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Short communication

Acid catalysed steaming for solubilization of bamboo grass xylan

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Abstract

Culms of bamboo grass (*Sasa senanensis* Rehd.) were treated with saturated steam at a range of temperatures between 170 and 197°C for 10 min in the presence of various acid catalysts in order to improve solubilization of the xylan in the substrate. The catalysts were Lewis acids, inorganic salts and organic acids. These compounds strongly modified the steam-hydrolysis and solubilization of bamboo grass xylan. The use of low levels of Lewis acids or $CaCl_2$ had the advantage of a substantial reduction in temperature requirement for the steaming treatment. The solubilized sugars were mainly composed of xylose, arabinose and low molecular weight xylo-oligosaccharides.

Keywords: Acid catalysed steaming; Xylan; Xylose; Xylo-oligosaccharides; Bamboo grass; *Sasa senanensis* Rehd

1. Introduction

Bamboo grasses, perennial grasses with woody culms, constitute the Bambusoideae, a subfamily of the Gramineae. They contain 20–30% hemicelluloses which are mainly composed of β-D-xylan. In *Sasa senanensis* Rehd. xylan, approximately every 6–7th and 100th xylose unit carries arabinosyl and glucuronosyl substituents, respectively (Yoshida et al., 1998). Because of the limited content of glucuronosyl and 4-*O*-methylglucuronosyl stubs, *S. senanensis* xylan is hydrolysed to afford a mixture of xylose, arabinose and xylo-oligosaccharides under relatively mild reaction conditions. Recent studies on solubilization of bamboo grass xylan (Aoyama et al., 1995; Aoyama, 1996) have shown that 55% of xylan present in the culm of *S. senanensis* could be recovered as a mixture of xylose and xylo-oligosaccharides by steaming and subsequent water extraction, and that pre-extraction with water before steaming significantly improved the recovery of xylo-oligosaccharides.

It has been demonstrated that impregnation of wood chips with various acid catalysts before steaming resulted in an increase in the recovery of hemicellulose sugars as well as enhancement of the enzymatic saccharification of steamed substrates (Mackie et al., 1985; Brownell et al., 1986; Sudo et al., 1986; Wayman et al., 1986; Dekker, 1987; Clark et al., 1989; Schwald et al., 1989; Rughani et al., 1990; Rughani et al., 1992; Ramos et al., 1992; Tsuda et al., 1998). The use of low levels of acid catalyst has the advantages of a substantial reduction in temperature and residence time requirements for the steaming treatment. In this study, culms of *S. senanensis* were treated with saturated steam in the presence of various acid catalysts. The catalysts included Lewis acids, inorganic salts and organic acids. The effects of the catalysts on the recovery yield of solubilized sugars were investigated.

2. Methods

The culms of *S. senanensis* were chopped in a hammer-mill and screened to remove particles smaller than 1 mm. The chemical composition of the stored material was 24.5% pentosan (including 21.6% xylan), 39.4% hexosan and 23.9% Klason lignin. About 5% glucan arose from starch and water-soluble glucose oligomers.

Compared with wood, the chemical characteristics of bamboo grass culms have higher contents of water solubles and ash, especially potassium and silicon (Kawase and Ujiie, 1985). In the previous work (Aoyama, 1996), we reported that the removal of basic salts from bamboo grass culms before steaming enhanced the hydrolysis rate of xylan and greatly improved the recovery of xylo-oligosaccharides. So, to

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remove basic salts and other water solubles, the chopped culms were pre-extracted with hot water, using the Japanese Industrial Standard method (JIS P8005-1959), and subsequently air-dried.

Before steaming, the substrates were sprayed with desired amounts of acid catalyst aqueous solution or water (control) and kept in a refrigerator for a few days. The catalysts were $AlCl_3$, $Al_2(SO_4)_3$, $FeCl_3$ $CaCl_2$, NH_4Cl, $(NH_4)_2SO_4$, acetic acid, maleic anhydride, succinic anhydride and phthalic anhydride. Although aqueous solutions of NH_4Cl and $(NH_4)_2SO_4$ are neutral, the solutions show strong acidic properties by the evaporation of NH_3 at elevated temperatures (Sudo et al., 1986). The initial moisture content of the substrates was 67% (DM basis). The moist samples were placed in a stainless steel basket and treated in an autoclave with saturated steam at temperatures ranging from 170 to 197°C for 10 min. After closing the steam inlet valve, the pressure was bled down to atmospheric at a controlled rate. The resulting steamed culms were air-dried and ground in a Wiley mill to pass through a 32 mesh (0.5 mm) screen. A portion of each type of the steamed and ground culm was extracted with hot water using the Japanese Industrial Standard method (JIS P8005-1959). For convenience, the following abbreviations were used in referring to the steamed culm: WS (hot water soluble fraction of the steamed culm); WI (hot water-extracted residue of the steamed culm). Loss for each type of steamed culm in Table 1 was calculated from the difference between the amount of xylan in the raw material (24.5% as xylose after complete acid hydrolysis) and that of the steamed culm (as the sum of xylose in WS and WI after complete acid hydrolysis). No attempt was made to determine the components in the steam condensate.

High performance liquid chromatography (HPLC) was used for partial mass balances for xylan on the acid catalysed steaming and subsequent hot water extraction and characterization of the solubilized sugars present in the hot water soluble fraction (WS). In order to determine the amount of xylan-derived sugars or xylan, the hot water soluble fraction (WS) and steamed and extracted residue (WI) were hydrolysed with 3% H_2SO_4. Since solubilized hemicelluloses are more readily hydrolysed than original cell-wall polysaccharides, hydrolysis of such oligo- and polysaccharides can be carried out in one stage using a dilute mineral acid. To 25 ml of each hot water-extract liquor (WS), 0.9 ml of 72% H_2SO_4 was added. The solution was refluxed for 1 h, and then the hydrolysate was quenched to room temperature. The cool hydrolysate was filtered to remove insoluble precipitates. After the addition of a known amount of erythritol (as an internal standard), the filtrate was neutralized with $Ba(OH)_2$, followed by centrifugation. The supernatant was concentrated *in vacuo*, transferred into a 10 ml volumetric flask and finally diluted to the scale of the volumetric flask with water for determination of xylose in the hydrolysate. For the steamed and extracted residue (WI), the samples for determination of xylan were prepared by the method of Effland (1977) and Pettersen et al. (1984). To about 300 mg of each steamed and extracted residue (WI), 3 ml of 72% H_2SO_4 was added. The mixture was placed in a 30°C water bath, stirring frequently to assure complete solution. After 1 h, the solution was diluted to 3%

Table 1
Recovery yield of xylan (as xylose) in the steamed culm of bamboo grass, *S. senanensis* Rehd.

Catalyst[a]	Recovery yield of xylan (%)[b]											
	170°C			179°C			191°C			197°C		
	WS[c]	WI[c]	L[c]	WS	WI	L	WS	WI	L	WS	WI	L
Uncatalysed	0.3	22.8	1.4	3.6	19.9	1.0	10.6	11.2	2.7	16.5	3.6	4.4
$AlCl_3$	7.6	14.6	3.5	13.4	8.5	2.6	13.0	2.1	9.4	7.2	–[d]	17.3
$Al_2(SO_4)_3$	6.7	14.0	3.8	11.4	6.2	6.9	12.0	–	12.5	4.0	–	20.5
$FeCl_3$	6.1	14.9	3.5	9.5	8.9	6.1	15.8	3.0	5.7	11.2	–	13.3
$CaCl_2$	1.8	16.7	6.0	7.2	12.3	5.0	17.1	3.2	4.2	15.3	–	9.2
NH_4Cl	1.4	17.6	5.5	4.6	14.4	5.5	10.0	6.1	8.4	8.2	1.9	14.4
$(NH_4)_2SO_4$	1.0	14.1	9.4	4.8	11.1	8.6	10.0	4.4	10.1	8.2	1.6	14.7
Acetic acid	1.1	15.9	7.5	4.6	12.8	7.1	13.8	7.2	3.5	14.9	2.6	7.0
Maleic anhydride	2.1	18.0	4.4	6.6	13.3	4.6	10.9	7.3	6.3	13.0	3.9	7.6
Succinic anhydride	1.3	18.3	4.9	5.1	17.0	2.4	10.2	8.0	6.3	12.4	4.1	8.0
Phthalic anhydride	1.4	15.2	7.9	4.9	11.3	8.3	6.9	4.8	12.8	8.9	1.9	13.7

[a]The amount of catalyst added was 10 mmol kg^{-1} substrate.
[b]Each figure (%) is based on dry substrate.
[c]WS: Water soluble fraction; WI: water insoluble fraction; L: loss (see Methods for determination).
[d]Not detected.

H_2SO_4 and treated in an autoclave with saturated steam at 120°C for 1 h. The hydrolysate was quenched to room temperature and insoluble precipitates filtered off. The filtrate was transferred into a 200 ml volumetric flask and diluted to the scale of the volumetric flask with water (stock solution). A known amount of erythritol was added to a suitable aliquot of the stock solution, and the solution was neutralized with $Ba(OH)_2$, followed by centrifugation. The supernatant was used for determination of xylose in the hydrolysate. An HPX 87P column (300 × 7.8 mm, Bio-Rad) was employed for analysis of neutral sugars in the hydrolysate. Xylo-oligosaccharides present in the hot water extract (WS) were determined using an HPX 42C column (300 × 7.8 mm, Bio-Rad). Each figure in Table 1 and Table 2 is the mean of two replicates.

3. Results and discussion

Mass balances for the xylan on acid catalysed steaming and subsequent water extraction are shown in Table 1. As expected, the catalysts strongly modified the hydrolysis and solubilization of the xylan. The addition of any of the acid catalysts resulted in increased losses of xylan, although the extent of xylan hydrolysis was greatly improved. In particular, by the addition of Lewis acids, $AlCl_3$, $Al_2(SO_4)_3$ or $FeCl_3$, extensive thermal decompositions of the formed sugars occurred at higher temperatures. The total xylose balance indicated that about 13–21% xylan-derived sugars were destroyed at 197°C in the presence of Lewis acids, whereas loss in the uncatalysed system was only 4.4%. When the substrate was steamed in the presence of ammonium salts or organic acids, a lesser extent of xylan hydrolysis was observed compared with Lewis acids or $CaCl_2$. An acid-catalysed system with $CaCl_2$ provided a good recovery of xylan-derived sugars (17.1% based on dry substrate at 191°C). These results indicated that the use of low levels of Lewis acids or $CaCl_2$ had the advantage of a substantial reduction in temperature requirement for the steaming treatment.

Table 2 shows the effects of catalyst concentration on both the amount of solubilized sugars and the chemical composition of soluble xylan fragments. The amount of steamed and extracted residue (WI) decreased as the concentration of catalyst increased. Xylan present in the substrate was almost completely hydrolysed during the catalysed steaming at higher concentrations of Lewis acids (20 mmol kg^{-1} or more). The chemical composition of solubilized sugars was also greatly affected by the addition of catalyst. The use of low levels of the catalysts resulted in increased yields of xylose and xylo-oligosaccharides, as shown in Table 2. When the steaming was carried out at 191°C in the presence of $AlCl_3$ or $Al_2(SO_4)_3$, the best recov-

Table 2
Recovery yields of hemicellulose after acid catalysed steaming of *S. senanensis* culm at 191°C for 10 min

Catalyst (mmol kg^{-1} substrate)	Recovered hemicellulose (%, DM basis)								
	Hot water extract (WS)[a]								Extracted residue (WI)[b]
	Xyl	Ara	X_2	XA	X_3	X_4	X_5	X_n^b	
Uncatalysed	3.4	1.4	2.1	0.3	1.1	0.4	0.2	2.9	11.2
$AlCl_3$ (5)	6.1	0.9	2.4	0.4	1.8	1.2	0.7	3.1	3.9
(10)	5.5	1.1	1.7	0.5	1.2	1.3	0.5	2.1	2.1
(20)	1.7	1.3	1.0	0.4	0.4	0.2	0.1	0.4	–[c]
(30)	0.4	0.8	0.2	0.4	0.1	–	–	–	–
$Al_2(SO_4)_3$ (5)	6.8	0.9	3.0	0.3	1.6	1.0	0.4	2.0	3.5
(10)	5.7	0.8	2.5	0.5	1.3	0.7	0.2	1.0	–
(20)	1.2	1.2	0.4	0.4	0.2	0.1	0.1	0.1	–
(30)	0.4	1.0	0.2	0.3	0.1	0.1	–	–	sz–
$FeCl_3$ (5)	4.2	1.1	3.5	0.5	2.3	1.5	0.8	2.0	3.7
(10)	4.0	0.9	3.3	0.4	2.7	2.0	1.1	1.7	3.0
(20)	5.7	1.3	2.4	0.3	2.4	1.1	0.5	1.6	–
(30)	5.2	1.1	2.0	0.3	1.1	0.9	0.6	0.2	–
$CaCl_2$ (5)	4.0	1.1	3.4	0.4	2.7	2.5	1.1	2.1	4.0
(10)	4.5	1.3	3.0	0.3	2.6	2.5	1.3	2.2	3.2
(20)	5.7	1.0	3.5	0.3	2.3	2.2	0.9	1.4	3.0
(30)	6.0	0.9	3.1	0.5	2.6	2.0	0.5	0.7	2.5

[a]Xyl: Xylose; Ara: arabinose; X_2: xylobiose; XA: *O*-β-D-xylopyranosyl-(1→2)-L-arabinofuranose; X_3: xylotriose; X_4: xylotetraose; X_5: xylopentaose; X_n: solubilized xylan ($n \geq 6$).
[b]Each sample was hydrolysed with 3% H_2SO_4 and xylose in the hydrolysate was determined by HPLC. Figures were expressed as xylose (see Methods for determination).
[c]Not detected.

eries for both xylose and xylo-oligosaccharides were obtained at a catalyst level of 5 mmol kg^{-1}. An additional increase in the concentration resulted in a decrease in the yield of these sugars. On the other hand, the optimum concentration for $FeCl_3$ or $CaCl_2$ necessary to maximize the yield of solubilized sugars was 10 mmol kg^{-1}. A catalysed steaming with $CaCl_2$ at a concentration of 10 mmol kg^{-1} provided the best recovery of xylan derived-sugars in a 17.7% yield, of which 67% could be recovered as a mixture of xylo-oligosaccharides.

In conclusion, various acid catalysts, Lewis acids, acidic inorganic salts and organic acids, strongly modified the steam-hydrolysis and solubilization of bamboo grass xylan. The use of low levels of Lewis acids or $CaCl_2$ had the advantage of a substantial reduction in temperature requirement for the steaming treatment. The resulting sugars were mainly composed of xylose, arabinose and low molecular weight xylo-oligosaccharides. Since xylo-oligosaccharides and arabinose diets have been shown to exert beneficial effects on diabetic symptoms (Imaizumi et al., 1991; Sanai et al., 1993), the solubilized sugars prepared from steamed bamboo grass could be used as a functional food additive.

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