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Enzymatic hydrolysis of delignified bagasse polysaccharides

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Abstract

Sugarcane bagasse, consisting of cellulose, xylan, and lignin, was chemically treated to generate bagasse samples with continuously decreasing content of lignin. These bagasse samples were hydrolyzed by cellulase and xylanase enzymes, produced earlier by *Penicillium janthinellum* NCIM 1171 in the same bagasse polysaccharides production medium. The hydrolysis was carried out by using different concentrations of the enzymes at two different temperatures, 30 and 50 °C, taking hydrolysis of Avicel as control. It was found that while the maximum hydrolysis for Avicel was 70% that of some of the bagasse polysaccharides was as high as 95%. The products of hydrolysis were glucose, xylose, and arabinose, as confirmed by high pressure ion chromatography (HPIC). It is interesting to note that arabinose, which constitutes about 10% of the weight of bagasse xylan, could also be released easily by the enzymes. Also, the initial rates of hydrolysis was found to be much higher for the bagasse polysaccharides, and in some cases about 90% of the hydrolysis occurred within 20 h. Amongst all bagasse samples, the sample with (Kappa no. 1.2, lignin content 0.18%) gave the highest degree of hydrolysis at 50 °C. Even the bagasse polysaccharide with Kappa no. 16.8 (lignin content 2.5%) underwent greater extent of hydrolysis than Avicel. Apparently, the delignified bagasse medium appears to be a facile medium for the combined hydrolytic action of the cellulase and xylanase enzymes. Considering that sugarcane bagasse is a waste biomass material available in abundance annually, this methodology can be used to value-add to this biomass to produce sugars, which can be fermented to produce biofuels like ethanol.

Keywords: Sugarcane bagasse; Bagasse polysaccharides; Chemical treatment; Cellulase; Xylanase; *Penicillium janthinellum*; Enzymatic hydrolysis

1. Introduction

Lignocellulosic biomass such as agricultural crop residues provide a low cost feedstock for biological production of fuels and chemicals, and offer economic, environmental and strategic advantages (Wyman, 1996). These materials generally contain 75–80% of cellulose and hemicellulose, which cannot be easily converted to simple monomeric sugars due to their recalcitrant nature. With the advent of modern genetics and engineering tools the cost of producing sugars from these recalcitrant fractions and converting them into products like ethanol has been significantly reduced. However, additional cost reductions are desirable to achieve competitiveness vis a vis the existing conventional fuels.

Enzymatic hydrolysis of such cellulosic material by cellulase enzymes is the most promising approach to get high product yields vital to economic success (Hinman et al., 1992; Lynd et al., 1996). The high cost of cellulase enzyme production hinders the application of these enzymes to bioethanol production (Himmel et al., 1999; Wooley et al., 1999). The use of such waste and low cost cellulosic biomass for enzyme production would significantly reduce the costs of the enzyme provided cellulase productivities are comparable to those obtained in presence of other carbon sources like lactose (Kadam, 1996). In a previous paper (Adsul et al., 2004), we have shown that high cellulase productivities can be achieved by the use of chemically pretreated biomass as carbon sources for specific microorganisms.

Lynd (1996) has summarized the desireable properties for an ideal lignocellulose material after chemical

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pretreatment, i.e. it should (a) produce reactive fibers, (b) yield pentoses in non-degraded form, (c) not release the compounds that significantly inhibit fermentation, (d) work in reactors of reasonable size with moderate cost, (f) produce no solid residues, (g) have a high degree of simplicity, and (h) be effective at low moisture contents. A number of pretreatment options are available: acid pretreatment, alkaline treatment, steam explosion, wet oxidation, organic solvent pretreatment, and hot water. Among all these methods, acid pretreatment is still the method of choice in several model processes.

The present study deals with the evaluation of delignified sugarcane bagasse as a source for the production of sugars (glucose, xylose, arabinose) using enzymes that were produced by treating delignified bagasse polysaccharides with a mutant of *Penicillium janthinellum* NCIM 1171 obtained from our own laboratory. This study shows the high potential of agricultural waste biomass as important sources of sugars for fermentation to biofuels like ethanol.

2. Experimental

2.1. Materials and methods

2.1.1. Chemicals

Cellulose-123 powder was obtained from Carl Schleicher and Schull Co. Dassel, FRG. *p*-Nitro phenyl β-D-glucopyranoside, carboxymethylcellulose (CMC), xylan (oat spelts), 3,5-dinitrosalysilic acid and Sigmacell were obtained from Sigma-Aldrich Co. St Louis, MO USA. Sodium azide was obtained from S.D.Fine-Chem (India). Avicel PH-101 obtained from Fluka chemie GmbH.

2.1.2. Delignified bagasse samples

Sugarcane bagasse was obtained from Tamil Nadu Pulp and Paper Mills, Chennai, India. The bagasse was made into 100 mesh fine powder by use of a laboratory blender at 3000 rpm. The delignification of bagasse was generally done using different quantities of sodium chlorite at pH 3.8 at 70 °C (samples D–F), hydrogen peroxide treatment (sample B), and alkali (sample C). Samples G and H were prepared by a more elaborate steam explosion treatment followed by bleaching sequences using a proprietary process. The details are shown in Table 1.

2.1.3. Microorganism and culture media

Mutant of *P. janthinellum* NCIM 1171 was obtained from National Collection of Industrial Microorganism (NCIM), National Chemical Laboratory, Pune, India. This culture was maintained on Potato Dextrose Agar (PDA) and sub-cultured once in every 3 months. PDA contained (g/l) extract from 200 g of potatoes, glucose, 20.0 g; yeast extract, 1.0 g; and agar, 20.0 g. Production medium described by Mandels and Weber (1969) was used for enzyme production.

2.1.4. Enzyme production and enzyme assay

The procedure for enzyme production and assays was followed as reported earlier (Adsul et al., 2004).

2.1.5. Enzyme units

One unit (IU) of enzyme activity was defined as the amount of enzyme required to liberate 1 μmol of glucose, xylose or *p*-nitrophenol from pNPG from the appropriate substrates per minute per milliliter of crude filtrate under assay conditions.

2.1.6. Enzymatic hydrolysis

The hydrolysis of the samples was carried out in 100 ml flask containing 50 ml citrate buffer (pH 4.5, 50 mM), 0.5 g different bagasse samples or other cellulosic materials (Avicel, Sigmacell and Cotton), 5 mg sodium azide and crude enzyme preparation. This mixture was incubated at 30 or 50 °C on with shaking at 150 rpm. The samples were analyzed for the reducing sugars after suitable time intervals. The enzyme preparation contains 3.5 IU/ml FPA, 4.0 IU/ml β-glucosidase, 75 IU/ml CMCase and

Table 1
Total reducing sugars obtained by enzymatic hydrolysis of 0.5 g (1%) of each bagasse and cellulose samples

Sample	Kappa no.	Total reducing sugars (mg) after first enzymatic hydrolysis using 0.5 g bagasse	Total reducing sugars from residues of first enzymatic hydrolysis (mg)	Total sugar obtained from 0.5 g of bagasse sample (mg)	Hydrolysis (%)
A	79.4	34.5	10.5	45.0	9.0
B	66.6	97.5	18.7	116.2	23.2
C	54.7	216.6	26.3	242.9	48.5
D	26	320.0	5.8	325.8	65.1
E	17.8	281.6	8.0	289.6	57.9
F	16.8	338.3	0.0	338.3	67.6
G	1.2	402.9	70.1	473.0	94.6
H	0.8	365.0	55.5	420.5	84.1
I	Cotton	16.7	40.4	57.1	11.4
J	Avicel PH101	143.5	164.1	307.6	61.5
K	Sigmacell	153.5	179.7	333.2	66.6

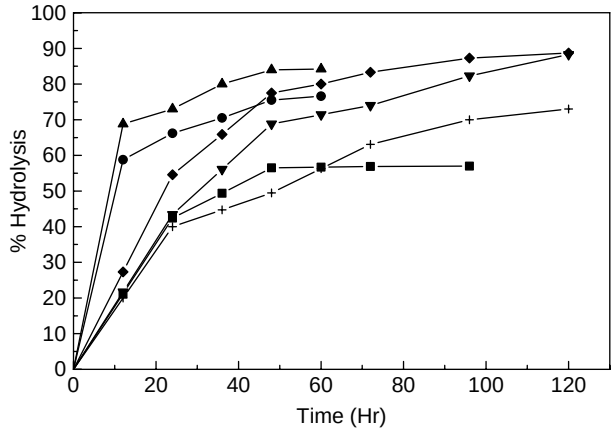

Fig. 1. Hydrolysis profile of sample H (Kappa no. 0.8) at 30 °C (x, +; 2x, ▼; 3x, ♦) and 50 °C (x, ■; 2x, ●; 3x, ▲) (x, 2x, and 3x denote the concentrations of cellulase and xylanase enzymes, mentioned in the experimental part).

80 IU/ml xylanase. This preparation is denoted further as x concentration of enzyme. Similarly, 2x and 3x denote multiples of the x concentration.

2.1.7. Analytical method

The reducing sugar was estimated as either xylose or glucose equivalent by dinitrosalicylic acid (DNS) method (Fisher & Stein, 1961). The Kappa number of the samples was determined by the method described in Ulman's Encyclopedia of Industrial Chemistry (Ulmann's Encyclopedia of Industrial Chemistry, 2002) and the lignin content was obtained by multiplying the Kappa number with 0.15 factor. (This factor is generally applied to only samples with low lignin content, say upto Kappa no. 20 or so.)

2.1.8. High pressure ion chromatography (HPIC)

A Dionex HPIC consisting of GS 50 quaternary gradient pump, ED-50 Electrochemical Detector, Rheodyne injector and chromeleon software was used. The column was a CarboPack PA-10, 4×250 mm (Analytical) column with a CarboPack PA-10 guard column. The mobile phase was 20 mM NaOH at a flow rate of 1 ml/min. The injection volume was 25 μl, and the observed pressure drop was 2700 psi

3. Results and discussion

Fig. 1 presents the data on enzymatic hydrolysis of delignified bagasse with Kappa no. 0.8 (lignin content 0.12%). The purpose behind using different concentrations of enzymes and different temperatures was to establish optimum conditions for the rapid hydrolysis of the bagasse sample. This would throw some light on the economic feasibility of the method being developed. Fig. 1 shows that 3x enzyme activities at 50 °C achieved approximately 90% hydrolysis.

In the case of Avicel, 3x concentration at 30 °C seems to be optimum for maximum hydrolysis of Avicel. We got approximately 55% hydrolysis within 60 h; the slower rate could be due to its microcrystalline structure.

One would have expected sample G (Kappa no. 1.2) to be less efficient than sample H with Kappa no. 0.8, but we actually found it to be slightly superior. It appears that residual lignins play a part in binding the enzymes, leading to higher local concentration of enzymes, and consequently higher hydrolysis rates. This fact has also been noted in prior literature (Palonen et al., 2004). This data is shown in Fig. 2, where over 95% hydrolysis occurs within 48 h at 50 °C. Indeed, in this case the initial rate of hydrolysis was such that about 90% of the hydrolysis occurred in less than 20 h.

However, as the lignin content increases beyond a certain limit, it begins to have an adverse effect on the rate

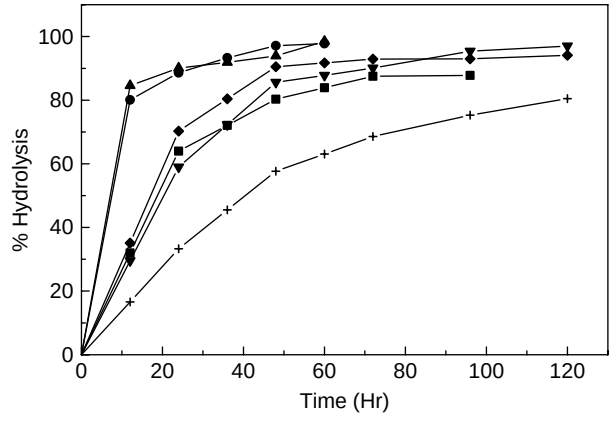

Fig. 2. Hydrolysis profile of sample G (Kappa no. 1.2) at 30 °C (x, +; 2x, ▼; 3x, ♦) and 50 °C (x, ■; 2x, ●; 3x, ▲) (x, 2x, and 3x denote the concentrations of cellulase and xylanase enzymes, mentioned in the experimental part).

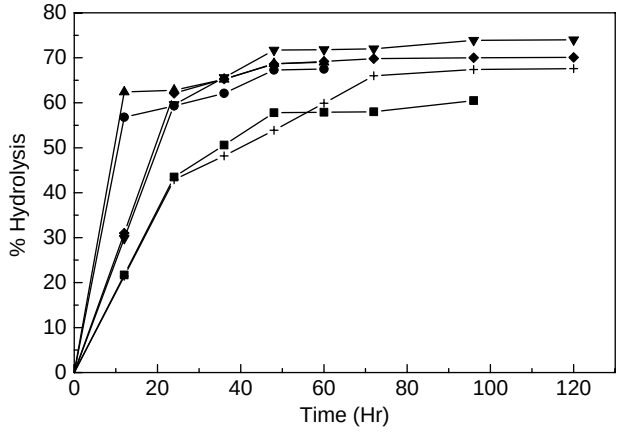

Fig. 3. Hydrolysis profile of sample F (Kappa no. 16.8) at 30 °C (x, +; 2x, ▼; 3x, ♦) and 50 °C (x, ■; 2x, ●; 3x, ▲) (x, 2x, and 3x denote the concentrations of cellulase and xylanase enzymes, mentioned in the experimental part).

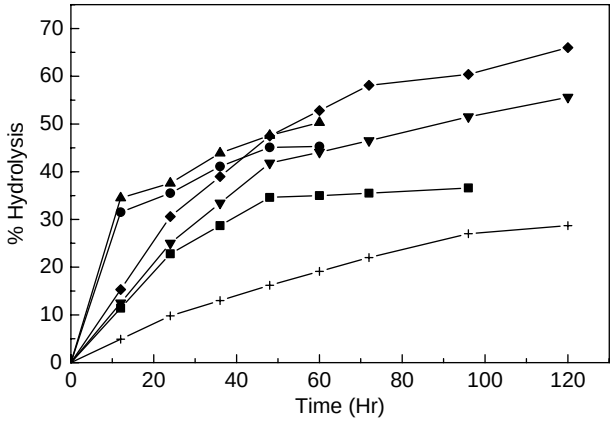

Fig. 4. Hydrolysis profile of avicel at 30 °C (x, +; $2x$, ▼; $3x$, ♦) and 50 °C (x, ■; $2x$, ●; $3x$, ▲) (x, $2x$, and $3x$ denote the concentrations of cellulase and xylanase enzymes, mentioned in the experimental part).

of hydrolysis, as now it not only acts as a phenolic (acidic) inhibitor, but also its crosslinked structure provides a steric barrier to the enzymes to access the polysaccharide components. This is seen in Fig. 3 for sample F (Kappa no. 16.8) where the total hydrolysis was limited to about 67% of the weight of the polysaccharide taken. As the lignin content rises further, the extent of hydrolysis decreases further, as shown in Table 1. Ultimately, for untreated raw bagasse, with a Kappa no. of 79.6, only 9% hydrolysis was achievable. For pure microcrystalline cellulose (Avicel and Sigmacell) the hydrolysis extent was limited to 61.5 and 66.6%, respectively, inspite of having no lignin content. This fact is attributable to their strongly crystalline nature, which prevents easy access to enzymes. Thus, the performance of Avicel (Fig. 4) and Sigmacell was comparable to bagasse polysaccharides with Kappa nos in the range 16–26. Considering the inexpensive nature of the bagasse polysaccharides, there is no doubt about their commercial potential for producing sugars like glucose, xylose, and arabinose. It is pertinent to point out here that the xylose content of bagasse xylan is 75–80% and that of arabinose, a relatively rare and expensive sugar, is about 10%. Both these major constituents of bagasse xylan were hydrolyzed easily by the enzymes produced and used by us. This is clearly shown by the HPIC data (Fig. 5), wherein the peaks matched completely with standards injected.

In conclusion, we have shown the vast techno-economical potential for using waste agricultural biomass like sugarcane bagasse for producing sugars, which can be used for producing biofuels like ethanol. We are continuing our work in that direction. Rare sugars like arabinose can also be produced by this method. This methodology is generic, and will be equally applicable to other biomass like wheat, rice and cereal straws, which are produced annually in most countries in abundant quantities. Such technologies have great implication for using ‘green raw materials’ and producing ‘green products’, much needed today.

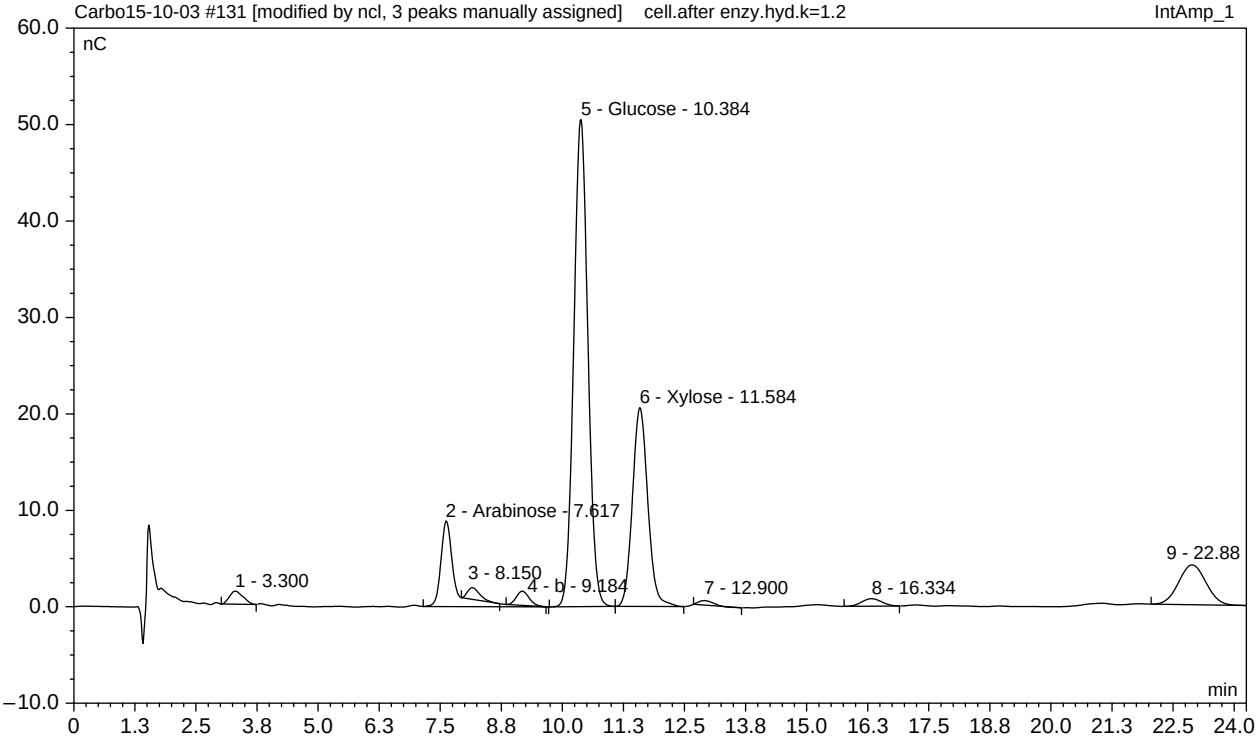

Fig. 5. HPIC analysis of the sugars produced by the hydrolysis of sample G, showing glucose, xylose and arabinose.

Acknowledgements

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