Evaluation of Ammonia Pretreatment for Enzymatic Hydrolysis of Sugarcane Bagasse to Recover Xylooligosaccharides

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Abstract: Sugarcane bagasse is a useful biomass resource. In the present study, we examined the efficacy of ammonia pretreatment for selective release of hemicellulose from bagasse. Pretreatment of bagasse with aqueous ammonia resulted in significant loss of xylan. In contrast, pretreatment of bagasse with anhydrous ammonia resulted in almost no xylan loss. Aqueous ammonia or anhydrous ammonia-pretreated bagasse was then subjected to enzymatic digestion with a xylanase from the glycoside hydrolase family 10 or a xylanase from the GH family 11. The hydrolysis rate of xylan in bagasse pretreated with aqueous ammonia was approximately 50 %. In contrast, in the anhydrous ammonia-pretreated bagasse, xylan hydrolysis was > 80 %. These results suggested that anhydrous ammonia pretreatment would be an effective method for preparation of sugarcane bagasse for enzymatic hydrolysis to recover xylooligosaccharides.

Key words: Ammonia pretreatment, sugarcane bagasse, hemicellulose, xylan, β-xylanase

INTRODUCTION

Sugarcane bagasse is a by-product of the sugar production process.Cogenerated energy derived from bagasse is used as an energy source to operate sugar factories. However, surplus bagasse tends to accumulate if the factory occupancy rate increases. Generally, the low energy density and light specific gravity of biomasses hinder their efficient utilization such as energy sources, as these features increase transportation costs. However, because bagasse has a high energy density compared to other biomass resources, it potentially is a desirable biomass resource.10–12

Plant cell walls, such as bagasse, are primarily comprised of cellulose, hemicellulose, and lignin. Often, biomass utilization processes such as bioethanol production use only cellulose, and the hemicellulose and lignin remain unused. Thus, these biomass utilization processes are not practical, as more than half of the resources are not exploited.

In order to increase the possible use of hemicellulose, our group has been studying the enzymatic degradation of hemicellulose.3,4,6,7,8,9,10,11,12,13,14 Biomass pretreatment is required prior to its enzymatic degradation. However, pretreatment methods to optimize enzyme-mediated recovery of hemicellulose have not been studied. In the present study, we investigated the relationship between ammonia pretreatment and enzymatic degradation of bagasse in order to find a suitable pretreatment method to allow efficient hemicellulose recovery from biomass resources.

MATERIAL AND METHODS

Biomass and pretreatment. Sugarcane bagasse was a kind gift from Mitsui Sugar Co., Ltd. Figure 1 illustrates the workflow of the pretreatment strategy. Aqueous ammonia pretreatment was performed by immersing 10 g dry bagasse in 3 % (w/w) or 5 % (w/w) aqueous ammonia, followed by incubation at 85 °C for 24 h. Thereafter, the treated bagasse was washed with running water until the pH became neutral, and the sample was subsequently dried at 60 °C. Non-aqueous ammonia treatment was conducted according to a method previously reported by Sakuragi et al.11,15 Briefly, 30
The reactions were incubated at 40 °C for 72 h, and were ly described method.

Bio-Rad, Hercules, CA, USA) and eluted with distilled wa‐

tance liquid chromatography and assessed using an IR de‐

IR-6100 spectrophotometer (JASCO, Tokyo, Japan) in

Materials were collected by centrifugation, followed by three

terminated by heating at 100 °C for 20 min. Insoluble mate‐

Fig. 1. The flowchart of ammonia pretreatment of bagasse fol‐

lowed by enzyme hydrolysis.

g dry bagasse was placed in a pressure vessel and filled with ammonia gas, and the sample was incubated at 100 °C for 2.5 h at 6 MPa.

Enzyme hydrolysis. Two xylanases derived from Strepto‐myces olivaceoviridis E-86 were used. One xylanase is a member of the glycoside hydrolase (GH) family 10 (GH10, SoXyn10A), and the other is a member of the GH family 11 (GH11, SoXyn11B). A description of the GH families is available at the CAZy website (http://afmb.cnrs-mrs.fr/CAZY). SoXyn10A and SoXyn11B were prepared ac‐

hence according to previously reported methods. Each enzyme reaction mixture contained 2 g bagasse, 50 mL distilled wa‐

ter, 40 mL McIlvaine buffer (pH 7.0), and 10 mL purified enzyme preparation (0.17 µmol SoXyn10A or SoXyn11B). The reactions were incubated at 40 °C for 72 h, and were terminated by heating at 100 °C for 20 min. Insoluble mate‐

rials were collected by centrifugation, followed by three washes with distilled water. The samples were then dried in an oven at 60 °C.

Analysis. The compositions (neutral sugars, organic acids, lignins and nitrogen content) of untreated and ammonia-pretreated bagasses were analyzed according to a previous‐ly described method. The Fourier transform infrared spectroscopy (FT-IR) spectrum was measured using an FT/IR-6100 spectrophotometer (JASCO, Tokyo, Japan) in transmittance mode from 4000 to 400 cm$^{-1}$ in a KBr disc. The KBr disc was prepared by dispersing the solid sample in KBr salt. For analysis of the solubilized hydrolysis products of control and pretreated bagasse samples, the reaction mixtures described above were subjected to high-performance liquid chromatography and assessed using an IR de‐

tector (LC-2000Plus, JASCO, Tokyo, Japan). The samples were analyzed using an HPX-87P column (7.8 × 300 mm, Bio-Rad, Hercules, CA, USA) and eluted with distilled wa‐

ter at a flow rate of 0.6 mL/min at 70 °C.

RESULTS AND DISCUSSION

Effect of ammonia pretreatment on bagasse composition.

Several pretreatment methods for biomass, such as acid, hydrothermal, ionic liquids and alkali have been reported. Acid and hydrothermal pretreatments remove hemicellu‐

lose from biomass, and are not suitable for enzymatic deg‐

radation of hemicellulose. There have been several attempts to produce xylooligosaccharides from mushroom waste beds and corn cobs using hydrothermal treatment. In these studies, more than 80 % of the hemicellulose fraction was recovered from corn cobs, but a significant amount of furfural was also detected.

Ammonia pretreatment is an alkali pretreatment method, and has been widely used in hemicellulose research. Ammonia fiber expansion (AFEX) pretreatment results in cel‐

lulose decrystallization, partial hemicellulose depolymeri‐

zation, removal of hemicellulose acetyl groups, cleavage of lignin–carbohydrate complex (LCC) linkages, lignin C–O–C bond cleavage, increased accessible surface area due to structural disruption, and increased wettability of treated bi‐

omass. There are a few studies evaluating ammonia pre‐

treatment of bagasse, but the aims of these studies were saccharification and conversion to ethanol or biogas, not re‐

covery of the hemicellulose fraction. Thus, we in‐

vestigated whether pretreatment of saccharine bagasse with aqueous or anhydrous ammonia was effective at recovering hemicellulose.

The composition of bagasse before and after aqueous or anhydrous ammonia pretreatment is shown in Table 1. In the aqueous ammonia pretreatment group, the post-treat‐

ment bagasse weight decreased with increased ammonia concentrations of 3 % to 5 %. Lignin content tended to de‐

crease in proportion to ammonia concentration. Hemicellu‐

lose content also slightly decreased when the ammonia con‐

centration was increased (Fig. 2). On the other hand, except for amide formation, no significant change in composition was observed with anhydrous ammonia pretreatment. Be‐

cause the acetyl group in bagasse was removed by aqueous ammonia treatment, the disassociated acetyl group from the biomass was likely dehydrated and condensed with ammo‐

nia to form acetamide in the anhydrous ammonia treated bagasse.

Effect of ammonia pretreatment on enzyme-mediated xy‐

lan degradation.

Next, control and pretreated bagasse was digested with GH10 or GH11 xylanases from S. olivaceoviridis E-86 (Ta‐

ble 1). The weights of the GH10 xylanase hydrolysate from aqueous or anhydrous ammonia-pretreated bagasse were 66.5 % (3 % aqueous ammonia), 64.5 % (5 % aqueous am‐

monia), and 59.7 % (anhydrous ammonia), and 59.7 % (anhydrous ammonia) relative to the weight of non-treated bagasse. Correspondingly, the xylan hydrolysis rates were 34 % (3 % aqueous ammonia), 38 % (5 % aqueous ammonia), and 80 % (anhydrous ammonia). In contrast, the weight of the GH11 xylanase hydrolysate from aqueous or anhydrous ammonia-pretreated bagasse was 62.6 % (3 % ammonia), 60.9 % (5 % ammonia), and 57.8 % (anhydrous ammonia) relative to the weight of non-
exhibited synergistic effects on hemicellulose hydrolysis after lime treatment of bagasse, only initial hydrolysis rates of the hydrolysis were investigated, and the final hydrolysis rates were not reported. Thus, it was unclear how much bagasse hemicellulose was broken down by these hemicellulases. The merit of using enzyme as a tool for selective release of hemicellulose from biomass is not only environmentally friendly and equipment friendly, but also allows for removal of heteropolysaccharide-containing pentose without generating compounds toxic to yeast, such as furfural. There are several reports of bagasse pretreatment with aqueous ammonia followed by saccharification and fermentation. In these cases, hemicellulose degradation products, together with cellulose degradation products, are fermented, resulting in inefficient fermentation. These approaches apparently do not take advantage of the enzyme substrate specificity, which precisely discriminates substrate structure for selective cleavage of hemicellulose. Because hemicellulose (xylan) is a heteropolysaccharide and it is difficult to control the cleavage sites with acid or hydrothermal treatment, the products obtained after these treatments are mixtures of xylene, arabinose, galactose, mannose and other compounds, precluding recovery and utilization of hemicellulose. In contrast, enzyme-mediated cleavage produces products exhibit a defined structure. This is advantageous, as the heterogeneous and complex structure of hemicellulose can be recovered in a homogeneous state, allowing for more convenient functional analysis of the products. It is known that the 19 xylan hydrolysis rate in all pretreated samples.

Interestingly, the amount of Glc in the SoXyn11B hydrolysate was significantly low (45.9 %) compared to the SoXyn11A hydrolysate (50.9 %). As discussed in a previous study, the compact wide shallow structure of the GH11 substrate binding cleft could facilitate more efficient substrate binding of the cellulose-xylan complex compared to GH10, resulting in increased cellulose solubilization from the biomass.

Although xylanase, arabinoxylanase and mannanase

| Treatment | Enzymes | Glc (%) | Xyl (%) | Gal (%) | Ara (%) | Man (%) | GlcA (%) | KL (%) | AcOH (%) | N (%) | Recovery of weight (%) |
|-----------|---------|---------|---------|---------|---------|---------|----------|--------|-----------|------|-----------------------|
| —         | —       | 37.3    | 22.6    | 0.9     | 2.2     | 0.6     | N.D.     | 17.8   | 0.8       | 0.3  | 100.0                 |
| 0 %       | —       | 33.3    | 22.0    | 1.4     | 2.6     | 0.4     | N.D.     | 19.2   | 0.8       | 0.2  | 91.7                  |
| 3 %       | —       | 39.1    | 24.7    | 1.1     | 2.7     | 0.5     | N.D.     | 11.5   | 0.0       | 0.2  | 75.4                  |
| 5 %       | —       | 39.9    | 24.8    | 1.0     | 2.7     | 0.5     | N.D.     | 10.0   | 0.1       | 0.2  | 73.0                  |
| Anhydrous | —       | 34.9    | 23.6    | 1.6     | 3.5     | 0.5     | N.D.     | 13.4   | 0.8       | 2.0  | 96.7                  |
| —         | GH10    | 31.9    | 22.2    | 1.4     | 2.6     | 0.5     | N.D.     | 20.8   | 0.7       | 0.2  | 93.6                  |
| 0 %       | GH10    | 34.6    | 22.7    | 1.4     | 2.6     | 0.5     | N.D.     | 19.2   | 0.7       | 0.2  | 87.8                  |
| 3 %       | GH10    | 44.1    | 18.6    | 1.2     | 2.0     | 0.6     | N.D.     | 14.2   | 0.0       | 0.3  | 66.6                  |
| 5 %       | GH10    | 45.9    | 14.0    | 1.0     | 1.7     | 0.6     | N.D.     | 11.5   | 0.0       | 0.2  | 60.9                  |
| Anhydrous | GH10    | 50.9    | 7.7     | 1.2     | 1.1     | 0.7     | N.D.     | 20.9   | 0.0       | 0.8  | 59.7                  |
| —         | GH11    | 35.8    | 24.2    | 1.3     | 2.2     | 0.5     | N.D.     | 19.7   | 0.7       | 0.2  | 90.8                  |
| 0 %       | GH11    | 35.7    | 23.4    | 1.6     | 2.8     | 0.5     | N.D.     | 19.0   | 0.7       | 0.2  | 87.3                  |
| 3 %       | GH11    | 49.4    | 15.6    | 1.3     | 1.9     | 0.7     | N.D.     | 13.8   | 0.0       | 0.3  | 62.6                  |
| 5 %       | GH11    | 51.0    | 14.7    | 1.2     | 1.7     | 0.6     | N.D.     | 11.5   | 0.0       | 0.2  | 60.9                  |
| Anhydrous | GH11    | 45.9    | 5.6     | 1.0     | 1.0     | 0.6     | N.D.     | 19.2   | 0.0       | 0.7  | 57.8                  |

—, no treatment; N.D., not detected.

Fig. 2. Xylan content in biomass.

The xylan content of each sample was calculated relative to that of untreated bagasse. 1: untreated; 2: incubated with DW at 85 °C for 24 h; 3: incubated with 3% ammonia solution at 85 °C for 24 h; 4: incubated with 5% ammonia solution at 85 °C for 24 h; 5: incubated with anhydrous ammonia at 6 MPa, 100 °C for 2.5 h; 6, SoXyn10A hydrolysate of 1; 7, SoXyn10A hydrolysate of 2; 8, SoXyn10A hydrolysate of 3; 9, SoXyn10A hydrolysate of 4; 10, SoXyn10A hydrolysate of 5; 11, SoXyn11B hydrolysate of 1; 12, SoXyn11B hydrolysate of 2; 13, SoXyn11B hydrolysate of 3; 14, SoXyn11B hydrolysate of 4; 15, SoXyn11B hydrolysate of 5.

Table 1. The compositions of bagasse before and after pretreatment.
nosyl-(1→3)-O-β-D-xylopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→4)-β-D-xylopyranose (A1X4) and O-β-D-xylopyranosyl-(1→4)-[O-4-O-methyl-α-D-glucuronopyranosyl-(1→2)]-O-β-D-xylopyranosyl-(1→4)-β-O-D-xylopyranosyl-(1→4)-β-D-xylopyranose (MeGlcA3Xyl4) as a branched oligosaccharide. The proportions of soluble products were analyzed after digestion of anhydrous ammonia-pretreated bagasse with SoXyn10A or SoXyn11B (Table 2). Xylose (6.9 %), xylobiose (26 %), xylotriose (9.3 %), xylotetraose (15 %), and a branched oligosaccharide mixture of A1X2 and A1X3 (14 %) were detected in the SoXyn10A reaction mixture supernatant. Xylose (3.4 %), xylobiose (20 %), xylotriose (18 %), xylotetraose (2 %), and the branched oligosaccharide A1X4 (35 %) were detected in the SoXyn11B reaction mixture supernatant. The highest yield of branched oligosaccharide was the SoXyn11B product A1X4, and the oligosaccharide yield was calculated as 8.4 g recovered from 100 g anhydrous ammonia-pretreated bagasse.

To determine why the remaining xylan in the hydrolysis residue could not be decomposed, FT-IR analysis was performed. The absorption peaks near 1,745 cm⁻¹ and 1,245 cm⁻¹, which were attributable to the acetyl group, were eliminated by ammonia pretreatment (Fig. 3A). A new peak around 1,650 cm⁻¹, corresponding to the formation of amide bonds, was observed in anhydrous ammonia-pretreated bagasse (Fig. 3A1), and the peak was completely eliminated by xylanase digestion (Fig. 3B1 and Fig. 3C1). These observations were consistent with the biomass composition analysis (Table 1). However, no significant features of the structure were found to be related to the remaining xylan in the hydrolysis residue.

**CONCLUSION**

The effect of ammonia pretreatment on xylan recovery efficiency from sugarcane bagasse was evaluated. Aqueous ammonia pretreatment, which is effective for xylan recovery from rice straw,20 was not as effective in bagasse, in spite of the decrease in biomass weight after aqueous ammonia pretreatment. In contrast, anhydrous ammonia pretreatment of bagasse had great potential for enhanced xylan recovery, as it did not result in hemicellulose loss, and the subsequent enzyme-mediated hemicellulose hydrolysis rate was significantly high. Furthermore, most of the lignin was retained in the biomass after hemicellulose hydrolysis, suggesting that the hemicellulose-lignin network was cleaved almost completely by anhydrous ammonia pretreatment. Therefore, it could be possible to refine cellulose, hemicellulose, and lignin from bagasse if the lignin is selectively

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**Table 2.** Proportion of oligosaccharides detected in the supernatant of enzyme reaction mixture.

| Substituted oligosaccharides | Xylose | Xylobiose | Xylotriose | Xylotetraose |
|-----------------------------|--------|-----------|------------|--------------|
| SoXyn10A                    | 14.0 % | 6.9 %     | 26.0 %     | 9.3 %        | 15.0 %       |
| SoXyn11B                    | 35.0 % | 3.4 %     | 20.0 %     | 18.0 %       | 2.0 %        |

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**Fig. 3.** FT-IR spectra of bagasse before and after enzyme hydrolysis.

FT-IR analysis of bagasse was conducted before and after enzyme hydrolysis. A, samples lacking enzyme; B, SoXyn10A hydrolysate; C, SoXyn11B hydrolysate. a, cellulose-h bonding; b, acetyl group (lignin & hemicellulose); c, amide bonds; d and e, aromatic ring of lignin; f, acetyl group. 1: incubated with anhydrous ammonia at 6 MPa, 100 °C for 2.5 h; 2: incubated with 5 % ammonia solution at 85 °C for 24 h; 3: incubated with 3 % ammonia solution at 85 °C for 24 h; 4: incubated with DW at 85 °C for 24 h; 5: untreated.
dissolved prior to hemicellulose hydrolysis.

As demonstrated in the present study, although it was possible to produce defined branched oligosaccharide structures with ammonia pretreatment, this strategy produced a mixture of linear oligosaccharides with different degrees of polymerization. If the size of the linear oligosaccharides can be controlled to produce a more homogeneous product, the utility of hemicellulose would likely be enhanced. This is a topic to be investigated in future studies.

In addition, we would like to emphasize that selective xylan hydrolysis led to the production of fine cellulose. Because cellulose nanofiber has great potential as a new material, the need hemicellulose coexisting with cellulose is increasing. Thus far, the use of hemicellulose has not been developed because there was no sample to examine, but we believe this study will contribute to the use of hemicellulose by enabling mass production of xylooligosaccharides with defined branched structures.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1) Y.R. Loh, D. Sujan, M.E. Rahman, and C.A. Das: Sugarcane—The future composite material: A literature review. Resour. Conserv. Recy., 75, 14–22 (2013).

2) T.L. Bezerra, and A.J. Ragauskas: A review of sugarcane bagasse for second-generation bioethanol and biopower production. Biofuels, Bioprod. Bioref., 10, 634–647 (2016).

3) S. Kaneko, A. Kuno, Z. Fujimoto, D. Shimizu, S. Machida, Y. Sato, K. Yura, M. Go, H. Mizuno, K. Taira, I. Kusakabe, and K. Hayashi: An investigation of the nature and function of module 10 in a family F/10 xylanase FXYN of Streptomyces olivaceoviridis E-86 by module shuffling with the Cex of Cellulomonas fimii and site-directed mutagenesis. FEBS Lett., 460, 61–66 (1999).

4) S. Kaneko, H. Ichinose, Z. Fujimoto, A. Kuno, K. Yura, M. Go, H. Mizuno, I. Kusakabe, and H. Kobayashi: Structure and function of a family 10 β-xylanase chimera of Streptomyces olivaceoviridis E-86 and Cellulomonas fimii Cex. J. Biol. Chem., 279, 26619–26626 (2004).

5) A. Kuno, D. Shimizu, S. Kaneko, Y. Koyama, S. Yoshida, H. Kobayashi, K. Hayashi, K. Taira, and I. Kusakabe: PCR cloning and expression of the F/10 family xylanase gene from Streptomyces olivaceoviridis E-86. J. Ferment. Bioeng., 86, 434–439 (1998).

6) S. Kaneko, A. Kuno, M. Muramatsu, S. Iwamatsu, I. Kusakabe, and K. Hayashi: Purification and characterization of a family G/11 β-xylanase from Streptomyces olivaceoviridis E-86. Biosci. Biotechnol. Biochem., 64, 447–451 (2000).

7) T. Maehara, Z. Fujimoto, H. Ichinose, M. Michikawa, K. Harazono, and S. Kaneko: Crystal structure and characterization of the glycoside hydrolase family 62 α-L-arabinofuranosidase from Streptomyces coelicolor. J. Biol. Chem., 289, 7962–7972 (2014).

8) H. Ichinose, S. Dietavitian, Z. Fujimoto, A. Kuno, L. Lo Leggio, and S. Kaneko: Structure-based engineering of glucose specificity in a family 10 xylanase from Streptomyces olivaceoviridis E-86. Process Biochem., 47, 358–365 (2012).

9) T. Maehara, H. Yagi, T. Sato, M. Ohnishi-Kameyama, Z. Fujimoto, K. Kamino, Y. Kitamura, F. St John, K. Yaoi, and S. Kaneko: GH30 glucuronoxylan-specific xylanase from Streptomyces turgidiscabies C56. Appl. Environ. Microbiol., 84, e01850–17 (2018).

10) S. Kaneko, S. Ito, Z. Fujimoto, A. Kuno, H. Ichinose, S. Iwamatsu and T. Hasegawa: Importance of interactions of the α-helices in the catalytic domain N- and C-terminals of the family 10 xylanase from Streptomyces olivaceoviridis E-86 to the stability of the enzyme. J. Appl. Glycosci., 56, 165–171 (2009).

11) K. Suzuki, M. Michikawa, H. Sato, M. Yuki, K. Kamino, W. Ogasawara, S. Fushinobu, and S. Kaneko: Purification, cloning, functional expression, structure, and characterization of a thermostable β-mannanase from Talaromyces trachyspermus B164 and its efficiency in production of mannooligosaccharides from coffee wastes. J. Appl. Glycosci., 65, 13–21 (2017).

12) S. Kaneko, H. Ichinose, Z. Fujimoto, S. Iwamatsu, A. Kuno, and T. Hasegawa: Substrate recognition of a family 10 xylanase from Streptomyces olivaceoviridis E-86: A study by site-directed mutagenesis to make an hindrance around the entrance toward the substrate-binding cleft. J. Appl. Glycosci., 56, 173–179 (2009).

13) H. Yagi, R. Takehara, A. Tamaki, K. Teramoto, S. Tsutsui, and S. Kaneko: Functional characterization of the GH10 and GH11 xylanases from Streptomyces olivaceoviridis E-86 provide insights into the advantage of GH11 xylanase in catalyzing biomass degradation. J. Appl. Glycosci., 66, 29–35 (2019).

14) H. Yagi, T. Maehara, T. Tanaka, R. Takohara, K. Teramoto, K. Yaoi, and S. Kaneko: 4-O-Methyl modifications of glucuronic acids in xylans are indispensable for substrate discrimination by GH67 α-glucuronidase from Bacillus halodurans C-125. J. Appl. Glycosci., 64, 115–121 (2017).

15) K. Sakuragi, K. Igarashi, and M. Samejima: Application of ammonia pretreatment to enable enzymatic hydrolysis of hardwood biomass. Polym. Degrad. Stab., 148, 19–25 (2018).

16) V. Lombard, H.G. Ramulu, E. Drula, P.M. Coutinho, and B. Henriссat: The Carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res., 42, 490–495 (2014).

17) F.A.F. Antunes, A.K. Chandel, R. Terán-Hilares, A.P. Ingle, M. Rai, T.S.S. Milessi, S.S. da Silva, and J.C. dos Santos: Overcoming challenges in lignocellulosic biomass pretreatment for second-generation (2G) sugar production: emerging role of nano, biotechnological and promising approaches. 3 Biotech, 9, 230 (2019).
18) S. Makishima, M. Mizuno, N. Sato, K. Shinji, M. Suzuki, K. Nozaki, F. Takahashi, T. Kanda, and Y. Amano: Development of continuous flow type hydrothermal reactor for hemicellulose fraction recovery from corncob. *Bioresour. Technol.*, **100**, 2842–2848 (2009).

19) N. Sato, K. Shinji, M. Mizuno, K. Nozaki, M. Suzuki, S. Makishima, M. Shiroishi, T. Onoda, F. Takahashi, T. Kanda, and Y. Amano: Improvement in the productivity of xylooligosaccharides from waste medium after mushroom cultivation by hydrothermal treatment with suitable pretreatment. *Bioresour. Technol.*, **101**, 6006–6011 (2009).

20) I. Kusakabe, T. Yasui, and T. Kobayashi: Enzymatic Hydrolysis-extraction of Xylan from Xylan-containing Natural Materials (1) Increase in Susceptibility for Enzymatic Action of Native Xylan in Natural Materials with Pretreatment (Studies on Xylanase System of *Streptomyces* Part V). *Nippon Nogeikagaku Kaishi*, **5**, 199–208 (1976). (in Japanese)

21) J.S. Kim, Y.Y. Lee, and T.H. Kim: A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresour. Technol.*, **199**, 42–48 (2016).

22) A.K Chamdel, F.A.F. Antunes, M.B. Silva, and S.S. da Silva: Unraveling the structure of sugarcane bagasse after soaking in concentrated aqueous ammonia (SCAA) and ethanol production by *Scheffersomyces* (Pichia) stipitis. *Biotechnol. Biofuels*, **6**, 102 (2013).

23) S. Cao and G.M. Aita: Enzymatic hydrolysis and ethanol yields of combined surfactant and dilute ammonia treated sugarcane bagasse. *Bioresour. Technol.*, **131**, 357–364 (2013).

24) M. Kim and D.F. Day: Enhancement of the enzymatic digestibility and ethanol production from sugarcane bagasse by moderate temperature-dilute ammonia treatment. *Appl. Biochem. Biotechnol.*, **171**, 1108–1117 (2013).

25) T. Shi, J. Lin, J. Li, Y. Zhang, C Jiang, X. Lv, Z. Fan, W. Xiao, Y. Xu, and Z. Liu: Pre-treatment of sugarcane bagasse with aqueous ammonia-glycerol mixtures to enhance enzymatic saccharification and recovery of ammonia. *Bioresour. Technol.*, **289**, 121628 (2019).

26) S.S. Hashemi, K. Karimi, and A.M. Karimi: Ethanolic ammonia pretreatment for efficient biogas production from sugarcane bagasse. *Fuel*, **248**, 196–204 (2019).

27) N. Beukes and B.I. Pletschke: Effect of lime pre-treatment on the synergistic hydrolysis of sugarcane bagasse by hemicellulases. *Bioresour. Technol.*, **101**, 4472–4478 (2010).

28) S. Yoshida, I. Kusakabe, N. Matsuo, K. Shimizu, T. Yasui, and K. Murakami: Structure of rice-straw arabinoxylans and specificity of *Streptomyces* xylanase toward the xylan. *Agric. Biol. Chem.*, **54**, 449–457 (1990).

29) P. Biely, M. Vršanská, M. Tenkanen, and D. Kluepfel: Endo-β-1,4-xylanase families: differences in catalytic properties. *J. Biotechnol.*, **57**, 151–166 (1997).