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Pressurised disc refining of wheat straw as a pre-treatment approach for agricultural residues: A preliminary assessment of energy consumption and fibre composition

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Highlights

- Pressure refining wheat straw potential good route to accessing fermentable sugars
- Scalable pre-treatment technology for agri-residues
- Electricity demand around 160 kWh per tonne refined fibre DM at commercial-scale
- Heat requirement 980 – 1,900 MJ per tonne refined fibre DM
- Non-fibre content of refined straw increased with increasing refiner pressure

Abstract

This preliminary study assesses a potential pre-treatment approach for agricultural residues, in order to improve enzyme access and cellulose digestibility that increased with increasing refining pressure. Wheat straw, an important European agri-residue, was chopped then refined at pilot-scale under different pressures (4 – 10 bar) and two refiner plate configurations. The most energy efficient runs used 0.94 – 0.96 kWh electricity; 8.9 – 11.0 MJ heat per kg dry matter fibre. A scaling factor specific to the machinery used in the trial suggested that wheat straw could be refined using

approximately 160 kWh electricity and 980 – 1,900 MJ heat per tonne DM yield at commercial-scale. Hemicellulose content in wheat straw at 31.8% decreased to the lowest level of 14.6% after refining at 10 bar. Pressurised disc refining did not appear to produce significant quantities of acetic acid, a key fermentation inhibitor, that could limit microbial fermentation.

Key words:

Wheat straw, energy, pre-treatment, pressurised disc refining, cellulose digestibility

Introduction

A range of biomass pre-treatments have been developed to deconstruct the C6 and C5 polymers in lignocellulosic materials, for conversion into fermentable sugars. There are limitations to these techniques, including the high energy demands required to achieve cell wall disruption and the formation of chemical species that inhibit yeast growth, which can reduce conversion efficiencies of the downstream fermentation process. Optimising pre-treatment is therefore a key step in the commercial viability of many biotransformation processes and remains a challenge, for example, in the production of advanced biofuels.

The main aim of any pre-treatment approach for lignocellulosic biomass is to improve the physical access to cellulose by a range of enzymes, predominantly cellulases, leading to improved digestibility and hydrolysis to glucose. Cellulases are multicomponent enzyme systems produced by *Trichoderma reesei*, a filamentous fungus, and composed of endoglucanases that hydrolyse cellulose chains randomly, cellobiohydrolases that hydrolyse cellobiose from the polymer ends and cellobiases that hydrolyse cellobiose to glucose (Henrissat et al., 1985).

Cellulose, a linear, crystalline homopolymer, consisting of 10-14,000 D-glucopyranose units linked by β -1,4-glycosidic bonds, is the predominant component in plants and is located primarily in the secondary cell wall. Cellulose has an extremely complex micro-structure, consisting of fibres, fibrils and microfibrils, held together by hydrogen bonding, which results in areas of highly orientated monomers (crystalline regions) and those which are less well orientated (amorphous regions) (O'Sullivan, 1997). The

cellulose component of any biomass matrix exists as part of a complex macro-structure, composed of hemicelluloses, lignin and other carbohydrate polymers and its isolation and downstream conversion to glucose has proved to be technically challenging over a number of decades. There are a number of issues associated with the efficient pre-treatment of cellulose that are linked to its structure. These include physical barriers due to the presence of lignin and the highly complex surrounding cellular structure reducing enzyme accessibility, coupled to its stereo-regular, highly crystalline configuration, leading to a highly recalcitrant material which requires conventional acid / alkaline hydrolysis, often at elevated temperature and pressure, in order to degrade it. The more amorphous regions of the cellulose polymer are more amenable to digestion by cellulases, compared to the linear, crystalline regions.

Along with improving cellulose digestibility, there are a number of associated challenges with any pre-treatment approach for lignocellulosic biomass. These include minimising degradation and loss of monosaccharides during processing; minimising the formation of decomposition products, including small molecular weight lignin fragments, organic acids and heterocyclics (e.g. furfural), which can inhibit downstream fermentation of glucose, and ensuring that the process is both cost-effective and scalable. A number of pre-treatment methods have been used in order to improve cellulose digestibility, including physical (milling), physico-chemical (steam explosion and autohydrolysis), chemical (acid/ alkaline, AFEX, organosolv), biological (fungal) and electrical, often in combination (Kumar et al., 2009). There are associated issues with many conventional approaches, including high energy costs, effluent generation and toxicity of some of the pre-treatment methods.

The thermomechanical pre-treatment of lignocellulosic biomass uses a combination of both steam at comparatively low pressure and mechanical disc refining, to improve cellulose digestibility. This approach softens the biomass using steam, facilitating easier processing for more recalcitrant materials and cellular disruption during the depressurisation stage, coupled with a mechanical processing step which opens up the macro-cellular structure through a combination of cutting, shearing and compressive forces (Gharehkhania et al., 2015), leading to improved enzyme accessibility.

Mechanical disk refining (MDR), typically with a hydrothermal or chemical pre-treatment step, has been proposed as one approach that may reduce the energy

intensity of biomass pre-treatment when viewed relative to sugar yield. Jones *et al.* (2014) reported that MDR at atmospheric pressure significantly improved enzymatic hydrolysis of chemically pre-treated hardwood pulp. Refining at industrial scale (42-inch disc plates) sugar conversion improved from 36% (for unrefined pulp) to 65% at 5 FPU/g of biomass. They reported refiner energy use at this scale as 67 kWh/t. Chen *et al.* (2014) demonstrated that MDR of corn stover, when employed after an initial dilute alkali deacetylation step, resulted in low concentrations of fermentation inhibitors. Their approach achieved up to 84% monomeric glucose yield after high solids enzymatic hydrolysis at moderate enzyme loadings (20 mg CTec 3 and 2.5 mg HTec3 / g cellulose). Milling energy for their 36-inch small-commercial scale refiner ranged from 128 – 468 kWh / ODMT (Oven Dry Metric Ton), and subsequent techno-economic analysis suggested that this approach had potential to be highly cost-effective in a bio-refinery scenario (Chen *et al.*, 2015).

Continuous pressurised MDR offers an alternative to atmospheric MDR and has the potential benefit of removing the need for a chemical pre-treatment step. Previous research has shown that any technology including steam refining which decreases the particle size has a profound effect on enzymatic accessibility (Schütt *et al.*, 2012). However, fewer studies have looked at pressurized MDR, even though the approach has been employed in the pulp and paper industry for many years in order to break up the fibre bundles for paper production. It is a proven and scalable technology, capable of processing up to 1,500 tonnes of biomass per day (Park *et al.*, 2016), and is generally run at lower temperatures and pressures than comparable technologies (such as steam explosion). Compared with atmospheric MDR, the technology carries an additional requirement for heat (to produce steam for pressure) but this is typically a lesser input, in terms of the carbon balance, when compared against the electricity demand of refining, especially when it is generated from biomass combustion (Skinner *et al.*, 2016).

There have been a limited number of reports on the application of pressurised MDR in relation to optimising cellulose accessibility to enzymes and facilitating hydrolysis to monomeric sugars. Koo *et al.* (2011) demonstrated that the approach enhanced enzymatic hydrolysis of hard and softwood residues, while Gonzalez *et al.* (2011) worked with wheat straw, corn stover and sweet sorghum bagasse. The latter found that carbohydrate yields using pressurised MDR were relatively similar for each

feedstock under the same refining conditions and enzyme loading. Fang *et al.* (2011) also working with wheat straw, investigated the effect of varying steam pressure and retention time on either auto-hydrolysis or dilute acid pre-treated biomass. Optimum conditions for their auto-hydrolysed sample were 198⁰C and 4 minutes retention time, yielding 88.4% total glucose.

The optimisation of pressurised MDR for biochemical conversion processes is still at an early stage and none of the published studies assessed wheat straw fibre throughput / sugar yield relative to energy consumption. Zhu *et al.* (2009) have reported a correlation between energy usage and glucose conversion for wood. Park *et al.* (2016) have reviewed refining variables that play a role in biomass throughput and conversion efficiency and reported that 100 kWh / ODMT may be a realistic figure for the optimised commercial-scale refining of lignocellulosic biomass.

Wheat straw is an attractive feedstock for second generation biofuels and platform chemical production, because it exhibits reasonably consistent levels of cellulose and other components across different varieties, that could lead to relatively high levels of monosaccharides (Collins *et al.*, 2014). A few studies have examined pressurised refining as an approach to improve the deconstruction of straw, in order to facilitate downstream hydrolysis of the cellulose fraction (Fang *et al.*, 2011; Han *et al.*, 2001; Pan *et al.*, 2008). The use of pressurised MDR could involve less severe conditions, resulting in lower concentrations of fermentation inhibitors and requiring lower energy inputs (Park *et al.*, 2016). The effect of pressure is an important parameter within many different physicochemical techniques used to breakdown wheat straw (Talebnia *et al.*, 2011). However, one potential problem is that lignin melts during pre-treatment at higher temperatures and is redeposited onto the surface of fibres (Gustafsson *et al.*, 2003). This could reduce the effectiveness of the hydrolysis step by limiting cellulase access to cellulose, during the hydrolysis step.

In this study, refiner energy consumption and resulting fibre composition are reported for a range of experimental conditions using wheat straw as a feedstock. Energy consumption relative to throughput of the refined fibres is investigated. This is done for different refiner plate configurations and refining pressures, with a view to establishing the potential processing energy requirement of MDR, when used as a pretreatment step for onward hydrolysis and conversion into a range of downstream products.

Materials and methods

Pressurised disc refining

Wheat straw was harvested in the east of England and transported to the BioComposites Centre's Biorefining Technology Transfer Centre (Anglesey, Wales). The straw was processed using a Pierret N40 forage chopper set to a 0.5 inch (12.7mm) cut length. The chopped straw was then fed into an Andritz Sprout-Bauer 12 inch (30.5cm) pressurised refiner through a hopper. The biomass initially entered a screw feeder or modular screw device where a biomass 'plug' was formed and the desired refining pressure reached. The plug was fed into a heated cooker screw for a nominal residence time of 60 seconds, and subsequently into a 60 litre digester for the same duration and at the selected pressure. The steam treated biomass was then fed through a screw feed into the refining zone, consisting of two 12-inch diameter refining plates, one of which was fixed in position while the other rotated. The refined fibre was then diverted into a blow line and into the collection area through a cyclone, where it was bagged and weighed ready for onward analysis.

The refining trials were run over three sessions, during which a total of 11 run samples were completed. Trial 1 used 'low intensity' refining plates, similar in design to the Andritz D2-516 plates and typical of those used in medium density fibreboard production. Refining runs were carried out at 4, 6, 8 and 10 bar, maintaining a disk speed of 2,500 rpm and a plate gap of 4 μm throughout. Trial 2 followed the same pressure, plate gap and disk speed conditions but used different refiner plates. These were Andritz D2-503 plates, which have a bi-directional pattern and a bar angle of 0° (bar width: 1.60 mm; groove width: 7.14 mm; bar height: 2.39 mm). They are designed with more emphasis on grinding. The final trial repeated the conditions used in trial 1 for 6, 8 and 10 bar. Run samples ranged from 15 – 44 minutes in duration.

Energy monitoring

Throughout the trials, electricity consumption was recorded for each piece of equipment using OWL +USB energy monitors and data loggers. The monitors were hardwired to the machinery and provided real-time energy usage data for each refiner run. Following the trials, the data was downloaded using proprietary software (OWL

Energy Monitoring Systems) and exported into Excel for analysis. Gas consumption, for the steam boiler, was recorded using a dedicated 50 mm gas flow meter (Aichi Tokei TBX series) attached to the boiler inlet pipe.

Fibre analysis

The samples were oven dried at 70°C for 24 h and the preparation for fibre analysis was performed as previously described by sequential extraction in the Ankom machine to determine neutral detergent fibre, acid detergent fibre and acid detergent lignin (Baker et al., 2015). The method differed from the standard Ankom procedure in determining the ash content separately in a furnace at 600°C for 4 h rather than at the end of the lignin analysis. The fibre concentrations were adjusted to reflect ash content. The material after enzyme hydrolysis was collected on a fine nylon mesh, transferred to a glass vessel and dried at 70°C for 24 h. Fibre analysis was performed on the dried material to determine the amount of fibre remaining after hydrolysis.

Hydrolysis

Cellulose digestion was achieved using 25 ml phosphate citrate buffer, pH 4.8 containing 525 µl Celluclast, 1.5L was added to duplicate samples of 0.5 g wheat straw or refined wheat straw. The amount of cellulases added was equivalent to 10 FPU, which was independently determined using the method of Ghose (1987) as previously described. The buffer solution containing Celluclast 1.5L was prepared as a bulk solution to ensure similar concentrations in each flask. These flasks were weighed and then incubated at 50 °C with shaking at 150 rpm. After 72 h, the flasks were weighed to determine the volume of moisture that had evaporated from the flasks and then 1 ml aliquots were taken from each flask. These samples were centrifuged at 13,000 rpm for 5 min and the sugar contents were determined by DNSA on the supernatants. Sugar analysis of 0.2 µm filtered samples revealed similar concentrations of sugars to those samples which had been centrifuged but had not been filtered.

Sugar contents were determined using a modification of Miller's method (1959) which involved using 100 µl of each supernatant and 300 µl DNSA solution [3,5-Dinitro-2-hydroxybenzoic acid (0.5 g) in 2 M NaOH (20 ml) and sodium potassium tartrate (100 ml of 20% (w/v))]. This was heated at 100 °C for 5 min with shaking at 300 rpm and then the samples were immediately placed on ice for 5 min. Into a microplate, 100 µl of

each sample was dispensed and measured at 540 nm. The sugar concentrations were calculated from a standard curve using glucose. A standard curve using xylose yielded similar results to glucose. The total sugar yield per g dry biomass was calculated by multiplying with the volume of buffer remaining.

The theoretical glucose is calculated from the percentages of hemicellulose and cellulose which are multiplied by 1.1111. The reason for this number is that each glucose unit in the polysaccharide has increased by one water molecule during the hydrolysis reaction. The theoretical pentose content, which mostly comprises of xylose, must be multiplied by 1.136 to reflect the increase during hydrolysis. An assumption is made based on a previous publication that the proportion of xylose present in the hemicellulose of wheat straw amounts to 74.4% (Collins *et al.* 2014; Reddy *et al.* 1983). Most of the other sugars such as galactose have molecular weights identical to glucose and only arabinose (a pentose) has a lower molecular weight although the concentrations of this are very low. The sum of these glucose concentrations are added to the amount of free sugars determined in the wheat straw samples; again an assumption is made that this is mostly glucose.

Acetic acid determination

Acetic acid concentrations were determined based on the inhibitory effect of acetic acid on the acetate assay when high concentrations of acetate added to the assays (BioVision), which showed an inverse linear relationship with acetic acid concentrations ranging from 0.04-0.1%. Outside these ranges, it was assumed that ratio values greater than one contained no acid compared with standard concentrations of acetic acid. Standard inhibition assays were prepared containing 92 μ l acetate buffer with 10 mM acetate and acetic acid concentrations ranging from 0-1% in 0.02% increments. The proportion of absorbance that was detected in the presence of acid (AD) was calculated from the absorbance measurement at 450 nm, in the presence of a known concentration of acid divided by the expected absorbance measurement for the sample without acid. The relationship between this calculated value and acetic acid concentrations was inversely linear between acetic concentrations from 0.04 - 0.1%. Outside these ranges, it was assumed that ratio values greater than one contained no acid. The assay was performed using the highest concentration of acetate at 10 mM acetate (1 ml). To 50 mg of refined wheat of sample, 1 ml of the buffer was added to the

pressure refined samples and 2 ml of buffer was added to 50 mg of milled wheat straw. The latter samples required a greater volume because much of the buffer was absorbed by the sample. The wetted samples were ground with pestle and mortar until only fine particles were present. The milled wheat straw required 2 ml acetate buffer containing 10 mM acetate in order to form a liquid suspension after grinding. The assays in pre-weighed Eppendorf tubes were performed using 92 μ l of the supernatant along with 2 μ l each of enzyme mix, substrate buffer, probe and ATP. The assay was incubated at 25 °C with shaking at 300 rpm in the dark for 40 min. The suspensions were centrifuged at 9800 xg for 5 min and aliquots were removed from the supernatant for measurement at 450 nm using a microplate reader. All the liquid was removed using a pipette and the wet weight of the biomass was determined. The tubes were dried at 70 °C for 24 h and reweighed to determine the dry weight of the biomass. All of the samples contained a minor yet similar concentration of acetate indicating that the initial concentration of acetate was slightly higher than 10 mM. Therefore, AD for each of the pressure refined samples was calculated by dividing by the absorbance determined with unrefined wheat straw containing 10 mM acetate rather than by dividing by the absorbance of the samples with 10 mM acetate, in order to factor in this variance. The concentrations of acetic acid in each of the tubes were found from the graph using the AD value at the point of intercept. The concentration of acetic acid in the wet and dry samples was calculated by multiplication of the acetic acid concentration, the total water content present in the extracted sample and the reciprocal of the total wet or dry mass of wheat straw in each sample.

Results and discussion

Energy analysis

Over the eleven refining runs, the refiner electricity consumption ranged from 0.82 – 1.41 kWh / kg DM fibre (table 1). The electrical consumption generally remained consistent over the different refining pressures when using the 516 plates, whereas consumption was higher at the lower refining pressures when using the 503 plates. Longer runs appeared to yield similar results when using the 516 plates although the 503 plates were not examined which showed much greater variation.

The most efficient configurations in terms of refiner energy consumption were runs 2 (516 plates, 6 bar pressure) and 8 (503 plates, 10 bar pressure) though further trials would be needed to establish whether differences observed between the runs were significant. When electricity usage by the forage chopper and steam boiler is factored in the observed pattern remained, with total electrical energy usage for runs 2 and 8 being 0.94 and 0.96 kWh / kg DM fibre yield respectively.

Table 1: Refining trial results; energy figures 'per kg DM fibre' [e = electricity, h = heat]. 516 = Andritz D2-516-style plates; 503 = Andritz D2-503 plates. Disk speed 2,500 rpm and plate gap width 4 μ m for all runs.

Run	Plate type	Pressure (bar)	Duration (mins)	Yield (kg DM)	Chopper (kWh/kg)	Boiler (e) (kWh/kg)	Boiler (h) (MJ/kg)	Refiner (kWh/kg)	Total elec. (kWh/kg)
1	516	4	15	6.21	0.03	0.09	6.86	1.01	1.13
2	516	6	15	7.28	0.03	0.09	8.09	0.82	0.94
3	516	8	15	6.50	0.03	0.13	11.25	0.86	1.02
4	516	10	15	6.86	0.03	0.15	13.66	0.90	1.09
5	503	4	15	4.63	0.03	0.10	-	1.41	1.55
6	503	6	15	4.81	0.03	0.14	-	1.20	1.37
7	503	8	15	5.43	0.03	0.13	12.11	1.05	1.20
8	503	10	15	7.21	0.03	0.12	10.99	0.81	0.96
9	516	6	30	15.32	0.03	0.09	8.63	0.92	1.04
10	516	8	30	15.66	0.03	0.10	10.35	0.90	1.04
11	516	10	44	23.08	0.03	0.11	11.58	0.92	1.06

The choice of refiner plates appeared to effect refining efficiency in terms of throughput of biomass per unit electricity. At lower pressures (4 – 8 bar), the 516 plates gave notably better results in our trials, while at 10 bar, the 503 plates performed marginally better (figure 1a). Again, further trials would be needed to establish whether this pattern was repeated. The total electrical energy burden of the forage chopper was 2 – 4% of total electricity usage across all runs, while for the steam boiler, usage rose with

increasing refining pressure. It was evident from a previous review that there was a minor increase in energy requirements during mechanical reduction, especially with wheat straw compared with other lignocellulosic substrates (Bhutto et al., 2017). At the lowest pressures (4 bar), steam boiler usage was equivalent to 7 – 8% of total electricity usage for those runs, while at 10 bar the contribution had risen to 10 – 14%. This suggests that equipment that is ancillary to the main refining machinery should not be overlooked when energy balances are being considered.

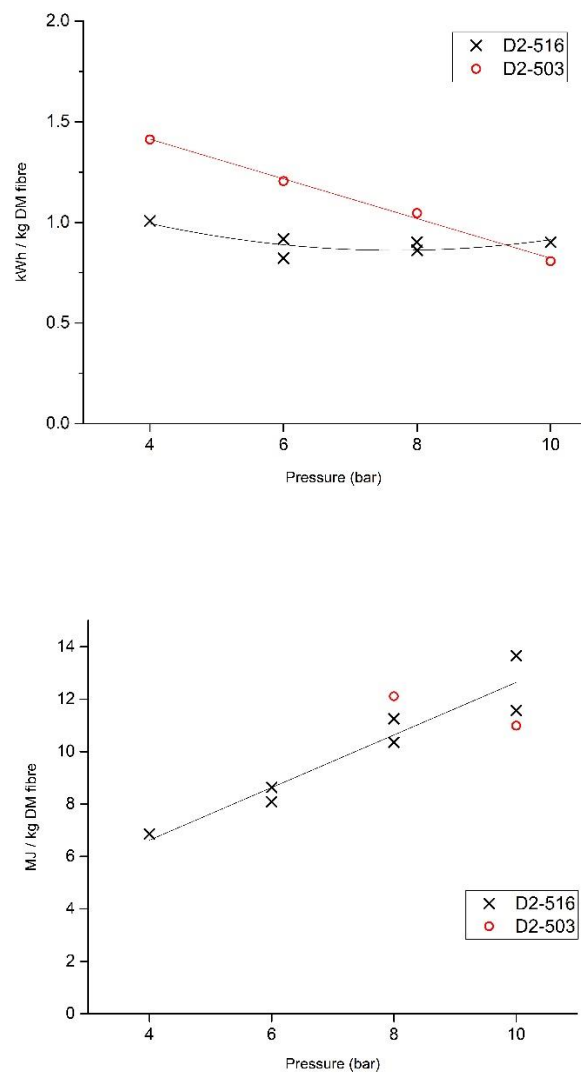


Figure 1: (A) Refiner electricity consumption and (B) heat energy required for steam generation at different refining pressures, per kg refined fibre (dry matter)

Gas consumption for heat energy (to produce steam) was collected for 9 of the trial runs. This was seen to increase with increasing pressure requirement, as may be expected. Across the 11 trial runs, heat energy consumption ranged from 6.9 to 13.7 MJ per kg DM fibre (figure 1b). For the two most electrically efficient runs (runs 2 and 8), the heat requirement was 8.1 and 11.0 MJ per kg respectively. This would make run 2 (516 plates, 6 bar pressure) the most energetically efficient of those trialled.

The equipment used in this study was pilot-scale plant designed for research purposes and therefore not reflective of optimised commercial-scale production in terms of achievable energy efficiency. However, data previously published using this equipment allows a tentative scaling factor to be calculated for this purpose. Skinner *et al.* (2016) assessed electrical energy consumption for softwood refining using this equipment and found that the plant used 2.0 kWh / kg DM fibre for this purpose (including the fibre drying components). This compares with data published by Rivela *et al.* (2007) for the same processes at modern, large-scale medium density fibreboard factories, which reported an equivalent figure of 0.346 kWh per kg DM fibre. This suggests that a 'tentative' scaling factor of 5.8 may be realistic for projecting from the plant used here to a commercial-scale operation. There are some limitations with this (in particular, it assumes that energetic economies of scale will apply equally to the refining and drying components assessed in these studies; though the large-scale data also included a minor input (chipping) that was not present in the pilot data), however it allows for an estimate of achievable energy efficiency to be made. Using this scaling factor, electricity for refining wheat straw at optimised, commercial-scale may be in the region of 160 kWh / tonne DM fibre, for the refiner and steam boiler combined.

At commercial scale, energy for heat (to generate steam) was 1.7x that required for electricity for refining (Rivela *et al.* 2007). The pilot-scale trials were broadly in keeping with this relationship, with energetic demand for heat relative to that for electricity ranging from 1.7x in the most heat-efficient scenario (run 1, at 4 bar), to x3.3 for the most heat-efficient scenario at high pressure (run 8, 10 bar). If the same scaling factor is

assumed, then this would tentatively suggest that heat demand at commercial-scale could be in the region of 980 – 1,900 MJ / tonne DM fibre.

Fibre analysis

The effects of pressure refining were evident from the fibre digestion analysis. The non-fibre content of the pressure refined wheat straw appeared to increase with increasing refining pressure, in comparison to the wheat straw before pre-treatment (figure 2). Statistical analysis revealed that the non-fibre component of the refined wheat straw, using the 516 plates at 4 bar, was significantly lower compared with the wheat straw before refining. In contrast, the non-fibre contents in the refined samples at 8 bar and 10 bar were significantly higher compared with original wheat straw before pressure refining. In addition, the non-fibre content of the refined samples at 10 bar was significantly higher compared with samples recovered at 8 bar. These results correlate well with another study using sugar cane bagasse, which showed an increase in extractive content (Wang et al., 2018). In contrast, other studies using wheat straw showed the opposite trend to our study where the non-fibre content showed a minor decrease and fibre content increased (Gonzalez et al., 2011; Han et al., 2009). The fibre content was measured directly in our study whereas in other studies the fibre content was determined from HPLC analysis of sugars after acid solubilisation. It is most likely that the non-fibrous portion contained soluble sugar compounds.

The hemicellulose content appeared to decrease with increasing refining pressures and statistical analysis indicated that the hemicellulose content in all of the pressure refined samples were significantly lower compared with un-treated wheat straw, except for one sample which was material refined at 4 bar using the 516 plates. The decrease in hemicellulose content was expected based on similar findings from a previous publication (Sun et al., 1998) indicating that hemicelluloses were being decomposed into smaller molecules that appeared in the non-fibrous component. A more recent study using a tubular reactor to reduce particle size reported that a higher proportion of xylan, 70%, was decomposed into xylosaccharides compared with corn stover or sugar cane bagasse (Rodríguez et al., 2019). Pressurised refining of wheat straw indicated that incremental increases of 2 bar resulted in significant decreases in hemicellulose contents at pressures above 6 bar. However, a comparison of experiments conducted

using two different refiner plate configurations (D2-516 and D2-503) revealed that there were no significant differences in hemicellulose contents. In general, the hemicellulose content determined from pressure refined wheat straw at 4 bar and 6 bar were not significantly different except the hemicellulose content using the 503 plates at 6 bar was significantly lower compared with the hemicellulose content recovered when the 516 plates at 4 bar.

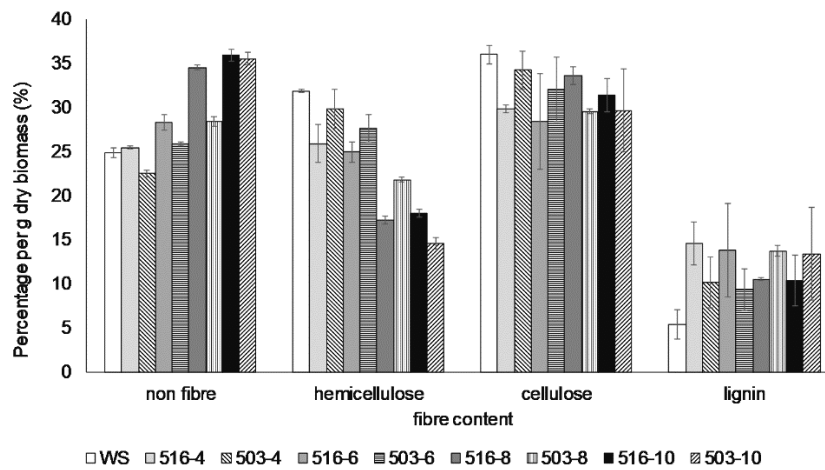


Figure 2: Fibre analysis of the reference unrefined wheat straw (WS), and the refined wheat straw using the 516 and 503 plates at pressures 4 – 10 bar.

Statistical analysis of cellulose contents revealed that none of the refined samples showed any significant differences between samples or compared with the wheat straw before pressure refining. Although the lignin content appeared to increase, only the pressure refined wheat straw using the 503 plates at 8 bar was significantly different, compared with the un-treated wheat straw before refining. The increase in lignin content could be attributed to the decrease in biomass due to the loss of volatile compounds through combustion. Previous studies have shown an increase in ash content indicating significant biomass loss during pressure refining (Fang et al., 2011). However, the effect of high temperature during processes such as steam explosion has been shown to have a minor effect in decreasing the ash content of wheat straw (Han et al., 2009). Calculations based on the increase in lignin content indicated that $8.8 \pm 1.5\%$ was lost using the 516 plates and $15.3 \pm 2.2\%$ was lost using the 503 plates. In addition,

there were no evident differences between the different refining conditions. The lower concentrations of ash contents led to much higher and variable predictions of mass losses. Another effect of pressurised refining which was not measured in this study are decreases in the diameter of fibres (Han et al., 2001, Pan et al., 2008), presumably due to the degradation of the middle lamella and S1 layer (Park et al., 2016; Valenzuela et al., 2012). In addition, the percentage of non-fibrous compounds which are composed of soluble, small molecular weight compounds that do not form any part of the structural integrity of the plant cell wall material, showed an increase particularly with higher refining pressures. It can be assumed that these compounds are formed through the decomposition of hemicellulose, which clearly showed a decrease, and cellulose which did not increase as with lignin during biomass loss of volatiles. It would appear that some cellulose was decomposed during mechanical refining whereas lignin remained largely intact.

The results show that increasing quantities of sugars were released from each gram of wheat straw with increasing refining pressure using both types of refining plates (figure 3a). The quantity of sugars associated with 516 plates was significantly higher at 8 bar and 10 bar compared with wheat straw processed at pressures below 6 bar. In contrast, the quantity of sugar associated with the 503 plates was significantly higher at 8 bar and 10 bar compared with the wheat straw that was not pressure refined. However, there were no significant differences between the two different plates even though there were apparent differences when using SPSS analysis. This may be because only duplicate samples were analysed and the analysis of higher number of replicates would have shown more significant. Statistical analysis using tTest revealed that samples processed at 6 and 8 bar were significantly higher when the 516 plates were used compared with the 503 plates. It is difficult to determine why sugar concentrations appeared higher when using the 516 plates but visual examination of the flasks containing the hydrolysis reactions showed clumps being formed with the fibres created using the 503 plates, whereas the fibres created using the 516 plates were evenly distributed. Experiments performed where better mixing was achieved revealed that there was little difference in sugar concentrations after hydrolysis of the fibres that were created using both plates (data not shown). Therefore, possibly the enzyme activity may have become reduced because mixing was significantly affected. The effects of processing indicated that significantly higher quantities of sugar was associated with

the wheat straw that was not pressure refined compared with the wheat straw pressure refined at 8 and 10 bar (figure 3b). However, there were no significant differences when the sugars resulting from both plates were compared. In comparison, mechanical refining of hardwoods under optimal conditions showed that 90% of sugars were released through hydrolysis, albeit with higher energy requirements of about 1.5 kWh/kg (Jones et al., 2017).

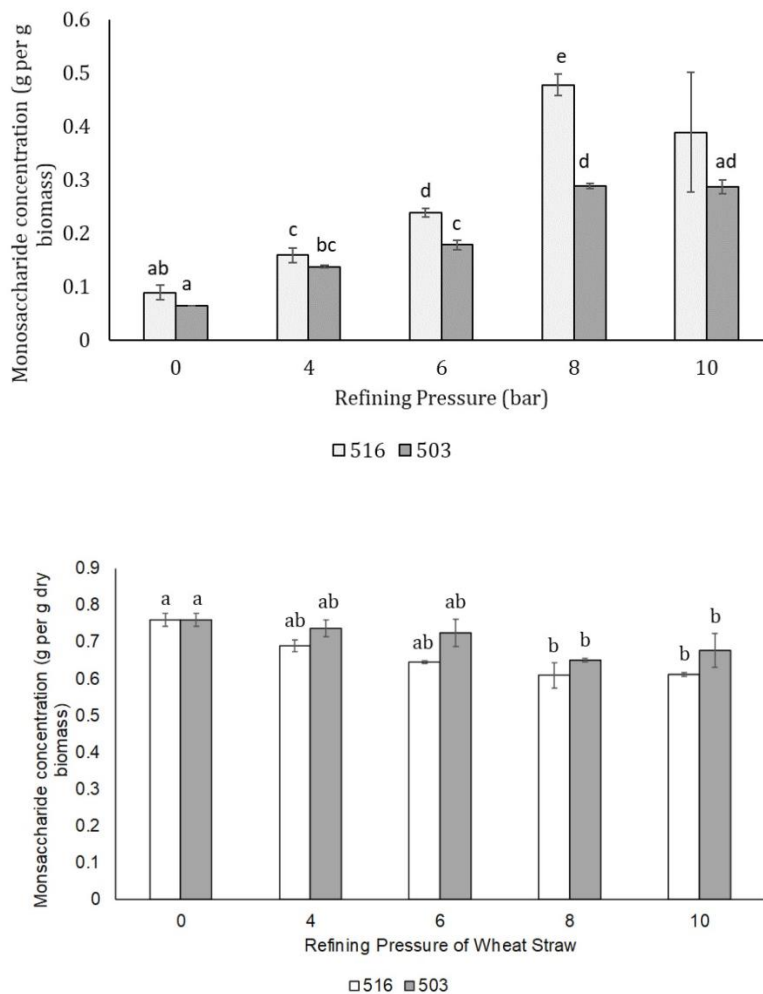


Figure 3: (A) Hydrolysis of wheat straw using Celluclast 1.5L for 72 h. Bars with the same letters are not significant and those with different letters are significant at $P < 0.05$. The pressure refined sample at 10 bar using plate 516 was excluded from the analysis because error was so large. (B) Total theoretical quantity of monosaccharides present in each gram of wheat straw. Bars with the same letters are not significantly different and those with different letters are significantly different at $P < 0.05$.

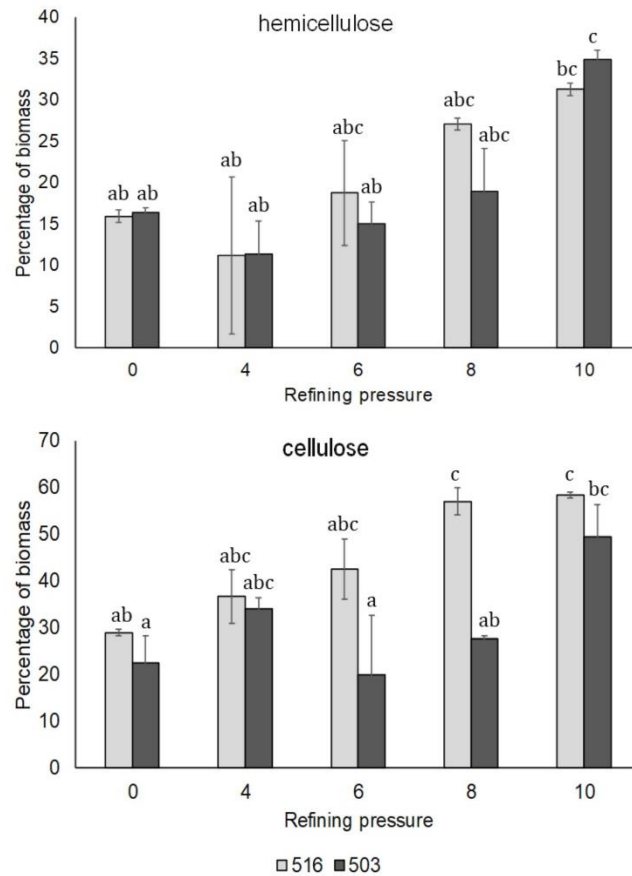


Figure 4: The proportion of hemicellulose and cellulose hydrolysed using Celluclast 1.5L as determined by fibre analysis. Bars with the same letter are not significantly different and bars with different letters are significantly different at $P < 0.05$.

Hydrolysis of hemicellulose gradually increased with refining pressure from about 15% with the non-pressure refined wheat straw to about 35% with the wheat straw pressure refined at 10 bar (figure 4). Twice as much cellulose was hydrolysed, which showed much clearer increases with refining pressure from about 30% with the un-refined wheat straw, to about 60% with wheat straw pressure refined at 10 bar using the 516 plates. The degree of hydrolysis of the wheat straw refined at 6 and 8 bar using the 503 plates was lower compared with the wheat straw pressure refined using the 516 under the same conditions. These results generally reflected the concentrations of monosaccharides that were detected after hydrolysis of the wheat straw under each of the pressure refining conditions. There are a number of important factors in the

hydrolysis of cellulose such as absorption and desorption of the enzymes and product inhibition of cellobiose by-products (Sun and Cheng, 2002). In addition, crystallinity index of one of the most important factors determining enzymatic hydrolysis (Peng et al., 2013). It must be assumed that some proportion of cellulases will enter into the vessels of milled wheat straw and under capillary forces are unlikely to detach. However, cellulases may be more readily detached under shear rotational forces when attaching to the surface of exposed pressure refined wheat straw fibres.

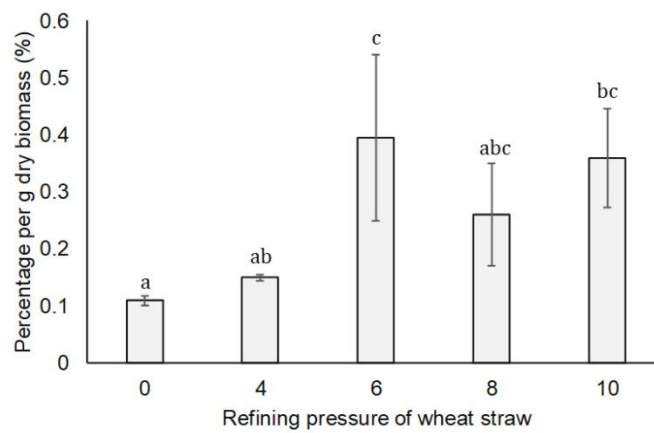


Figure 5: Concentration of acetic acid per g dry biomass in samples processed using the 503 plates. Statistical analysis using Tukey's posthoc test showed no significant differences due to the high error bars. Therefore, statistical analysis was performed using LSD posthoc test, which indicated possible differences. Bars with the same letters are not significantly different and those with different letters are significantly different at $P < 0.05$.

The acetic acid concentration, produced through partial hydrolysis of the hemicellulose fraction in the wheat straw during the refining process, appeared to be much higher at pressures above 6 bar compared with the unrefined wheat straw (figure 5). These values are similar to those obtained in a pre-hydrolysis study which involved autoclaving wheat straw at 140°C for up to 1 h (Brezániová et al., 2016). Much higher values were obtained when only wheat straw was processed at temperatures above 180°C (Brezániová et al., 2016; Ibbett et al., 2014). The concentrations of other inhibitors in wheat straw processed under similar conditions to our study, such as furfural, are at similar concentrations to acetic acid, whereas formic acid and HMF are

lower (Brezániová et al., 2016; Ibbett et al., 2014). The concentrations of furfural become a little higher than acetic acid concentrations when temperatures are raised further (Park et al., 2016). Therefore, it would appear that the measurement of acetic acid concentrations is a good indicator of inhibitor formed during the mechanical process and this is likely to have the greatest effect on the growth of yeast under anaerobic conditions (Palmqvist, and Hahn-Hägerdal, 2000). It appeared that monosaccharides released through hydrolysis increased with pressure refining conditions until 8 bar. Indeed, statistical analysis showed that these monosaccharides were significantly higher at $P > 0.05$ with the pressure refined samples using the 516 plates at 8 and 10 bar compared with the unrefined wheat straw. However, it would appear that the concentrations of acetic acid produced during the refining process are low and are unlikely to inhibit microbial conversion of the released monosaccharides during downstream fermentation.

Conclusion

Significantly higher fibre breakdown and subsequent hydrolysis occurred at the highest pressures examined in this study (10 bar) and at these pressures energy requirements between the two plates were similar. Under the most energy efficient conditions, 0.94 – 0.96 kWh electricity and 8.9 – 11.0 MJ heat were used per kg DM fibre at pilot-scale. A tentative scaling factor based on published data specific to the pilot machinery used here, and comparable commercial-scale data for the same operation, suggests that an optimised plant could refine chopped straw for approximately 160 kWh electricity and 980 – 1,900 MJ heat per kg DM yield.

E-supplementary data for this work can be found in e-version of this paper online.

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References

1. Baker, P. W., Charlton, A., Hale, M. D., 2015. Increased delignification by white rot fungi after pressure refining Miscanthus. *Bioresour. Technol.*, 189, 81-86.
2. Bhutto, A.W., Qureshi, K., Harijan, K., Abro, R., Abbas, T., Bazmi, A.A., Karim, S., Yu, G., 2017. Insight into progress in pre-treatment of lignocellulosic biomass. *Energy*, 122, 724-745.
3. Brezániová, Z., Fišerová, M., Stankovská, M., Gigac, J., Opálená, E., Puškelová, J. (2016). Effect of prehydrolysis on enzymatic hydrolysis of two-stage pretreated wheat straw. *Wood Res.* 61, 697-708.
4. Chen, X., Shekiro, J., Pschorn, T., Sabourin, M., Tao, L., Elander, R., Park, S., Jennings, E., Nelson, R., Trass, O., Flanagan, K., Wang, W., Himmel, M.E., Johnson, D., Tucker, M.P. 2014. A highly efficient dilute alkali deacetylation and mechanical (disc) refining process for the conversion of renewable biomass to lower cost sugars. *Biotechnol. Biofuels*, 7, 98.
5. Chen, X., Shekiro, J., Pschorn, T., Sabourin, M., Tucker, M.P., Tao L. 2015. Techno-economic analysis of the deacetylation and disk refining process: characterizing the effect of refining energy and enzyme usage on minimum sugar selling price and minimum ethanol selling price. *Biotechnol. Biofuels* 8, 173.
6. Collins, S. R., Wellner, N., Bordonado, I. M., Harper, A. L., Miller, C. N., Bancroft, I., Waldron, K.W., 2014. Variation in the chemical composition of wheat straw: the role of tissue ratio and composition. *Biotechnol. Biofuels* 7, 121.
7. Fang, H., Deng, J., Zhang, X., 2011. Continuous steam explosion of wheat straw by high pressure mechanical refining system to produce sugars for bioconversion. *BioResources* 6, 4468-4480.
8. Gharehkhani, S., Sadeghinezhad, E., Kazi, S. N., Yarmand, H., Badarudin, A., Safaei, M. R., Zubir, M. N. M., 2015. Basic effects of pulp refining on fiber properties—A review. *Carbohydr. Polym.* 115, 785-803.
9. Ghose, T. K. 1987. Measurement of cellulase activities. *Pure Appl. Chem*, 59, 257-268.

10. Gonzalez, R., Jameel, H.; Chang, H., Treasure, T., Pirraglia, A., Saloni, D., 2011. Thermo-mechanical pulping as a pretreatment for agricultural biomass for biochemical conversion. *BioResources* 6, 1599-1614.
11. Gustafsson, J., Lehto, J.H., Tienvieri, T., Ciovica, L., Peltonen, J., 2003. Surface characteristics of thermomechanical pulps; the influence of defibration temperature and refining. *Colloid Surface A* 225, 95–104.
12. Han, G., Kawai, S., Umemura, K., Zhang, M., Honda, T., 2001. Development of high-performance UF-bonded reed and wheat straw medium-density fiberboard. *J. Wood Sci*, 47, 350-355.
13. Han, G., Cheng, W., Deng, J., Dai, C., Zhang, S., Wu, Q., 2009. Effect of pressurized steam treatment on selected properties of wheat straws. *Ind. Crops Prod.* 30, 48-53.
14. Henrissat, B., Driguez, H., Viet, C., Schülein, M., 1985. Synergism of Cellulases from *Trichoderma reesei* in the Degradation of Cellulose. *Nat. Biotechnol.* 3, 722–726.
15. Ibbett, R., Gaddipati, S., Greetham, D., Hill, S., Tucker, G., 2014. The kinetics of inhibitor production resulting from hydrothermal deconstruction of wheat straw studied using a pressurised microwave reactor. *Biotechnol. Biofuels* 7, 45.
16. Jones, B.W., Venditti, R., Park, S., Jameel, H. 2014. Comparison of lab, pilot, and industrial scale low consistency mechanical refining for improvements in enzymatic digestibility of pretreated hardwood. *Bioresour. Technol.* 167, 514–520.
17. Jones, B.W., Venditti, R., Park, S., & Jameel, H., 2017. Optimization of pilot scale mechanical disk refining for improvements in enzymatic digestibility of pretreated hardwood lignocellulosics. *BioResources*, 12, 4567-4593.
18. Kim, S.M., Dien, B.S., Singh, V., 2016. Promise of combined hydrothermal/chemical and mechanical refining for pretreatment of woody and herbaceous biomass. *Biotechnol. Biofuels* 9:97.
19. Koo, B-W., Treasure, T.H., Jameel, H., Phillips, R.B., Chang H., Park, S., 2011. Reduction of Enzyme Dosage by Oxygen Delignification and Mechanical Refining for Enzymatic

Hydrolysis of Green Liquor-Pretreated Hardwood. *Appl. Biochem. Biotech.* 165, 832–844.

20. Kumar, P, Barrett, D.M., Delwiche, M.J., Stroeve, P., 2009. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Ind. Eng. Chem. Res.* 48, 3713-3729.

21. Miller, G. L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.*, 31, 426-428.

22. O'Sullivan, A.C., 1997. Cellulose: the structure slowly unravels. *Cellulose* 4, 173–207.

23. Palmqvist, E., Hahn-Hägerdal, B., 2000. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. *Bioresour. Technol.* 74, 25-33.

24. Pan, M.Z., Zhou, D.G., Mei, C.T., Deng, J., Wang, X.M., Zhang, T.S. 2008. Effects of thermomechanical refining conditions on the morphology and thermal properties of wheat straw fibre. *Holzforschung* 62, 338-343.

25. Park, J., Jones, B., Koo, B., Chen, X., Tucker, M., Yu, J-H., Pschorn, T., Venditti, R., Park, S., 2016. Use of mechanical refining to improve the production of low-cost sugars from lignocellulosic biomass. *Bioresour. Technol.* 199, 59–67.

26. Peng, H., Li, H., Luo, H., Xu, J., 2013. A novel combined pretreatment of ball milling and microwave irradiation for enhancing enzymatic hydrolysis of microcrystalline cellulose. *Bioresour. Technol.* 130, 81-87.

27. Reddy, N. R., Palmer, J. K., Pierson, M. D., Bothast, R. J., 1983. Wheat straw hemicelluloses: Composition and fermentation by human colon Bacteroides. *J. Agric. Food Chem.* 31, 1308-1313.

28. Rivela, B., Moreira, M. T., Feijoo, G., 2007. Life cycle inventory of medium density fibreboard. *Int. J. Life Cycle Assess.* 12, 143-150.

29. Rodríguez, F., Sanchez, A., Amaya-Delgado, L., 2019. Xylooligosaccharides production from lignocellulosic biomass using a pilot-scale pretreatment continuous tubular reactor. Modelling and experimental validation. *Ind. Crops Prod.* 134, 62-70.
30. Schütt, F., Westereng, B., Horn, S.J., Puls, J., Saake, B., 2012. Steam refining as an alternative to steam explosion. *Bioresour. Technol.* 111, 476-481.
31. Skinner, C.; Stefanowski, B.K.; Heathcote, D.; Charlton, A.; Ormondroyd, G.A., 2016. Life cycle assessment of pilot-scale wood fibre production using mechanical disc refining at different pressures. *Int. Wood Prod. J.* 7, 149-155.
32. Sun, Y., Cheng, J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresour. Technol.* 83, 1-11.
33. Sun, R., Lawther, J.M., Banks, W.B., 1998. Isolation and characterization of hemicellulose B and cellulose from pressure refined wheat straw. *Ind. Crops Prod.* 7, 121-128.
34. Talebnia, F., Karakashev, D., Angelidaki, I., 2010. Production of bioethanol from wheat straw: an overview on pretreatment, hydrolysis and fermentation. *Bioresour. Technol.* 101, 4744-4753.
35. Valenzuela, P., Bustos, C., Lasserre, J.P., Gacitúa, W., 2012. Fracture in *Eucalyptus nitens* wood: effect of nanomechanical properties, ultrastructure and anatomical level. *Maderas. Cienc Tecnol.* 14, 225-238.
36. Wang, Z., Dien, B. S., Rausch, K. D., Tumbleson, M. E., Singh, V., 2018. Fermentation of undetoxified sugarcane bagasse hydrolyzates using a two stage hydrothermal and mechanical refining pretreatment. *Bioresour. Technol.* 261, 313-321.
37. Zhu, J.Y., Wang, G.S., Pan, X.J., Gleisner, R., 2009. Specific surface to evaluate the efficiencies of milling and pretreatment of wood for enzymatic saccharification. *Chem. Eng. Sci.* 64, 474-485.
38. Xu, X., Jayaraman, K., Morin, C., Pecqueux, N., 2008. Life cycle assessment of wood-fibre-reinforced polypropylene composites, *J. Mater. Process. Tech.*, 198, 168-177.

