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Application of Polysaccharide Enzymes in Sugar Cane Bagasse

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ABSTRACT

Commercial cellulase, hemicellulase and pectinase enzymes were applied to hydrolyse sugar cane bagasse. Cell wall polysaccharides of this substrate were hydrolysed extensively. Maximum hydrolysis is obtained when commercial hemicellulase and pectinase are combined with cellulases. The digests were chromatographed by high performance liquid chromatography in order to obtain information on the structure features of bagasse polysaccharides.

INTRODUCTION

Agricultural by-products containing cellulose, hemicellulose, pectin and lignin, if available in large amounts at low price, appear to be some of the most useful raw materials for producing renewable fuel, food and chemical feedstocks (Chapman & Lynch, 1985; Juanbarò & Puigjaner, 1986; Ladisch & Tsao, 1986; Sudo *et al.*, 1986; Waldron & Eveleigh, 1986; Schwald *et al.*, 1988). Enzymatic hydrolysis of the cellulose is one of the most promising processes for converting these by-products (Anderson, 1984; 1985; Araujo & D'Souza, 1986), but its performance is restricted by the chemical structure itself and cellulose's association in nature with other polysaccharides (Beldman *et al.*, 1985; Beldman, 1986; Ayyad, 1989), and therefore bioconversion yields thus obtained are generally low unless polysaccharide enzymes other than cellulase are used. Combinations of polysaccharide degrading enzymes are known to

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act synergistically in the degradation of the cell wall matrix (Voragen & Pilnik, 1981; Ayyad & Ragab, 1989).

The purpose of the present work is to investigate the enzymatic hydrolysis of sugar cane bagasse. This process is attractive for the production of fermentable sugars and for the distribution of the cell wall matrix, resulting in liquefaction of the materials. We have selected a mainly cellulosic agroindustrial by-product that has an insignificant cost in most countries. In Egypt, there are still more than 200 000 tons produced every year without industrial use (Mohamed, 1988).

MATERIALS AND METHODS

Substrate: fresh bagasse supplied by Egyptian Co. for sugar and distillation, Egypt, was used. The dry bagasse (drying loss = 5·7%) was hammer milled to particles smaller than 5 mm. Alcohol insoluble solids were prepared by four successive extractions with 96% isopropanol at 40°C from other sub-samples and then fractionated into pectin, hemicellulose, cellulose and lignin as described by Voragen *et al.* (1983).

Analytical methods: drying loss, nitrogen, starch and ash were determined by the standard methods (AOAC, 1985). Uronic acids with *m*-hydroxydiphenyl were prepared using the method of Ahmed and Labavitch (1977). Sugar composition was determined by GC analysis of the alditol acetate derivatives of the sugar obtained by Seaman hydrolysis described by Selvendran *et al.* (1979). Enzymatically solubilized sugars were assayed using the Nelson–Somogyi method (Nelson, 1944) for reducing end groups, and the Dubois method for the total sugars (Dubois *et al.*, 1956). Soluble products were analysed by HPLC (Spectra Physics SP 8700), equipped with an Aminex HPX-87P column (300 × 7·8 mm, Bio Rad Labs, Richmond, CA, USA) and a guard column (50 × 4·6 mm). Sugars were detected with an ERMA-ERC 7510 refractive index detector thermostatted at 40°C (Voragen *et al.*, 1986).

Enzymatic treatments: several commercial enzyme preparations were tested for their ability to hydrolyse sugar cane bagasse polysaccharides (Table 1).

A suspension of bagasse (10% w/w, on dry weight) in 0·1 M sodium acetate buffer (pH 4·5) was incubated with the (1% w/w) enzyme of choice for 24 h at 40°C.

RESULTS AND DISCUSSION

The composition of the dried bagasse is presented in Table 2. Cellulose, hemicellulose and lignin are the main polysaccharides at 35·4, 20·6 and

18·6% respectively. The amount of pectic substances, expressed as uronic acids, was low (3·3%). Starch was only present in minimal amounts (1·5%). Ash and protein contents were 8·3 and 3·8% respectively. If the sum of starch and cellulose contents presented in Table 2 is compared with the amount of glucose found by GC analysis (Table 3), it can be seen that different methods of analysis gave almost the same results. As expected, next to glucose, xylose, arabinose and galactose contents were 11·8, 6·4 and 4·3% respectively. Rhamnose/fucose and mannose were only found in very low amounts (0·2 and 0·4% respectively).

The data listed in Fig. 1 show the solubilization and hydrolysis of bagasse polysaccharides after incubation with various enzymes or a combination of these enzymes. Differences in polysaccharide hydrolysis by the various enzyme preparations are observed. A maximum

TABLE 1
Commercial Enzyme Preparations Used for Enzymatic Hydrolysis of Sugar Cane Bagasse

Enzyme	*Supplier*
Biocellulase	Bicon Ltd, Cork, Eire
Maxazyme CL2000	Gist Brocades NV, Delft, The Netherlands
Ultra SP	Novo Industri AIS, Bagsvaerd, Denmark
Celluclast	Novo Industri AIS, Bagsvaerd, Denmark
Cellulase 9108	Rapidase, Seclin, France
Hemicellulase AS	Rapidase, Seclin, France
Pectinase	Rapidase, Seclin, France

TABLE 2
Chemical Composition of Sugar Cane Bagasse

Component	*%*
Drying loss	5·7
Protein (N × 6·25)	3·8
Uronic acids	3·3
Cellulose	35·4
Hemicellulose	20·6
Starch	1·5
Lignin	18·6
Ash	8·3
Soluble sugars	2·8

TABLE 3
Neutral Sugar Composition of Sugar Cane Bagasse

Sugar	*%*
Rhamnose/fucose	0·2
Arabinose	6·4
Xylose	11·8
Mannose	0·4
Galactose	4·3
Glucose	41·3
Total sugars	53·8

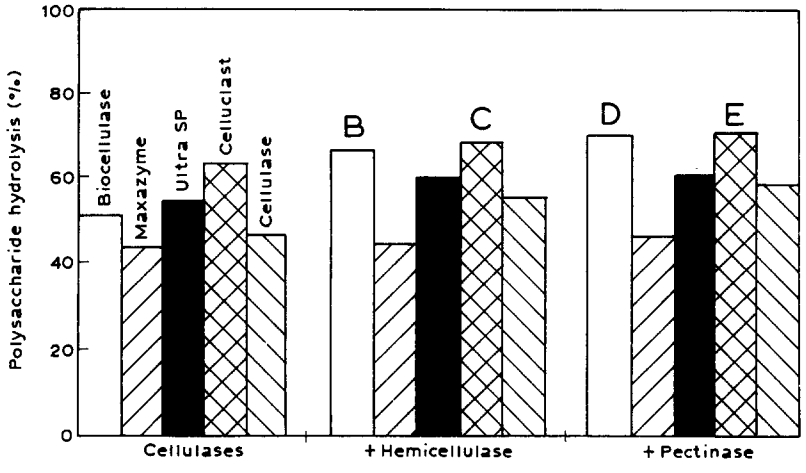

Fig. 1. Degradation limits of sugar cane bagasse by various enzyme preparations. B, Biocellulase + Hemicellulase; C, Celluclast + Hemicellulase; D, B + Pectinase; E, C + Pectinase.

hydrolysis rate of 62·2% was obtained with Celluclast enzyme, while a minimum rate (43·1%) was found in the case of samples treated with Maxazyme enzyme. To overcome possible incomplete enzyme systems, Hemicellulase was added to the various cellulase preparations. In the case of the Maxazyme preparation, this had only a minor effect (2·6%) on the hydrolysis rate of bagasse polysaccharides. But this effect increased to levels of 31·0, 11·5, 8·4 and 19·2% respectively when Bicon Biocellulase, Ultra SP, Celluclast and Rapidase-cellulase preparations were mixed.

On the basis of the amount of total sugars, which were measured in the soluble fraction: 69·1, 60·5, 71·3 and 58·1% of the polysaccharides were hydrolysed by a mixture of Rapidase pectinase with the previously listed mixture preparations respectively. Addition of the same Pectinase to the Maxazyme preparation combined with Hemicellulase resulted in a minor effect.

Increasing the incubation temperature from 40 to 50°C resulted in more solubilized sugars as shown in Fig. 2.

However, Beldman *et al.* (1985) showed that increasing the cellulase enzyme concentration from 1 to 2% resulted in only slightly more solubilized sugars from spent grain.

Because of their high degradation limits, products of enzymatic breakdown with Bicon Biocellulase and Celluclast after combination with Hemicellulase or Hemicellulase and Pectinase have been submitted, in comparison with acid hydrolysis (0·5 M H_2SO_4), to high performance liquid chromatography. The products of the digestion of sugar cane bagasse by these enzymes had much the same elution patterns. HPLC analysis obtained after acid treatment showed that the arabinosyl linkages of bagasse are highly susceptible to this hydrolysis. These linkages are known to be more degradable than the glycosidic linkages between both glucosyl and galactosyl residues (BeMiller, 1967). Because of this differential hydrolysis, arabinose was released mainly as monomer (Fig. 3(a)). Next to arabinose, xylose and several oligomers were produced. Under these conditions less glucose was liberated. This suggests that cellulose was little degraded by this acid treatment.

Besides some oligomers, glucose was the main monomeric sugar found in the digest of the combination of Biocellulase and Hemicellulase (Fig. 3(b)). Xylose and arabinose were detected in large amounts, less fructose, and only a minimal amount of cellobiose was recorded. This

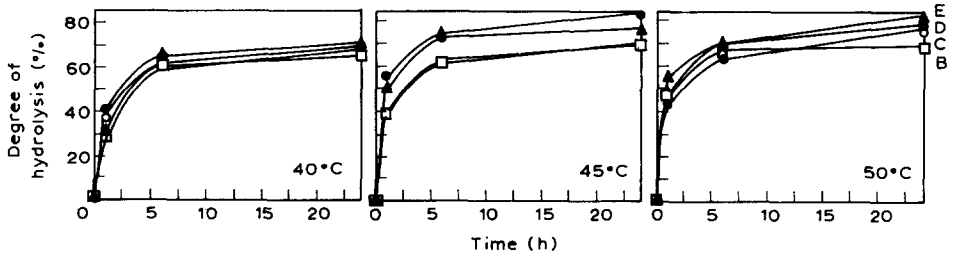

Fig. 2. Effect of incubation temperature on the hydrolysis rate of bagasse polysaccharides by various enzyme preparations. For key see Fig. 1.

implies that the combination of these two enzymes is not deficient in cellobiase activity. It is not clear why the oligomers appearing in the chromatogram are not further hydrolysed by the enzymes to smaller products. It may be that these oligomers are heteroglycans which are only degraded by special enzymes (Beldman, 1986).

Incubation of the sugar cane bagasse with a mixture of Celluclast and Hemicellulase showed that no oligomers were detected (Fig. 3(c)), glucose, xylose and arabinose were presented in quite large amounts besides some cellobiose (8% of the glucans of the digest).

HPLC analysis of sugars obtained after addition of Pectinase to the mixtures of Cellulases and Hemicellulase indicates that the hydrolysis products were almost completely in monomeric form. Actually there were only the monomers glucose, xylose, galactose and arabinose, with a minimal amount of rhamnose; no oligomers were found (Fig. 3(d) and (e)). Essentially no cellobiose was detected. This indicates that the overall susceptibility to enzymatic hydrolysis was somewhat improved by the combination of Pectinase with the Cellulase and Hemicellulase. Extrusion resulted in additional soluble sugars, mainly because cell wall polysaccharides were increased.

It must be concluded that the interregions contribute to the structural stability of the polysaccharide backbones. In other words, these regions could play an important role in controlling polysaccharide substance degradation. Therefore, it must be considered that glycosidases other than cellulolytic enzymes are involved in the hydrolysis of the inter-regions in polysaccharide substances.

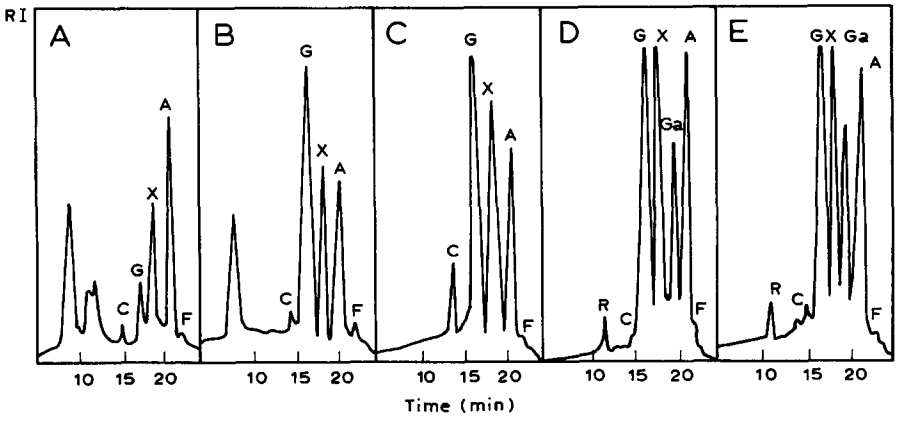

Fig. 3. HPLC analysis of products released from sugar cane bagasse by 0·5 M H_2SO_4 (a), and various polysaccharide enzyme preparations. C, cellobiose; R, rhamnose; A, arabinose; X, xylose; Ga, galactose; G, glucose; F, fructose. For key see Fig. 1.

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