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Changes in the composition of the main polysaccharide groups of oil seed rape straw following steam explosion and saccharification

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ABSTRACT

The composition of oil seed rape straw treated by steam explosion with increasing severity was investigated before and after saccharification. Chemical changes were monitored by FTIR-ATR spectroscopy. Sugar contents were determined after acid hydrolysis. Discriminant analysis of the spectra before and after digestion showed the main compositional changes are losses of carbohydrates and a subsequent increase in the proportion of lignin. Construction of partial least squares (PLS) predictive models for the concentration of eight cell wall sugars indicated different fates for cellulose, hemicelluloses and pectic substances. No cellulose was lost during steam explosion and the amount digested to glucose increased linearly with severity. Pectin was partially degraded during steam explosion, but a bound fraction remained which was only released during saccharification. Hemicelluloses were gradually destroyed in the steam explosion process, and the extent of subsequent saccharification was most strongly associated with the breakdown of xylan-like hemicelluloses.

1. Introduction

Oil seed rape (OSR) straw is a cheap and abundant source of lignocellulose. The greatest demand for straw is for burning in power stations but it can also be used for second generation (2G) ethanol production. OSR straw contains a diversity of plant organs including stems, rachis, pods and pedicels, and many tissue types, some highly lignified and some friable and unlignified.

In 2G biorefining, hydrothermal pre-treatments such as steam explosion are used to change the physical and chemical properties of the raw material. This has the effect of increasing the proportion of cellulose that can be enzymatically digested to release glucose for subsequent fermentation to ethanol [1,2]. The severity of the steam explosion is a function of the

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duration of the treatment, and the temperature of the steam, which is directly related to steam pressure [3].

There are several mechanisms in which steam explosion alters the chemical and physical properties of lignocellulosic materials. Physical changes include a general 'opening up' of the cell wall structure: lignin melts, cellulose crystallinity changes and the surface area of the material increases [4]. The extreme conditions also drive chemical reactions: the most labile components are the acetyl groups of acetylated polysaccharides which are hydrolysed to acetic acid. The steam and acid cause a partial acid hydrolysis of the polysaccharides: pectins and hemicelluloses are more readily hydrolysed than cellulose, and at high temperature the pentoses and hexoses released are dehydrated to furfural or hydroxymethyl furfural (HMF), respectively [5]. The extent to which these factors influence the proportion of cellulose accessible for enzymatic hydrolysis (i.e., digestible to glucose) is a keen area of current research.

In a previous study [6], the effects of varying the steam-explosion regime on OSR straw were studied. The results showed that severity acts as a useful summary of the impact of steam explosion. Using mid-infrared spectroscopy, changes in the composition of pre-treated straw were found to vary steadily with the severity of the pre-treatment. The severity factor explained a large proportion of the total variance in the infrared spectra. Conversely, it was possible to produce good predictions of pre-treatment severity from the infrared spectra of steam-exploded samples. The results also showed a strong linear relationship between the severity of pre-treatment and parameters describing the rate of methane production during 81 days of anaerobic digestion. The predictive relationships are of potential importance in monitoring and controlling biorefining processes.

This paper concerns the saccharification stage of 2G bioethanol production. It describes an investigation of the impact of steam explosion severity on the chemical composition of OSR straw; the proportion of cellulose digested to glucose; and the composition of the recalcitrant residue. A total of 15 different pre-treatment conditions were studied comprising all combinations of five different temperatures (10° increments between 190 and 230 °C) and three time durations (5 min increments between 5 and 15 min). The steam exploded samples were digested by a commercial cellulase preparation, Celluclast®, for 22 h and the amount of released glucose recorded. The chemical composition of the straw was assessed by two methods, first after steam-explosion, and again after digestion. Gas chromatography of alditol acetates was used to directly measure the sugar composition of the polysaccharides, and mid-infrared spectroscopy was used as an indirect assessment of overall chemical composition. Multivariate regression was used to investigate whether the mid-infrared spectra contains sufficient information to give good predictions of composition or saccharification. A correlation-based (i.e. univariate) approach was used to investigate associations between these outcomes and variations in the mid-infrared spectra in order to identify compounds related to saccharification efficiency. A schematic diagram representing the processing and measurement stages used in this study is provided as Supplementary Material (Fig. S4).

2. Materials and methods

2.1. Raw material

Commercially prepared rape straw (*Brassica napus*) bedding material was sourced from a local supplier (*Rapport*, Hemp Technology Limited, Suffolk, IP19 8QJ). Harvested in autumn 2009, the dried stems were treated on a hemp decortication processing line to soften and fragment the straw to a maximum length of 35 mm. The dust-extracted material was packaged in heat-sealed 20 kg polypropylene bags and stored after purchase at 10 °C until steam-explosion on 16 March 2010. The dry matter content of the straw was $\omega = 87.7\%$.

2.2. Steam-explosion pre-treatment

Pre-treatment was performed at the steam explosion facility (Cambi AS, Asker, Norway) located in the Norwegian University of Life Sciences (UMB) in Ås, Norway as described in Ref. [7]. Rape straw (500 g for each run) was steam exploded at temperatures ranging from 190 °C to 230 °C using intervals of 10 °C. For each temperature, samples were kept in the reactor for 5, 10 or 15 min leading to a total of 15 temperature/time combinations. The pressure vessel was preheated for 10 min at the desired temperature before each run. The pre-treated material was vacuum-bagged and frozen.

2.3. Enzymic digestions

Digestibility was assessed at 50 kg m^{-3} substrate concentration. The frozen steam-exploded straw was thawed and the dry matter content determined. A small amount of the wet material (*ca.* 1.5 g dry weight equivalent) was placed in a 60 cm^3 polystyrene container and dispersed in Na Succinate buffer pH 5 and then 0.4 $cm^{-3}$of Celluclast® and 0.1 cm^3 Novozyme 188 were added. The total volume was adjusted to 30 cm^3. Reaction mixtures were incubated at 50 °C for 22 h with rotary stirring at 3.33 Hz. The liquor was filtered off (Whatman Paper No. 4) and part of the wet residue retained for subsequent compositional analysis (see Sections 2.4 and 2.5). Glucose in the liquor was measured in duplicate using the GOPOD assay (Megazyme.com) in which glucose oxidase converts glucose to gluconate and H_2O_2, which reacts to form a coloured dye (absorbance measured at 510 nm). The glucose measurements were expressed as a weight fraction of the dry steam-exploded material.

2.4. Polysaccharide analyses

Straw sugar composition was measured after steam-explosion, and after enzyme digestion (15 samples each). Samples were dried at 40 °C and freeze milled for 3 min using a SPEX 6700 freezer mill (SPEX SamplePrep, 2 Dalston Gardens, Stanmore, HA7 1BQ, UK). Triplicate samples were dispersed in 200 mm^3 $\omega_{H_2SO_4} = 72\%$ at 20 °C for 2.75 h, then 2.2 cm^3 water was added and the samples were hydrolysed at 100 °C for 2.5 h. The hydrolysed sugars (rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose) were reduced and acetylated and analysed as alditol acetates by gas chromatography [8]. Uronic