Intensification of Cellulolytic Hydrolysis of Rice Husk, Rice Straw, and Defatted Rice Bran by Sodium Hydroxide Pretreatment

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Abstract

The ability of sodium hydroxide (NaOH) pretreatment to intensify the digestibility of lignocellulosic from rice straw (RS), rice husk (RH), and defatted rice bran (DRB) for cello-oligosaccharides and xylo-oligosaccharides productions using commercial cellulases was investigated. Initially, 10 g of biomass was soaked with 300 mL of 2% NaOH for 6 days at room temperature. The total pentosan contents of NaOH-pretreated rice straw (NP-RS), rice husk (NP-RH), and defatted rice bran (NP-DRB) were measured and compared to non-treated biomass showing increases from 21.74 to 26.42%, 19.89 to 28.00%, and 11.33 to 19.94%, respectively, while the percentage yield mass after NaOH-pretreated biomass decreased from 100 to be 44.8, 68.7, and 24.3, respectively. In addition, the NaOH pretreatment strongly affected the arabinose/xylose ratio (A/X) of DRB which was decreased from 1.08 to 0.82. Moreover, arabinoxylan contents were increased from 11.0 to 18.3% for RS, 11.5 to 18.6% for RH, and 5.8 to 14.3% for DRB. After mentioned processes, non-treated biomass and NaOH-pretreated biomass were used to produce oligosaccharides at 50 °C for 4 h by using Cellulase SS and Cellulase XL. The results exhibited that non-treated biomass was less hydrolyzed by both enzymes. Cellulase SS showed greater hydrolysis effect on NP-RS, NP-RH, and NP-DRB than Cellulase XL. High Performance Anion Exchange Chromatography results confirmed that the hydrolysates from both cellulolytic enzymes had similar sugar patterns mainly found as cellobiose and xylobiose. Moreover, the component with an arabinose substituted onto xylose backbone was found in a small content. Hence, this study has confirmed the capability of cellulolytic enzymes for production of mixed oligosaccharides which could be further used for the prebiotic properties.

Keywords: NaOH pretreatment, Cello-oligosaccharide, Xylo-oligosaccharide, Cellulase, Rice production wastes

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1. Introduction

Rice production in Thailand represents a significant portion of the Thai economy. After rice processing, a large amount of by-products including rice straw (RS), rice husk (RH), as well as defatted rice bran (DRB) after rice bran oil production was left. The major components in these biomasses are lignocelluloses consisting of cellulose, hemicellulose and lignin accounting for 22–39% 26–37% and 10–24%, respectively (Rahnama *et al*., 2013; Di Blasi *et al*., 1999; Hernandez *et al*., 2005). Among these lignocelluloses, cellulose was revealed as the most abundant renewable constituent followed by hemicellulose (Yang *et al*., 2011; Yan *et al*., 2009).

Cellulose is an unbranched homopolymer of glucose units. Unlike cellulose, hemicellulose is a heterogenous polymer having branches with short side chains composed of different sugar units arranged in different proportions and with different substituents. Arabinoxylan (AX) is a hemicellulose with a linear xylose backbone consisting of arabinose side chain (Kulkarni *et al*., 1999). These polymers are associated with each other in a hetero-matrix form by intra-polymer and inter-polymer linkage. All of these act as a barrier shielding the polysaccharides from enzymatic hydrolysis (Li *et al*., 2004). The alkali pretreatment by diluted NaOH has been widely used to improve the digestibility of lignocellulosic materials, resulting in reduction of crystallinity, disruption of lignin structure, and also increase in the internal surface area (Fan *et al*., 1987). Millett *et al*., (1976) reported that pretreated lignocelluloses by soaking with diluted NaOH can improve the digestibility in ruminants. Fan *et al*., (1982) revealed that the increasing external surface area of the pretreated biomass could enhance the rate of enzymatic hydrolysis. Cellulolytic enzymes, such as endo-cellulase, endo-glucanase, and cellobiohydrolase act in concert to breakdown cellulose polymer (Guo *et al*., 2008) to glucose, cellobiose, and cello-oligosaccharides (Okeke *et al*., 2015). However, Endo-β-1,4-xylanase which is xylanolytic enzymes degrades the main chain of linear xylan or AX to arabinoxylo-oligosaccahrides (AXOS) (Collins *et al*., 2005). The commercial cellulase enzymes normally originate from single and/or different species of microbes that can produce all the necessary cellulolytic and xylanolytic enzymes to enable production of an ideal cocktail with a single hydrolysis. The cocktail enzymes can reduce the cost of enzymes and consequently the cost of biomass conversion to varieties of oligosaccharide production.

Hence, this study aimed to investigate the ability of NaOH pretreatment to improve the digestibility of lignocellulosic materials from RS, RH, and DRB for oligosaccharides productions using commercial cocktail cellulase enzymes; Cellulase SS and Cellulase XL.

2. Materials and Methods

2.1 Materials

RS and RH were obtained from Phitsanulok and Pichit, Thailand. DRB was obtained from a rice bran oil company in Ayutthaya, Thailand. RS was size-reduced to 20 mm long and DRB was sieved into 60 sieving mesh to remove the undesirable contamination. RS, RH, and DRB were dried in a hot air oven until moisture contents were lower than 10%, and then stored at 4 °C until use. Commercial cellulase enzymes, Cellulase XL-531 and Cellulase SS were kindly provided by NAGASE Chemtex Corporation (Osaka, Japan). Arabinose, glucose, and galactose were purchased from Sigma Aldrich (USA). Xylose, mannose and cellobiose were purchased from Merck (Germany), Xylobiose and xylotriose were purchased from Wako Pure Chemicals Industries Ltd. (Japan). All chemicals and solvents used in this research were analytical grades.

2.2 NaOH pretreatment of biomass

One hundred grams of biomass were separately soaked in 2% NaOH for 6 days. After completed pretreatment, the alkali solution from RH and RS were decanted through muslin cloth. In case of DRB, the biomass was separated by centrifugation (7,000 rpm for 30 min at room temperature). Then the residues were washed several times with distilled water until being neutral. The NaOH-pretreated biomasses were dried at 45 °C for 24 h, then ground, filtered through 60 mesh sieving size to ensure their uniformity, and finally obtained as the pretreated-rice husk (NP-RH), pretreated-rice straw (NP-RS), and pretreated-defatted rice bran (NP-DRB). These NaOH-pretreated biomasses were analyzed in the structural carbohydrates compared with non-treated biomasses.

2.3 Analysis of biomass structural carbohydrates

To analyze the structural carbohydrates in a solid biomass according to National Renewable Energy Laboratory method (NREL, Laboratory Analytical Procedure Issue Date: 12/08/2006), four hundred mg of non-treated and NaOH-pretreated biomasses were mixed with 4.5 mL of 72% sulfuric acid with stirring for 30 min. The slurry of individual sample was then diluted with distilled water to a final concentration of 4% and autoclaved for 1 h at 121 °C. After cooled down at room temperature, the hydrolysate was filled up to 100 mL, then the pH was adjusted to 5.0–6.0 using barium carbonate. The total sugar as pentosan content was measured by the orcinol-HCl method using xylose as a standard (Fernell and King, 1953). Ion chromatography (IC) analysis for monosaccharide content was performed with Dionex CarboPac PA-1 column (250 mm × 4 mm) with a guard column (50 mm × 4 mm) at flow rate of 1.0 mL/min. The post-column pump flow rate was controlled at 0.5 mL/min for 300 mM NaOH. A stepwise linear gradient was applied over 20 min by 100% distilled water (Solution A)

and was applied over 16 min by mixing solutions of 200mM NaOH (Solution B) and 200 mM NaOAc in 170 mM NaAc (Solution C). Eluted oligosaccharides were monitored by pulsed amperometric detection using an Au electrode. Peaks of monosaccharides were assigned by using xylose, arabinose, mannose, galactose, and glucose as standards. The monosaccharides were quantified with a calibration curve for each sugar standard using linear regression. A/X ratio was calculated from %arabinose divided by %xylose content. Finally, the AX content was calculated from the concentration of the polymeric sugars from the concentration of the corresponding monomeric sugars, using anhydro correction of 0.88 for xylose and arabinose (NREL, Laboratory Analytical Procedure Issue Date: 12/08/2006).

2.4 Hydrolysis by commercial cellulases

Hydrolysis of non-treated and NaOH-pretreated biomasses was performed with Cellulase SS or Cellulase XL-531 for oligosaccharides production. 1 g of each biomass was suspended in 20 mL of 100 mM sodium acetate buffer (pH 5.0). Fifty microliter of each commercial cellulase was added and then incubated at 50 °C with stirring at 170 rpm for 4 h. The reaction was stopped by boiling for 30 min, then the production of oligosaccharides were determined by DNS method (Miller, 1959) using xylose as a standard. Oligosaccharides hydrolysates were determined qualitatively by IC, Dionex CarboPac PA-200 column (250 mm × 4 mm) with a guard column (50 mm × 4 mm) at constant flow rate of 0.4 ml/min. The condition and eluents informations were found in a study by McCleary *et al*., (2015). Peaks of oligosaccharides were assigned by using arabinose, xylose, xylobiose, xylotriose, 1,3-arabinosyl-xylobiose and 1,2-arabinosyl-xylotriose as standards.

One Unit of xylanase activity is defined as the amount of enzyme required to release one µmole of xylose reducing sugar equivalents per minute from wheat arabinoxylan (5 mg/mL) in sodium phosphate buffer (100 mM), pH 6.0.

Data were analyzed by one way Analysis of variance (ANOVA). Significant differences ($p \leq 0.05$) between samples were evaluated using Duncan's new multiple range test. Replication was performed in the experiment.

3. Results and Discussion

3.1 Sugar composition of biomasses

The carbohydrate portions of non-treated and NaOH-pretreated biomass after degraded by diluted sulphuric acid at 121 °C for 1 h were analyzed by HPAEC. Sugar compositions are expressed as % w/w (Table 1). After NaOH pretreatment, the total sugars as pentosan content of RS, RH, and DRB were increased from 21.7 to 26.4%, 19.9 to 28.0%, and 11.3 to 20.0%, respectively.

Table 1. Composition of structural carbohydrates of biomass before and after pretreated by NaOH

Biomass	Total sugar as pentosan content (%)	Arabinose (%)	Xylose (%)	AX content (%)	A/X ratio
Rice Straw	21.74 ± 0.32	2.25	10.28	11.0	0.22
NP-RS	26.42 ± 0.13	3.38	17.45	18.3	0.22
Rice Husk	19.89 ± 0.57	1.57	11.47	11.5	0.14
NP-RH	28.00 ± 0.89	2.48	18.64	18.6	0.13
Defatted Rice Bran	11.33 ± 0.13	3.43	3.19	5.8	1.08
NP-DRB	19.94 ± 0.00	7.35	8.93	14.3	0.82

Note: NP-RS = sodium hydroxide pretreated rice straw, NP-RH = sodium hydroxide pretreated rice husk, NP-DRB = sodium hydroxide pretreated defatted rice bran

However, the chromatograms of RS, RH, and DRB demonstrated that glucose was the most abundant sugar component. Furthermore, xylose and arabinose were found as the secondly abundant sugar components of the hemicellulose materials. There were approximately 11 g/100 g of xylose and 3 g/100 g of arabinose in dry weight. These results showed good agreement with the finding that non-wood hemicellulose contains high amounts of xylose (Scheller and Ulvskov, 2010). The results that AX content in RS, RH, and DRB exhibited 11%, 11.5%, and 5.8%, respectively, were consistent with the previous report indicating the AX contents in RH and DRB as 8.3–9.2% and 4.8–5.1%, respectively (Hashimoto *et al*., 1987). After NaOH pretreatment, the AX contents of RS, RH, and DRB were increased from 11.0 to 18.3%, 11.5 to 18.6%, and 5.8 to 14.3%, respectively. Furthermore, the NaOH pretreatment reduced arabinose content in DRB resulting in A/X ratio decreasing from 1.08 to 0.82. However, the yields of RH, RS, and DRB after NaOH pretreatment appeared to

be 68.7, 44.8, and 24.3%, respectively (Figure 1). Changing in structural carbohydrates proportion and yield in biomass during NaOH pretreatment could result disrupts the lignin structure and breaks the linkage between lignin and the other carbohydrate fractions in lignocellulosic biomass by the penetration of the sodium ions act as a counter charge to carboxylate ions (Mcmillan, 1992), thus making the carbohydrates in the heteromatrix more accessible to enzymatic hydrolysis of the carbohydrate fraction of the pretreated biomass. Harun and Geok (2016) found that after pretreated rice straw at different NaOH concentration and time indicated increasing the xylan and arabinan composition (pentosan content) was observed only at 2% NaOH concentration with long pretreatment time and the content of lignin ash and extractives was also significantly decreased after pretreatment with NaOH.

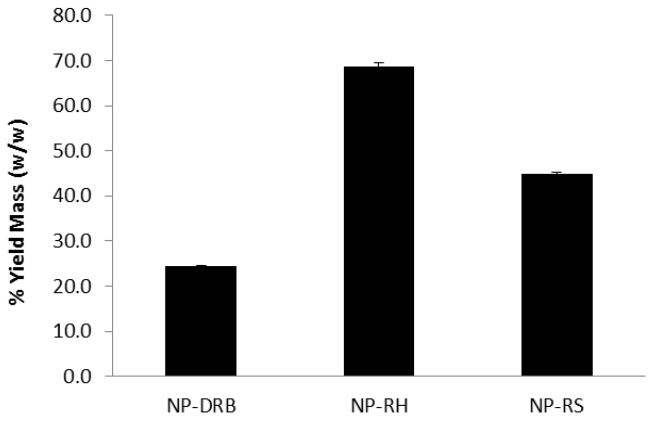

Figure 1 Percentage yield mass after NaOH pretreatment of RS, RH, and DRB

3.2 Sugar productivity and profile by Cellulase SS and Cellulase XL-531

The sugar productivities by Cellulase SS and Cellulase XL-531 were compared between different substrates, non-treated and NaOH-pretreated biomass. Figure 2 demonstrated the total reducing sugar after hydrolyzed by cellulase enzymes at 4 h of hydrolysis time at 50 °C and pH 5.0 in sodium acetate buffer. Under the aforementioned conditions, the most efficient hydrolysis was on NP-RS with Cellulase SS treatment (approximately 42% of the total reducing sugar), whereas NP-DRB followed by Cellulase XL-531 treatments produced 17% of the total reducing sugar.

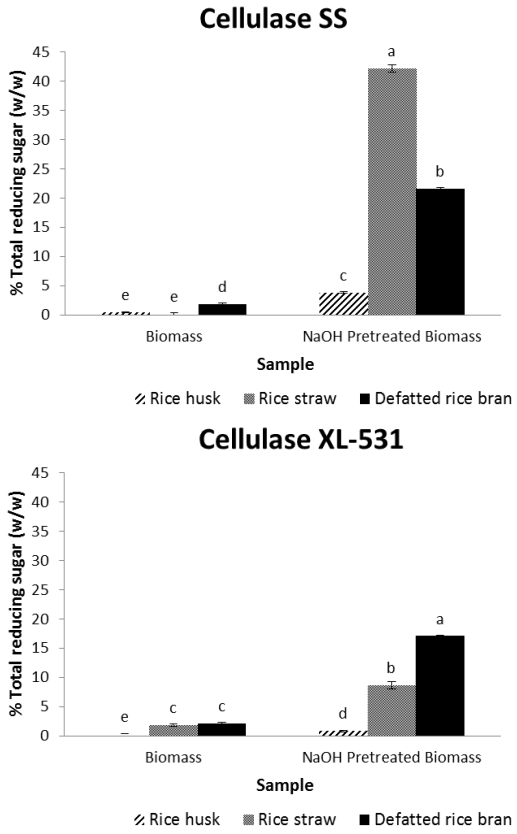

Figure 2 Enzymatic production of oligosaccharides from non-treated biomass and NaOH-pretreated RH, RS and DRB at using Cellulase SS and Cellulase XL-531 (50 °C, 4 h). Values are reported as mean; same lower case letters indicate no significant difference ($p > 0.05$); n=2.

Moreover, the total reducing sugar contents of RS, RH, and DRB hydrolysates after NaOH-pretreatment were increased from 0.16% to 42.1%, 0.39% to 3.78%, and 1.79% to 21.65% with Cellulase SS treatment while the hydrolysates of Cellulase XL-531 showed the changes of 1.84% to 8.64%, 0.36% to 0.81%, and 2.14% to 17.06%, respectively. The non-treated biomasses were found to be poorly hydrolyzed by both enzymes. This could result from the presence of lignin and other extractives densely packed with cellulose and hemicellulose chain (Deguchi, Tsujii and Horikoshi. 2006) through intra-polymer and inter-polymer linkage. All of them act as a barrier shielding the polysaccharides from enzymatic hydrolysis (Li et al., 2004). In this study, NaOH pretreatment may cause the disruption of hydrogen bonding in cell wall matrix resulting in swelling of hemicellulose and decrystallization of cellulose. Furthermore, Cellulase SS was greater in hydrolyzing NP-RS (42.18% of total reducing sugar) than Cellulase XL-531 (8.64% of total reducing sugar) . These could be because of presence of β-glucanase activity in Cellulase SS which can break down into glucose. From HPAEC analysis (Figure 3B and 3E) it could be confirmed that 42% of sugars from NP-RS obtained with Cellulase SS produced monosaccharides mainly of xylose and glucose. Interestingly, Cellulase SS produced 1,2-arabinosyl-xylotriose, a kind of AXOS. Moreover, NP-DRB was hydrolyzed

with Cellulase SS (21.64% of total reducing sugar) to produce several kinds of oligosaccharides (Figure 3A) such as xylobiose, cellobiose, in a higher degree than NP-DRB (17.06% of total reducing sugar) with Cellulose XL-531. Besides, Cellulase XL-531 also produced higher arabinose content than Cellulase SS because the former contained α-L-arabinofuranosidase activity (Kumagai *et al*., 2012) which can easily hydrolyze arabinose side chain of DRB. Although the %total reducing sugar of NP-RH was lower than NP-RS and NP-DRB with both enzymes, they still contained some of xylobiose and cellobiose (Figure 3B and 3E). From the result, these enzymes play different roles cooperatively in the hydrolysis of the cell wall (Kumar, Singh and Singh, 2008) due to the presence of several kinds of enzyme activities. In this study, Cellulase SS and Cellulase XL-531 seemed to have cleaved the polymer chain into fragments containing more than 2 units of sugar and breakdowned into some di-saccharides. It has been reported that xylo-oligosaccharide (XOS) and AXOS activate an immune response in human and promoted growth of lactic acid bacteria in human intestine (Sheu *et al*., 2008; Chung *et al*., 2007; Okazaki *et al*., 1990). Moreover, cellobiose was found to have a potential to improve intestinal microflora conditions (Sanz, Gibson and Rastall, 2005). However, as the commercial enzymes usually contain high amounts of exo-type enzymes (Kumagai *et al*., 2012), monosaccharides such as glucose and xylose may be produced in the hydrolysates. Therefore, a purification step for discarding these monosaccharides would be required.

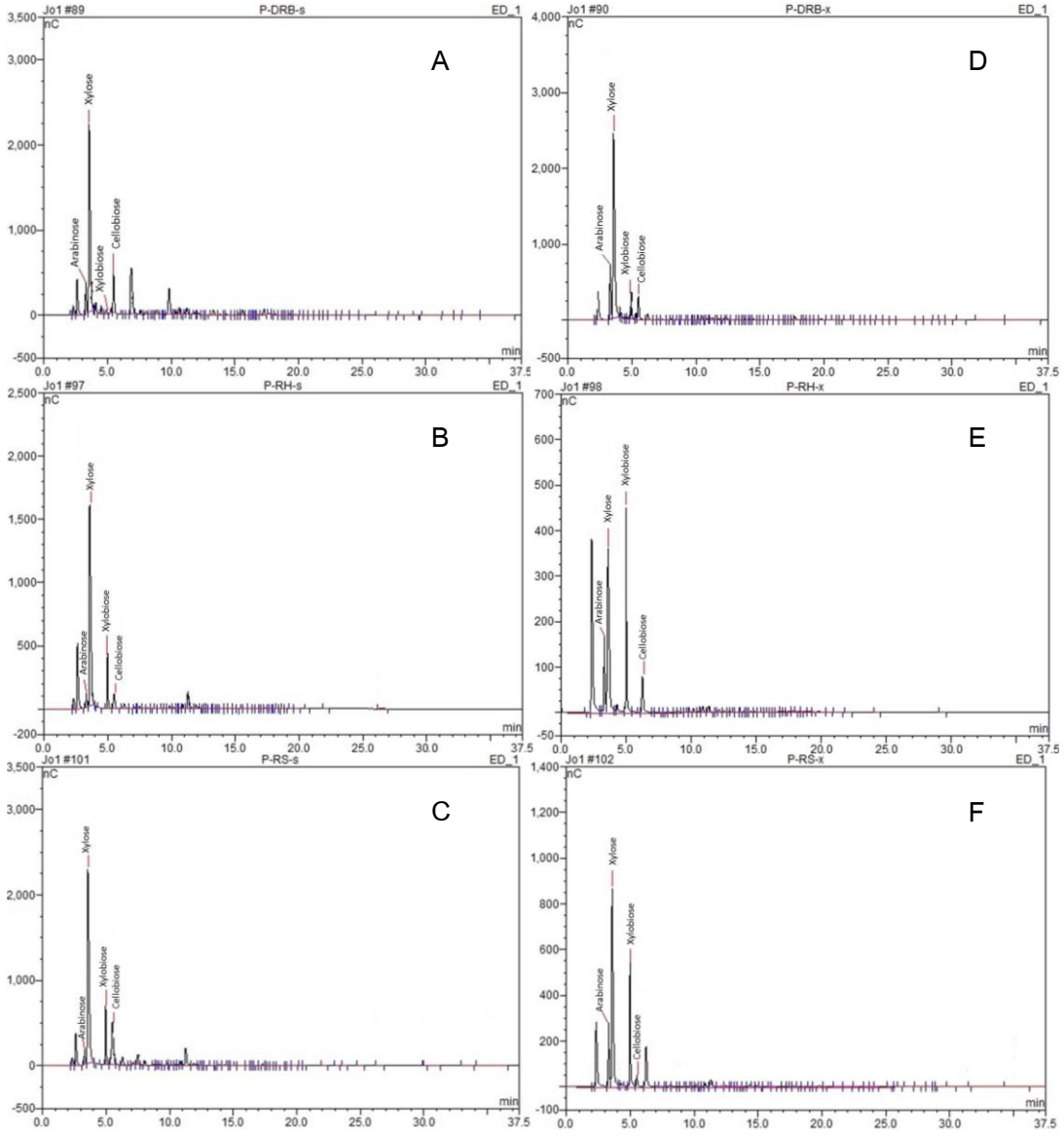

Figure 3 IC Chromatograms of enzymatic hydrolysates at 50 °C for 4 h. (A) NP-DRB by Cellulase SS, (B) NP-RH by Cellulase SS, (C) NP-RS by Cellulase SS, (D) NP-DRB by Cellulase XL-531, (E) NP-RH by Cellulase XL-531, (F) NP-RS by Cellulase XL-531. The standard of xylose, xylobiose, xylotriose, 1,3-arabinosyl-xylobiose, 1,2-arabinosyl-xylotriose, arabinose and cellobiose were used as standards.

4. Conclusion

In this study, we expected to make a high value-added product like prebiotics from rice industrial wastes by simple method. The NaOH pretreatment was found to be able to decrease A/X ratio of DRB and increase the content of AX of all biomass. Hence, NaOH pretreatment improved the digestibility of biomass by enzymes for the complete degradation of the biomass into oligosaccharides. The commercial cellulases treatment is a promising method for producing mixtures of short oligosaccharides mainly consisting of xylobiose, cellobiose and some kinds of AXOS. However, the degradation products also contained a monosaccharide

comprising glucose and xylose. A purification step would be required for a high yield of oligosaccharides.

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