

23. K.-E. Eriksson, Biotechnol. Bioeng. 20(3), 317 (1978)

24. A.I. Hatakka, Eur. J. Appl. Microbiol. Biotechnol. 18, 350 (1983)

25. H. Binder, J. Chromatogr. 189, 414 (1980)

26. G. Bonn, R. Pecina, E. Burtscher, and O. Bobleter, J. Chromatogr. 287, 215 (1984)

27. D. Sternberg, Appl. Environ. Microbiol. 31(5), 648 (1976)

28. T.K. Ghose and V.S. Bisaria, Biotechnol. Bioeng. 21(1), 131 (1979)

29. L.E.R. Berghem, L.G. Pettersson and U.-B. Axiö-Frederiksson, Eur. J. Biochem. 53, 55 (1975)

30. D.W. Sundstrom, H.E. Klei, R.W. Coughlin, G.J. Biederman and C.A. Brouwer, Biotechnol. Bioeng. 23(3), 473 (1981)

31. R. Pecina, E. Burtscher, G. Bonn and O. Bobleter, Fresenius Z. Anal. Chem. (1986), in press