Comparative Analysis of Sugarcane Baggase Using Different Methods

Protibha Nath Banerjee
Department of chemistry, School of physical sciences, The University of Dodoma, Dodoma, Tanzania
e-mail: pbanabo@yahoo.com, protibha.banerjee@udom.ac.in

Abstract - To find reliable analytical methods to ensure an accurate analysis of structural compounds in plants is always challenging due to structural diversity of constituents in different lignocellulosics. The non-cellulosic carbohydrate in sugarcane baggase was analysed by acid methanolysis followed by gc analysis. Our results showed that 3 hours of acid methanolysis is more sufficient to obtain high yield of monomeric pentoses and uronic acid units. To determine total carbohydrate content the optimum condition for acid hydrolysis was found by the comparison of reaction kinetics using different acids (sulfuric, trifluoroacetic and *ortho*-phosphoric acid) at different concentrations and different reaction time followed by HPLC and GC analysis. The lignin content was determined by conventional klason method as well as by AcBr method. The effect of perchloric acid on lignin determination by AcBr method has also been evaluated and the optimum condition for sugarcane baggase has been found. Acetyl group content in non-cellulosic carbohydrate was analysed after mild alkaline hydrolysis followed by hplc.

Keywords— Analytical methods, HPLC, GC, Methanolysis, Non-cellulosic carbohydrates, Sugarcane bagasse

1 INTRODUCTION

Traditional methods for the characterization of the carbohydrate composition in biomass are usually based on two stage hydrolysis experiment followed by HPLC analysis. (e.g. TAPPI & NREL methods). However, in case of sugarcane baggase containing mainly xylan, where xylose units are linked to 4-O-methyl glucuronic acid (4OMeGlcA), the analytical procedure sometimes underestimate the (pentoses and 4OMeGlcA) carbohydrate composition due to acid resistance of 1→2 linkages between 4OMeGlcA and xylose units. Moreover, due to drastic condition employed during acid hydrolysis stage, the hydrolyzed monomeric sugar units, especially pentoses and uronic acids, can also be easily degraded. The hydrolytic procedure and the subsequent analysis have been pointed out as problematic by various groups.[1,2] Other commonly used method for the depolymerisation of exclusively amorphous hemicelluloses involves acid methanolysis[3,4,5] when applied directly on wood samples without pretreatment such as delignification. However, little work on depolymerisation by acid methanolysis followed by the analysis of liberated monosaccharides on sugarcane baggase has been done (Ericka et al). Lignin, an integral chemical component of cell wall of lignocellulosic biomasses is commonly associated with the reduced digestibility of fiber. Simple, but time-consuming Klason lignin method used 72% sulfuric acid are gravimetric and results are affected by solubilisation of acid soluble lignin and (or) by contamination with proteins bound to lignin[6] or other condensation products like polyphenolics from the cell contents. Alternatively lignin can be determined spectrophotometrically using AcBr method which was developed by Johnson et al and modified by Iiyama et al [7] where perchloric acid was used to accelerate the dissolution of the samples.

Hence the objectives of this research was to evaluate

- the optimum condition for the analytical hydrolysis of sugarcane bagasse using different acids (sulfuric, trifluoroacetic acid, and *ortho*-phosphoric acids) of different concentration and different reaction time,
- the optimum condition for acid methanolysis of hemicelluloses and pectin in sugarcane bagasse,
- the effect of the various concentration of perchloric acid and the time of digestion on lignin determination in sugarcane baggase by AcBr method, method for accurate acetyl group determination..

2 EXPERIMENTAL

2.1 Biomass sample

Sugarcane baggase from the experimental sugar factory of National Sugar Institute, Kanpur (India) was washed with water, air dried and then dried in oven at 65°C for 24 hours. The oven dried bagasse was grounded in a Wiley mill to particles passing a 20-mesh screen. The sample was then extracted with ethanol/toluene in accordance with Tappi Method T204 om-88 (Tappi, 1988a, b).

2.2 Acid methanolysis followed by GC analysis

The dried extracted-free sugarcane baggase (10 mg) was transferred to a pear shaped flask and dried in a vaccum oven at 40°C for 1 hour. Two milliliters of 2M HCl in anhydrous methanol was added to each flask (Sundberg etal, 1996) and the samples were then kept at 105°C for 1, 2, 3 and 5 hours, respectively. Four different calibration solutions containing equal amount of the sugar monomers and uronic acids (except 4-O-MeGlcA) were also subjected to acid methanolysis under similar condition. The vessels (2, 3, 5 hrs) were shaken every hour to ensure uniform methanolysis. All samples were cooled to room temperature and neutralized by addition of 200 µl of pyridine. 4 ml of 0.1 mg/ml of sorbitol solution in methanol was added as internal standard to all the samples, the content was mixed and 1 ml of clear solution was then transferred to another flask. The methanol was then evapo-

rated in a stream of nitrogen, dried, silylated and analysed by GC according to Sundberg et al. 1996.

2.3 Acid hydrolysis followed by HPLC and GC

Hydrolysis experiments on extractive-free sugarcane baggase were carried out with three different acids (sulphuric, orthophosphoric and trifluoroacetic acids) of different concentrations (0.125M, 0.25M and 0.5M), different reaction time (30min, 45min, 60min and 120min) and different temperature (100°C and 120°C). 2 ml of hydrolysed samples were drawn out, filtered with 0.45 µ nylon filter cartridge and directly analysed for sugars by HPLC using instrument model 1260 Infinity (Agilent technologies), equipped with a refractive index detector, and column Supelguard H (5 cm X 4.6 mmID) + Supelcogel C-610H (30 cm X 7.8 mm ID) coupled at 30°C. The samples were analysed with 0.1% phosphoric acid at a flow rate of 0.5 ml/min. Same hydrolysates was neutralized with barium carbonate (pH = 6.5), filtered with 0.45 µ glass filter, concentrated at 35°C under vacuum, freeze dried, silylated and analysed for monomeric sugars by GC. Extractive-free sugarcane baggase was also autoclaved at 100°C and 120°C with 0.125, 0.25 and 0.5 M trifluoroacetic acid. The content was cooled-down, 2 ml of hydrolysed samples were drawn out, filtered with 0.45 µ nylon filter cartridge and directly analysed for sugars by HPLC. Same hydrolysates was evaporated under vacuum, freeze dried, silylated and analysed for monomeric sugars by GC using the same condition as described for sulphuric acid hydrolysis.

2.4 Lignin analysis by AcBr method and Klason method

Acetyl Bromide method: Extractive-free sugarcane bagasse (4 mg) was weighed in screw capped testtubes. Acetyl bromide (5 ml, 25% in glacial acetic acid) and different doses (0.05, 0.1 and 0.2 ml) of perchloric acid (70%) was added to the tubes and heated at 70°C for 10, 20 and 30 min. The tubes were cooled and transferred to a 50 ml volumetric flask containing 10 ml NaOH (2M) and 12 ml glacial acetic acid, the level was adjusted with glacial acetic acid and absorbance was measured immediately at 280nm.

Klason method *was* performed using 1 g of extractive-free ***s***ugarcane bagasse and 72% sulphuric acid according to convenient procedures [7]. The soluble lignin concentration in the filtrate was determined by measuring the absorbance at 205 nm.

2.5 Determination of Acetyl and Ash content

The acetyl content was determined after mild alkaline hydrolysis of sugarcane baggase with sodium hydroxide followed by the analysis of liberated acetic acid by HPLC. The sugarcane baggase was found to contain 0.89% of acetyl group. Ash content in sugarcane baggase was determined by *Tappi* Method T 211 om – 93 and was found to contain 3% ash.

3 Results and Discussion

3.1 Acid methanolysis followed by GC analysis

Acid methanolysis of sugarcane bagasse was carried out at 105°C for 1, 2, 3 and 5 hours and the results are shown in Fig.1 and Fig.2. It can be seen from Fig.1, that after 2 hours methanolysis the yield of total non-cellulosic carbohydrates decreases. This may be due to degradation of labile arabinose. However, the yield of xylose units have a maximum upto 3 hours. Total 156 mg/g of xylose was released during methanolysis which acounts for nearly 68% of xylose in hemicellulose. However, after 3 hours of methanolysis the concentration of all the monomeric sugars in solution starts decreasing and hence the optimum condition of methanolysis for sugarcane bagasse is recommended to be 3 hours at 105°C.

3.2 Acid methanolysis followed by GC analysis

Acid hydrolysis method is well known to analyze the total carbohydrates content of biomass sample. The summative results of the acid hydrolysis of sugarcane baggase are shown in Figs.3-7. Due to paucity of space only results for 120°C are exhibited. The acid hydrolysis of sugarcane baggase using 0.25M sulphuric acid for 60 min at 120°C liberated highest concentration of sugars (Fig.4). Total 733 mg/g o.d. baggase, i.e. nearly 73% of carbohydrate in baggase was released. However, for the same sample the HPLC values are lower. This can be due to the poor sensitivity of HPLC detectors compared to GC. Phosphoric acid hydrolysis at 120°C using 0.25 M for 120 min resulted in the release of mainly xylose (Fig.7-8) while arabinose was predominant at 100°C when treated with 0.125 M phosphoric acid for 120 min. Total 165mg of xylose per g of o.d.baggase was released during hydrolysis with 0.25 M phosphoric acid at 120°C for 120 min. This accounts for 71% of xylose in hemicellulose. Altogether 224 mg sugar/g of bagasse, i.e. 22.4% of hemicelluloses in sugarcane baggase was released under this condition. Hydrolysis of sugarcane baggase with trifluoroacetic acid also resulted in the release of mainly xylose. Maximum yield of xylose units was obtained when bagasse was treated with 0.25 M Trifluoroacetic acid at 120°C for 45 min. Thus, hydrolysis of sugarcane baggase with phosphoric acid and trifluoroacetic acid resulted in the release of sugars mainly from xylan.

3.3 Lignin analysis by AcBr method and Klason Method

Lignin in sugarcane baggase was found to be 21-22% by Klason method while AcBr method gave nearly 9% more lignin content than the Klason method. Due to lack of model lignin of sugarcane baggase the calibration with birch MWL was used for the calculation of lignin content by AcBr method. This can be the reason for the higher value of lignin in sugarcane baggase by AcBr method. However, from the absorption values (Fig.15) at 280 nm it can be seen that maximum absorption is recorded when the sugarcane baggase was digested with acetyl bromide (5 ml, 25% in acetic acid) and perchloric acid (0.05 ml) for 30 min.

3.4 Determination of Acetyl and Ash content

The acetyl content was determined after mild alkaline hydrolysis of sugarcane baggase with sodium hydroxide followed by the analysis of liberated acetic acid by HPLC. The sugarcane baggase was found to contain 0.89% of acetyl group. Ash content in sugarcane baggase was determined by *Tappi* Method T 211 om – 93 and was found to contain 3% ash.

4 Conclusion

Acid methanolysis and acid hydrolysis with different acids of different concentration and different reaction time at two different temperatures were compared for the depolymerisation of sugarcane bagasse. A combination of methanolysis and acid hydrolysis is recommended for the complete analysis of carbohydrates followed by the subsequent analysis of the released monosaccharide. However, acid methanolysis should be preferred for the analysis of hemicellulosic content of sugarcane bagasse. It is recommended to carry out methanolysis of sugarcane bagasse for 3 hours as further reaction time results in the degradation of mainly pentoses. Acid methanolysis and hydrolysis with phosphoric and trifluoroacetic acid gave nearly similar selectivity to non-cellulosic carbohydrate depolymerisation.

Acknowledgment

The author thanks Prof R.B.Nigam, Ex-Director-National Sugar Institute (India) for providing the sugarcane bagasse sample.

References

[1] **Jacobs, A**., Report from the NSP Network group for carbohydrate analysis. In : 2nd chemical Analysis Seminar, Stock, Sweden (2003).

[2] **Willfor, S**.; Pranovich, A.; Tamminen, T.; Puls, J.; Laine, C.; Suurnakki, A.; Sake, B.; Uotila, K.; Simolen, H.; Hemming, J.; Holmbom, B. , Industrial Crops And Products, 29 (2009), 571 – 580.

[3] **Bertaud, F**.; Sundberg, A.; Holbom, B. Carbohydrate Polym. 48 (2002) 319 – 324

[4] **P.N.Banerjee**, A.Pranovich, D.Dax, Stefan Willfor; Non-Cellulosic heteropolysaccharide from sugarcane bagasse - Sequential extraction with pressurized hot water and alkaline peroxide at different temperatures, Bioresource Technology, vol – 155 (2014), 446-450.

[5] **P. N. Banerjee** & S. Bhatt ;Structural studies of a new acidic polysaccharide of apricot seeds, Natural Product Research, 21(6), 507 – 514 (2007)

[6] **Soest, P.V**; J. Nutritional Ecology of the Ruminants; O&B Books: Corvallis. OR (1982), 118 – 138.

[7] **Iiyama, K**.; Wallis, A.F.A; Wood Sci.Tecnol. , 22, (1988), 271 – 280.

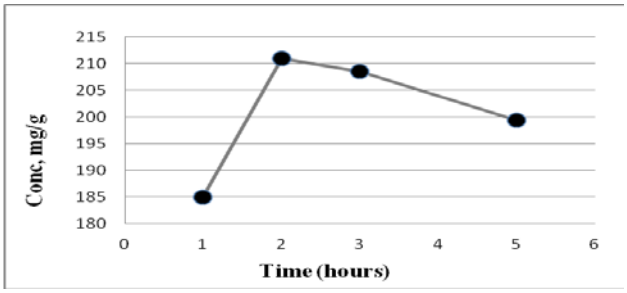

Fig 1: Total carbohydrates from methanolysis and GCanalysis

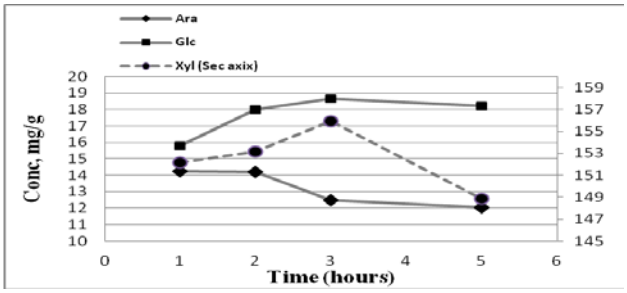

Fig 2: Individual sugars from methanolysis and GC analysis

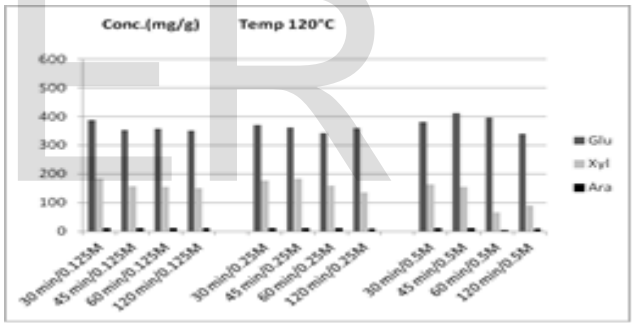

Fig 3: HPLC results from Sulphuric acid hydrolysis at at 120°C

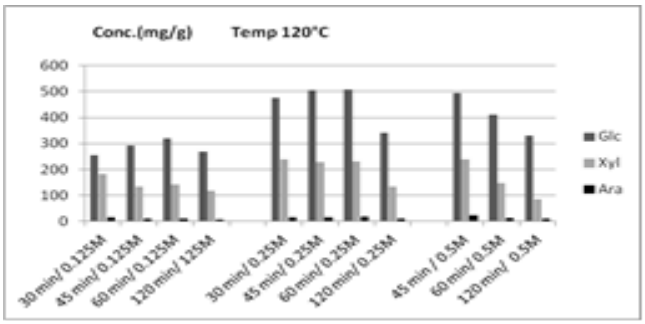

Fig 4: HPLC results from Sulphuric acid hydrolysis at 120°C

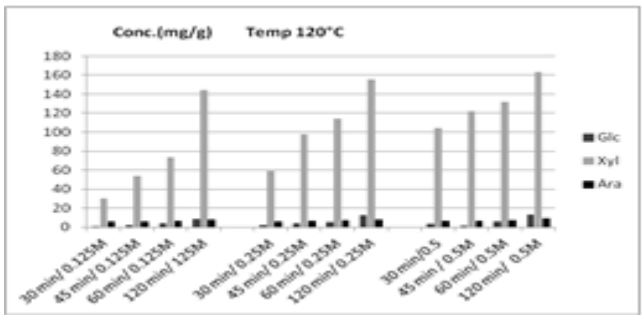

Fig 5: HPLC results from Phosphoric acid hydrolysis at 120°C

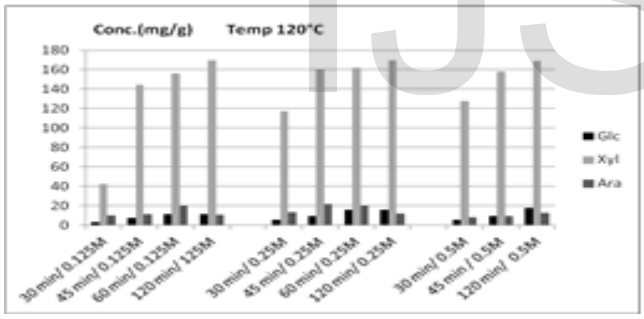

Fig 6: GC results from Phosphoric acid hydrolysis at 120°C

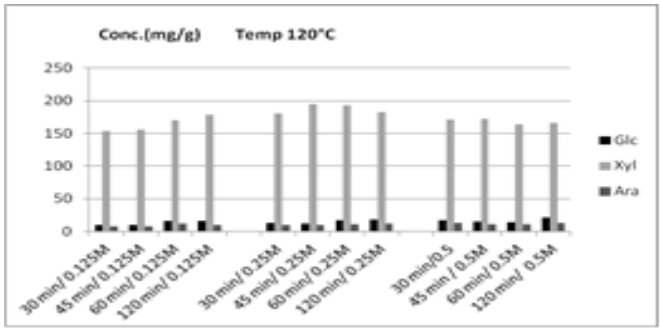

Fig 7: HPLC results from TFA hydrolysis at 120°C

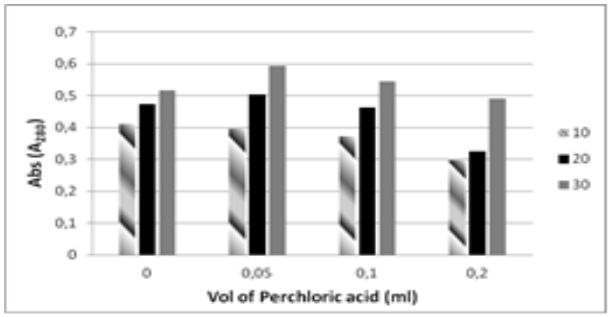

Fig 8: Absorption values from AcBr method (A_{280})

Author Information
Protibha Nath Banerjee is working as a Lecturer in the Department of Chemistry, School of physical sciences, The University of Dodoma, Dodoma, Tanzania