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Hydrolysis of Polysaccharides with 77% Sulfuric Acid for Quantitative Saccharification

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Abstract: Classical standard hydrolysis of polysaccharides with 72% sulfuric acid was modified in 2 manners. In order to avoid treatment in an autoclave at 120 °C under pressure, wood or pulp material was first swollen in cold 77% acid followed by hydrolysis steps in diluted acid solutions. Further, the neutralization of the hydrolyzate with dilute barium hydroxide was carried out in heated mother liquor ensuring a crystalline precipitate of barium sulfate. Digestion enables the separation of clear aliquots by decantation in large amounts for analysis by HPLC. The modified procedure allows hydrolyses of polysaccharides with low losses as indicated by correction factors between 1.07 and 1.1 for 5 sugars, i.e. glucose, xylose, mannose, galactose and arabinose.

Key Words: quantitative saccharification, acid hydrolysis, polysaccharides, wood, pulp, HPLC

Kantitatif Sakkarafikasyon Amacıyla Polisakkaritlerin % 77'lik Sülfürik Asit ile Hidrolizi

Özet: Polisakkaritlerin % 72'lik sülfürik asitle klasik hidrolizi 2 açıdan değiştirilmiştir. Otoklavda basınç altında 120 °C deki işlem yerine odun veya selüloz örneği ilk önce soğukta % 77'lik asit ile muamele edilmiş ardından hidrolize, seyreltik asit koşullarda devam edilmiştir. Daha sonra hidrolizat seyreltik barium hidroksit ile nötralize edilerek, oluşan barium sülfatın ana çözeltiden iyi bir şekilde çökmesi sağlanmıştır. Böylece berrak ana çözeltiden dekantasyonla belli bir miktar nötral çözelti alınarak HPLC de analiz edilmiştir. Modifiye hidroliz yöntemiyle odun polisakkaritlerinin çok küçük kayıplarla hidrolizi mümkün hale gelmekte ve ilgili düzeltme faktörleri hidroliz ürünü beş şeker, glukoz, ksiloz, mannoz, galaktoz ve arabinoz için 1.07-1.1 arasında bulunmaktadır.

Anahtar Sözcükler: Kantitatif Sakkarifikasyon, Asit Hidrolizi, Polisakkaritler, Odun, Kağıt Hamuru, HPLC

Introduction

In wood and pulp analyses the determination of polysaccharides, which comprise the major part of these materials, is of great importance. The main constituent among the polysaccharides considered here are cellulose and methylglucuronoxylan in hardwoods, or galactoglucomannan in softwoods.

Although ongoing research suggests that the direct determination of polysaccharides in wooden tissue is possible by means of spectrometric methods (FTIR), these are still under investigation and few individual examples are known (Schultz et al., 1985; Faix et al., 1989; Rodrigues et al., 1998, 2001). The most widely applied methods for the quantification of polysaccharides prerequisite to break down these polymers into monosaccharides are known as hydrolysis. The resulting individual sugars (glucose, xylose, mannose, galactose and arabinose) can then be determined in different ways, for example, by HPLC or after derivatization by GC. The hydrolyses of polysaccharides can be performed mostly in the presence of mineral acids, and 72% sulfuric acid is generally used.

Since hydrolysis is associated with some unavoidable side reactions, mass losses occur and therefore the reaction conditions should be selected carefully. On the other side, the neutralization of the hydrolyzates may further decrease the yield of sugars and therefore the losses are accounted for either by correction factors for each of the 5 sugars or by the treatment of sugar standards throughout the procedure in exactly the same

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way. TAPPI standard T 249 cm-85 is applied in many laboratories working on the estimation of the carbohydrate composition of wood and pulp. Some changes in the conditions of the hydrolysis procedure and in the determination of sugars were suggested to minimize losses (Kaar et al., 1991; Puls, 1993; Wright and Wallis, 1996; Davis, 1998).

Polysaccharides can also be hydrolyzed in the presence of trifluoroacetic acid (TFA). The TFA method has been introduced with 2 major advantages: causing smaller losses and omitting the step of neutralization since the TFA can be removed by evaporation (Fengel et al., 1978; Fengel and Wegener, 1979). The conditions of TFA hydrolysis should be varied depending on the nature and composition of lignocellulosic material and, in case of incomplete hydrolysis, a pretreatment step is necessary (Fengel and Przyklenk, 1993).

In this study, the technique of 2-step hydrolysis with sulfuric acid is somewhat changed with the aim of enabling complete hydrolysis under atmospheric pressure. With exact temperature control and without pressure, hydrolysis could be carried out more carefully. The old method of Jayme and Knolle (1960) is modified in both reaction conditions and acid concentrations. Furthermore, the digestion applied to the neutralization with barium hydroxide ensures clear supernatant solutions that can be separated easily by decantation.

Materials and methods

Samples of extracted soft-and hardwoods, unbleached and bleached kraft pulps and cotton linters were chosen as materials for the determination of their polysaccharide composition.

Hydrolysis

Solutions of 77.0 ± 0.1 and 25.0 ± 0.1% sulfuric acid were prepared from concentrated sulfuric acid (Merck, sp gr 1.84). Both acids were put in the refrigerator in the 0 °C compartment 1 h before they were used. About 100 mg samples were weighed into 50 ml round bottom flasks with grounded necks (NS 29/32). Glass rods with a diameter of 5 mm and of a suitable length were put in the round bottom flasks so that the top of the rod emerged about 2 mm. Hollowed ground stoppers were then used to ensure tight closure. The stoppered flasks were kept for about 15 min in the refrigerator at 5 °C before the acid was added.

Draining along the glass rod, 1.00 ml of cold 77% acid was slowly added to each flask with a volumetric pipette and mixed thoroughly with the material for about 1 min. The stoppered flasks were then put in a small refrigerator, where the temperature was kept constant at –5.0 ± 0.5 °C. The samples swollen in cold acid were kept for 12-14 h (overnight) and the next day 1.00 ml of cold 25% acid was transferred into each flask and stirred well with rods. Closed tightly with stoppers, the samples were allowed to warm up to ambient temperature before they went into an oven where a constant temperature of 55 ± 0.5 °C was maintained. The treatment in the warm oven lasted 2 h and, about 10-15 min after the start, each flask was opened once, stirred briefly, closed and put back in the oven. At the end of the period, the flasks were taken out, left to cool down to room temperature and then 10.00 ml of cold, distilled water was slowly drained along the glass rod into the flasks. The flasks containing the hydrolyzates (about 12% sulfuric acid solutions) were attached to reflux condensers standing over a water bath with a constant temperature of 95 ± 0.5 °C. This last stage of hydrolysis took 1 h.

After cooling to ambient temperature, the hydrolyzates were filtered off through fritted glass crucibles of medium porosity. Round bottom flasks and the crucibles were rinsed several times and filtrates and washings were transferred into a 100 ml volumetric flask, which was then filled to the mark with distilled water. By using a 50 ml volumetric pipette, half of the acidic hydrolyzates were transferred into a 250 ml beaker, the empty weight of which was noted to the nearest 10 mg. The amount of barium hydroxide, which is about 20-30 mg less than that required to neutralize 50 ml of acidic solution, was weighed in another 150 ml beaker and dissolved with 100 ml of distilled water (approximately 2.56 g of $Ba(OH)_2$ 8 H_2O is needed to neutralize sulfuric acid in 50 ml of dilute hydrolyzate). Both beakers, 250 ml with 50 ml of hydrolyzate and 150 ml with barium hydroxide solution, were put in a water bath set at 80-85 °C for about 5 min before the neutralization began. One drop of methyl red indicator was added to the hydrolyzate and by stirring with a glass rod the first 25 ml portion of 100 ml base was slowly poured. After 2-3 min of stirring the second 25 ml base was added and the other third and fourth portions were

added to the hydrolyzate in the same way. Although most of the acid was neutralized, the end point was reached by adding 0.05 M $Ba(OH)_2$ dropwise (light pink color of the $BaSO_4$ suspension disappears). This last step should be carried out slowly and carefully. It is also important to stir well.

The entire neutralization step in the water bath takes about 20 min. The beakers were taken out of the bath, and the glass rods were rinsed with 1-2 ml of distilled water and allowed to cool. Digestion results in well-separated barium sulfate precipitates within 5-10 min. However at least 1 h is necessary to cool the supernatant to room temperature.

Before each beaker with neutral solution and precipitate was weighed, some drops of water condensed on the inside wall of the beaker over the liquid should be remowed with a clean paper tissue. To determine the exact weight of neutralizate, the weight of $BaSO_4$ formed should also be considered. This calculation is performed by subtracting the empty weight of each beaker and 1.9 g (the weight of precipitate) from those with solution and precipitate.

Since the exact weight of the neutralizate was known, about half of the supernatant (75-80 ml) was decanted in a 250 ml round bottom evaporator flask and weighed to the nearest 10 mg. In this way, the exact ratio of the amount of sample, which will be analyzed by HPLC, to the hydrolyzed sample, was determined. The neutralizate was then evaporated to dryness in a rotary evaporator with water bath at 40 °C, dissolved in 10 or 20 ml of ultra pure water, and filtered through a 0.45 µ membrane. Then 10 µl were chromatographed on an Aminex column (HPX87P with micro-guard cartridges, Bio-rad) connected to HPLC equipment (Waters Associates: 600 system controller with pump, 717 plus automatic sample injector, 410 refractive index detector, 746 data module, mobile phase: ultra pure water from Millipore Milli-Q system, flow rate: 0.5 ml min^{-1}, column temperature: 82 °C). Five standard sugars in applicable composition to wood or pulp samples were run through the entire steps of hydrolysis and neutralization.

The carbohydrate composition of each specimen was assessed with 2 replicates and by a minimum of 2 but often 3 injections from each replicate. Two standard injections were performed before and after each injection of specimen. The reproducibility was between 0.5 and 1.0% for higher amounts of sugars (>10%), glucose, xylose and mannose for instance. In the case of sugar yields less than 2-3%, the reproducibility was adversely affected and increased to 3-5% and in cases where the yield of an individual sugar was around 0.5 to 1%, the reproducibility increased up to 10%.

Results and discussion

Since the cellulose and individual polyoses in wood and pulps exhibit different resistance towards hydrolysis, it is a compromise in the end where a complete breakdown of the strong cellulose chain has to be achieved under appropriate conditions; on the other hand, losses in easily hydrolyzable polyoses should be as small as possible. Losses during acid hydrolysis are caused by side reactions. Further on, during the neutralization of hydrolyzates the reversion of monosaccharides may occur if the end point is missed and the solution becomes alkaline.

In order to suppress side reactions during hydrolysis, the conditions selected should be as mild and protective as possible for the product monosaccharides. Hydrolysis at elevated temperatures and under pressure indeed takes place quickly but the side reactions are also accelerated. In the hydrolysis method with 77% sulfuric acid, the highest temperature was 95 °C and no excess of pressure was applied. With appropriate dilutions the complete hydrolysis of cellulose is ensured. Thus, the amount of cellobiose in chromatograms is generally low (≤0.5%). On the other hand, the correction factors for the sugars from polyoses (Table 1) indicate that about 10% of galactose is lost in the worst case.

Generally evaluated, these factors are as good as those given in the work by Pettersen et al. (1984) and even better than those factors suggested by Fengel and Wegener (1979) for careful hydrolysis with TFA.

Table 1. Hydrolysis loss factors during 77% sulfuric acid hydrolysis.

Glucose	Mannose	Galactose	Xylose	Arabinose
1.07	1.09	1.10	1.09	1.08

Announced first as the most gentle method, enzymatic hydrolysis does not deliver higher results than acid hydrolysis since the total amount of neutral sugars was 2-5% lower by enzymatic hydrolysis (Tenkanen et al., 1995, 1999).

Table 2 shows the composition of some soft- and hardwoods as well as pulps and cotton linters as one example of pure cellulose. The summative analyses deliver satisfactory results and for pure cellulose a theoretical yield of 111% is achieved.

Table 2. The carbohydrate composition of some selected lignocellulosic materials and cellulose.

	Cellobiose %	Glucose %	Xylose %	Galactose %	Arabinose %	Mannose %	Sum	
							Monosac.	Polysac.
Woods								
Oak (*Q. vulcanica*) sap	0.3	49.4	20.4	1.2	0.8	2.1	74.2	66.4
(*Q. vulcanica*) heart	0.3	48.8	19.8	1.2	0.8	2.5	73.4	65.7
Carob (*C. ciliqua*) sap	0.3	49.8	19.8	1.1	1.0	1.8	73.8	66.0
(*Ceretonia ciliqua*) heart	0.2	52.2	20.6	1.2	1.1	2.3	77.3	69.4
Maple (*A. campestre*)	0.2	52.2	14.5	1.0	1.3	1.5	70.7	63.3
Pine (*P. nigra*)	0.3	49.2	5.0	2.3	3.0	14.7	74.4	66.8
Fir (*A. equitrojani*)	0.4	48.1	6.9	2.8	2.2	13.0	73.4	65.0
Spruce (*P. orientalis*)	0.4	49.1	7.5	2.5	2.3	14.4	76.2	68.4
Pine (mixed chips)*	0.3	45.0	7.0	2.7	2.4	13.7	71.0	63.7
Pulps								
unbleached kraft*	0,4	85.2	8.7	0	0	7.6	101.9	91.5
bleached kraft*	0.4	90.2	9.2	0	0	8.0	107.8	96.8
unbleached kraft§	0.3	83.2	7.4	1.3	1.8	8.4	102.4	92.1
Cotton linters	0.2	110.8	0	0	0	0	111.0	99.9
Hemp bast fibers	1.5	76.6	3.3	2.1	1.6	4.9	90.0	80.9

* factory produced (mixed chips: 80% Aleppo pine 20% black pine), § produced in laboratory (black pine)

References

Davis, M.W. 1998. A rapid modified method for compositional carbohydrate analysis of lignocellulosics by high pH anion-exchange chromatography with pulsed amperometric detection (HPAEC/PAD). Journal of Wood Chemistry and Technology 18: 235-252.

Faix, O., H. Schubert and R. Patt. 1989. Continuous process control of pulping by FTIR spectroscopy. International Symposium on Wood and Pulping Chemistry. May 22-25, Raleigh, pp. 1-8.

Fengel, D. and M. Przyklenk. 1993. Vorbehandlungen von cellulosehaltigen Proben für die Totalhydrolyse mit Trifluoressigsäure. Holz Roh- Werkstoff 51: 294.

Fengel, D. and G. Wegener. 1979. Hydrolysis of polysaccharides with trifluoracetic acid and its application to rapid wood and pulp analysis. *In*: Brown Jr., R.D. Jurassek, L. (Eds.): Hydrolysis of cellulose; mechanisms of enzymatic and acid catalysis. Adv. in Chem. Series no: 181. Am. Chem. Soc. Washington, 145-158.

Fengel, D., G. Wegener, A. Heizmann and M. Przyklenk. 1978. Analyse von Holz und Zellstoff durch Totalhydrolyse mit Trifluoressigsäure. Cellulose Chemistry and Technology 12: 31-37.

Jayme, G. and H. Knolle. 1960. Zur quantitativen Auswertung von Papierchromatogrammen durch Direktphotometrie im UV-Licht. Bestimmung von Zuckern. Z. Analyt. Chem. 178: 84-100.

Kaar, W.E., L.G. Cool, M.M. Merriman and D.L. Brink. 1991. The complete analysis of wood polysaccharides using HPLC. Journal of Wood Chemistry and Technology 11: 447-463.

Pettersen, R.G., V.H. Schwandt and M.J. Effland. 1984. An Analysis of the Wood Sugar Assay Using HPLC: A Comparison with Paper Chromatography. Journal of Chromatographic Science 22: 478-484.

Puls, J. 1993. Substrate analysis of forest and agricultural wastes. *In*: Bioconversion of Forest and Agricultural Plant Residues. Ed. J.N. Saddler. pp. 13-32. C.A.B. International, Wallingford, U.K.

Rodrigues, J., O. Faix and H. Pereira. 1998. Determination of lignin content of *Eucalyptus globules* wood using FTIR spectroscopy. Holzforschung 52: 46-50.

Rodrigues, J., J. Puls, O. Faix and H. Pereira. 2001. Determination of monosaccharide composition of *Eucalyptus globules* wood by FTIR spectroscopy. Holzforschung 55: 265-269.

Schultz, T., M. Templeton and G. McGinnis. 1985. Rapid determination of lignocellulose by diffuse reflectance Fourier transform infrared spectrometry. Anal. Chem. 57: 2867-2869.

Tenkanen, M., T. Hausalo, M. Siikaaho, J. Buchert and L. Viikari. 1995. Use of enzymes in combination with anion exchange chromatography in the analysis carbonhydrate composition of kraft pulps. Proc. 8th Intl. Symp. Wood Pulping Chem. Helsinki, III: 189-194.

Tenkanen, M., G. Gellerstedt, T. Vuorinen, A. Teleman, M. Perttula, J. Li and J. Buchert. 1999. Determination of hexenuronic acid in softwood kraft pulps by three different methods. J. Pulp Ppaer Sci. 25: 306-311.

Wright, P.J. and A.F.A. Wallis. 1996. Rapid determination of carbohydrates in hardwoods by high performance anion exchange chromatography. Holzforschung 50: 518-524.