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# Optimizing Dilute-Acid Pretreatment of Rapeseed Straw for Extraction of Hemicellulose

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**Abstract** Biological conversion of biomass into fuels and chemicals requires hydrolysis of the polysaccharide fraction into monomeric sugars prior to fermentation. Hydrolysis can be performed enzymatically or with mineral acids. In this study, dilute sulfuric acid was used as a catalyst for the pretreatment of rapeseed straw. The purpose of this study is to optimize the pretreatment process in a 15-mL bomb tube reactor and investigate the effects of the acid concentration, temperature, and reaction time. These parameters influence hemicellulose removal and production of sugars (xylose, glucose, and arabinose) in the hydrolyzate as well as the formation of by-products (furfural, 5-hydroxymethylfurfural, and acetic acid). Statistical analysis was based on a model composition corresponding to a $3^3$ orthogonal factorial design and employed the response surface methodology to optimize the pretreatment conditions, aiming to attain maximum xylan, mannan, and galactan (XMG) extraction from hemicellulose of rapeseed straw. The obtained optimum conditions were: $H_2SO_4$ concentration of 1.76% and temperature of 152.6 °C with a reaction time of 21 min. Under these optimal conditions, 85.5% of the total sugar was recovered after acid hydrolysis (78.9% XMG and 6.6% glucan). The hydrolyzate contained 1.60 g/L glucose, 0.61 g/L arabinose, 10.49 g/L xylose, mannose, and galactose, 0.39 g/L cellobiose, 0.94 g/L fructose, 0.02 g/L 1,6-anhydro-glucose, 1.17 g/L formic acid, 2.94 g/L acetic acid, 0.04 g/L levulinic acid, 0.04 g/L 5-hydroxymethylfurfural, and 0.98 g/L furfural.

**Keywords** Rapeseed straw · Response surface methodology (RSM) · Sulfuric acid · Pretreatment

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## Introduction

The effect of rapidly developing countries, such as China, will drive increasing global energy demand. This "China effect" and inevitable depletion of the world's oil reserves has prompted much research into finding alternative sources of fuels and chemicals. The most likely renewable resource is lignocellulosic biomass, i.e., plant materials such as agriculture and forest wastes. Cellulosic biofuels could significantly reduce $CO_2$ generation because there is no net increase in $CO_2$ released to the atmosphere as a result of their life cycle [1, 2]. Furthermore, Farell et al. [3] suggested that only lignocellulosic ethanol offers considerable greenhouse gas emission reductions compared to fossil fuel. The potential for non-oil-producing countries to derive a substantial portion of its transportation fuel needs from domestically grown biomass holds a broad variety of benefits for the country. These benefits have been widely recognized and include improved air quality, strengthened rural and agricultural economies, decreased reliance on foreign supplies of petroleum, improved balance of trade, reduced greenhouse gas emissions, enhanced national security, and increased sustainability of the transportation system [4, 5].

Lignocellulosic biomass is the most abundant organic material on earth and also promising raw material for bioenergy production [6, 7]. Rapeseed straw, an agricultural residue in the process of bio-oil extraction, is an abundant and low-cost lignocellulosic material in many European and Asian countries similar to US corn stover. Utilization of the rapeseed straw gives an added value for this material and a solution for the removal of this abundant waste, solving a problem of the bio-oil industry and increasing the economical yield of the process. Therefore, a double effect is obtained, economic and ecologic. The most frequent use for rapeseed straw is energy production by combustion in many Asian countries. This adds to the problem of air pollution, increasing the emissions of $CO_2$ [8, 9]. Other alternative uses of rapeseed straw are the production of chemical compounds such as acetic acid, furfural, or 5-hydroxymethylfurfural (5-HMF) and the biotechnological production of ethanol [10–12]. Rapeseed straw is formed by three main fractions (cellulose, hemicelluloses, and lignin). Cellulose and hemicellulose are both polymers built up by long chains of sugar monomers, which after pretreatment and hydrolysis can converted into fermentable sugars and produce a myriad of fuels and chemicals by microbial fermentation [13].

The technology for conversion of lignocellulosic biomass resources to fuels and chemicals, such as ethanol, has been under development for decades. Currently, commercial enterprises are developing commercial operations with lignocellulose-derived ethanol. Although the technology has advanced considerably over the past decades, a much greater understanding of the process fundamentals is needed [14, 15]. There are many process configurations possible for converting biomass to ethanol. One of the well-studied technologies that is currently being commercialized is to use a dilute-acid-catalyzed pretreatment followed by enzymatic hydrolysis and fermentation to produce ethanol. The objective of pretreatment is to alter the biomass structure in order to make the cellulose and hemicelluloses more accessible to hydrolytic enzymes that can generate fermentable sugars [16]. Effective pretreatment technologies need to meet several important criteria, essentially minimal energy, high sugar yield, high sugar concentration, and acceptable capital and operating costs [17, 18]. Hydrothermal pretreatments, including steam explosion, hot water autocatalyzed pretreatments, and dilute-acid pretreatment, have been extensively studied in the literature [17, 19–22] and typically employ hot water or dilute acid to hydrolyze the hemicellulose. For the case of dilute-acid pretreatment, it typically employs 0.4–2% (*w*/*v*) of acid (nitric, sulfur dioxide, phosphoric acid, and mainly sulfuric acid) at temperatures of

160–220 °C to recover hemicelluloses and enhance digestion of cellulose. The severity of the hydrothermal pretreatment processes is often described by a severity factor or modified severity factor [12, 23–26]. However, this kind of pretreatment is not much carried out with rapeseed straw, which partly accounts for the lack of literature concerning its pretreatments. Recently, Lu et al. [27] pretreated rapeseed straw with sulfuric acid solution for 5 to 20 min at 180 °C and reported that, by enzymatic hydrolysis, 75.12% total xylan and 63.17% total glucan were converted to xylose and glucose, respectively.

In this work, the dilute-acid hydrolysis of rapeseed straw was optimized through the utilization of statistical experimental design. Evaluation criteria for optimization of the pretreatment conditions were based on high xylan, mannan, and galactan (XMG) recovery and low inhibitor contents in the hydrolyzates. In addition, this paper reports the compositional analysis of hydrolyzate liquors and solids, XMG, and glucose mass balance closures and digestibility results from the acid-pretreated rapeseed straw. The treated samples were further analyzed with SEM in order to determine optimal pretreatment conditions. The purpose of this study was to gain a more accurate understanding of the quantities of acid required for effective hydrolysis and the reactivity trade-offs with reaction time and temperature that will enable overall process optimization.

## Materials and Methods

### Raw Material

Rapeseed (*Brassica napus*) straw was supplied by the Bioenergy crop research center, National Institute of Crop Science, Rural Department of Administration (Muan, Jeonranam-Do, Korea). The straw was ground to an average size of 10~40 mesh (0.42~2.00 mm) using a laboratory knife. The screened straw chips were air dried for 24 h at 45±5 °C and then used directly in acid-pretreatment studies. Some of the screened chips ground to an average size of 30~40 mesh (0.595~0.420 mm) were used for determination of total moisture and carbohydrate content in biomass. The moisture content of milled straw was 4.63%.

### Quantitative Saccharification and Hydrolysis

Primary hydrolysis of 300 mg subsamples was performed with 3.0 ml 72% (*w*/*w*) $H_2SO_4$ for 120 min at 30 °C. Hydrolyzates were adjusted to pH 1 with 84 mL distilled water, which equates to 4% sulfuric acid. A secondary hydrolysis was performed for 60 min at 121 °C. Following the secondary hydrolysis, samples were immediately centrifuged at 5,000 rpm for 20 min to remove particles. The resulting supernatant was decanted, and a portion of this was syringe filtered (Gelman, 0.2 μm pore size), and then concentrations of dissolved monomer sugar were determined using high-performance liquid chromatography (HPLC) chromatography. Samples of hydrolyzate were hydrolyzed using the same procedure as the secondary hydrolysis: 4% sulfuric acid (pH=1) for 60 min at 121 °C. For calculation of sugar yields, a conversion factor was considered. The conversion factor for dehydration on polymerization to glucan was 162/180 for glucose, to XMG was 132/150 for xylose, mannose, and galactose (xmg), and to arabinan was 132/150 for arabinose. The conversion factor for XMG ignores the factor for mannose and galactose because xylose is the dominant building unit of the hemicelluloses of most woods and annual plants. Here, XMG (capitalized) represents the sum total of the oligomeric sugar (xylan + mannan + galactan) and xmg (lowercase) represents monomeric sugar.

## High-Performance Liquid Chromatography

The composition of reaction products was quantitatively analyzed by HPLC. The Breeze HPLC system (Waters Co., Milford, MA, USA) used for carbohydrate measurement had a Bio-Rad Aminex HPX-87H column (300 mm×7.8 mm) and Cation H micro-guard cartridge (30×4.6 mm; Bio-Rad Laboratories Inc., Hercules, CA). The column was maintained at 60 °C, with a 5 mM $H_2SO_4$ eluent at a flow rate of 0.6 mL/min. All of the sugar peaks detected by a refractive index detector (Waters 2414, Waters Co., Milford, MA, USA) were identified and quantified by comparison to retention times of authentic standards. The Bio-Rad Aminex HPX-87H analytical column allows the concurrent analysis of liquid sample for the presences of acetic and lactic acids as well as sugar degradation products. Quantification of the samples was calculated by HPLC from the peak areas of the standard solutions for three sugars: D (+)-glucose, D (+)-arabinose, and D (+)-xylose. Fucose was used as an internal standard. A reference mixture of carbohydrates typically contains the pure reference compounds in a range from 1 to 2 g/L. In addition, a high degree of correlation exists between the sugar peak area ($X$) and the sugar mass ($Y$), as indicated by a linear regression. The correlation coefficients ($R^2$) between ($X$) and ($Y$) are 0.994–0.999 (data not shown).

## Klason Lignin and Ash

Primary hydrolysis of 1,000 mg milled chips was performed with 15 ml 72% ($w/w$) $H_2SO_4$ for 120 min at room temperature. Hydrolyzates were diluted to 4% ($w/w$) $H_2SO_4$ with distilled water and then boiled gently for 4 h under a flux condenser. After 4 h, samples were filtered through a porcelain Büchner funnel containing Whatman filter (Qualitative Filter Paper Grade 4, 20–25 μm, 125 mm ∅, Whatman). Residues were extensively washed with hot water, dried, and measured gravimetrically. The ash content was determined by placing ~0.5 g sample in a tarred crucible, ignited at 550 °C for 24 h, cooled in a desiccator, and weighed.

## Acid Pretreatment and Design of Experiments

The pretreatment experiments were performed using sealed bomb tubular reactors. The vessels were 20 cm long and 2.54 cm in diameter and constructed out of 316 stainless steel tubing, capped at either end with Swagelok fittings, giving an internal volume of 30 $cm^3$. The stainless steel reactor was loaded with 0.5 g of air-dried rapeseed straw. The residual moisture in the air-dried straw was accounted for in the determination of the amount of acid solution to be added, which gave a final ratio of 15 mL liquor per gram oven-dry straw. The reaction conditions tested were temperature (140 and 170 °C), concentration of acid (0.25–2.5%, $w/v$), and reaction time (0–30 min). The temperature, acid concentration, and residence time for the experimental design are given in Table 2. A total of 17 runs were carried out, with runs 9–11 as triplicate.

## Response Surface Methodology

Data analysis was carried out using SAS package (version 8.0, SAS Institute, Cary, NC) in Statistical Analysis System. A second-order model was employed to fit the data individually for the responses $Y$ (XMG yield) by the general model [28] with three factors: $X_1$ (reaction temperature), $X_2$ (acid concentration), and $X_3$ (residence time), with each factor

coded to be in the levels of −1.732, 0, and +1.732 (Table 2). The relationship of the independent variables and response was calculated by the second-order polynomial Eq. 1:

$$Y = A_0 + \sum A_i X_i + \sum A_{ij} X_i X_j + \sum A_{ii} X_i^2 + e_i \quad (1)$$

where $Y$ is the response variable; $A_0$, $A_i$, $A_{ii}$, and $A_{ij}$ are the regression coefficient variables for intercept, linear, quadratic, and interaction terms, respectively; $X_i$ and $X_j$ are independent variables; and $e_i$ is error constant. The model was evaluated in terms of statistically significant coefficients, $R^2$, and $P$ value.

### Enzyme and Enzymatic Hydrolysis

Extensively washed pretreated residues under the optimized condition were tested for enzymatic digestibility using National Renewable Energy Laboratory (NREL) standard Laboratory Analytical Procedure #009 [29]. Commercial cellulase, Cellulase (Novozyme A/S Bagsvaerd, Denmark), and Novozym 188 (Novozyme A/S Bagsvaerd, Denmark) purchased from Sigma Aldrich were used. Hydrolysis experiments were conducted in 150 mL Erlenmeyer flasks with a total working volume of 20 mL at substrate concentration of 1% (*w*/*v*). The prewarmed flask contained 50 mM sodium citrate buffer (pH=4.8), 80 μg/mL of tetracycline, and 60 μg/mL of cycloheximide to minimized microbial contamination. The enzyme loading was 60 FPU/g of glucan, supplemented with β-glucosidase to alleviate end-product inhibition by cellobiose. Triplicate reaction flasks were incubated at 50 °C with 120 rpm rotation and compared to controls that contained 1% α-cellulose and untreated straw. One-milliliter samples were withdrawn at various time intervals, and the samples were centrifuged at 15,000 rpm for 10 min. Glucose concentrations in the sample supernatant were determined by HPLC.

## Results and Discussion

### Composition of Rapeseed Straw

The composition of this material was analyzed according to the NREL Chemical Analysis and Testing Standard Procedures [29]. Table 1 shows the results of an analysis (based on a 105 °C dry weight) of the composition of raw rapeseed straw. The composition of straw chips was determined to be 37.8% glucan, 18.6% XMG, and 0.9% arabinan. The results agree well with the recent analysis data reported by Lu et al. [27]. Analysis of the rapeseed straw shows that theoretical fermentable sugars accounted for more than 62.1% on a dry matter basis, similar to that of other major lignocellulosic biomass types, such as rice straw, wheat straw, and corn stover, indicating that rapeseed straw has a great potential as a biofuel feedstock.

### Optimization of Acid Pretreatment by Response Surface Methodology

The coded and uncoded values of factors in the central composite design (CCD) are shown in Table 2. Experimental design and XMG yield are shown in Table 3. The statistical significance of the second-order model equation was verified by an $F$ test [analysis of variance (ANOVA)]. The following second-order polynomial prediction was obtained from regression analysis of XMG production data.

**Table 1** Chemical composition of rapeseed straw on dry basis.

| Components | Dry solids (%, *w*/*w*) |
|---|---|
| As fermentable sugars (theoretical yield) | |
| Glucose | 42.0 |
| xmg[a] | 19.5 |
| Subtotal | 62.1 |
| | |
| As major constituents of rapeseed straw | |
| Glucan | 37.8 |
| XMG[b] | 17.6 |
| Arabinan | 0.9 |
| Klason lignin | 28.1 |
| Extractives (hexane) | 5.7 |
| Ash | 7.3 |
| Others | 2.7 |
| Subtotal | 100 |

[a] Monomeric sugar (=xylose + mannose + galactose)

[b] Oligomeric sugar (=xylan + mannan + galactan)

$$Y = 3.551 - 0.331x_1 + 0.778x_2 + 0.452x_3 - 0.753{x_1}^2 - 0.488x_1x_2 - 0.265{x_2}^2 - 0.601x_1x_3 - 0.44x_2x_3 - 0.422{x_3}^2 \quad (2)$$

where $Y$ (XMG yield) is the predicted response, $x_1$ the coded value of variable $X_1$ (temperature), $x_2$ the coded value of variable $X_2$ (acid concentration), and $x_3$ the coded value of variable $X_3$ (residence time). The ANOVA results showed that this model was appropriate since the $R^2$ value was 95% ($R^2$ implies that the sample variation of 95% for XMG production is attributable to the independent variables, and only 5% of the total variation cannot be explained by the model). Also, the statistics' $P$ value for the overall regression is significant at the 5% level, which further supports that the model is adequate in approximating the response surface of the experimental design. Figure 1 presents the results of a series of dilute-acid pretreatments evaluated through a $3^3$ full factorial design. According to response surface methodology (RSM), the optimum temperature, acid concentrations, and residence time were determined. The experimental optimum yield for XMG production was found to be 78.9% at 152.6 °C, 1.76% acid, for 21 min.

The graphical representation provides a method for visualizing the relationship between the response and the interactions among test variables in order to determine

**Table 2** The coded and uncoded values of factors in CCD.

| Variables | Symbol | Coded variable level | | | | |
|---|---|---|---|---|---|---|
| | | −1.732 | −1 | 0 | 1 | 1.732 |
| Temperature (°C) | $X_1$ | 161.3 | 165 | 170 | 175 | 178.7 |
| Acid concentration (wt.%) | $X_2$ | 0.82 | 1.00 | 1.25 | 1.50 | 1.68 |
| Reaction time (min) | $X_3$ | 6.34 | 10.00 | 15.00 | 20.00 | 23.66 |

**Table 3** Three-variable, three-level fractional factorial design for the rapeseed straw hydrolysis.

| Run # | Independent variables (coded level) | | | Responses[a] |
|---|---|---|---|---|
| | $X_1$ ($x_1$) | $X_2$ ($x_2$) | $X_3$ ($x_3$) | XMG yield (%, *w*/*w*) |
| 1 | 175 (1) | 1.5 (1) | 15 (0) | 31.4 |
| 2 | 175 (1) | 1.25 (0) | 20 (1) | 19.2 |
| 3 | 175 (1) | 1.0 (−1) | 10 (−1) | 57.7 |
| 4 | 170 (0) | 1.5 (1) | 10 (−1) | 51.3 |
| 5 | 170 (0) | 1.0 (−1) | 20 (1) | 41.9 |
| 6 | 165 (−1) | 1.5 (1) | 20 (1) | 54.5 |
| 7 | 165 (−1) | 1.0 (−1) | 15 (0) | 57.4 |
| 8 | 165 (−1) | 1.25 (0) | 10 (−1) | 56.9 |
| 9 | 170 (0) | 1.25 (0) | 15 (0) | 52.7 |
| 10 | 170 (0) | 1.25 (0) | 15 (0) | 57.9 |
| 11 | 170 (0) | 1.25 (0) | 15 (0) | 55.1 |
| 12 | 178.7 (1.732) | 1.0 (−1) | 10 (−1) | 40.7 |
| 13 | 161.3 (−1.732) | 1.0 (−1) | 10 (−1) | 39.4 |
| 14 | 165 (−1) | 1.68 (1.732) | 10 (−1) | 61.2 |
| 15 | 165 (−1) | 0.82 (−1.732) | 10 (−2) | 39.5 |
| 16 | 165 (−1) | 1.0 (−1) | 23.66 (1.732) | 52.4 |
| 17 | 165 (−1) | 1.0 (−1) | 6.34 (−1.732) | 38.8 |

$x_1=(X_1-170)/5$, $x_2=(X_2-1.25)/0.25$, $x_3=(X_3-15)/3$.

[a] Observed experimental data.

the optimum conditions (Fig. 1). The contour plots for the estimated XMG production surface were constructed over the optimized variables ($X_1$=152.6 °C, $X_2$=1.76%, and $X_3$=21 min). Figure 1a shows the effect of temperature and acid concentration on XMG. At higher temperatures (185 °C), the yield of XMG decreased gradually with the increase in acid concentration but increased at the optimal temperature range (170–175 °C). It can be seen that when the acid concentration was below 0.5%, the effect of residence time on XMG production was not significant (Fig. 1b). The effect of temperature and residence time on XMG production at an acid concentration of 1.25% is shown in Fig. 1c. Figure 1c shows that increasing the temperature and residence time within the range tested improved XMG production and that maximum XMG production is achieved at a temperature in the range of 165–170 °C and a residence time in the range of 20–25 min.

## Dilute-Acid Pretreatment Results

Figure 2 shows the soluble sugar yields of dilute-acid pretreatment experiments. The effects of residence time (ranging from 0 to 27 min) on dilute $H_2SO_4$ (1.76%, *w*/*v*) hydrolysis of rapeseed straw was evaluated at the temperature of 152. 6 °C. As can be seen in Table 4, the compositions of the hydrolyzates were strongly influenced by the residence time employed during hydrolysis. In particular, the concentrations of xmg, acetic acid, furfural, and HMF, selected at various residence time, varied from 1.54 to 10.49 g/L, from 0.55 to 2.94 g/L, from 0.28 to 1.06 g/L, and from 0.02 to 0.08 g/L, respectively (Table 4). The maximum

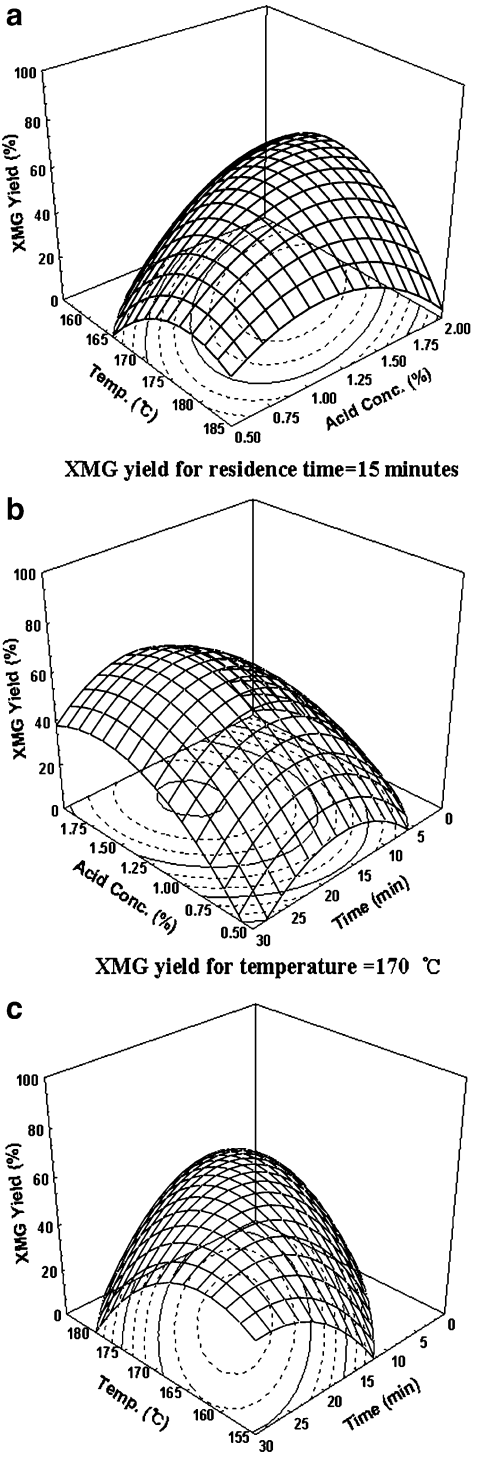


**Fig. 1** Response surface and contour plot of the combined effects of (**a**) temperature and acid concentration, (**b**) residence time and acid concentration, and (**c**) temperature and residence time

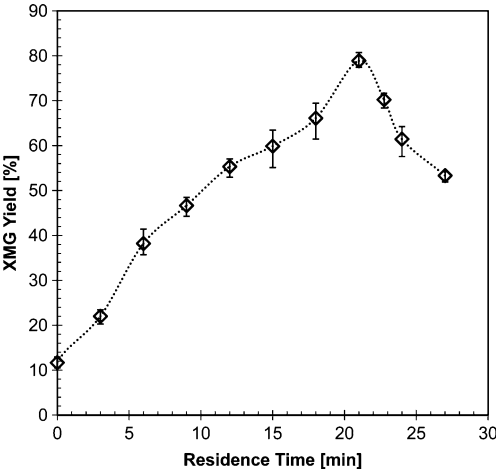


**Fig. 2** XMG yield resulting from optimal temperature (152.6°C) and acid concentration (1.76%, $H_2SO_4$) as function of residence time

xmg concentration of 10.5 g/L was obtained by hydrolyzing at 1.76% $H_2SO_4$ and 152.6 °C for 21 min, representing 78.9% of the maximum XMG yield (Fig. 2). The experimental XMG yield is slightly higher than the predicted yield. Optimization and comparison of multiple variables has been successfully carried out in the current study using RSM. At the operating conditions, 2.94 g/L of the potential acetic acid was also detected. Under the hydrolysis conditions used, approximately 52% of the mass of the straw was extracted. The total extraction mass balance was 89.3% for glucan and 89.2% for XMG. These values are shown in Table 5. Thus, XMG equivalents released into the hydrolyzate from the optimal conditions are relatively high. The high XMG yields obtained in the present study are highly desirable from the standpoint of maximizing ethanol yields in a 5carbon-to-ethanol process.

**Table 4** The concentration of chemical composition in hydrolyzate as function of residence time.

| Time (min) | Glucose (g/L) | xmg (g/L) | Fructose (g/L) | Arabinose (g/L) | Formic acid (g/L) | Acetic acid (g/L) | Levulinic acid (g/L) | 5-HMF (g/L) | Furfural (g/L) |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0.39 | 1.54 | 0.17 | 0.23 | 0.12 | 0.55 | nd | 0.02 | 0.25 |
| 3 | 0.30 | 2.97 | 0.23 | 0.56 | 0.08 | 0.85 | nd | nd | nd |
| 6 | 0.57 | 5.52 | 0.35 | 0.54 | 0.17 | 1.48 | nd | 0.03 | 0.28 |
| 9 | 0.78 | 6.30 | 0.47 | 0.55 | 0.34 | 1.72 | nd | nd | 0.23 |
| 12 | 0.80 | 7.13 | 0.49 | 0.54 | 0.40 | 1.94 | 0.03 | nd | 0.29 |
| 15 | 0.98 | 7.34 | 0.61 | 0.51 | 0.62 | 2.08 | 0.03 | nd | 0.43 |
| 18 | 1.27 | 8.12 | 0.75 | 0.46 | 0.82 | 2.37 | 0.04 | 0.04 | 0.88 |
| 21 | 1.60 | 10.49 | 0.94 | 0.61 | 1.17 | 2.94 | 0.04 | 0.04 | 0.98 |
| 22.5 | 1.61 | 9.36 | 0.86 | 0.54 | 1.11 | 2.67 | 0.06 | 0.07 | 1.01 |
| 24 | 1.38 | 7.76 | 0.80 | 0.50 | 0.98 | 2.31 | 0.09 | 0.08 | 1.06 |
| 27 | 1.53 | 8.25 | 0.95 | 0.49 | 1.08 | 2.51 | 0.08 | 0.12 | 1.21 |

Reaction temperature=152.6 °C, acid concentration=1.76% (*w*/*v*) $H_2SO_4$.

nd=not detected.

**Table 5** Composition of the solid and hydrolyzate of rapeseed straw.

| Sample | Solid remaining (%) | Solid | | | | Liquid | | EMB[b] | |
|---|---|---|---|---|---|---|---|---|---|
| | | Glucan (%) | XMG (%) | Klason Lignin (%) | Ash (%) | Glucan (%) | XMG (%) | Glucan (%) | XMG (%) |
| Untreated | 100 | 37.8 | 17.6 | 28.1 | 7.3 | – | – | – | – |
| Treated | 47.9 | 65.3 | 3.8 | 28.6 | 0.4 | | | 89.3 | 89.2 |
| Treated[a] | | 31.3 | 1.8 | 13.7 | 0.2 | 2.5 | 13.9 | | |
| Component retention (%) | 82.8 | 10.2 | 40.5 | 2.9 | | | | | |

The straw chips are pretreated for 21 min with 1.76% (*w*/*v*) $H_2SO_4$ at 156.2 °C.

[a] Data are based on the oven dry untreated biomass.

[b] Extraction Mass Balance (EMB) = $\frac{\sum C_{Li}+\sum C_{Si}}{\sum C_{Ri}}$; where $C_i$ is the mass of each sugar component as determined through HPLC chromatography, the subscripts L, S and R refer to the extracted liquid, extracted solids and raw straw fractions, respectively.

## Decomposition of Sugars

Furfural, the product of decomposition of xylose, increased with residence time at optimized temperature and acid concentration. This clearly indicates that pentose sugars (xylose and arabinose) derived from hemicellulose were further degraded through the hydrolysis. At 1.76% added $H_2SO_4$, a maximum of 0.1 g/L of the 5-HMF and 1.21 g/L of the potential furfural was found in the hydrolyzate. The maximum occurred at reaction time of 27 min (Table 4). Little 5-HMF was detected in the hydrolyzate after hydrolysis of the straw. The main reason for this may be the small quantities of glucose and glucan that appear in the acid hydrolyzate. The data on these minor constituents are, however, subject to little errors because their quantities are small. However, going to higher severity might somewhat reduce both the xylan remaining in the hydrolyzate and the loss. Tested reaction times were selected such that the longest reaction time was sufficiently long to result in a

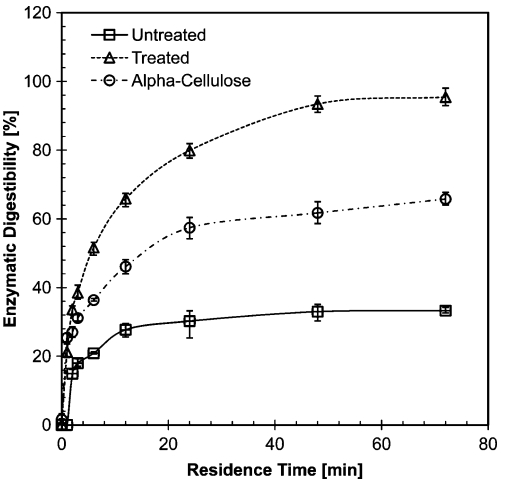


**Fig. 3** Three-day enzymatic digestibility (60 FPU/g cellulose) for each of the pretreated straw and the control samples

decreased XMG yield, ensuring that a maximum yield had been at least bracketed by each experimental condition of temperature and pH.

Enzymatic Hydrolysis of Acid-Treated Rapeseed Straw

The enzyme digestibility test of the pretreated straw under the optimized operating conditions was carried out in order to evaluate the extent of susceptibility. The enzymatic digestibility of treated rapeseed straw is summarized in Fig. 3. The digestibility of the pretreated biomass was significantly improved over the control (untreated biomass). The highest digestibility of the treated biomass is 95.4% after 72 h at 60 FPU/g of glucan in our experiments, while the digestibility of the untreated sample was only 27.1% at the same condition. The reason for this is the fact that the flow rate significantly affects the removal of hemicellulose which hinders the enzymatic hydrolysis of lignocellulose [13]. Jørgensen et al. and Kim et al. [30, 31] also found that lignin is able to embed in the cellulose and to form covalent bonds to some hemicellulose, thereby offering protection against microbial and chemical degradation. The enhanced digestibility of the straw by optimal acid hydrolysis is thought to be due to better exposure of cellulose to enzymes through increased removal of hemicellulose and particularly lignin.

## Conclusion

Pretreatment is a necessary element in bioconversion of lignocellulosics to fuels and chemicals. Although various forms of chemical pretreatment of cellulosic materials have been proposed, their effectiveness varies, depending on the substrate. Hence, an optimal pretreatment must be established for each substrate. Rapeseed straw is an attractive raw material as a biofuel feedstock due to its high content of fermentable sugars (more than 60%). The maximum xmg concentration of 10.5 g/L was obtained by hydrolyzing at 1.76% $H_2SO_4$ and 152.6 °C for 21 min, representing 78.9% of the maximum XMG yield. The highest enzyme digestibility of the treated rapeseed straw is 95.4% after 72 h at 60 FPU/g of glucan in this experiment. Optimization and comparison of multiple variables has been successfully carried out in the laboratory study using RSM. The statistical optimization method, which incorporates reaction time, temperature, and acid concentration, did prove to provide a useful means of trading off the combined effects of these three variables on total XMG yields.

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