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Reduction of surface area of lignin improves enzymatic hydrolysis of cellulose from hydrothermally pretreated wheat straw

M. H. Sipponen,* V. Pihlajaniemi, O. Pastinen and S. Laakso

Enzymatic hydrolysis of cellulose after pretreatment of wheat straw (WS) was investigated for the first time in relation to lignin surface area (SA). Lignin SA in solid residues from WS autohydrolysis (AH) and successive NH₃ (aq) extraction was determined using cationic dye adsorption. AH at increasing severity decreased up to 45% and 53% of WS lignin SA and specific surface area (SSA), respectively. Cellulose-to-glucose conversion from AH solid fractions from 24 h reaction with 15 FPU g⁻¹ cellulase activity increased linearly from 31% to 91% with decreasing lignin SA. When AH solid fractions were extracted with NH₃ (aq), both lignin SA and SSA increased in the corresponding solid residues, SSA up to 92%. As a consequence, cellulose-to-glucose conversion decreased in spite of the lower proportion of lignin in the solid residues after the NH₃ (aq) extraction. Up to 85% sugar yield was obtained from the single-stage AH process but when combined with NH₃ (aq) extraction the two-stage process yielded at most 71% of the original straw sugars. These results show that, independent of the lignin content, reduction of surface area of lignin improves the enzymatic hydrolysis process.

Introduction

Production of biofuels from renewable lignocellulosics is complicated by lignin that impedes enzymatic hydrolysis of carbohydrates. Without pretreatment, lignin content of forages shows a negative linear correlation with digestibility.¹ The detrimental effect of lignin on enzymatic hydrolysis of carbohydrates has been attributed to inhibition of enzymes by lignin,² unproductive adsorption of cellulases on lignin,³ and steric hindrance caused by lignin or lignin-carbohydrate complexes to cellulases.⁴⁻⁶

Hydrothermal pretreatments that reduce recalcitrance of lignocellulosic biomass to enzymatic hydrolysis do not normally decrease lignin content in the solid fraction. Instead, enrichment of lignin occurs particularly as a result of hemicellulose dissolution in a process referred to as autohydrolysis (AH).⁶ AH cleaves lignin-carbohydrate linkages, and consequently improves enzymatic hydrolysis of cellulose. Lignin has been said to undergo both depolymerization and repolymerization,⁷ the first resulting from cleavage of aryl ether bonds under acidic conditions.⁸ Although degradation of cellulose does not usually occur in AH,⁷ hemicellulose degradation can be drastic. The degradation products take part in further reactions, which in acid-catalyzed conditions generate so-called pseudo-lignin⁹ that has been suggested to impede enzymatic hydrolysis of pretreated lignocellulosics.¹⁰ Previously, the amount of pseudo-lignin in the aqueous ammonia extracts from wheat straw (WS) AH solid residues increased linearly with increasing AH severity.¹¹ It might thus be difficult to avoid generation of pseudo-lignin completely if high enzymatic hydrolysis yield from the solid residues is desired. However, increased enzymatic carbohydrate conversion after AH at high severity has been reported in spite of droplets of altered lignin observed on WS.¹² The effect of lignin on enzymatic hydrolysis process after AH is not yet fully understood. Here, the key questions are whether formation of surface deposited pseudo-lignin is detrimental altogether, and could enzymatic hydrolysis of cellulose be improved by removal of pseudo-lignin from AH solid residues. In the current paper, WS solid residues from AH only, or with successive NH₃ (aq) extraction were characterized for composition and surface area (SA) of lignin. The determination of lignin SA was made according to the newly developed method that is based on specific adsorption of the cationic dye Azure B on lignin.¹³ Changes in lignin SA and specific surface area (SSA) revealed the effect of AH severity on lignin. For the first time, the lignin SA was correlated to enzymatic hydrolysis of cellulose. Discussion of the results opens up a new perspective for the investigation of existing and novel lignocellulose fractionation processes.

Experimental

Fractionation of wheat straw by autohydrolysis and aqueous ammonia treatments

Preparation of the solid residues from WS AH treatment and successive aqueous ammonia extraction has been described...
elsewhere\textsuperscript{11} and outlined in Fig. 1. Briefly, AH was conducted at 170–200 °C maximum temperature and a part of the solid residues was extracted with NH\textsubscript{3} (aq) either at moderate intensity (140 °C, 5% NH\textsubscript{3}) or high intensity (160 °C, 20% NH\textsubscript{3}) conditions. The solid residues were separated by filtration, washed until neutrality, and stored at 4 °C until enzymatic hydrolysis experiments.

**Analytical procedures**

Analytical results were calculated based on dry matter content of the sample, as determined gravimetrically by drying at 105 °C to constant weight. The analytical determinations were carried out in duplicate, and the mean values were calculated.

**Compositional analysis**

The solid lignocellulose fractions were milled using a type Pulverisette 14 mill (Fritsch, Germany) to pass through a 200 μm screen prior to the two-stage sulfuric acid hydrolysis.\textsuperscript{14} Gravimetric analysis of the acid-insoluble residue, isolated by filtration on a Whatman GF/F membrane and corrected for its ash content gave a fraction termed Klassen lignin. Mono-saccharides were analyzed from the sulfuric acid filtrate after neutralization with CaCO\textsubscript{3} by high-performance liquid chromatography (HPLC) using the system described in the literature.\textsuperscript{15}

**Enzymatic hydrolysis of solid residues from wheat straw**

Enzymatic hydrolysis of solid residues (0.5 g) was performed in 100 mL conical flasks at 2% solids concentration (w/v) in 50 mM sodium phosphate buffer (pH 4.8). Reactions catalyzed by an enzyme mixture containing 15 FPU g\textsuperscript{-1} Econase CE cellulase preparation (AB Enzymes, Finland) supplemented with cellobiase preparation Novozym 188 (Novozymes, Denmark) at 81 CBU g\textsuperscript{-1}, and GC 140 xylanase preparation (Genencor, Denmark) at 1020 IU g\textsuperscript{-1} were carried out in an oscillation (100 rpm) water bath (B.Braun, Germany) at 50 °C. Enzyme activities were determined according to literature.\textsuperscript{16,17} One activity unit (U) refers to the amount of the enzyme that releases 1 μmol of glucose or xylose under the assay conditions. Tetracycline (0.04 mg mL\textsuperscript{-1}) and cycloheximide (0.03 mg mL\textsuperscript{-1}) were used to inhibit bacterial growth. Hydrolysis was followed by sampling at 24 h and 72 h for sugar analysis. Hydrolysis yield and sugar yield were calculated using the following formulas:

\[
\text{Hydrolysis yield} \%(\%) = \left(\frac{\text{m}_{\text{sugar AH}} + \text{Y}_{\text{SR}}}{\text{m}_{\text{WS}}}ight)/0.741
\]

Here, \(\text{m}_{\text{sugar AH}}\) is the total amount of monosaccharides released in enzymatic hydrolysis of the solid residue sample (\(\text{m}_{\text{SR}}\)), \(\text{m}_{\text{sugar AH}}\) (g) is the total amount sugar (as monosaccharides) released from WS in AH, \(\text{Y}_{\text{SR}}\) (%) is the yield of solid residue from either AH only, or with successive extraction with NH\textsubscript{3} (aq) relative to the amount \(\text{m}_{\text{WS}}\) (g) of WS weighed to AH, and 0.741 is the proportion monosaccharides released in analytical acid hydrolysis of WS as described in 2.2.1.

**Characterization of lignin surface area by Azure B adsorption**

Surface area of lignin (SA) and specific surface area of lignin (SSA) were determined according to the literature.\textsuperscript{13} Briefly, solid residues (100 mg) were agitated in 0.1 g L\textsuperscript{-1} Azure B solution at pH 7 at 25 °C, and after 24 h the absorbance was measured from the liquid phase passed through 0.45 μm PTFE filters. Lignin surface area (SA) and specific surface area (SSA) were calculated based on the amount of surface-accessible acidic hydroxyls:

\[
\text{Lignin SA} = \left(\frac{\text{mmol of sugar}{g}}{g}\right) = 0.91F_{WS}\frac{1.64q_{e}}{305.83} \times 397
\]

\[
\text{Lignin SSA} = \left(\frac{\text{m}^2}{g \text{lignin}}\right) = \text{SA/proportion of lignin}
\]

Here, 0.91 is a correction factor for non-specific binding, \(F_{WS}\) is the correction factor based on the total lignin content of the solid fraction, 1.64 is the ratio of maximum equilibrium adsorption capacity to equilibrium adsorption capacity of WS in the assay conditions, \(q_{e}\) is the equilibrium adsorption capacity of solids residues in the assay conditions, 397 (m\textsuperscript{2} mmol\textsuperscript{-1}) is the area covered by Azure B, and proportion of lignin (%) is the
sum of Klassen lignin and acid-soluble lignin contents in the solid residues.

Results and discussion
Effect of autohydrolysis and aqueous ammonia treatments on solid residues

Composition and appearance of straw solid fractions from AH and NH₃ (aq) treatments was investigated in order to reveal changes in proportions of carbohydrate and lignin components. Both of the treatments removed hemicelluloses from the straw solids, and after the most severe AH only 2.9% xylan remained in the solid residues (Table 1). Due to partial dissolution of both lignin and hemicellulose, NH₃ (aq) extractions enriched cellulose content in the solid residues to 68% and 74% after moderate (M) and high (H) intensity extractions, respectively. The maximum reduction in Klassen lignin content (49%) was obtained from AH solid fractions produced at log R₀ = 3.81 compared to 23% reduction at log R₀ = 4.39. At higher severities (log R₀ ≥ 4.10) accumulation of pseudo-lignin and formation of condensed lignin could explain the increasing amount of lignin in the solid fraction and its resistance against NH₃ (aq) extraction. This biphasic behavior with respect to AH severity has previously been shown only for wood materials. Therefore, possible structural changes in WS lignin that may have occurred in AH and NH₃ (aq) extraction were studied more closely.

Visual observation revealed that AH had produced dark brown matter, which may have originated from relocalization of lignin from disrupted lignin–carbohydrate network onto particle surfaces and by formation of surface-deposited pseudo-lignin. SEM micrographs confirmed the presence of spheres on AH solid fractions (Fig. 2). Physical fluidization of lignin by melting at temperatures above its glass-transition temperature may have caused aggregation and formation of denser material that precipitated on solid surfaces. Only the high intensity NH₃ (aq) extraction removed the spheres from the solid residues (Fig. 2), consistent with their lower lignin content than after the

![Fig. 2](image-url) Scanning electron micrographs of untreated wheat straw (1-a to 1-c) and solid residue fractions of straw obtained from autohydrolysis at 190 °C before (2-a to 2-b) and after NH₃ (aq) extraction in moderate (3-a to 3-c) and high (4-a to 4-c) severity conditions.

### Table 1

<table>
<thead>
<tr>
<th>Solid fraction</th>
<th>Treatment severity</th>
<th>KL (%)</th>
<th>ASL (%)</th>
<th>Glucose (%)</th>
<th>Xylose (%)</th>
<th>Arabinose (%)</th>
<th>24 h Cellulose conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WS</td>
<td>—</td>
<td>21.8 ± 0.2</td>
<td>1.8 ± 0.0</td>
<td>39.8 ± 0.2</td>
<td>23.5 ± 0.4</td>
<td>2.7 ± 0.0</td>
<td>14.7 ± 0.8</td>
</tr>
<tr>
<td>170°C</td>
<td>3.10</td>
<td>22.1 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>41.2 ± 0.6</td>
<td>21.9 ± 1.1</td>
<td>2.1 ± 1.0</td>
<td>31.8 ± 0.2</td>
</tr>
<tr>
<td>170°C</td>
<td>3.15</td>
<td>21.5 ± 0.1</td>
<td>1.3 ± 0.0</td>
<td>41.5 ± 0.8</td>
<td>23.7 ± 0.1</td>
<td>1.8 ± 0.3</td>
<td>31.2 ± 0.1</td>
</tr>
<tr>
<td>180°C</td>
<td>3.47</td>
<td>23.1 ± 0.0</td>
<td>1.2 ± 0.1</td>
<td>47.2 ± 2.1</td>
<td>19.3 ± 1.5</td>
<td>1.5 ± 0.8</td>
<td>40.9 ± 0.4</td>
</tr>
<tr>
<td>180°C</td>
<td>3.52</td>
<td>23.2 ± 0.1</td>
<td>1.1 ± 0.0</td>
<td>47.2 ± 1.5</td>
<td>18.5 ± 0.1</td>
<td>0.8 ± 0.4</td>
<td>45.4 ± 0.4</td>
</tr>
<tr>
<td>190°C</td>
<td>3.81</td>
<td>24.8 ± 0.1</td>
<td>0.9 ± 0.0</td>
<td>52.1 ± 1.8</td>
<td>11.1 ± 0.7</td>
<td>1.3 ± 0.7</td>
<td>70.4 ± 2.4</td>
</tr>
<tr>
<td>190°C</td>
<td>4.06</td>
<td>28.2 ± 0.1</td>
<td>0.9 ± 0.0</td>
<td>56.3 ± 2.1</td>
<td>7.7 ± 0.6</td>
<td>0.6 ± 0.6</td>
<td>76.9 ± 0.6</td>
</tr>
<tr>
<td>195C</td>
<td>4.10</td>
<td>24.6 ± 0.0</td>
<td>0.9 ± 0.1</td>
<td>53.6 ± 1.8</td>
<td>4.2 ± 0.4</td>
<td>ndf</td>
<td>89.3 ± 0.6</td>
</tr>
<tr>
<td>200C</td>
<td>4.39</td>
<td>26.3f</td>
<td>1.0f</td>
<td>55.3f</td>
<td>2.9f</td>
<td>ndf</td>
<td>91.5 ± 1.5</td>
</tr>
<tr>
<td>M170°C</td>
<td>M</td>
<td>18.2 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>38.4 ± 1.3</td>
<td>19.9 ± 0.8</td>
<td>1.5 ± 0.1</td>
<td>66.5 ± 1.0</td>
</tr>
<tr>
<td>M180°C</td>
<td>M</td>
<td>19.3 ± 0.2</td>
<td>1.1 ± 0.0</td>
<td>55.6 ± 0.9</td>
<td>15.0 ± 0.5</td>
<td>0.5 ± 0.1</td>
<td>63.3 ± 0.0</td>
</tr>
<tr>
<td>M190°C</td>
<td>M</td>
<td>20.4 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>65.2 ± 0.4</td>
<td>9.3 ± 1.0</td>
<td>1.6 ± 0.2</td>
<td>64.3 ± 1.7</td>
</tr>
<tr>
<td>M195C</td>
<td>M</td>
<td>22.1 ± 0.1</td>
<td>0.6 ± 0.0</td>
<td>67.6 ± 2.0</td>
<td>3.9 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>72.3 ± 1.2</td>
</tr>
<tr>
<td>M200C</td>
<td>M</td>
<td>24.0 ± 0.0</td>
<td>0.5 ± 0.0</td>
<td>67.0 ± 1.6</td>
<td>3.2 ± 0.8</td>
<td>0.9 ± 0.3</td>
<td>66.4 ± 0.3</td>
</tr>
<tr>
<td>H170°C</td>
<td>H</td>
<td>14.6 ± 0.2</td>
<td>1.0 ± 0.0</td>
<td>61.0 ± 1.1</td>
<td>17.9 ± 0.5</td>
<td>0.8 ± 0.0</td>
<td>69.3 ± 1.6</td>
</tr>
<tr>
<td>H180°C</td>
<td>H</td>
<td>12.1 ± 0.2</td>
<td>0.9 ± 0.0</td>
<td>66.8 ± 2.2</td>
<td>15.0 ± 0.4</td>
<td>0.8 ± 0.5</td>
<td>78.7 ± 1.4</td>
</tr>
<tr>
<td>H190°C</td>
<td>H</td>
<td>12.7 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>72.5 ± 4.0</td>
<td>9.0 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>84.7 ± 1.1</td>
</tr>
<tr>
<td>H195C</td>
<td>H</td>
<td>15.2 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>74.6 ± 3.3</td>
<td>4.4 ± 0.2</td>
<td>0.3 ± 0.3</td>
<td>79.4 ± 1.7</td>
</tr>
<tr>
<td>H200C</td>
<td>H</td>
<td>20.3 ± 0.2</td>
<td>0.6 ± 0.0</td>
<td>70.7 ± 1.2</td>
<td>2.1 ± 0.1</td>
<td>0.9 ± 0.5</td>
<td>60.1 ± 0.2</td>
</tr>
</tbody>
</table>

a KL, Klassen lignin; ASL, acid-soluble lignin. The content of glucose, xylose and arabinose are expressed as anhydrous sugars. The amount of galactose in the solid residues (not shown) was less than one percent. b Autohydrolysis severity was calculated from log R₀ = log(t × exp([T – Tm]/(0.607 (140°C))) (Overend and Chornet, 1987). c Analyzed as sum of arabinose plus mannose. d nd, not detected. f Values from single determination.
moderate intensity extraction (Table 1). Previously, presence of lignin-containing droplets was shown in micrographs of solid residues from dilute acid treatment of maize stalk.\textsuperscript{16,18} Ahead of the current study the proportion of pseudo-lignin in the NH\textsubscript{3} (aq) extracts from the AH solid residues was estimated to be as high as 55%.\textsuperscript{11} However, altered lignin has not been quantified in the AH solid residues, and its effect on enzymatic hydrolysis or surface area of lignin has not been elucidated before.

**Effect of autohydrolysis and NH\textsubscript{3} (aq) extraction on specific surface area of lignin**

Native WS lignin is expected to undergo structural changes in the pretreatment conditions. Effect of AH and NH\textsubscript{3} (aq) extractions on lignin structure was studied by cationic dye adsorption in order to estimate possible changes in surface area (SA) of lignin. Azure B is a basic violet-blue dye that leaves lignin-free tissues unstained,\textsuperscript{12} but instead binds to acidic hydroxyl groups of lignin.\textsuperscript{13} Increasing severity (log \textit{R}0) decreased lignin SA of the AH solid residues from 84 m\textsuperscript{2} of the untreated straw to 47 m\textsuperscript{2} after the highest AH severity (Fig. 3a). In contrast, moderate intensity NH\textsubscript{3} (aq) extraction (5% NH\textsubscript{3}, 140 °C) increased lignin SA compared to the corresponding AH solid residues. However, the high intensity NH\textsubscript{3} (aq) extraction (20% NH\textsubscript{3}, 160 °C) decreased lignin SA at severities up to log \textit{R}0 = 3.81. These differences in lignin SA are in accordance with the earlier results that showed higher relative proportion of pseudo-lignin in the aqueous ammonia extracts from the moderate intensity extraction than from the high intensity extraction.\textsuperscript{11} As a result of the moderate intensity NH\textsubscript{3} (aq) extractions that dissolved higher proportions of pseudo-lignin, the resulting solid residues were enriched in lignin containing acidic hydroxyl groups. This is also indicated by only slight decreases in the lignin content of the solid residues after moderate intensity extraction (Table 1).

When surface area of lignin was calculated relative to the proportion of lignin in the solid residues, lignin specific surface area (SSA) decreased linearly as a function of AH severity (Fig. 3b). Compared to the lignin SSA of AH solid residues, NH\textsubscript{3} (aq) extraction increased the lignin SSA of the corresponding solid residues, high intensity extraction more than moderate intensity extraction. These results indicate that AH had drastic overall effect on surface properties of lignin in solid residues obtained at log \textit{R}0 ≥ 4.1, showing lignin SSA that were less than half than in untreated WS (354 m\textsuperscript{2} g\textsuperscript{-1}). Although these changes could be attributed to lignin repolymerization reactions that form non-labile biphenyl ether (S-O-4) structures, SEM investigation of the straw solid residues and compositional analysis of the aqueous ammonia extracts suggested that phase transition of lignin (melting) and accumulation of pseudo-lignin were the main changes causing lower lignin SSA. Removal of pseudo-lignin could be conducted using NH\textsubscript{3} (aq) that dissolves it more selectively than native lignin from the AH solid residues,\textsuperscript{11} thus exposing acidic OH groups of lignin on the surfaces. Previously, supplementation of cellulose with artificially generated pseudo-lignin was detrimental to enzymatic hydrolysis of cellulose.\textsuperscript{10,22} However, the direct effect of lignin surface structure after hydrothermal treatment on enzymatic hydrolysis has not been elucidated. It was thus studied if the presence of altered lignin formed during AH affected enzymatic cellulose-to-glucose conversion from the solid residue samples.

**The effect of lignin surface area on enzymatic hydrolysis of cellulose**

AH and NH\textsubscript{3} (aq) extractions of straw produced solid residues that contained altered levels of lignin surface area (SA). In order to elucidate the effect of lignin on enzymatic hydrolysis of cellulose, Klason lignin (KL) content or lignin SA of the solid residues was correlated with the degree of cellulose conversion. Increasing KL content showed a positive linear correlation after AH ($R^2 = 0.68$) and, in contrast, a weak negative correlation after successive NH\textsubscript{3} (aq) extraction (Fig. 4). The highest 24 h cellulose conversions (89% and 91%) resulted from AH at high severity (log \textit{R}0 = 4.1 and log \textit{R}0 = 4.4), despite the 13% and 30% higher lignin content in the solid residues relative to untreated WS (Table 1). This apparent contradiction of improved hydrolysis of cellulose in presence of higher amounts of lignin has not been completely understood before. However, degree of cellulose conversion from 24 h or 72 h hydrolysis of

![Figure 3](image-url)
AH solid residues showed negative linear correlations ($R^2 = 0.92$, $R^2 = 0.97$) with lignin SA (Fig. 4). Similarly, NH$_3$ (aq) extractions also led to a negative correlation with SA (Fig. 4). In contrast to prior indication that enzymatic digestibility is controlled by lignin content, these results showed that lignin SA, rather than the lignin content, governs degree of cellulose conversion from AH solid residues. Prior to the current study, Kristensen and co-workers concluded that improved enzymatic digestibility was a result of re-localization of lignin and removal of hemicellulose rather than physical disruption of cell walls in hydrothermal treatment of WS. More specifically, lignin probably undergoes complex changes involving phase-transition, reaction, and dissolution during hydrothermal pretreatment. However, the nature of lignin surface properties after AH has not been elucidated with respect to cellulose hydrolysis before the current study. Extraction of AH solid residues with aqueous ammonia led mainly to lower enzymatic cellulose conversions than obtained without extraction (Table 1). This was despite delignification was the apparent result of the NH$_3$ (aq) extractions, showing enrichment of up to 74% cellulose in the solids residues (Table 1). Moreover, based on the SEM micrographs, solid residues after the NH$_3$ (aq) extractions contained defibrillated material that was absent in the AH solid residues (Fig. 2). NH$_3$ (aq) extraction improved cellulose conversion only after AH at low severity (Table 1). Thus, while the effect of lignin removal might be ascribed as formation of additional cellulose surface area, it appears in contrast that the most important factor of AH on cellulose hydrolysis is the reduction of lignin SA.

### The effect of autohydrolysis and aqueous ammonia treatments on sugar yield

Sugar yield from lignocellulosic feedstock deserves a closer look with respect to the studied fractionation processes. Sugar yields were calculated from the two processes where prior to enzymatic hydrolysis, straw was subjected to AH only or AH with successive NH$_3$ (aq) extractions. If only the sugars released by enzymatic hydrolysis are considered, the maximum sugar yield (59%) was obtained from the single-stage AH at log $R_0 = 3.81$ severity (Fig. 5a). When sugars in AH liquors were additionally taken into account, up to 85% sugar yield was obtained from the process comprising AH only (Fig. 5b). The successive NH$_3$ (aq) extractions did not improve the sugar yield compared to the single-stage AH process, except after AH at the lowest severity.

### Conclusions

High severity AH decreased the surface area of lignin in the solid residues almost by half compared to untreated straw, leading to nearly complete hydrolysis of cellulose. Extraction of AH solid residues with NH$_3$ (aq) increased the surface area of
autohydrolysis the sum of sugar from enzymatic hydrolysis and sugar released in on sugar released only by enzymatic hydrolysis of solid residues (a) or (white rectangles) or high intensity (black triangles). Sugar yield based

Sugar yield from wheat straw carbohydrates as a function of autohydrolysis severity with different process combinations prior to enzymatic hydrolysis process. Funding from Neste Oil Corporation and the Finnish Funding Agency for Technology and Innovation (Tekes) within the Microbial Oil project is gratefully acknowledged.

Fig. 5 Sugar yield from wheat straw carbohydrates as a function of autohydrolysis severity with different process combinations prior to enzymatic hydrolysis of the solid fraction: autohydrolysis (gray circles), autohydrolysis followed by NH₃ (aq) extraction at moderate intensity (white rectangles) or high intensity (black triangles). Sugar yield based on sugar released only by enzymatic hydrolysis of solid residues (a) or the sum of sugar from enzymatic hydrolysis and sugar released in autohydrolysis(b).

Notes and references


Acknowledgements

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