

# Hydration Properties of Briquetted Wheat Straw Biomass Feedstock

Heng Zhang,<sup>a,\*</sup> Maria Fredriksson,<sup>a,b</sup> Jozef Mravec,<sup>c</sup> and Claus Felby<sup>a</sup>

Biomass densification elevates the bulk density of the biomass, providing assistance in biomass handling, transportation, and storage. However, the density and the chemical/physical properties of the lignocellulosic biomass are affected. This study examined the changes introduced by a briquetting process with the aim of subsequent processing for 2<sup>nd</sup> generation bioethanol production. The hydration properties of the unprocessed and briquetted wheat straw were characterized for water absorption *via* low field nuclear magnetic resonance and sorption balance measurements. The water was absorbed more rapidly and was more constrained in the briquetted straw compared to the unprocessed straw, potentially due to the smaller fiber size and less intracellular air of the briquetted straw. However, for the unprocessed and briquetted wheat straw there was no difference between the hygroscopic sorption isotherms, which showed that the amount of cell wall water was not affected by the briquetting process and that the sugar yield was similar after a combined hydrothermal pretreatment and enzymatic hydrolysis. The factors which offset the benefits introduced by the briquetting process need to be further examined to optimize the processing parameters and enzyme recipe for better use of the wheat straw biomass feedstock.

*Keywords:* Briquetting; Water absorption; Wheat straw; Pretreatment; Enzymatic hydrolysis; Sorption isotherm

*Contact information:* a: Department of Geosciences and Natural Resource Management, University of Copenhagen, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark; b: Division of Building Materials, Lund University, P.O. Box 118, SE-221 00 Lund, Sweden; c: Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark; \* Corresponding author: heng@ign.ku.dk

## INTRODUCTION

Lignocellulosic biomass is the most available renewable carbon source that has the potential to replace fossil fuels. The major obstacle of converting lignocellulosic biomass into biofuels is the recalcitrant nature of the plant cell walls, which requires physical or chemical treatment prior to enzymatic degradation and fermentation.

Wheat (*Triticum* spp.) has a large potential for 2<sup>nd</sup> generation biofuel production due to its wide distribution (Talebnia *et al.* 2010). However, the low bulk density of wheat straw (80 kg m<sup>-3</sup> to 100 kg m<sup>-3</sup>) causes difficulties in handling, transportation, storage, and feeding into a conversion process (Tumuluru *et al.* 2011). A number of densification technologies have been developed for more efficient biomass handling and improved biomass uniformity, including the pellet mill, briquette press, screw extruder, tabletizer, and agglomerator (Smith *et al.* 1977; Tumuluru *et al.* 2011), which can raise the bulk density to 450 kg m<sup>-3</sup> to 700 kg m<sup>-3</sup> (Kaliyan *et al.* 2009). Densification methods apply mechanical force to create inter-particle bonding through short-range forces or the

formation of solid bridges, *i.e.* mechanical entanglement (Kaliyan and Morey 2010). Short-range forces are typically valence forces, electrostatic forces, and magnetic forces that adhere solid particles to each other when the solid particles are close enough, but the increase of the particle size and inter-particle distance can weaken the strength of particle bonding (Kaliyan and Morey 2010). Solid bridges are formed either by diffusion of the molecules between the contact areas, crystallization, chemical reaction, and hardening of binders or by solidification of melted components mainly during the cooling of the densification process (Kaliyan and Morey 2010). Natural binders in lignocellulosic biomass, *e.g.* lignin, protein, fat, and water-soluble carbohydrates show auto-adhesive properties under densification processes at elevated temperature and pressure (Kaliyan and Morey 2010). The strength and durability of densified biomass are influenced by the densification system variables, namely, temperature, pressure, die geometry, and speed, as well as feedstock moisture content, particle size, and chemical composition (Smith *et al.* 1977; Kaliyan and Morey 2010). Due to the physical and chemical changes, densified biomass responds differently to alkaline or hydrothermal pretreatments and enzymatic hydrolysis compared to undensified biomass (Li *et al.* 2014).

Various thermal-chemical pretreatment approaches have been reported in the past decades (Mosier *et al.* 2005b; Jørgensen *et al.* 2007), of which hydrothermal pretreatment is highlighted for less degradation of hemicelluloses, non-addition of chemicals, and economical use of water (Mosier *et al.* 2005a; Kabel *et al.* 2007). Optimization of the sugar recovery and thus enzymatic hydrolysis require sufficient amounts of water to solubilize the hemicelluloses (Petersen *et al.* 2009). It has been demonstrated that water plays a critical role during the pretreatment and enzymatic hydrolysis of lignocellulosic biomass (Felby *et al.* 2008; Selig *et al.* 2012, 2014; Zhang *et al.* 2014; Weiss *et al.* 2016), which can affect the diffusion of enzymes and products to or from the reaction sites, either as a medium, or as a reactant, during the enzymatic hydrolysis reaction (Roberts *et al.* 2011).

Sorption isotherms, *i.e.* the relationship between the moisture content of a sample and the ambient relative humidity (RH), can be measured by a sorption balance. This method has been widely applied in food science, material science, and pharmaceutical applications, and has also been used for natural fibers (Teoh *et al.* 2001; Hill *et al.* 2009; Dubina *et al.* 2011; Arya *et al.* 2013). In a sorption balance, the RH is changed stepwise in pre-programmed steps and the mass of the sample is monitored with a high resolution. Sorption isotherms have previously been determined for wheat straw (Duggal and Muir 1981) and wheat straw pellets (Theerarattananoon *et al.* 2011) but *via* other methods.

Water exists in distinct locations in the cell wall matrix, as has been reported for various biomass types by the use of low field nuclear magnetic resonance (LF-NMR) (Menon *et al.* 1987; Araujo *et al.* 1993; Capitani *et al.* 1998; Elder *et al.* 2006). The spin-spin ( $T_2$ ) relaxation time reflects how tightly the water hydrogen nuclei associate to the cell wall matrix and the different locations of the water, and the distribution of the states of water changes with hydrothermal pretreatment or enzymatic hydrolysis (Capitani *et al.* 1998; Felby *et al.* 2008; Zhang *et al.* 2014; Weiss *et al.* 2016).

In this study, the hydration properties of the briquetted straw were explored, and the effects of the briquetting process on its performance during pretreatment and enzymatic hydrolysis were also investigated. The biomass-water interaction was studied using a sorption balance and LF-NMR, and high performance liquid chromatography (HPLC) was used for the detection of released carbohydrates after enzymatic hydrolysis. In addition, the material was chemically characterized using Fourier transformation

infrared (FT-IR) spectroscopy, and microscopic analyses were performed to visualize cell wall changes and to determine the particle sizes after briquetting.

## EXPERIMENTAL

### Materials

#### *Unprocessed and briquetted wheat straw preparation*

The wheat straw biomass was harvested in Denmark in 2013 at a location between Randers and Hadsund, and then was stored under roof in bales for 3 months to 6 months. The density of the unprocessed straw was less than  $100 \text{ kg m}^{-3}$ , and the moisture content, defined as mass of water divided by total mass of biomass, ranged from 10% to 18%. The straw might contain sands or stones in a size range from 5 mm to 200 mm, which were removed by a stone trap equipped on a shredder and a hammer mill. The straw was air-dried to a maximum 15% moisture content prior to further processing. The fiber length of the straw was then reduced to typically 15 mm to 40 mm after homogenization on the shredder. A mechanical briquetting press BP6510HD (C.F. Nielsen A/S, Baelum, Denmark), was used to compress the straw feedstock into briquettes. The briquetting press includes a 75 kW main motor, which provides a processing capacity at  $1200 \text{ kg h}^{-1}$  to  $1800 \text{ kg h}^{-1}$ . The final briquetted product has a diameter of approximately 90 mm, with a density of approximately  $1000 \text{ kg m}^{-3}$  to  $1200 \text{ kg m}^{-3}$ . The briquettes were stored at room temperature prior to further analyses. The unprocessed wheat straw in this article refers to the wheat straw obtained directly after the homogenization from the shredder.

### Methods

#### *Hydrothermal pretreatment*

The briquettes (7 kg each) were soaked in water and pressed with a piston to obtain 40% dry matter (DM), which is defined as the dry mass divided by the total mass. The samples were transferred into a customized reactor connected to a steam generator (BioFuel Technology A/S, Hobro, Denmark). The reactor was heated to  $190 \text{ }^\circ\text{C}$  in 30 s and kept at this temperature for 13 min. The liquid fraction was collected by pressing, and the solid fraction was sealed in plastic bags and stored at  $-20 \text{ }^\circ\text{C}$  for further experiments and analyses. The unprocessed wheat straw was pretreated likewise.

#### *Water absorption test*

Two grams of unprocessed or briquetted straw were mixed with  $500 \text{ cm}^{-3}$  distilled water in a  $600 \text{ cm}^{-3}$  beaker. Pictures were taken at time points 0 min, 1 min, 3 min, 5 min, 7 min, 10 min, 15 min, and 30 min after the straw was placed in water (S1). Note that both the unprocessed and briquette samples were collected directly from the production site without further fiber size reduction.

#### *Fourier transform infrared (FT-IR) spectroscopy*

The FT-IR measurements were performed on ball-milled samples of unprocessed and briquetted straw using a Nicolet 6700 spectrometer from Thermo Fisher (Hvidovre, Denmark) equipped with a PIKE Diamond attenuated total reflectance (ATR) unit (Thermo Fisher, Hvidovre, Denmark). Spectra were obtained from  $600 \text{ cm}^{-1}$  to  $4000 \text{ cm}^{-1}$  using 64 scans, Happ-Genzel apodization, and at a resolution of  $4 \text{ cm}^{-1}$ . Measurements were performed on oven-dried samples with a DM content of 90%. Each sample was measured in five replicates.

### *Particle size measurement and microscopic analysis*

The macroscopic particle size was determined by scanning the hydrated material in a square petri dish using CanoScan 8800F flatbed scanner (Canon, Tokyo, Japan). Length measurement was performed using ImageJ program (<https://imagej.nih.gov/ij/index.html>). For light microscope (LM) analysis, the hydrated unprocessed and briquetted straw samples were cut using a razor blade to approximately 200- $\mu\text{m}$ -thick sections. The sections were dipped to Wiesner stain, prepared as a saturated solution of phloroglucinol (Sigma-Aldrich, Broendby, Denmark) in 20% HCl or directly in water. The sections were observed using a LM Olympus BX41 (Olympus, Ballerup, Denmark) equipped with ColorViewI camera (Ballerup, Denmark). Laser scanning confocal microscopy (LSCM) was performed on a Leica SP5 (Leica Microsystems A/S, Broenshoej, Denmark) using ultraviolet (UV) (405 nm) and an Argon laser (488 nm). The sections were briefly stained for 5 min in Calcofluor White M2R (Fluorescent Brightener 28, Sigma-Aldrich, Broendby, Denmark) solution in water (1 g L<sup>-1</sup>), washed, and then mounted in water on a glass slide. The Calcofluor signal was observed using a 405 nm laser, recording the emission within 430 nm to 470 nm. The lignin fluorescence was observed using a 488 nm laser, recording the emission within 520 nm to 560 nm.

### *Enzymatic hydrolysis*

Enzymatic hydrolysis was performed on the samples of hydrothermally pretreated unprocessed and briquetted straw with Cellic Ctec2 (Novozymes A/S, Bagsværd, Denmark), at a solid loading of 5% DM and an enzyme loading of 5 FPU (filter paper units)  $\cdot\text{g}^{-1}$  DM. Then, 1 g dry mass of straw samples was mixed with water, pH 5.0 sodium citrate buffer, and enzyme preparation to obtain 5% DM in 50 cm<sup>3</sup> plastic bottles. The sealed bottles were incubated in a drum tumbler at 50 °C, 2.72 rad s<sup>-1</sup>, for 96 h. Hydrolysis was terminated by boiling the bottles for 10 min. Each pretreated unprocessed or briquetted straw sample was tested in triplicates.

The monosaccharides (D-glucose, D-xylose, and L-arabinose) released from enzymatic hydrolysis were measured on a Dionex Ultimate HPLC system (Dionex, Germering, Germany). The separation was performed on a column at 80 °C with 5 mM H<sub>2</sub>SO<sub>4</sub> as eluent and a flow rate of 0.6 cm<sup>3</sup> min<sup>-1</sup>, coupled to a refractive index (RI)-detector. The samples were diluted in eluent and filtered through a 0.45  $\mu\text{m}$  nylon filter before injection.

### *Sorption isotherms*

Sorption isotherms were measured at 20 °C using a sorption balance (DVS Advantage, Surface Measurement Systems Ltd., London, UK) where the mass of the specimen was monitored with a resolution of 0.1  $\mu\text{g}$ . The method is further described by Williams (1995). One sample of unprocessed straw and one sample of briquetted straw were prepared and cut to include all parts of the straw (*e.g.* leaf blade, sheath, node, and internode). Before the measurements, the samples were placed in deionized water for at least one hour. This gave an initial moisture content that was substantially higher than the equilibrium moisture content at the first relative humidity level (95%) and therefore ensured pure desorption. The samples were equilibrated to the following RH-levels: 95%, 85%, 75%, 65%, 50%, 40%, 25%, 10%, and 0%. The RH was then increased using the same steps. An equilibrium criterion (dm/dt) of 0.0005% over a 10 min period was used

for all steps except at 0% RH, where a time criterion of 48 h was used. The moisture content ( $u$ ) at each RH level was then determined as,

$$u = \frac{m_{eq} - m_{dry}}{m_{dry}} \quad (1)$$

where  $m_{eq}$  is the equilibrium mass (kg) at each relative humidity level and  $m_{dry}$  is the dry mass (kg).

#### *Low field nuclear magnetic resonance (LF-NMR)*

Five samples of the unprocessed and briquetted straw were dispensed respectively in Milli-Q water at 10% DM, and incubated at 40 °C for 1 h prior to measurement. This short incubation time was applied because it provided more relevant data for industrial processing, as the biomass feedstock pre-soaking step in a 2<sup>nd</sup> generation bioethanol process will be equally short.

The LF-NMR analyses were conducted using a Bruker mq 20-Minispec (Bruker Daltonik GmbH Life Sciences, Bremen, Germany), with a 0.47 Tesla permanent magnet (20 MHz proton resonance frequency), operating at 40 °C. The spin-spin ( $T_2$ ) relaxation times were determined using the Carr-Purcell-Meiboom-Gill (CPMG) sequence. Then, 8000 echoes were collected with a pulse separation of 0.8 ms, the acquisition of 32 scans, and a 5 s recycle delay. The CPMG relaxation curves were then evaluated using two methods: the discrete multi-exponential fitting (Pedersen *et al.* 2002) and the inverse Laplace transformation method, CONTIN (Provencher 1982). For the discrete multi-exponential fitting, the CPMG-data was fitted to Eq. 2 using up to seven components,

$$y = C_1 e^{-\frac{t}{T_{2,1}}} + \dots + C_n e^{-\frac{t}{T_{2,n}}} + C_{n+1} \quad (2)$$

where  $y$  is the vector with measured signal intensity,  $n$  is the number of exponential components,  $C_n$  is the amplitude of the  $n^{\text{th}}$  exponential,  $t$  is the acquisition time axis, and  $T_{2,n}$  is the  $T_2$  relaxation time of the  $n^{\text{th}}$  exponential.

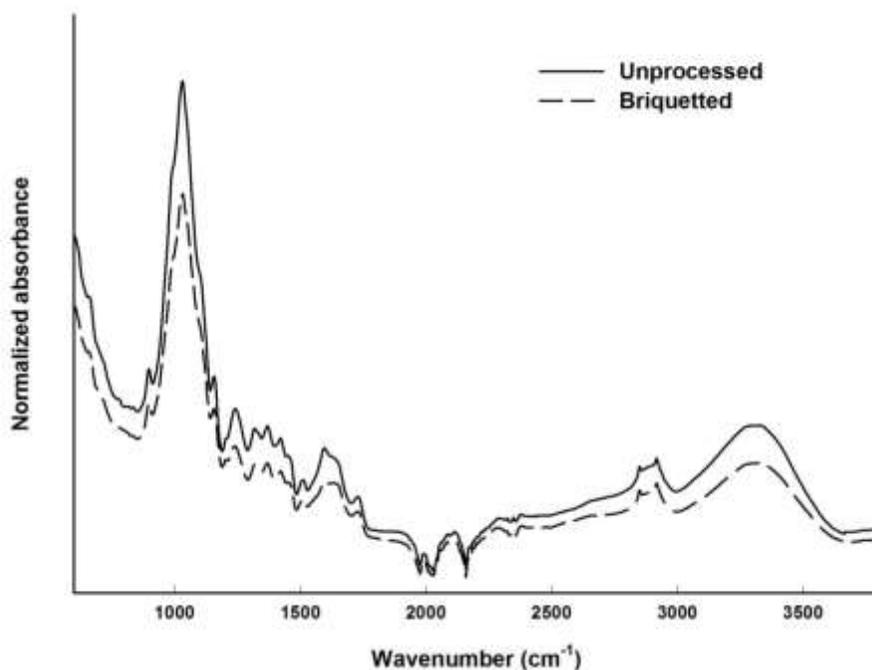
Four components were chosen due to an additional component that did not give a noticeable decrease in the residual sum of squares. For CONTIN, the CPMG relaxation curves up to 5000 ms were used. The average CONTIN distributions,  $T_2$ , and amplitudes for the discrete multi-exponential fitting were determined for the unprocessed and briquetted straw, respectively.

## RESULTS AND DISCUSSION

### **Chemical Characterization of Wheat Straw Biomass**

The FT-IR spectra of the unprocessed and briquetted straw were identical in the fingerprint bands at 1000  $\text{cm}^{-1}$  (C-O), 1510  $\text{cm}^{-1}$  and 1590  $\text{cm}^{-1}$  (aromatic ring stretch), 1750  $\text{cm}^{-1}$  (C=O), and 2850  $\text{cm}^{-1}$  and 2910  $\text{cm}^{-1}$  (C-H), which are ascribed to cellulose, lignin, hemicellulose, and wax, respectively (Fig. 1) (Kristensen *et al.* 2008). The carbon-carbon double bond in the region from 1600  $\text{cm}^{-1}$  to 1680  $\text{cm}^{-1}$  was reduced for the briquetted straw compared to the unprocessed straw, indicating the chemical changes of pectins during the briquetting process (Stuart 2004). The torus in bordered pits connected with pectin strings to the lignified cell walls might be damaged due to the compression forces, which could improve the water absorption property of wheat straw (Choat *et al.* 2008).

The chemical composition of wheat straw was not affected by the briquetting process, as shown by the FT-IR results.



**Fig. 1.** FT-IR spectra of the unprocessed and briquetted wheat straw samples

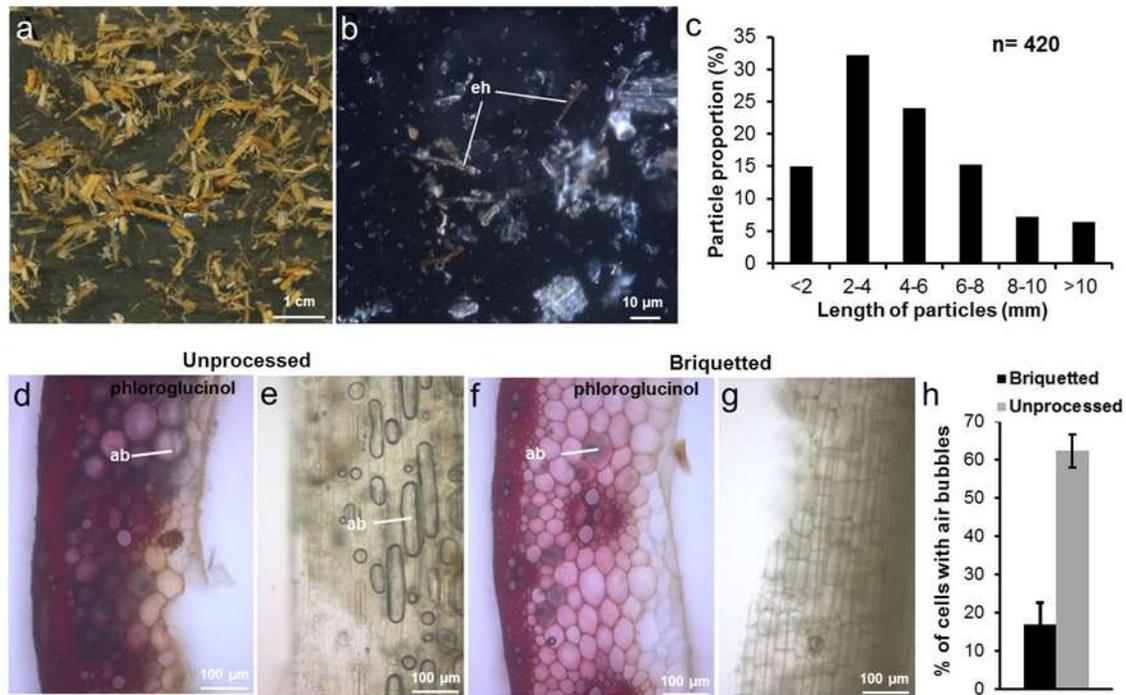
### Characterization of the Particles Forming Briquettes

The structure of the particles forming the briquettes was assessed visually after 30 min of hydration. After hydration, the distinguishable relatively intact pieces of wheat material could be observed including leaves, leaf sheath, internodes, nodes, and char (Fig. 2a). The washed-out microscopic particles were isolated from the liquid by brief centrifugations and observed under a microscope. These particles were a heterogeneous mixture of small shreds of tissue and epidermal hairs (Fig. 2b). The measurements showed that the largest portion of the macroscopic particles was between 2 mm and 4 mm in length, few (approximately 5%) were longer than 10 mm (Fig. 2c), compared to the unprocessed fibers (15 mm to 40 mm).

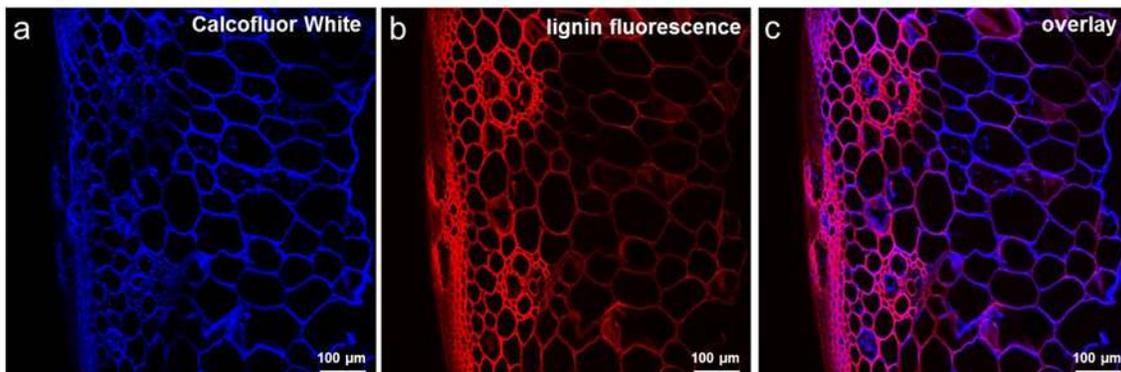
Microscopic analysis of hand sections using both LM and LSCM revealed well preserved tissue morphology of a compressed and hydrated wheat internode (Figs. 2 and 3). The typical lignin-specific red staining of phloroglucinol was observed in xylem vessels and sclerenchyma cells (interfascicular fibers) and also in the parenchyma cells, but weaker (Fig. 2f). The same result was obtained by recording the emission within the lignin fluorescence spectra (Fig. 3b).

The most noticeable difference was in the amount of bubbles trapped inside the cells. Unlike briquetted straw, the unprocessed samples (both leaves and internodes) contained a considerable amount of air bubbles filling the whole intracellular spaces in the cells (approximately 15% of the briquetted compared to approximately 60% of the unprocessed) (Figs. 2e, 2g, and 2h). These results suggested that the air evacuation and

straw fiber size reduction during briquetting may be the features that account for better water absorption in the briquettes.



**Fig. 2.** (a) Scan of the hydrated particles used for the length measurements; (b) Analyses of microscopic particles, note the epidermal hairs (eh); (c) Quantification of the length of the particles (d, e, f, g), microscopic analyses of the hydrated material: (d, f) internodes, (e, g) leaves, (d, f) sections of hydrated internodes; (d, e) Unprocessed wheat straw; (f) Briquettes stained with phloroglucinol (red), note the air bubbles trapped (a, b) in intracellular space in unprocessed samples in d and e; (h) Quantification of the percentage of parenchymatic cells with air bubbles, at least 100 cells from 5 independent sections were counted; Error bars represent standard deviation (SD)



**Fig. 3.** Analyses of hand sections of hydrated briquetted straw using LSCM; (a) Signal of the staining with  $\beta$ -(1,4)-glucan specific dye Calcofluor White M2R; (b) Scan of the fluorescence signal emanating (488 nm laser used for excitation, emission recording between 520 nm to 560 nm) from lignin and other phenolics; (c) Overlay of the two channels; note the preserved cell morphology

## Hydrothermal Pretreatment and Enzymatic Hydrolysis Yield

The pH values of the liquid fractions of the unprocessed and briquetted straw were  $3.81 \pm 0.05$  and  $4.24 \pm 0.01$ , respectively. It is known that acetyl groups on hemicellulose, mainly arabinoxylan in wheat straw, are cleaved off during hydrothermal pretreatment, which can catalyze partial solubilization of hemicellulose into oligosaccharides (Kabel *et al.* 2007). The higher the pH value of the briquetted straw indicated either less physical/chemical changes or better buffering capacity of the briquetted straw compared to the unprocessed straw when exposed to hydrothermal treatment.

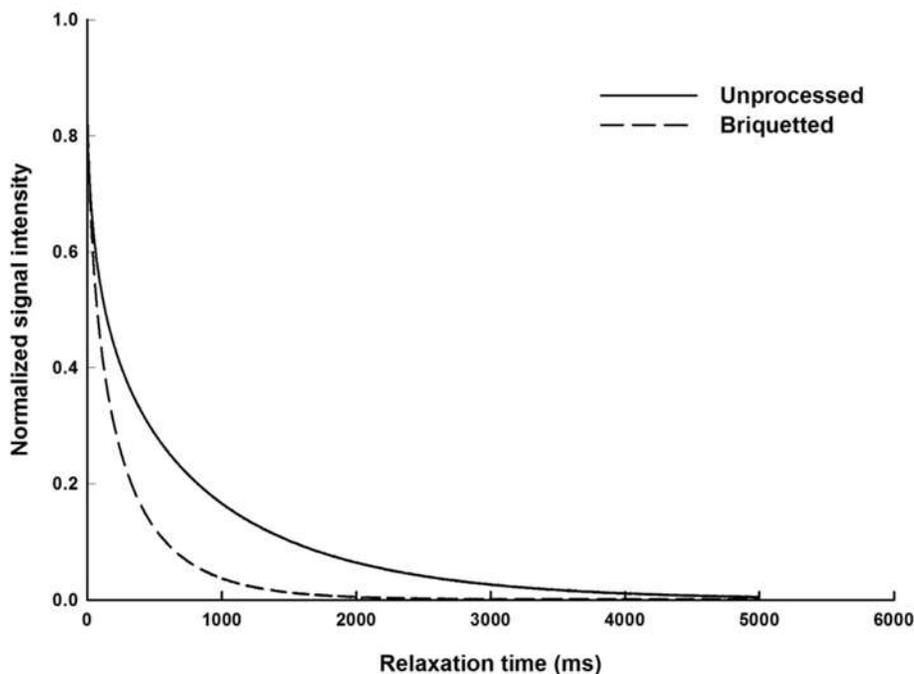
The unprocessed straw gave nearly an identical glucose yield (0.33 g/g DM) and xylose yield (0.15 g/g DM), compared to the briquetted straw (glucose yield at 0.31 g/g DM and xylose yield at 0.14 g/g DM) after a 96 h enzymatic hydrolysis. The briquetting process applied mechanical forces on the wheat straw fiber, which is known to introduce supramolecular structural changes, also known as dislocations (Thygesen *et al.* 2011; Hidayat *et al.* 2012). It has been shown that cellulose in dislocations was more susceptible to enzymatic degradation (Thygesen *et al.* 2011). However, the identical sugar yield between the unprocessed and the briquetted straw after the combined hydrothermal pretreatment and enzymatic hydrolysis suggested that the briquetting process increased the recalcitrant level of the wheat straw biomass towards enzymatic hydrolysis. Thus, it potentially offset the benefits of smaller fiber sizes and more dislocations in cellulosic fibrils introduced by the briquetting process. This might be due to the flow and entanglement of lignin and water soluble carbohydrates during the briquetting process, which may reduce the enzymatic accessibility of the carbohydrates (Kaliyan and Morey 2010). The results published by Li *et al.* (2014) demonstrated a 15% lower glucan conversion from the briquetted corn residues than the undensified samples by applying a more critical pretreatment (200 °C, 15 min) and hydrolysis conditions (20 FPU/g glucan).

## Water-biomass Interaction

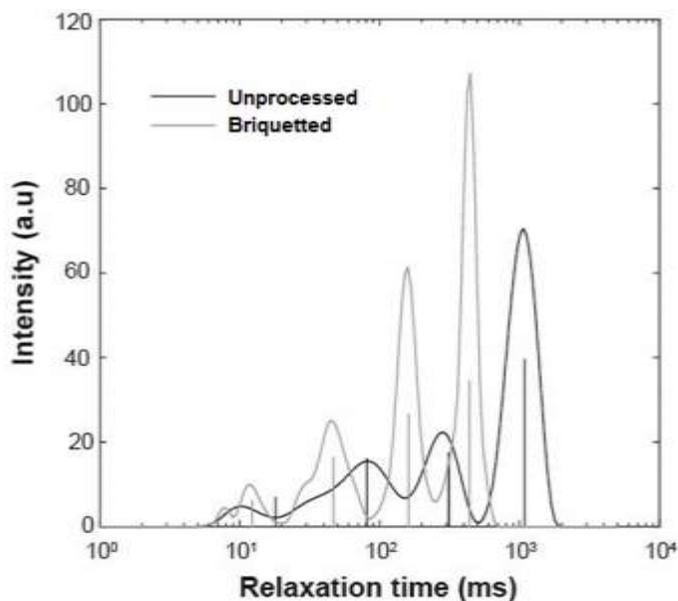
The unprocessed and briquetted straw behaved very differently in the water absorption test (S1). In contrast, the briquetted straw began to absorb water and swelled rapidly upon immersion in water. The fibers started to detach from the lump, part of which sank to the bottom of the beaker, within 1 min. The water turned into a yellowish color after approximately 10 min and a noticeable amount of fibers settled at the bottom. The briquetted lump completely lost its structure in approximately 15 min without any mixing. The test continued for 30 min, and there was no change in terms of fibers sinking or the color of the water. Consequently, the unprocessed straw fibers kept floating in the water during the 30 min and the color of the water did not change at all. This meant that the briquetted straw was able to absorb water more rapidly than the unprocessed straw when it was subjected to hot steam during the hydrothermal pretreatment.

The briquetting process reduced the fiber size and formed a complex interlocked fiber structure, which transformed the unprocessed straw into cylindrical briquettes, elevating biomass density from approximately  $100 \text{ kg}\cdot\text{m}^{-3}$  to  $1000 \text{ kg}\cdot\text{m}^{-3}$ . In addition, air was also pushed out from hollow culm as well as the tissues, *e.g.* pith parenchyma cells. The rapid water absorption feature of the briquetted straw was beneficial in hydrothermal pretreatment from a processing point of view, as it was technically easier to load the briquettes to the reactor compared to the unprocessed straw and may have not required mixing, as the fibers fall apart spontaneously during swelling.

Figure 4 shows the LF-NMR  $T_2$  relaxation curves of the unprocessed and briquetted straw at 10% DM after 1 h of water absorption. The  $T_2$  relaxation curve of the briquetted straw declined faster than the unprocessed straw, which indicated that the briquetted straw constrained water better compared to the unprocessed straw.

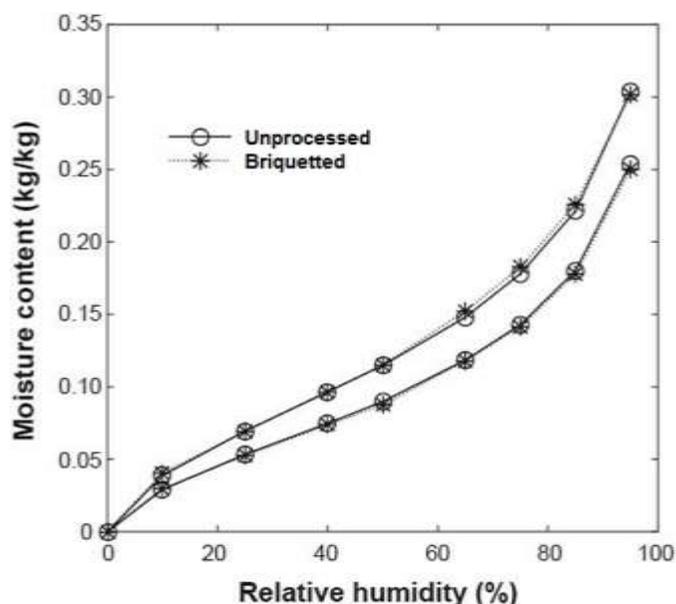


**Fig. 4.** Average CPMG  $T_2$  relaxation curves for the unprocessed and briquetted wheat straw samples; the data represent average values for five samples



**Fig. 5.** The continuous  $T_2$  distribution evaluated using CONTIN and the fit to four discrete components for the unprocessed and the briquetted wheat straw, respectively; the data represent average values for five samples

The results from the discrete multi-exponential fit, as well as the curve fit using CONTIN, are shown in Fig. 5. Both curve fit methods gave similar results in terms of the number of peaks and their  $T_2$ . The unprocessed straw samples gave three major  $T_2$  relaxation peaks, centered at 77 ms, 226 ms, and 1014 ms, which shifted to shorter relaxation times after briquetting (46 ms, 159 ms, and 445 ms). This strongly suggested that water was more constrained in the briquetted straw compared to the unprocessed sample, which is possibly due to the smaller fiber size of the briquetted straw (Selig *et al.* 2014; Weiss *et al.* 2016). Normally, there is also a peak at approximately 1 ms to 3 ms representing bound water in the cell wall (Felby *et al.* 2008). However, because of the long pulse separation (0.8 ms) and the high moisture content of the samples, this peak was not visible in the present study. Thus, the peaks in Fig. 5 represent more free water, such as lumen water, water between fibers, and excess water. The sorption isotherms for the unprocessed and briquetted straw during both desorption and absorption are shown in Fig. 6. The sorption isotherms were similar and thus the briquetting process did not affect the amount of water vapor absorbed by the straw in the hygroscopic moisture range, *i.e.* the amount of water bound in the cell wall.



**Fig. 6.** Desorption and absorption isotherms of the unprocessed and briquetted wheat straw samples; the upper curves are desorption curves and the lower curves are absorption curves

## CONCLUSIONS

1. The results demonstrated the distinctive hydration property of the briquetted wheat straw at both the macro- and micro-scales, compared to the unprocessed wheat straw. The amount of water bound to the cell wall was not affected by the briquetting process; however, the lumen water and water between the fibers were more constrained in the briquetted straw than the unprocessed straw, which benefited the briquettes in the pretreatment and bioconversion processes.

2. The physical/chemical changes introduced by the briquetting process were examined extensively using microscopic and spectroscopic methods. The results suggested that the reduced fiber sizes and air evacuation accounted for better water absorption of the briquetted straw, compared to the unprocessed straw.
3. Identical sugar yields were given from the unprocessed straw and the briquetted straw in the combined hydrothermal pretreatment and enzymatic hydrolysis. The flow and entanglement of lignin and cell wall carbohydrates during the briquetting process was hypothesized to offset the features and benefits of the briquettes.
4. Well-preserved plant cell morphology is important for the current conversion technology in a bioethanol plant. The plant tissues/organs were kept intact after the briquetting process, demonstrated by LM, and two major cell wall polymers, lignin and cellulose, were visualized using LSCM.

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