Ethanol from a biorefinery waste stream: saccharification of amylase, protease and xylanase treated wheat bran.

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Abstract

Biorefining aims to exploit the full value of plant material by sequentially extracting and valorising its components. Many studies focus on the saccharification of virgin biomass sources, but it may be more efficient to pre-extract high-value components before hydrolysis to fermentable sugars. In the current study, a bran residue from de-starched, protein depleted and xylanase treated wheat bran has been subjected to hydrothermal pretreatment, saccharification and fermentation procedures to convert the residue to ethanol. The most effective pretreatment conditions (>190°C, 10 min) and saccharification conditions were identified following bench-scale liquid hot water pretreatment. Pre-extraction of enzymatically-hydrolysable starch and xylan reduced the release of furfural production, particularly when lower pretreatment severities were used. Pilot-scale steam explosion of the lignocellulosic residue followed by cellulase treatment and conversion to ethanol at a high substrate concentration (19%) gave an ethanol titre of ≈25 g/L or a yield of 93% of the theoretical maximum.

Keywords: Bioethanol, Biorefining, FTIR, Pre-processing, Simultaneous Saccharification and Fermentation, Wheat Bran.
1. Introduction

Wheat is a major global commodity, with annual production ~600Mt (faostat.fao.org/prodstat). Around 65% of wheat is estimated to be milled for flour production, potentially generating ~90Mt of wheat bran. Only a small portion of this is currently utilised - mostly as animal feed and in fibre-enriched foods in a wide range of products (Pruckler, Siebenhandl-Ehn, Apprich, Holtinger, Haas, Schmid, et al., 2014). Wheat bran has excellent potential as a readily available standard composition feedstock for downstream biorefining following flour milling. Indeed wheat bran has been used to produce dietary fibre for food supplementation and as a source of bioactive oligosaccharides (Delcour, Rouau, Courtin, Poutanen, & Ranieri, 2012).

To extract components of varying functionality from bran, a range of procedures have been developed. These primarily target higher value, proteinaceous, phenolic or bioactive carbohydrate components (Javed, Zahoor, Shafaat, Mehmooda, Gul, Rasheed, et al., 2012) which can be enzymatically hydrolysed from the material (Robertson, Castro-Marinas, Collins, Faulds, & Waldron, 2011; Treimo, Westereng, Horn, Forssell, Robertson, Faulds, et al., 2009). However, once wheat bran has been exhausted of starch and protein and enzymatic release of oligosaccharides has been achieved, the residual, largely cellulosic, material still represents ~40% of the original bran. This material could be used as a reliable feedstock for industrial biotechnology (Apprich, Tirpanalan, Hell, Reisinger, Bohmdorfer, Siebenhandl-Ehn, et al., 2014).

Saccharification of fermentable sugars from biomass typically focusses on the exploitation of virgin biomass sources (Mosier, Wyman, Dale, Elander, Lee, Holtzapple, et al., 2005). However, in many cases, extraction of high value components is economically attractive before converting the residual ‘waste’ carbohydrate to fermentable sugars. Also, the removal of certain components may be preferred to improve the subsequent bioconversion to fermentable sugars. These processes will influence the structure, composition and extractability of the feedstock.

Upstream processing is also likely to modify the concentration of key components which may
have implications for their downstream use. This is particularly important when producing ethanol, where high titres are desirable for efficient fermentation (Elliston, Collins, Wilson, Roberts, & Waldron, 2013).

Previous work concerning the exploitation of wheat bran as a feedstock for industrial biotechnology has focussed on the pretreatment and hydrolysis of the native material (Reisinger, Tirpanalan, Pruckler, Huber, Kneifel, & Novalin, 2013), or after starch removal (Merali, Collins, Elliston, Wilson, Kasper, & Waldron, 2015; Palmarola-Adrados, Choteborska, Galbe, & Zacchi, 2005) but not in conjunction with other pre-extraction procedures. Also, although the suitability of the pretreated material for this process can be indirectly estimated by the abundance of common fermentation inhibitors (Palmarola-Adrados, Choteborska, Galbe, & Zacchi, 2005; Reisinger, Tirpanalan, Pruckler, Huber, Kneifel, & Novalin, 2013), substrates are rarely fermented. This aspect may be particularly important for substrates such as wheat bran, which contains an abundance of low-molecular weight phenolic acids (Merali, Collins, Elliston, Wilson, Kasper, & Waldron, 2015), which are difficult to quantify but could inhibit downstream processes (Palmqvist & Hahn-Hagerdal, 2000).

If the residual lignocellulosic residue can be converted to a bulk product such as ethanol then this would complete the controlled and holistic bioconversion of plant biomass in the food chain. In the study reported, optimum pretreatment and saccharification conditions have been established for wheat bran which was pre-processed to sequentially release starch, protein and oligosaccharides from component arabinoxylans (AX). The effects of pretreatment on the chemical and polymeric structure of wheat bran was assessed directly and by fourier transform infra-red spectroscopy (FT-IR). Pretreatment was conducted at a range of severities and a single-addition simultaneous saccharification and fermentation (SSF) trialled at both small (circa 20 mL) and small-pilot scales (10L).

2. Materials and methods

2.1. Processing wheat bran before saccharification
Wheat bran (original bran) provided by G.R.Wright and Sons Ltd. (Enfield, UK) was de-starched by heating (400 g bran/L, 95°C, 45 min) followed by hydrolysis (1 h) with α-amylase from *Bacillus licheniformis* (Sigma 9000-85-5, 0.5 units /g bran). Starch removal was confirmed using potassium iodide/iodine solution and the reactor cooled to 60°C. The pH was adjusted using ammonium carbonate (pH = 8.5) and protein was extracted (60 °C, overnight) using protease from *Bacillus licheniformis* (Sigma 9014-01-1, 2.4U/g). The residue was rinsed four times with water, added back to the reactor and brought to a new substrate concentration (200 g/L). The residual material was finally treated with xylanase from *Thermomyces lanuginosus* (Sigma 37278-89-0, 7.5kU/kg DW, 50°C, overnight) before rinsing with water (thrice) after which it was freeze-dried to constant mass (91.6 ± 0.7% DW). All subsequent experiments were carried out using this solid, ‘lignocellulosic residue’ (Figure 1).

**Insert: Figure 1** – Flow diagram illustrating the processing of wheat bran to sequentially release of components of various chemistries. The residual lignocellulosic material was then exposed to varying pretreatment and hydrolysis conditions.

### 2.2. Small-scale liquid hot water pretreatment, saccharification and SSF.

Dilute slurries of the freeze-dried, lignocellulosic residue (15 mL, 2% DW) were subjected to hydrothermal pretreatments at a range of severities (140-210°C, 10-40 min) using a Biotage Initiator+ Microwave Synthesiser. The pretreated slurries were transferred to 15 ml falcon tubes (Corning, UK), rinsed three times with ddH$_2$O and once with sodium acetate/acetic acid buffer (0.1M, pH = 5) before making up to 14 mL with buffer. The pretreated material was hydrolysed by incubating (96 h, 50°C, 100 RPM) with Cellic® CTec 2 (15 µL, Novozymes). The activity of the cellulase batch used was 170 filter paper units/mL, determined following Ghose (1987). Buffer salts preclude the use of HPLC to determine monomeric glucose concentrations in digests, therefore a commercially available glucose specific oxidase/peroxidase assay was used instead (Megazyme, Ireland).
For simultaneous saccharification and fermentation (SSF), rinsed hydrothermally pretreated substrate was transferred to 20 mL screw-capped glass vials, suspended in yeast nitrogen base (2%, w/w) and autoclaved. A robust *Saccharomyces cerevisiae* strain (NCYC 2826) was grown from a slope culture (25°C, 2 days), inoculating a 0.5 L of yeast mould (YM) media, flocculated by centrifugation and made back to volume with nitrogen base. Each sample was aseptically inoculated with 1 mL of yeast and CTec 2 (15 µL) and incubated for 96 h (35°C, 100 RPM). After incubation, digests were boiled (100°C, 5 min), filtered (<0.2 um) and ethanol quantified by HPLC. Ethanol released from a substrate blank (yeast inoculum + cellulase) was subtracted from all samples.

2.3. Large scale simultaneous saccharification and fermentation

A large sample of lignocellulosic residue (7.3 Kg DW) was steam exploded (190°C, 10 min) in four batches using a Cambi™ steam explosion pilot plant (Wood, Elliston, Collins, Wilson, Bancroft, & Waldron, 2014). Samples of the liquor, containing thermally soluble components, were collected for analysis. The remaining material was washed thrice with water, separating the water-insoluble material through a nylon mesh bag (100 µm) in a low speed centrifuge. The steam exploded residue (1.3 Kg DW) was mixed with double-strength nitrogen base (1.5 L) in a high-torque bioreactor (Elliston, Collins, Wilson, Roberts, & Waldron, 2013). The reactor was heated (90 °C) and cooled to 35 °C before adding 500 mL of concentrated yeast inoculum (NCYC 2826) and dosed with 6% CTec 2 to give a final substrate concentration of 19% DW. Ethanol concentration found in the liquid was assayed after incubation (96h) in a continually stirred reactor (35 °C).

2.4. Compositional analysis of solids

Klason lignin composition was determined gravimetrically by acid hydrolysing 100 mg of sample with 72% H₂SO₄ (1.5 mL, 1h, RT) followed by dilution to 1M and incubation at 100°C, 2.5 h. The acid-insoluble residue was collected following filtration through a dry, pre-weighed sintered glass funnel (porosity 4). The residues were dried to constant mass (40°C), reweighed
and ashed in a muffle furnace (see below). The amount of acid-insoluble ash was subtracted to give the quantity of klason lignin in the material.

The ash composition of different substrates was ascertained by heating 1 g of material in dry, pre-weighed crucibles (120 °C, 2 h → 250 °C, 4 h → 500 °C, 24 h; ramping at 5, 2 and 5 °C/min respectively) and noting the weight of the residual matter.

Sugar composition of each sample (2-10 mg) was determined by acid hydrolysis (72% H₂SO₄, 3h, RT, followed dilution to 1M, 100°C, 2.5 h). Portions of the resulting solutions were analysed by gas (GC) or liquid chromatography (HPLC). For GC, acid-hydrolysates were analysed following Blakeney et al. (1983). For HPLC, samples were neutralised using 2M CaCO₃, centrifuged to precipitate the salt (2500 RPM, 3 min), and filtered (<0.2 um, Acroprep™ Filter Plates). Sugar concentrations were determined using a Flexar® FX-10 UHPLC by (Perkin-Elmer, UK) equipped with an Aminex HPX-87P carbohydrate analysis column (Bio-Rad Laboratories Ltd, UK) and refractive index detector (85 °C, mobile phase Milli-Q water, flow rate 0.6 mL/min).

Starch concentration in the original material was determined using a commercially available amylglucosidase / α-amylase based assay kit (Megazyme, Ireland).

2.5. Fourier transform infra-red spectroscopy (FT-IR)

FT-IR spectra were collected for each substrate using a dynamic alignment attenuated total reflectance FT-IR spectrophotometer (Bio-Rad FTS 175C, Bio-Rad Laboratories, Cambridge, USA), equipped with a GoldenGate™ ATR accessory. Spectra were truncated to the 800-1800 cm⁻¹ region, baseline corrected and area normalised before analysis.

2.6. Xylanase digestions following pretreatment

Release of xylan was determined by hydrolysing a sample (100 mg) of freeze-dried hydrothermally pretreated material. The sample was hydrated with 20% ethanol (200 µL, 30
min, RT), diluted with 3 mL H₂O and equilibrated to 50 °C (15 min) before adding 100 µL of xylanase from *Thermomyces lanuginosus*, diluted to 7.5U/ml with bovine serum albumin (BSA, 0.5 mg/ml). BSA was used to mask protein binding sites on labware that might lower xylanase activity. After incubation (6h, 50°C) the xylanase was inactivated (100°C, 15 min), solid pelletized (2000 RPM, 3 min) and supernatant discarded. The pellet was washed twice with H₂O, freeze dried and weight recorded. The sugar composition of the residual material following xylanase treatment was determined by GC.

### 3. Results and Discussion

#### 3.1. Effect of pre-processing on bran composition

Wheat bran was processed by sequentially removing components of varying chemistries. The original bran was initially treated with α-amylase to remove starch, followed by a protease from *Bacillus licheniformis* to remove the majority of protein (est. 70%). Maltose from starch (~15%) and peptides from protein (~12%, of which ca. 70% can be digested) could be used as nutrient rich bystreams – as nitrogen source for culture media in industrial bioreactors and/or incorporation as feed additives. The resultant (dietary) fibre residue accounted for ~53% of the original bran. This fibre residue was then treated with a xylanase from *Thermomyces lanuginosus* to solubilise component arabinoxylans (AX) as oligosaccharides with potential bioactive properties. The xylanase treatment solubilised ~20% of the fibre residue / 10% of the original bran. Amylase, protease and xylanase treatments altered the composition of the original bran, removing starch (100%), a portion of the non-starch glucan (-39%), xylan (-57%), arabinan (-61%), ash (-62%) and other components including protein (-52%). Similar quantities of Klason lignin were found in the lignocellulosic residue as found in the original bran (~5-6% w/w original bran), showing that enzymatic pre-processing did not solubilise a significant quantity of phenolic components (Table 1). Glucan and AX were retained in similar proportions to that of the original material (Table 1). The pre-extraction of potentially useful components before pretreatment, alters the chemical composition of the bran which could have important implications for downstream processing of the lignocellulosic residue.
Table 1 – Composition of original bran and lignocellulosic residue following amylase, protease and xylanase treatment. Values were calculated with a relative standard deviation < 5%.

3.2. Effect of pretreatment severity on the composition of the lignocellulosic residue

Here, two autocatalytic pretreatment methodologies were used to increase the efficiency of enzymatic saccharification - liquid hot water (LHW) and steam explosion. Both methods employ similar mechanisms for biomatrix opening, where heated water induces biomass depolymerisation (Pedersen and Meyer, 2010). Heating the biomass using steam, rather than hot water is more applicable to industry, whereas LHW is more amenable to lab-scale optimisation. The most notable difference between these two methods is release of pressure following heating – either gradually in the case of LWH pretreatment, or rapid depressurisation with steam explosion. Although it is plausible that this depressurisation may cause further disruption of the biomass, empirical evidence has shown that this has almost no effect on saccharification performance (Brownell, Yu, & Saddler, 1986). Therefore, small-scale hydrothermal pretreatment was used to establish suitable pretreatment conditions at a larger scale.

Hydrothermal (liquid hot water) pretreatment resulted in the hydrolysis and loss of non-cellulosic components (AX) from the pre-processed bran at temperatures between 160 °C and 205 °C, 10 min (Figure 2A). However, the amount of glucan present in the hydrothermally pretreated material remained similar for all pretreatment severities used in this study (Figure 2A). On a weight/weight basis, glucan was concentrated in the solid fraction following pretreatment, as other non-cellulosic components were removed. These changes are consistent with other studies, which have shown that autocatalytic pretreatments tend to hydrolyse xylans in favour of other, more stable components such as cellulose (Hendriks & Zeeman, 2009).
Pretreatment at temperatures < 170 °C did not improve enzymatic hydrolysis. At higher, temperatures, an increasing proportion of glucan retained in the hydrothermally pretreated solid could be solubilised by cellulase (Ctec2). Pretreatment conditions > 190 °C, 10 min gave similar glucose yields after saccharification (Figure 2C), leaving only a small fraction of inaccessible, insoluble glucan post-saccharification (Figure 2B).

These results are comparable to those obtained using original (Palmarola-Adrados, Choteborska, Galbe, & Zacchi, 2005) and de-starched wheat bran (Reisinger, Tirpanalan, Pruckler, Huber, Kneifel, & Novalin, 2013) which showed that optimum glucan yield could be achieved at pretreatment severities of $R_O \approx 3.36$ ($R_O = \log_{10}(\text{Time} \times \exp((\text{Temp}-100)/14.75))$, (Overend & Chornet, 1987)) - 180 °C, 10 min (Reisinger, Tirpanalan, Pruckler, Huber, Kneifel, & Novalin, 2013) or 170 °C, 20 min (Palmarola-Adrados, Choteborska, Galbe, & Zacchi, 2005) without the addition of acid catalysts. Here, optimal glucan solubilisation was achieved at similar severities (185-190 °C, 10 min, $R_O \approx 3.50-3.65$).

To ascertain whether more AX could be extracted following hydrothermal pretreatment, a second hydrolysis was conducted using xylanase from *Thermomyces lanuginosus*. The sugar composition following xylanase treatment was almost identical to that of the hydrothermally pretreated material (data not shown). This confirmed that hydrothermal pretreatment did not improve AX accessibility to xylanases further. The likely reason for this is that phenolic components are the primary limitation to AX accessibility by xylanases in this material (Robertson, Castro-Marinas, Collins, Faulds, & Waldron, 2011). Although autocatalytic pretreatments such as liquid hot water and steam explosion cause some redistribution of lignin into droplets (Li, Pu, Kumar, Ragauskas, & Wyman, 2014) this may not have been sufficient to significantly increase enzymatic AX accessibility. Therefore alkali pretreatments, which can solubilise phenolic components, may be more effective for the fractionation of this material (Pedersen & Meyer, 2010).

**Insert: Figure 2** – Sugar composition of the lignocellulosic residue after hydrothermal pretreatment at varying severities followed by enzymatic hydrolysis with cellulase. The main
carbohydrate components (Black, Glucan; White, Xylan; Grey, Arabinan) after hydrothermal pretreatment (A) and cellulase hydrolysis (B) were quantified by acid hydrolysis and HPLC. Soluble, monomeric glucose released following cellulase hydrolysis was also determined using the glucose oxidase/peroxidase assay (C). Samples of non-hydrothermally pretreated lignocellulosic residue were also analysed before (A) and after (B and C) cellulase digestion (Control).

To gain a more detailed insight into the polymeric composition of the hydrothermally pretreated lignocellulosic residue, FT-IR spectra were collected from both the hydrothermally pretreated materials and the cellulase resistant residues (Figure 3). These spectra give a fuller picture of how the composition of the lignocellulosic residue changes in response to pretreatment and hydrolysis, beyond those of more specific techniques.

Increasing pretreatment severity resulted in a loss of carbohydrate-associated bonds, particularly those associated with non-cellulosic components (=1080 cm\(^{-1}\)) including those found in original, untreated wheat bran such as arabinoxylans (970, 1040 cm\(^{-1}\)) and β-1-4 linked glycans (895 cm\(^{-1}\))(Robert, Marquis, Barron, Guillon, & Saulnier, 2005). These losses were countered by an increase in absorbance of wavenumbers associated with more recalcitrant non-cellulosic carbohydrates (1055 cm\(^{-1}\)), cellulose (1165 and 1318 cm\(^{-1}\)) proteins (1705 and 1734 cm\(^{-1}\)) (Szymanska-Chargot & Zdunek, 2013), and lignin-associated bonds (1455 and 1514 cm\(^{-1}\))(Boeriu, Bravo, Gosselink, & van Dam, 2004). These changes are consistent with other studies, which have shown that autocatalytic pretreatments tend to hydrolyse xylan in favour of other more stable components such as cellulose (Hendriks & Zeeman, 2009).

Comparing spectra collected from the cellulase-resistant pellet following hydrothermal pretreatment and enzymatic hydrolysis showed further loss of carbohydrate-associated bonds (1100-900 cm\(^{-1}\) region), as would be expected by saccharification. Non-hydrolysable components concentrated in the undigested pellet included esterified carbohydrates (1408 cm\(^{-1}\)), protein (1553cm\(^{-1}\)) and phenolic components (1184 cm\(^{-1}\)). These components could hinder
the accessibly of cellulase to cellulose and could therefore be targets for cellulase cocktail improvement for the hydrolysis of this substrate (Hu, Arantes, Pribowo, & Saddler, 2013).

Insert: Figure 3 – FT-IR Spectra of the lignocellulosic residue pretreated in liquid hot water at a range of severities. Spectra of the water-insoluble material were taken before (black) and after (grey) cellulase treatment. A sample of the lignocellulosic residue before enzymatic saccharification was also analysed (Control).

3.3. Fermentation inhibitor production from the lignocellulosic residue during hydrothermal pretreatment

When biomass is pretreated at high temperatures, the cell wall can begin to break down to form furfural derivatives and other products that could inhibit downstream processes (Jonsson, Alriksson, & Nilvebrant, 2013). When the original, untreated wheat bran, containing ≈9% starch, is hydrothermally pretreated, detectable quantities of hydroxymethylfurfural (HMF) begins to form at severities > 180 °C, 10 min (Reisinger, Tirpanalan, Pruckler, Huber, Kneifel, & Novalin, 2013). However, in this study, much higher temperatures were needed to release detectable amounts of HMF from the lignocellulosic residue (> 210 °C, 10 min; Table 2). The most likely reason for this is that much of the HMF produced at low pretreated severities (<210°C) is derived from more available hexose sources, such as starch. Therefore, in this instance, pre-extraction of readily hydrolysable forms of glucan may favour to downstream processes.

Similarly, the primary degradation product generated from pentose-sugar decomposition, 2-furfural (2FA), was only released in detectable quantities at pretreatment severities > 180 °C, 10 min (R₀ ≈ 3.36, Table 2) as opposed to 160°C, 20 min (R₀ ≈ 3.07) when pretreating de-starched wheat bran in a similar way (Palmarola-Adrados, Choteborska, Galbe, & Zacchi, 2005). This would also suggest that enzyme-labile xylan is more readily hydrolysed at lower pretreatment temperatures and downstream processes would benefit from their removal.
3.4. Simultaneous saccharification and fermentation of pre-processed wheat bran

The suitability of biomass for bioethanol production is not only reliant on the amount of sugar available for fermentation, but also the effect of other compounds released during upstream processing (Palmqvist & Hahn-Hagerdal, 2000). To understand the effect of pretreatment severity on the suitability of the substrate for fermentation, samples of the hydrothermally pretreated lignocellulosic residue were exposed to SSF conditions and ethanol yields quantified (Figure 4a).

Under the most effective conditions trialled (185°C, 10 min), a maximum of 74% of the glucan present in the lignocellulosic residue before pretreatment, could be converted to ethanol (Figure 4a). As the severity of hydrothermal pretreatment increased to 185°C, 10 min, ethanol yields also increased (Figure 4a) – reflecting trends in saccharification yields produced using the same material (Figure 2). However, if the lignocellulosic residue was hydrothermally pretreated at severities >200°C, 10 min, ethanol yields began to decline (Figure 4a), which did not match trends in glucose release (Figure 2). Thermal decomposition of glucan and production of fermentation inhibitors at temperatures exceeding 200°C is the likely reason for this trend (Jacquet, Quievy, Vanderghem, Janas, Blecker, Wathelet, et al., 2011; Palmqvist & Hahn-Hagerdal, 2000).

Insert: Figure 4 – (a) Ethanol yields released from the lignocellulosic residue pretreated at a range of severities and exposed to SSF conditions. Yields are presented as percentages of the maximum theoretical ethanol yield if all glucose present in the pre-processed material was converted to ethanol.

(b) Dose response curve for the lignocellulosic residue, steam exploded at 190°C, 10 min and hydrolysed using various doses of CTec2 (96 h). Cellulase cocktails were substituted to varying degrees (5-15%) with HTec2. Glucose yield at any cellulase dose can be estimated following the equation: Glucose yield (%) = 85.76 − 81.51*(0.2423^% cellulase dose).
3.5. Cellulase dose response curve for steam exploded pre-processed bran

To discover what cellulase concentration was sufficient to hydrolyse the steam exploded lignocellulosic residue, portions of the steam exploded solid (2% solid DW) that had been pretreated at a near-optimum severity (190 °C, 10 min), were hydrolysed at a range of cellulase doses (Figure 4b). This demonstrated that cellulase doses > 2% (weight cellulase/weight substrate) were sufficient to achieve maximum glucan conversion of 86% based on the amount in the hydrothermally pretreated lignocellulosic residue, within 96 h. As the pretreated substrate contained ≈37 ± 2% glucose (DWB) this equates to an approximate cellulase dose of 5% g enzyme/g cellulose, which is similar to doses recommended by the manufacturer.

The lignocellulosic residue contained small, but significant, quantities of xylan after pretreatment at 190 °C, 10 min. Therefore a supplementary xylanase cocktail (HTec2) was also tested to see if this could increase yields further. However, various substitutions (5, 10 and 15% v/v) of a hemicellulose-degrading accessory enzyme (Cellic HTec2, Novozymes) had no significant effect on saccharification yields or cellulase economy (Figure 4b). The most likely reason for this is that xylan removal to ≈20% of the xylan found in the pretreated material (Figure 2) is sufficient to prevent xylan from hindering cellulose hydrolysis. Alternatively, the final portion of recalcitrance xylan was resistant to enzymatic hydrolysis, even when a hemicellulase cocktail, containing enzymes of varying activities was used.

3.6. Large scale digest at high substrate concentration (≈20%)

Efficient ethanol production requires biomass to be hydrolysed and fermented at a high substrate concentration (>15% DM) (Modenbach & Nokes, 2012). Here, a larger quantity of the lignocellulosic residue (7.3 Kg) was steam exploded at conditions that produced high glucan yields, but few inhibitory compounds (190 °C, 10 min). This material was hydrolysed and fermented simultaneously with sufficient cellulase (6% DW), to assess the suitability of this material at a larger scale. This larger-scale SSF was performed in a high-torque bioreactor to ensure mixing of the substrate at this concentration (Elliston, Collins et al., 2014). The final
ethanol titre produced from the reactor was $25.27 \pm 0.01\text{g ethanol/L}$, or an equivalent of a 93% glucan conversion to ethanol.

This demonstrates that, when using a single-addition SSF regime, high glucan conversion to ethanol ($< 90\%$) can be achieved using optimal conditions but product titre is limited by the comparatively low glucan abundance in the original, previously extracted and pretreated material ($\approx15\%$ FW). To gain higher ethanol titres using this material, multiple additions of substrate after liquefaction would almost certainly be needed (Elliston, Collins, Wilson, Roberts, & Waldron, 2013). Alternatively, fermentable sugars released from the lignocellulosic residue following wheat bran biorefining may be a more suitable feedstock to produce chemicals with a higher end-product toxicity and therefore a lower product titre is needed.

4. Conclusions

To realise the full value of biomass, components of varying value must be sequentially released and converted into useful products. Low value but high volume chemicals, such as ethanol, are likely to be produced in the latter stages of the process. Processing of wheat bran to remove starch, protein and xylan influenced latter stages of pretreatment, saccharification and fermentation. Most notably, the removal of more labile carbohydrates (enzymatically-available starch and xylan) decreased the production of furfural derivatives at lower pretreatment severities. A single-addition SSF conducted at a high substrate concentrations (19%), achieved a high conversion efficiency to ethanol (93% theoretical) but ethanol titre was limited to $\approx25\text{g/L}$. This illustrates the potential benefits and limitations of using the lignocellulosic residue produced from wheat bran biorefining for ethanol production.

Competing interests

The author(s) declare that they have no competing interests.

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