Enzymatic hydrolysis and fermentation of lime pretreated wheat straw to ethanol

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Abstract

BACKGROUND: The objective of this work is to develop an efficient pretreatment method that can help enzymes break down the complex carbohydrates present in wheat straw to sugars, and to then ferment all of these sugars to ethanol.

RESULTS: The yield of sugars from wheat straw (8.6%, w/v) by lime pretreatment (100 mg g\(^{-1}\) straw, 121 °C, 1 h) and enzymatic hydrolysis (45 °C, pH 5.0, 120 h) using a cocktail of three commercial enzyme preparations (cellulase, β-glucosidase, and xylanase) at the dose level of 0.15 mL of each enzyme preparation g\(^{-1}\) straw was 568 ± 13 mg g\(^{-1}\) (82% yield). The concentration of ethanol from lime pretreated enzyme saccharified wheat straw (78 g) hydrolyzate by recombinant *Escherichia coli* strain FBR5 at pH 6.5 and 35 °C in 24 h was 22.5 ± 0.6 g L\(^{-1}\) with a yield of 0.50 g g\(^{-1}\) available sugars (0.29 g g\(^{-1}\) straw). The ethanol concentration was 20.6 ± 0.4 g L\(^{-1}\) with a yield of 0.26 g g\(^{-1}\) straw in the case of simultaneous saccharification and fermentation by the *E. coli* strain at pH 6.0 and 35 °C in 72 h.

CONCLUSION: The results are important in choosing a suitable pretreatment option for developing bioprocess technologies for conversion of wheat straw to fuel ethanol.

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Keywords: wheat straw; ethanol; lime pretreatment; enzymatic saccharification; separate hydrolysis and fermentation; simultaneous saccharification and fermentation

INTRODUCTION

In the USA, the production of corn grain based ethanol reached 4.9 billion gallons in 2006, a fraction of the 140 billion gallons of transportation fuel used annually.\(^1\) The goal is to displace 30% of the nation’s current gasoline use with ethanol by 2030, and this will require production levels equal to roughly 60 billion gallons a year. If all corn grain now grown in the USA is converted to ethanol, it can satisfy approximately 15% of current gasoline needs. Thus, developing ethanol as a fuel, beyond its current role as a fuel oxygenate, will require the development of lignocellulose as feedstock because of its abundance. In particular, agricultural residues (corn stover, wheat straw, rice straw), agricultural processing byproducts (corn fiber, rice hulls, sugar cane bagasse), and energy crops (switchgrass) can be used as low-cost sources of sugars for biofuel production. Environmentally friendly methods for pretreatment, efficient and rapid enzymatic saccharification to fermentable sugars, high productivity fermentation of mixed sugar streams, and cost-effective recovery of dilute products need to be developed in order to use these materials economically as feedstocks for the production of biofuel and other value-added commodity chemicals.

Wheat straw can serve as a low-cost feedstock for the production of fuel alcohol. It contains 35–45% cellulose, 20–30% hemicellulose, and 8–15% lignin.\(^2\) Based on FAO data, 627.1 million metric tons of wheat were produced in the world in 2004 (US production, 58.7 million metric tons).\(^3\) The average yield of wheat straw is 1.3–1.4 kg kg\(^{-1}\) of wheat grain.\(^2\) Research has been done on the separation of cellulose, hemicellulose, and lignin components from wheat straw and structural characterization of the hemicellulose fraction.\(^4–7\) A few reports are also available on the production of ethanol from wheat straw hydrolyzates\(^8–10\)

Lignocellulosic biomass is generally resistant to enzymatic degradation in its native state. Various pretreatment options such as dilute acid, alkali, and steam explosion are available for pretreating lignocellulosic biomass, but no single method has yet been found suitable for commercial application. In previous studies, dilute acid and alkaline peroxide were evaluated as pretreatment options for wheat

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\(^1\) Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.
straw, enzymatic saccharification, and fermentation of the hydrolyzate to ethanol.\textsuperscript{11,12} Lime pretreatment has been studied on various biomass substrates such as switchgrass, corn stover, wood, and municipal wastes.\textsuperscript{13–15} Lime offers certain advantages; it is inexpensive ($0.06 \text{ kg}^{-1}$), safe to handle, and can be recovered easily. In the present study, conditions for obtaining a high sugar yield from wheat straw using lime as pretreatment option and enzymatic hydrolysis were examined.

Any lignocellulosic biomass, upon hydrolysis, generates hexose and pentose sugars.\textsuperscript{16} The utilization of all these sugars is essential for the economical production of ethanol. The conventional ethanol fermenting yeast (\textit{Saccharomyces cerevisiae}) or bacterium (\textit{Zymomonas mobilis}) cannot ferment multiple sugar substrates to ethanol. \textit{Escherichia coli} can metabolize a wide variety of sugars. The authors’ research unit has developed a recombinant \textit{E. coli} (strain FBR5) that can ferment mixed multiple sugars to ethanol.\textsuperscript{17} The strain carries the plasmid pL1297, which contains the genes from \textit{Z. mobilis} necessary for efficient conversion of pyruvate into ethanol. It selectively maintains the plasmid when grown anaerobically. As far as is known, no research paper is available on ethanol production from lime pretreated and enzyme hydrolyzed wheat straw (separate hydrolysis and fermentation, SHF). The simultaneous saccharification and fermentation (SSF) of lime pretreated wheat straw is also described.

\textbf{MATERIALS AND METHODS}

\textbf{Materials}

Wheat straw was purchased from a local farmer. It was dried in a forced-air oven at 55°C for 24 h and milled in a hammer mill to pass through a 1.27 mm screen. The milled wheat straw was stored at room temperature. Celluclast 1.5 L, Novozyme 188, glucose, xylose, arabinose, Tween 20, furfural, and hydroxymethyl furfural (HMF) were purchased from Sigma Chemical Co., St Louis, MO. Viscostar 150 L was supplied by Dyadic Corp., Jupiter, FL. Aminex HPX 87P column (300 × 7.8 mm), Aminex HPX 87H column (300 × 7.8 mm), deashing cartridge (30 × 4.6 mm), Carbo-P micro-guard cartridge (30 × 4.6 mm), and cation H micro-guard cartridge (30 × 4.6 mm) were purchased from Bio-Rad Laboratories, Inc., Hercules, CA. All other chemicals used were of analytical grade.

\textbf{Lime pretreatment}

Unless otherwise stated, milled wheat straw (8.6\% w/v) and lime (0.86\%, w/v) were slurried in water, mixed, and autoclaved at 121°C for 1 h. The pH of the lime pretreated wheat straw was adjusted to 5.0 using concentrated HCl before performing enzymatic hydrolysis.

\textbf{Enzyme assays}

Carboxymethyl cellulase (CMCase), β-glucosidase, xylanase, β-xylosidase, α-L-arabinofuranosidase and ferulic acid esterase activities were assayed using 1\% (w/v) carboxymethyl cellulose, 4 mmol L\textsuperscript{-1} p-nitrophenyl β-D-glucoside, 1\% (w/v) oat spelt xylan, 2 mmol L\textsuperscript{-1} p-nitrophenyl β-D-xyloside, 1 mmol L\textsuperscript{-1} p-nitrophenyl α-L-arabinofuranoside, and 0.9 mmol L\textsuperscript{-1} methyl ferulate, respectively, as substrate by the procedures described previously.\textsuperscript{18} One unit (U) of each enzyme activity is defined as the amount of enzyme that produces 1 μ mole of product in the reaction mixture per minute under the assay conditions used.

\textbf{Enzymatic hydrolysis}

Enzymatic hydrolysis of the lime pretreated wheat straw was performed by shaking gently (100 rpm) at 45°C after adjusting the pH to 5.0 with HCl and adding a cocktail of three commercial enzyme preparations at a dosage of 0.05 mL g\textsuperscript{-1} of wheat straw of each enzyme for 72 h, unless otherwise stated. Samples (1 mL) were withdrawn and kept at −20°C before analysis.

\textbf{Bacterial strain and preparation of inoculum}

Recombinant \textit{E. coli} strain FBR5 was maintained in glycerol vials at −20°C for use as a working stock. It was plated on Luria broth (LB; 10 g tryptone, 5 g yeast extract, and 5 g NaCl) containing 4.0 g xylose and 20 mg tetracycline solidified with 15 g agar L\textsuperscript{-1}. Plates were incubated at 35°C. Cells from a single well-isolated colony were inoculated into a 125 mL flask containing 100 mL of LB supplemented with 2 g xylose and 2 mg tetracycline. Cultures were incubated at 35°C and 100 rpm for 24 h and used as seed culture for fermentation experiments.

\textbf{Fermentation experiments}

Batch culture experiments were carried out in pH-controlled 500 mL flakers with a working volume of 350 mL under semianaerobic conditions essentially as described previously.\textsuperscript{19} Lime pretreated wheat straw hydrolyzate was used as substrate. The medium was prepared by dissolving 10 g tryptone and 5 g yeast extract in 1 L hydrolyzate and autoclaving at 121°C for 15 min. A 4 mol L\textsuperscript{-1} KOH solution was used for pH control. Samples were withdrawn periodically to determine cell density, ethanol, organic acids, and residual sugars and stored at −20°C before analysis. Base consumption and pH were also recorded. For SHF experiments, fermentation was performed at pH 6.5, 35°C and 130 rpm using the liquid portion of the hydrolyzate after separating it from the solids by filtration over a glass fiber filter (1.0–1.5 μm pore size, 75 mm diameter, Nalgene, Rochester, NY). For SSF experiments, 2 L fermenters (Biostat B, B.
Braun Biotechnology International, Allentown, PA) with working volumes of 1.5 L were used at pH 6.0 and 35 °C at an agitation rate of 150 rpm. The lime pretreated whole wheat straw hydrolyzate was added to the fermenter as substrate after adjusting the pH to 6.0 with concentrated HCl before adding enzyme cocktail and inoculum. Inoculum size was 5% (v/v) in both cases.

**Analytical methods**

Sugars, furfural, HMF, acetic acid, ethanol, and succinic acid were analyzed using high pressure liquid chromatography (HPLC). The separation system consisted of a solvent delivery system (P2000 pump, Spectra-Physics, San Jose, CA) equipped with an autosampler (Model 717, Waters Chromatography Division, Millipore Corp., Milford, MA), a refractive index detector (Model 410 differential refractometer, Waters), a dual λ absorbance detector (Model 2487, Waters), and a computer software based integration system (Chromquest 4.0, Spectra-Physics). Two ion moderated partition chromatography columns (Aminex HPX-87P with de-ashing and Carbo-P micro-guard cartridges, Aminex HPX 87H with cation H micro-guard cartridge) were used. The Aminex HPX-87P column was maintained at 85 °C, and the sugars were eluted with filtered (Milli-Q, Millipore Corp, Bedford, MA) deionized water at a flow rate of 0.6 mL min⁻¹. The Aminex HPX-87H column was maintained at 65 °C, and the sugars, organic acids, furfural, HMF, and ethanol were eluted with 10 mmol L⁻¹ HNO₃ prepared using filtered deionized water at a flow rate of 0.6 mL min⁻¹. Peaks were detected by refractive index or UV absorption (277 nm) and were identified and quantified by comparison with retention times of authentic standards (glucose, xylose, galactose, arabinose, furfural, HMF, acetic acid, succinic acid, and ethanol). Cell growth of the bacterium was monitored by measuring the optical density of the appropriately diluted culture broth at 660 nm in the case of SHF experiments.

**RESULTS AND DISCUSSION**

**Effect of lime dose and duration of pretreatment on enzymatic hydrolysis**

Wheat straw (moisture, 8.92 ± 0.08%) used in this study contained 44.24 ± 0.28% cellulose and 25.23 ± 0.11% hemicellulose, which made up the total carbohydrate content of 69.47 ± 0.39%. The detailed composition of wheat straw has been reported in a previous paper. Three commercial enzyme preparations were used in this study: Celluclast (cellulase preparation), Novozyme 188 (β-glucosidase preparation), and Viscostar 150 L (xylanase preparation). The activity levels of cellulase (carboxymethyl cellulase), xylanase, β-glucosidase, β-xylanosidase, α-L-arabinofuranosidase, and ferulic acid esterase in each of these enzyme preparations are presented in Table 1. Initially, the effects of lime doses (25, 50 and 100 mg g⁻¹ straw) on the pretreatment of wheat straw (8.6%, w/v) for 6 min, 30 min, and 1 h at 121 °C were evaluated. The resultant yield of glucose and total sugars in terms of mg g⁻¹ wheat straw after enzymatic saccharification using a cocktail of three commercial enzyme preparations (cellulase, β-glucosidase, and xylanase) at a dose level of 0.05 mL of each enzyme preparation g⁻¹ substrate at 45 °C and pH 5.0 for 72 h is shown in Fig. 1(A) and 1(B), respectively. The glucose as well as total sugars yields increased with increasing lime concentration for pretreatment and also depended upon the duration of pretreatment. However, the effect of lime dose was much more pronounced than the effect of pretreatment time. The yield of total sugars (g⁻¹) was increased from 247 ± 6 mg to 451 ± 3 mg (83% increase) with an increase of lime dose from 25 to 100 mg g⁻¹ straw. Using the same lime dose (100 mg g⁻¹ straw), the yield of total sugars increased from 410 ± 4 mg to 451 ± 3 mg (10% increase) when increasing the pretreatment time from 6 min to 1 h. The maximum yield of total sugars (451 ± 3 mg g⁻¹ straw; glucose, 252 ± 6 mg; xylose, 173 ± 3 mg; arabinose, 27 ± 2 mg; 65% conversion) was achieved at 100 mg lime g⁻¹ straw and 1 h pretreatment time. Thus, it was decided to use 100 mg lime g⁻¹ of straw and pretreatment time of 1 h for subsequent pretreatment studies. No galactose (detectable limit of sugars, 25 μg mL⁻¹ by HPLC) was detected in any of the hydrolyzates even though acid pretreatment released galactose (16 mg g⁻¹) from wheat straw. No furfural and HMF (detectable limit, 1 μg mL⁻¹) were detected in any of the lime pretreated wheat straw hydrolyzates.

Enzymatic hydrolysis (45 °C, pH 5.0, 72 h) of pretreated wheat straw hydrolyzates. Enzymatic hydrolysis (45 °C, pH 5.0, 72 h) of pretreated wheat straw without lime (1 h, 121 °C, control using water

**Table 1. Activity level of three commercial enzyme preparations used in lime treated wheat straw hydrolysis at pH 5.0 and 50 °C**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Celluclast (Cellulase)</th>
<th>Novozyme 188 (β-Glucosidase)</th>
<th>Viscostar 150 L (Hemicellulase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxymethyl cellulase</td>
<td>1,510</td>
<td>39</td>
<td>986</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>74</td>
<td>330</td>
<td>3</td>
</tr>
<tr>
<td>Xylanase</td>
<td>905</td>
<td>605</td>
<td>32,950</td>
</tr>
<tr>
<td>β-Xylanosidase</td>
<td>15</td>
<td>8</td>
<td>68</td>
</tr>
<tr>
<td>α-L-Arabinofuranosidase</td>
<td>8</td>
<td>29</td>
<td>58</td>
</tr>
<tr>
<td>Ferulic acid esterase</td>
<td>13</td>
<td>82</td>
<td>0</td>
</tr>
</tbody>
</table>

Ethanol from wheat straw
instead of lime) using the same enzyme cocktail generated 126 ± 1 mg glucose, 67 ± 1 mg xylose, and 17 ± 0 mg arabinose (total sugars, 210 ± 2 mg, 30% conversion) g⁻¹ straw.

**Effect of pH and temperature on enzymatic hydrolysis of lime pretreated wheat straw**

The effects of pH (3.5–6.5) and temperature (25–70°C) on the enzymatic hydrolysis of lime pretreated (100 mg g⁻¹ straw, 121 °C, 1 h) wheat straw (8.6%, w/v) using the three-enzyme combination mentioned above at an enzyme (each one) preparation dose level of 0.05 mL g⁻¹ substrate were investigated. The results for total sugars released and 6, 24, and 72 h reaction times are presented in Figs 2(A) and 3(A). Figures 2(B) and 3(B) show the release of glucose, xylose, arabinose and total sugars after 72 h for various pH and temperatures, respectively. At 6 h reaction time, the combined enzyme cocktail worked well over a broad pH range of 3.5–6.0 (Fig. 2(A)). However, at 72 h, the enzyme preparation showed a sharp optimum pH at 5.0. The relative sugar yields at pH 6.0 and 6.5 were 73 and 38% of the maximum level observed at pH 5.0. The results in Figs 2(A) and 2(B) clearly indicate that the enzyme cocktail worked better at pH levels below the optimum pH 5.0 than above it. With regard to temperature, the three-enzyme combination worked optimally at 45–50 °C and 6 h reaction time (Fig. 3(A)). The yield of total sugars was optimal at 45°C and 24 h reaction time. However, at 72 h, the sugar yield was optimal (100%) at 37–45 °C and decreased to 87% at 50 °C, 72% at 55 °C, and 44% at 60 °C (Fig. 3(A)). Taken collectively, pH 5.0 and temperature 37–45 °C are optimal for wheat straw hydrolysis by the cocktail of three enzymes.

The effect of nine different combinations of the three commercial enzyme preparations on the hydrolysis of lime pretreated wheat straw at 45 °C, pH 5.0 and 72 h reaction time is presented in Table 2. It is evident that for maximum release of sugars, the three-enzyme combination performed better than any two-enzyme combinations. The time course of each sugar (glucose, xylose, and arabinose), as well as total sugars release g⁻¹ of wheat straw by the three-enzyme cocktail using each enzyme preparation at a dose level of 0.15 mL g⁻¹ substrate at 45 °C and pH 5.0, is presented in Fig. 4. The yield of total sugars after 120 h was 568 ± 13 mg (glucose, 332 ± 8 mg; xylose, 203 ± 4 mg; arabinose, 33 ± 1 mg) g⁻¹ straw, which is about 82% of the total carbohydrates present in the
The data presented are averages of two individual experiments.

**Table 2.** Effect of enzyme combinations on enzymatic hydrolysis of lime pretreated wheat straw at 45°C and pH 5.0 for 72 h

<table>
<thead>
<tr>
<th>Cellulase (µL·g⁻¹)</th>
<th>Novozyme (µL·g⁻¹)</th>
<th>Viscostar (µL·g⁻¹)</th>
<th>Glucose (mg·g⁻¹)</th>
<th>Xylose (mg·g⁻¹)</th>
<th>Arabinose (mg·g⁻¹)</th>
<th>Total sugars (mg·g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>15</td>
<td>15</td>
<td>163 ± 1</td>
<td>146 ± 2</td>
<td>22 ± 1</td>
<td>331 ± 1</td>
</tr>
<tr>
<td>150</td>
<td>15</td>
<td>15</td>
<td>232 ± 21</td>
<td>165 ± 6</td>
<td>25 ± 1</td>
<td>422 ± 28</td>
</tr>
<tr>
<td>15</td>
<td>150</td>
<td>15</td>
<td>193 ± 4</td>
<td>155 ± 3</td>
<td>26 ± 0</td>
<td>374 ± 7</td>
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<tr>
<td>15</td>
<td>15</td>
<td>150</td>
<td>246 ± 12</td>
<td>171 ± 4</td>
<td>28 ± 0</td>
<td>445 ± 16</td>
</tr>
<tr>
<td>150</td>
<td>15</td>
<td>150</td>
<td>247 ± 21</td>
<td>174 ± 10</td>
<td>26 ± 1</td>
<td>448 ± 32</td>
</tr>
<tr>
<td>15</td>
<td>150</td>
<td>150</td>
<td>285 ± 1</td>
<td>180 ± 2</td>
<td>27 ± 1</td>
<td>491 ± 2</td>
</tr>
<tr>
<td>150</td>
<td>150</td>
<td>15</td>
<td>294 ± 4</td>
<td>189 ± 2</td>
<td>27 ± 0</td>
<td>512 ± 6</td>
</tr>
<tr>
<td>150</td>
<td>150</td>
<td>150</td>
<td>308 ± 5</td>
<td>190 ± 2</td>
<td>30 ± 0</td>
<td>528 ± 7</td>
</tr>
</tbody>
</table>

Wheat straw (8.6%, w/v) was pretreated with lime (0.86%, w/v) at 121°C for 1 h. The pH was adjusted to 5.0 and enzyme combinations were added. The data presented are averages of two individual experiments.


Figure 3. Effect of temperature on the enzymatic hydrolysis of lime pretreated (100 mg·g⁻¹, 121°C, 1 h) wheat straw (8.6%, w/v) at pH 5.0 using a cocktail of three commercial enzyme preparations (cellulose, β-glucosidase, and hemicellulase), each enzyme at a dose level of 0.05 mL·g⁻¹ straw. The data presented are averages of two individual experiments. (A) Yield of total sugars at (a) 6, (b) 24, and (c) 72 h; (B) yield of glucose (●), xylose (○), arabinose (▼), and total sugars (△) at 72 h.

Figure 4. Time course of enzymatic hydrolysis of lime pretreated (100 mg·g⁻¹, 45°C, 1 h) wheat straw (8.6%, w/v) using a cocktail of three commercial enzyme preparations (cellulose, β-glucosidase, and hemicellulase), each enzyme preparation at a dose level of 0.15 mL·g⁻¹ straw at 45°C and pH 5.0. The data presented are averages of two individual experiments: ●, glucose; ○, xylose; ▼, arabinose; and △, total sugars.

straw. However, the total sugars yield was 389 ± 10 mg (glucose, 228 ± 5 mg; xylose, 139 ± 4 mg, arabinose, 22 ± 1 mg)·g⁻¹ straw (56% conversion) after 6 h. After 24 h, the total sugar yield was 440 ± 12 mg·g⁻¹ straw (63% conversion). These data clearly indicate that both cellulose and hemicellulose saccharification rates decreased over time. The longer the reaction time, the slower the reaction rate.

Twee 20 is known to enhance the enzymatic hydrolysis of cellulose. The effect of Twee 20 (0, 1.25, and 2.5 g·L⁻¹) on the enzymatic action at two enzyme dose levels (0.05 and 0.15 mL of each enzyme preparation g⁻¹ substrate) was tested. The yield of each sugar, as well as total sugars, is shown in Fig. 5. It is clear that the effect of Twee 20 was more pronounced at the low enzymes dose (0.05 mL of each enzyme preparation g⁻¹ substrate) with 10% increase in the release of total sugars. At the low enzymes dose, glucose release was increased by 13% and xylose release by 6%. This indicates that Twee 20 may have a greater effect on cellulose hydrolysis than hemicellulose degradation. At the high enzymes dose (0.15 mL of each enzyme g⁻¹ of straw), the increase was only 1.4% over the control (without Twee 20). The exact mechanism of enhancement of enzymatic hydrolysis of cellulose by Twee 20 is not clear. It plays an important role in preventing the non-specific binding of cellulosases to lignin residues, allowing more enzymes to be available for the conversion of cellulose, resulting in a higher conversion rate. At the high enzymes dose, Twee 20 did not have any significant effect.
is shown in Fig. 6. The cell density (A_{660 nm}) reached a maximum of 7.4 ± 0.5 at 19 h, after which it declined to 7.0 ± 0.5 at 24 h in the case of SHF. There is little growth (A_{660 nm}, 0.44 ± 0.02) of recombinant *E. coli* strain FBR 5 in the control medium where water was substituted for the hydrolyzate. No detectable ethanol, succinic acid, or acetic acid was found to be produced in the control medium by the strain. From the sugar utilization pattern shown in Fig. 6, glucose was utilized first. The bacterium started to utilize xylose after almost all the glucose disappeared from the fermentation broth. Arabinose was slowly utilized from the beginning but finished before xylose. Similar patterns of mixed sugar utilization were also observed in cases of fermentation of both dilute acid and alkaline peroxide pretreated enzymatically saccharified wheat straw hydrolyzates by the recombinant bacterium.\textsuperscript{11,12}

A critical problem in the fermentation of dilute acid hydrolyzates is the inability of the fermentative microorganism to withstand inhibitory compounds formed during pretreatment, and usually a detoxification step is needed to improve fermentability.\textsuperscript{22} This was also true with the fermentation of the dilute acid hydrolyzates of wheat straw and rice hulls.\textsuperscript{11,18} The inhibitor problem is not pronounced in the case of lime pretreatment of wheat straw. Unlike corn fiber hemicellulose, which is very resistant to hydrolysis

### Table 3. Ethanol production from lime pretreated wheat straw hydrolyzate by recombinant *Escherichia coli* strain FBR5 at 35 °C

<table>
<thead>
<tr>
<th>Hydrolyzate</th>
<th>Fermentation time (h)</th>
<th>Total sugars (g L⁻¹)</th>
<th>Ethanol (g L⁻¹)</th>
<th>Ethanol (g g⁻¹ sugar)</th>
<th>Ethanol (g g⁻¹ straw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separate hydrolysis and fermentation (SHF)</td>
<td>24</td>
<td>44.6 ± 1.2</td>
<td>22.5 ± 0.6</td>
<td>0.50</td>
<td>0.29</td>
</tr>
<tr>
<td>Simultaneous saccharification and fermentation (SSF)</td>
<td>72</td>
<td>–</td>
<td>20.6 ± 0.4</td>
<td>0.48</td>
<td>0.26</td>
</tr>
</tbody>
</table>

The medium contained hydrolyzates from 78 g wheat straw L⁻¹. For pretreatment, wheat straw (8.6%, w/w) was treated with lime (0.86%, w/w) at 121 °C for 1 h. Separate enzymatic hydrolysis was performed using cellulase (Cellulast), β-glucosidase (Novozyme 188), and xylanase (Viscostar 150 L) at 45 °C and pH 5.0 for 120 h. Each enzyme preparation used 0.15 mL g⁻¹ wheat straw. Fermentation experiments were performed at pH 6.5 for SHF and pH 6.0 for SSF. The data presented are averages of two individual experiments.
using commercial enzymes, wheat straw hemicellulose can be easily hydrolyzed enzymatically by using a single xylanase preparation (Viscostar) after lime pretreatment. The structure of corn fiber hemicellulose is more complex than that of wheat straw, with highly branched side chains. Pan et al. reported that wheat straw contained 0.48% ferulic acid and 0.42% p-coumaric acid, and these phenolic acids had a tendency to dissolve in alkaline solution. This is probably the reason why the hemicellulose preparation (Viscostar 150 L) performed well in saccharifying hemicellulose in lime pretreated wheat straw. This research demonstrates that wheat straw can easily be converted to fermentable sugars with a very good yield by lime pretreatment and enzymatic saccharification, and the generated hydrolyzate can be efficiently fermented to ethanol by using recombinant bacterium without any detoxification step needed for dilute acid pretreated enzymatically saccharified wheat straw hydrolyzate.

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