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The promotional effect of water-soluble extractives on the enzymatic cellulose hydrolysis of pretreated wheat straw

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HIGHLIGHTS

- Water-soluble extractives from wheat straw enhance enzymatic cellulose hydrolysis.
- Promotional effect from unpurified extract, no additional processing required.
- Possibly positive effect on enzyme deactivation and cellulose accessibility.
- Up to 85% relative increase in digestibility of organosolv pretreated pulp.
- Promotional effect caused by components larger than 1 kD, possibly proteins.

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ABSTRACT

Enzymatic cellulose hydrolysis of pretreated wheat straw pulp to glucose is enhanced when the hydrolysis is performed in the presence of an aqueous extract of the wheat straw. A relative digestibility increase of about 10% has been observed for organosolv, alkaline and dilute acid pretreated wheat straw pulp (enzyme dose 2.5 FPU/g pulp). At lower enzyme doses, the extract effect increases leading to an enzyme dose reduction of 40% to obtain a glucose yield of 75% within 48 h using organosolv wheat straw pulp. Possibly, cellulase deactivation by irreversible binding to pulp lignin is reduced by competition with proteins in the extract. However, since the extract effect has also been demonstrated for lignin-lean substrates, other effects like improved accessibility of the pulp cellulose (amorphogenesis) cannot be excluded. Overall, this contribution demonstrates the positive effect of biomass extractives on enzymatic cellulose digestibility, thereby reducing costs for 2G biofuels and bio-based chemicals.

1. Introduction

The valorization of lignocellulose into bio-based platform chemicals and biofuels is one of the most important developments to meet concerns over global climate change and fossil fuel depletion. Efficient enzymatic conversion of (hemi)cellulose into monomeric sugars is one of the major challenges for an economically feasible lignocellulosic biorefinery (Wyman, 2007; Klein-Marcuschamer et al., 2012). The highly crystalline fibrillary structure of the cellulose encased in a matrix of lignin and hemicellulose renders it resistant to enzymatic depolymerization. Therefore, pretreatment of lignocellulosic biomass is required to increase the accessibility of the cellulose for (hemi)cellulolytic enzymes (Park et al., 2010; Hall et al., 2010). Lignin removal or redistribution is one of the most important factors for improving the accessibility, enhancing the hydrolysis rate and sugar yield (Van Dyk and Pletschke, 2012; Mansfield et al., 1999).

Residual lignin in pretreated biomass can negatively affect enzymatic hydrolysis by reducing enzyme activity due to non-productive binding of enzymes to its surface (Gao et al., 2014; Varnai et al., 2010; Yang and Pan, 2016). Several possible measures have been reported for minimizing enzyme deactivation by non-productive adsorption to lignin including addition of surfactants such as polyethylene glycol (Kristensen et al., 2007; Borjesson et al., 2007), Tween 20 (Zheng et al., 2008), Tween 80 (Tu and Saddler, 2010), metal ions (Akimkulova et al., 2016), and proteins such as the model protein BSA (Yang and Wyman, 2006; Pan et al., 2005; Brethauer et al., 2011) or biomass proteins (Han and Chen, 2007, 2010). Addition of non-hydrolytic proteins may not only minimize enzyme deactivation, but could possibly also loosen the highly ordered and tightly packed regions of the cellulose by amorphogenesis leading to increased access of cellulase enzymes to the cellulose (Arantes and Saddler, 2010; Han and Chen, 2007;

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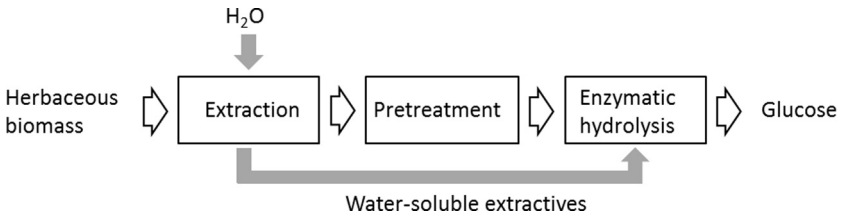

Fig. 1. Schematic representation of the process.

Coughlan, 1985). Han and Chen (2007) isolated a non-hydrolytic protein (Zea h) from fresh postharvest corn stover which was found to increase the cellulose hydrolysis rate and glucose yield substantially. However, the use of purified proteins is costly, which might negate the overall net cost savings generated by the lower enzyme dosage requirements.

In this study, we examined the effect of adding an unpurified protein-containing aqueous extract of biomass directly to the enzymatic saccharification of pretreated biomass pulp (Fig. 1). Extraction prior to pretreatment prevents the degradation of proteins caused by the high severity generally used for pretreatment of biomass. In this study, we explore whether similar effects as reported with purified proteins can be obtained with an unpurified extract in spite of possible inhibiting effects of matrix components such as salts. This paper focuses on EtOH organosolv, dilute acid and alkaline pretreatment of wheat straw. For various other combinations of herbaceous feedstocks, pretreatment processes and extracts tested, we refer to Smit and Huijgen (2014) and the Supporting Information.

2. Materials and methods

2.1. Materials

For compositional analysis the following chemicals were used: H_2SO_4 from Boom 72% p.a, $BaCO_3$ (Merck, EMSURE® ACS grade), and the sugar standards glucose (Sigma >99.5%), xylose (Fluka >98%), mannose, arabinose, galactose and rhamnose monohydrate (all Fluka, HPLC grade, >99%).

Ethanol 96% v/v was obtained from Nedalco, sodium azide 99.5%, sulfuric acid 98%, sodium hydroxide 98% from Sigma, sodium acetate trihydrate and glacial acetic acid 100% from Merck, o-toluidine 99% from Aldrich, Avicel PH-101, thiourea 99% and sodium chlorite 80% from Sigma-Aldrich and Pierce™ BCA protein assay kit from Thermo Scientific. The commercial enzyme mixtures Accellerase TRIO and 1500 were obtained from DuPont Industrial Biosciences (Leiden, NL) for (hemi)cellulose hydrolysis. Wheat straw was grown and harvested in 2013 in The Netherlands.

2.2. Biomass pretreatment

Ambient dry wheat straw was cut using a Retsch SM2000 cutter mill equipped with a 1 cm sieve. The moisture content of the straw was determined using a halogen moisture analyzer (Mettler Toledo HR83, Columbus, OH). 1.5 kg of wheat straw (moisture content 11.5% w/w) was placed in a glass pipe fitted with an 185 μm filter supported by a glass frit. 2 kg of demineralized water (50 °C) was added and the sample was heated in a convection oven at 50 °C for 60 min. The glass pipe was placed in vertical position and demineralized water (50 °C) was added to the top until 2 parts by weight of extract was obtained per part of dry weight straw from the bottom (i.e., 3 kg). A subsample of the extract was concentrated using a 1 kD Pall Minimate ultrafiltration module. The extract, concentrate and filtrate were preserved with sodium azide (0.02% w/v final concentration) and stored at 4 °C.

Organosolv pretreatment was performed (Table 1) with the wet extracted straw (moisture content 75.0% w/w) in an autoclave reactor (20 L Kiloclave, Büchi Glas Uster AG, Switzerland) using conditions based on earlier work (Wildschut et al., 2013). A mixture of extracted straw, 60% (w/w) aqueous ethanol (taking into account the straw moisture content) and 24 mM sulfuric acid (liquid/solid ratio of 10 L/kg dry weight extracted straw) was heated to 190 °C and kept isothermal for 60 min while stirring with an anchor stirrer at 500 rpm. After cooling below 25 °C, the slurry was measured for pH and filtered over a Whatman GF/D filter. The solids were first washed with 60% w/w aqueous ethanol (5 L/kg initial dry weight straw) followed by a wash with water to remove ethanol from the solids. A subsample was dried at 50 °C to determine pulp yield, moisture content and composition. 550 g of wet pulp (100 g dry weight) was bleached with 10 g of sodium chlorite and 6.6 mL glacial acetic acid in 1.5 L of demineralized water at 70 °C while stirring. The pulp was filtered over a 256 mm Whatman GF/D filter and the bleaching step repeated twice to a total of 3. The bleached pulp was extensively washed with demineralized water and stored at 4 °C.

Similar to the above mentioned extraction, extraction at smaller scale was performed on wheat straw cut to <4 mm. The filtrate was preserved with sodium azide and stored at 4 °C without a concentration step. Smaller scale dilute acid and alkaline pretreatments with the extracted straw were performed in 125 mL batch reactors (acid digestion bomb type 4748, SS 316 with Teflon liner, Parr Instrument Company, Moline, IL) as described in Table 1. 23.3 g (6 g dry weight) extracted wet straw was mixed with water and catalyst to a final liquid/solid ratio of 10 L/kg dry weight extracted straw. The batch reactors were placed in a heating block for 180 min (roughly equals a time on target temperature of 60 min, see Huijgen et al. (2011)), while being stirred using a magnetic bar (500 rpm). The solids were recovered by filtration, washed with water (10 L/kg initial dry weight straw) and stored at 4 °C. A subsample was dried at 50 °C to determine pulp yield, moisture content and composition.

Table 1
Substrates: applied pretreatment processes.

Substrate code	Pretreatment	T (°C)	t (min)	Solvent	Catalyst
OS-190	Organosolv	190	60	60% w/w aqueous ethanol	24 mM H_2SO_4
DA-140	Dilute acid	140	60	Water	80 mM H_2SO_4
DA-160		160	60	Water	40 mM H_2SO_4
DA-180		180	60	Water	20 mM H_2SO_4
AL-121	Alkaline	121	60	Water	2.5 wt% NaOH