Surface Accessibility of Hydrothermal Pretreated Biomass Using Simons’ Staining Technique

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Introduction

Simons’ Stain (SS) technique was a potentially useful semiquantitative methodology to estimate the surface accessibility of lignocellulosic substrates to the cellulase hydrolysis. This methodology showed the potential to assess the effectiveness of pretreatments. Studies have employed a mixture of Direct Blue-1 (DB-1) and Direct Orange-15 (DO-15) to measure the behavior of adsorption on the pretreated biomass. Direct Blue-1 has a smaller molecular size and a weaker affinity for cellulose than Direct Orange-15. When their mixture was applied to the cellulose sample, Direct Orange-15 molecules will preferentially be adsorbed on the cellulose surface than Direct Blue-1. While, the Direct Blue-1 molecules tend to be adsorbed on the surface of small pores. This different behavior suggests the pore structure and the pore size population distribution of the cellulose samples. Previous study showed the degree of polymerization of cellulose has been suggested to be one of major factors to influence the different enzymatic hydrolysis yield between leaf and internode after hydrothermal pretreatment. While, several argument has also been raised to provide conclusion that this is mainly due to the differences in degree of polymerization. One may argue that surface area of these fraction of plan could be the major factor to influence the different enzymatic hydrolysis yield. In this study Simons’ Staining technique has been employed to estimate the surface adsorption of two dye, Direct Blue-1 and Direct Orange-15. Hydrothermal pretreated leaf and internodes in switchgrass biomass have been evaluated for the surface accessibility for enzymatic hydrolysis.

Keywords: Leaf and internode, Simons’ Staining, Surface accessibility

Analytical procedure:

Results and Discussion

Chemical profiles of leaves and internode in biomass

Table 1. Chemical profiles of leaf and internode in biomass.

<table>
<thead>
<tr>
<th>Populations/Morphology</th>
<th>Mass yield %</th>
<th>Araban %</th>
<th>Galactan %</th>
<th>Glucan %</th>
<th>Xylan %</th>
<th>Lignin %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-SW2 leaves</td>
<td>27</td>
<td>1.8</td>
<td>1.7</td>
<td>80</td>
<td>18.5</td>
<td></td>
</tr>
<tr>
<td>Pre-SW2 internodes</td>
<td>70</td>
<td>4.0</td>
<td>1.4</td>
<td>33.5</td>
<td>20.4</td>
<td>22.3</td>
</tr>
<tr>
<td>Pre-SW2 leaves</td>
<td>48</td>
<td>0.0</td>
<td>1.1</td>
<td>25.7</td>
<td>2.6</td>
<td>38.8</td>
</tr>
<tr>
<td>Pre-SW2 internodes</td>
<td>51</td>
<td>0.2</td>
<td>1.2</td>
<td>64.1</td>
<td>4.0</td>
<td>31.8</td>
</tr>
</tbody>
</table>

Note: a. Mass percentages of morphological fraction
b. Yield percentages after hydrothermal pretreatment

Enzymatic hydrolysis of leaves and internode in biomass

The enzymatic hydrolysis conditions were as follows: 2 g of pretreated switchgrass (OD) was treated with cellulase (at a loading of 49 FPU/g cellulose) and Novozyme 188 (at a loading of 40 IU/g cellulose) in a 100 mL acetate buffer solution (0.1 M, pH 4.8) at 50°C with various time, 0.5, 1, 2, 4, 8, 16, 32, 48, and 63 h.

The glucose content of the filtrate of the enzymatic hydrolysis solution was measured using High-Performance Liquid Chromatography (HPLC) using an Agilent 1200 HPLC series system, equipped with an Aminex® HPX-42C column (300 mm x 7.8 mm) and a refractive index detector (RID). The gravimetric yield was based on the glucan content of the pretreated leaf and internode portions of SW2

Simons’ Staining Technique

DB-1 and DO-15 were prepared for Simons’ Staining measurement

Figure 3: Chemical Structures of Direct Blue-1 (a) and Direct Orange-15 (b)

The extinction coefficients for DB-1 and fractionated DO-15: 
$e_{DB1}=36.2 \text{ g}^{-1} \text{ cm}^{-1}$, $e_{DB15}=2.72 \text{ g}^{-1} \text{ cm}^{-1}$, $e_{DO15}=0.186 \text{ g}^{-1} \text{ cm}^{-1}$.

Simons’ Staining solution: A phosphate-buffered saline solution (PBS, 0.1 M) mixed with DO-15 (10 mg/mL) and DB-1 (10 mg/mL) in a series of increasing volumes (0.25, 0.50, 0.75, 1.0, 1.5, 2.0 mL) to 10 mL volumetric flask. Six samples (100 mg) were weighed into 50 mL polypropylene tube and filled with each Simons’ Staining solutions (10 mL). These mixtures were incubated at 70°C for 6 h with 200 rpm.

Equation 1.1 and 1.2 were used to determine the concentrations of DB-1 and DO-15 after the reaction for each sample. The amount of DB-1 and DO-15 adsorbed was determined by the difference between the adjusted initial concentration and the reacted concentration.

$A_{DO15} = A_{DO15} - A_{DO15+DB}$ Equation 1.1
$A_{DB1} = A_{DB1} - A_{DB1+DO}$ Equation 1.2

Equation 1.3 was used to determine the maximum absorbance

$C=\frac{[A]}{K_{ads} + [A]_{max}}$ Equation 1.3

$[C]$ (mg/mL) is free DB-1 or DO-15 concentration at equilibrium; $[A]$ (mg DB-1 or DO-15/g substrate) is the amount of DB-1 or DO-15 adsorbed; $[A]_{max}$ is the maximum amount of DB-1 or DO-15 adsorbed onto the sample (mg/g). $K_{ads}$ is the adsorption equilibrium constant.

Table 2. The adsorbed Direct Blue-1 and Direct Orange-15 in SW2

<table>
<thead>
<tr>
<th>Sample</th>
<th>$A_{DO15}$ mg/g</th>
<th>$A_{DB1}$ mg/g</th>
<th>Total mg/g</th>
<th>DO/DB</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaves SW2</td>
<td>34</td>
<td>23</td>
<td>57</td>
<td>1.5</td>
</tr>
<tr>
<td>internodes SW2</td>
<td>27</td>
<td>14</td>
<td>41</td>
<td>1.9</td>
</tr>
<tr>
<td>pre-leaves SW2</td>
<td>77</td>
<td>26</td>
<td>10 x 10^3</td>
<td>3.0</td>
</tr>
<tr>
<td>pre-internodes SW2</td>
<td>63</td>
<td>26</td>
<td>89</td>
<td>2.4</td>
</tr>
</tbody>
</table>

The $R^2$ values for the estimation of adsorbed DO-15 were 0.91, 0.82, 0.98, and 0.95 for leaves, internodes, pretreated leaves and pretreated internodes. The $R^2$ values for the estimation of adsorbed DB-1 were 0.88, 0.75, 0.89, and 0.60 for leaves, internodes, pretreated leaves, and pretreated internodes.

Conclusions

- Hydrothermal pretreatment improve the cellulose-to-glucose yield for both leaves and internodes. Pretreated leaves have greater cellulose-to-glucose yield after enzymatic deconstruction
- The Simons’ Staining measurement demonstrated that hydrothermal pretreatment improves the surface accessibility for both leaf and internode fractions
- The OIB value of pretreated switchgrass suggests that hydrothermal pretreatment increase the porosity of switchgrass. This result also suggests the pretreated leaf has slightly greater porosity than pretreated internode fractions of switchgrass.

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