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Chemical characterization of hydrothermally pretreated and enzyme-digested wheat straw: An evaluation of recalcitrance

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ABSTRACT

There is great interest in understanding changes that occur to cell wall constituents during saccharification of pretreated lignocellulose, particularly in relation to recalcitrance of the residues. This paper reports the effects of hydrothermal pretreatment followed by enzyme hydrolysis on the extractability and properties of recalcitrant wheat straw polymers. The results show that the undigested residue had lost much of its archestructure. Compositional analysis portrayed a considerable loss of cross-linking di-ferulic acid phenolics, hemicellulosic and cellulosic sugars. The remaining cellulosic and non-cellulosic polysaccharides were much more readily extractable in alkali and molecular profiling revealed the presence of low M_w oligomers in the fractions suggesting the partial enzyme hydrolysis of hemicelluloses and cellulose. Simultaneous saccharification and fermentation of the pretreated and enzyme-digested residues surprisingly resulted in ethanol yields of up to 99% of the theoretical. This is discussed in relation to the "recalcitrant" nature of the original pretreated and enzyme digested biomass.

1. Introduction

Meeting EU targets for renewable transport fuels by 2020 will necessitate a large increase in bioenergy feedstocks (Glithero, Wilson, & Ramsden, 2013). Lignocellulosic biomass is an abundant, renewable source of cellulosic glucose and worldwide attention has focused on the major biotechnological applications of this underutilized resource, including biofuel production (Mosier et al., 2005). Despite the potential promise of cellulosic ethanol and other cellulose-derived biofuels, major obstacles need to be addressed to make the process feasible and economically viable for large scale applications (Wyman, 1996). A major problem concerns the resistance of lignocellulose to saccharification. This is due to a number of compounding factors, including steric hindrance and cellulose crystallinity. The steric hindrance to cellulolysis is associated with hemicellulosic and sometimes pectic polymers which interfere with access of hydrolytic enzymes, compounded by their interpolymeric cross linking with themselves and lignin. The latter also acts to bind to many cell-wall degrading enzymes making them inactive (Rahikainen et al., 2011; Tejirian & Xu, 2011). There is substantial evidence that the lignin polymer is cross-linked to cellulose via hemicellulose and simple phenolics such as diferulic acid (Ralph, Grabber, and Hatfield (1995). A consequence of cross-linking, despite its importance to plant growth and development is reduced digestibility of the polysaccharides (Grabber, Ralph, & Hatfield, 1998). To improve enzymatic digestibility, interactions between lignin and polysaccharide components of the cell wall are disrupted initially

Abbreviations: AIR, alcohol insoluble residue; DiFA, diferulic acid; DM, dry matter; HT, hydrothermal pretreatment; HTED, hydrothermally pretreated enzyme digested; HPSEC, high performance size exclusion chromatography; HW, hot water extraction; M_w, molecular weight; SSF, simultaneous saccharification and fermentation.

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through a combination of thermophysical/chemical pretreatments (Wyman et al., 2005; Yang & Wyman, 2008).

Some attention has been paid to understanding the effects of the pretreatments on the physicochemical characteristics of the lignocellulose. Such investigations have included assessments to varying degrees of change to the polymeric components within the cell walls (DeMartini et al., 2011; Holopainen-Mantila et al., 2013; Kabel, Bos, Zeevalking, Voragen, & Schols, 2007; Lawther, Sun, & Banks, 1996; Merali et al., 2013; Sun, Lawther, & Banks, 1996; Sun et al., 2005). However to date, there are only a few studies concerning the nature of changes that occur to the cell-wall constituents during the saccharification stage, particularly in relation to the recalcitrance or resistance to digestion of the remaining insoluble residues. Hansen et al. (2014) evaluated the extractability and digestibility of plant cell wall polysaccharides during hydrothermal and enzymatic degradation of wheat straw stems and leaves. Cell wall materials before pretreatment, after pretreatment and after saccharification were sequentially extracted using increasing strengths of alkali, and evaluated using microarray polymer profiling. The results suggested that arabinoxylans and mixed-linkage glucans were loosely bound in the cell wall, and shielded other more tightly bound non-cellulosic polysaccharides until solubilized by pretreatment. They did not, however, detect any ferulic acid moieties which have been implicated in interpolymeric cross-linking in wheat straw (Ralph et al., 1995). Also, they looked at changes resulting from only one pretreatment severity.

Previously, Merali et al. (2013) investigated the effects of two hydrothermal pretreatments on the composition and polymeric nature of cell-wall polysaccharides of wheat straw. We demonstrated that under conditions that augment enzymatic saccharification, significant changes occur in the cell wall hemicelluloses, lignin, and cross-linking phenolics. Of particular note was the reduction in the molecular weight (M_w) of the remaining arabinoxylans and the considerable reduction in ferulic and diferulic acids. In the current study, we report the carbohydrate yields and profiles of cell-wall polymers that remain after subsequent, extensive enzymatic digestion of the hydrothermally pretreated wheat straw. In order to further understand resistance to cellulases, the pretreated and enzyme-digested biomass was evaluated for changes in ultrastructure and then sequentially extracted in increasing concentrations of alkali (Redgwell & Selvendran, 1986) prior to characterization of extracted components for composition and molecular weight profiles. The recalcitrance of the pretreated and enzyme-digested material was further evaluated by subjecting it to simultaneous saccharification and fermentation (SSF).

2. Materials and methods

2.1. Raw material

Control (untreated) and HT wheat straw was provided by Biogold (wheat straw was obtained from Hõbetse farm, Pärnu, Estonia and was grown similar to all conventional cropping). For the control, wheat straw was cut into approximately 2 cm long pieces, dry milled (Krups F20342, Cedex, France) and sieved (1 mm sieve). The sieved wheat straw was oven dried at 50 °C and stored in air-tight containers at room temperature until required for analysis.

2.2. Hydrothermal pretreatment

For each pretreatment 400 g (dry weight) of knife-milled (GRINDOMIX GM 300, Tallinn, Estonia) wheat straw (particle size 0.4–10 mm) was loaded into reactor (solid-to-water-ratio 1:10) and water was pumped through the packed material bed at a circulation flow rate of 4–5 L/min. The reactor (inner diameter 106 mm, material AISI 316) had a total volume of 6 L, with an electric heater and liquid phase circulation pump (Micropump Series-2200, Tallinn, Estonia). The wheat straw was then HT at two holding temperatures (190 and 200 °C) for 15 and 20 min respectively. Following HT the solid biomass was separated from the liquor by filtration and both fractions were frozen after dry mater evaluation.

2.3. Enzymatic hydrolysis

Prior to hydrolysis, the solid fraction of the HT wheat straw was washed with water (55 °C, 2.1 L/kg (wet weight)) and filtered through 90 μm mesh cloth. Enzymatic hydrolysis was performed in 10 g/L substrate concentration (dry matter, DM) using mixture of Celluclast 1.5 L (10 FPU/g substrate DM) and Novozymes 188 (100 nkat β-glucosidase activity/g substrate DM) at pH 5 and 45 °C in total volume of 10 L. The hydrolysis mixtures were supplemented with 0.01% (w/v) Na-azide for prevention of microbial growth. The hydrolysis was followed by sampling (1 mL) and quantification of reducing sugars with the PAHBAH assay (Lever, 1972). The hydrolysis was allowed to proceed for 72 h after which solids and liquids were separated by centrifugation (3963×*g*, 30 min). Enzymes in the liquid fractions were inactivated by heating (95 °C, 10 min). The solids were stored frozen (−20 °C) until analyzed.

2.4. Analysis of raw and HT material

2.4.1. Dry matter (DM) determination

The DM of the samples was determined by weighing triplicate samples in an infra-red balance (Mettler PM200, Leicester, UK) heated to 100 °C.

2.4.2. Preparation of alcohol insoluble residues (AIRs)

AIRs were prepared from the control and the HT and enzyme-digested wheat straw as described in Merali et al., 2013.

2.4.3. Cell wall fractionation

Sequential extraction (in progressively stronger alkali) was conducted by a modification of the method of Redgwell and Selvendran (1986) in degassed aqueous solution and in the presence of $NaBH_4$ to minimize alkaline peeling. HT AIRs together with the control (2 g) were suspended in hot water (60 °C, 200 mL) and cell wall components extracted (shaking, 2 h, 25 °C). The extracts were centrifuged (10,000×*g*, 1 h) and the supernatant filtered through Whatman GF/C filter paper (Whatman, Maidstone, UK) and freeze-dried. The insoluble residue was further extracted with 0.5 mol/L KOH with 20 mmol L^{-1} $NaBH_4$ for 2 h (shaking, 25 °C) centrifuged (10,000×*g*, 60 min) and the supernatant filtered. The filtrate was first neutralized with acetic acid, extensively dialyzed (tubing size 30/32", Medicell International, London, UK, 7 days, changing 3× times daily) and then freeze-dried. The residue was further extracted as above in 1 mol/L and 4 mol/L KOH containing 20 mmol L^{-1} $NaBH_4$, filtered and neutralized. All the filtrates were freeze-dried as above following dialysis. The freeze-dried extracts, insoluble residues and AIRs were biochemically analyzed in duplicate.

2.4.4. Carbohydrate analysis

Sugars were released from the fractions by hydrolysis with H_2SO_4 (w = 72%) for 3 h, followed by dilution to 1 mol/L (Saemen hydrolysis). Hydrolyzed monosaccharides were analyzed as their alditol acetates by GC as described in Merali et al., 2013.