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Fungal pre-treatment of forestry biomass with a focus on biorefining: A comparison of biomass degradation and enzyme activities by wood rot fungi across three tree species

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ABSTRACT

The fungal enzyme activities and breakdown of wood components may be influenced by the fibrous and structural composition across different wood species. White-rot fungi were inoculated into wood chips of *F. excelsior*, *A. pseudoplatanus* or *Q. robur* and incubated for 28 days revealing that most fungi appeared to successfully colonize the different types of wood chips. Fibre analysis revealed that fungi causing the highest mass losses in *F. excelsior* and *A. pseudoplatanus* were those that degraded more cellulose compared with hemicellulose or lignin. Fungal degradation leading to high mass losses of *Q. robur* was more complicated as lignin degrading activities became more important. The structural composition in terms of the largest vessel sizes only showed an inverse correlation with remaining moisture content and not with mass loss or fibre degradation. These results provide an insight into fungal degradation of wood from three common tree species, and the link between the compositional characteristics of each wood type and the ease of degradation. This could have an impact on future biological pre-treatment strategies and valorisation approaches for forestry residues in integrated biorefineries.

1. Introduction

The utilization of forestry waste products in the advanced (lignocelluosic) biofuels sector, or in the recovery of potentially useful value added speciality chemicals remains challenging due to the highly recalcitrant nature of these residues. This is as a result of the small internal pore spaces in wood cell walls as a result of the highly cross-linked lignin component and the linear, crystalline nature of the cellulose backbone in lignocellulose materials, which limits the accessibility to enzymes during pre-treatment and prior to downstream bioprocessing. Currently, there are a range of chemical and physical pre-treatment approaches used for different biomass feedstocks, in order to facilitate optimised downstream fermentation for the production of biofuels and platform chemicals, but each have their limitations [1]. Fungal degradation of wood will reduce the structural integrity and increase the porosity of wood allowing easier physical disruption and greater access of chemicals and enzymes further into the wood structure. The application of biological pre-treatments, using edible fungi in particular, should be considered as a sequential strategy in the biomass processing of challenging forest biomass substrates [2].

The cellular construction and chemical composition which form the wood structures varies between different tree species and this has an impact on the utilisation of biological pre-treatments, including fungal decomposition [3]. Understanding the relationship between the cellular structure of different wood species and the relative ease of fungal mediated degradation, could therefore have a major impact on future pre-treatment strategies for forestry residues and their valorisation within integrated biorefineries [4]. *Fraxinus excelsior* (ash), *Acer pseudoplatanus* (sycamore) and *Quercus robur* (oak) are tree species commonly found in Europe, yet no study has compared fibre analysis of these species. However, separate values obtained from different studies reveal that cellulose forms a considerable component of most hardwoods, and in these particular tree species constitutes between 38-50% [5, 3, 6, 7]. In some of these studies, the Klason lignin contents reported were 26% for *F. excelsior* and *A. pseudoplatanus* which was higher compared with *Q. robur* which ranged from 22-24%. Factors affecting fungal decay of *Q. robur* have shown that moisture

content is important and that wood sources from different geographical locations seemed to yield similar rates of decay [8].

The vessels are an important anatomical feature which provides wood rot fungi with a conduit into the centre of wood, as demonstrated with *Phellinus flavomarginatus* which has been shown to enter the wood chips of *Eucalyptus grandis* through fibre vessels and pits [9]. Besides providing fungi with access into the wood structure, the vessels facilitate gaseous exchange. Richter and Dallwitz [10] have provided the expected ranges of vessel diameters across different species which are 54-120 µm for *F. excelsior*, 44-65 µm for *A. pseudoplatanus* and 130-290 µm for *Q. robur*, clearly showing that the vessel diameters in *Q. robur* are larger. A previous study also revealed that the vessel diameters of *F. excelsior* and *A. pseudoplatanus* were similar but much less compared with *Q. robur* [11]. *A. pseudoplatanus* differs from *F. excelsior* in that the vessel diameters are similar throughout the seasons and is described as being diffuse porous. In contrast, *F. excelsior* and *Q. robur* are described as ring porous because wider vessels are formed early in the growing season and narrower vessels are formed near the end of the growing season.

The aim of this study was to obtain a comprehensive understanding into the degradation *F. excelsior*, *A. pseudoplatanus* and *Q. robur* by white rot fungi, which are commonly found wood substrates readily found in the European region. Only one study has made a comparison in white rot fungal degradation of several types of wood chips revealing higher enzyme production on one wood species [12]. There was particular emphasis on *Lentinula edodes* (used in Shiitake production) in comparison to other white rot fungi in order to obtain a greater understanding in how this fungus utilizes other wood substrates compared with its preferred wood substrate, *Q. robur*. ~~In particular, *Lentinus edodes*, an edible fungus was included as this fungus~~ *L. edodes* is becoming increasing popular worldwide [13] and could be effectively used in the pretreatment of woody substrates.

2. Methods

2.1 Preparation of microcosms

The fresh wood from three different tree species were examined: *F. excelsior* (ash), *A. pseudoplatanus* (sycamore) and *Q. robur* (oak) that were coarsely cut into small chips. Glass jars containing ~~sterilized~~ wood chips (100 g) were sealed with a screw capped lid each containing a 2.5 cm central hole that was filled with non-absorbent cotton wool to allow gaseous exchange. The jars were autoclaved at 121 °C and 15 p.s.i for 1 h and autoclaving was determined to have no effect on the moisture content. Duplicate jars were inoculated with an agar square containing one of the fungi growing on 2% malt agar. The fungi used in this study were white rot wood decaying basidiomycete fungi: *Lentinula edodes* (LE), *Phlebiopsis gigantea* (PG), *Ganoderma tsugae* (GT); *Trametes versicolor* (TV1) *Ceriporiopsis subvermispora* D98698 (CS), *Phanerochaete chrysosporium* S596 (PC) and two fungal isolates. The two fungal isolates isolated from an *Q. robur* log in Treborth Botanical Gardens, Bangor, Gwynedd, UK were revealed by DNA sequencing revealed to be highly similar to *Trametes versicolor* (referred to in following text as *T. versicolor* 2) and *Phlebia radiata* (PR), respectively. The glass jars were incubated in the dark in an environmentally controlled room at 22 °C and 65 ± 5% humidity for 4 weeks. This period allowed complete fungal colonisation which occurred from the central inoculation point within the microcosm and mycelia spread to the outer vessel walls. After incubation, the glass jars were weighed and were samples removed to determine enzyme activities. The remaining material was oven dried (103 °C for 24 h) to determine dry mass loss, then ball milled prior to Klason lignin analysis and fibre analysis of hemicellulose, cellulose and acid detergent lignin.

2.2 Analysis of microcosms and original plant material

The wood chips were air dried for one week and the sizes of the wood chips were determined by rigorously shaking wood chips for 10 minutes through >3.15 mm, 1.4 mm, 600 µm, 250 µm and 250 µm sieves. The contents collected on each of the sieves were weighed. The sizes of the largest vessels were determined by thinly cutting cross sections of wood and staining with safranin. The thin sections were viewed under a phase contrast microscope and at least 10 of the largest vessels were measured using a micrometre. Fibre analysis was achieved by oven drying wood chips (103 °C for 24 h), ball milling the chips and weighing 0.525 ± 0.025 g into each Ankom bag which was then heat sealed. The hemicellulose and cellulose contents were determined by extractions methods using neutral detergent fibre (NDF) and acid detergent fibre (ADF), respectively as previously described [14]. Lignin was extracted from each wood sample remaining after the ADF extraction by the Klason method. Each filter bag was immersed in 7 ml of 72% (v/v) sulphuric acid for 2 h at 20 °C with periodic agitation to facilitate mixing. The acid solution was diluted to 4% (v/v) with 196 ml distilled water and autoclaved at 121 °C for 1 h, washed thoroughly with hot water ~40 °C, dried at 103 °C for 20 h and weighed. The ash content was determined by heating 1 g of the milled wood in a muffle furnace (4 h at 600 °C). The percentage of ash was subtracted from the percentages of hemicellulose, cellulose and lignin. The percentage of hemicellulose remaining was calculated by subtracting ADF from NDF extracts. The percentage of cellulose remaining was calculated by subtracting the lignin and percentages of ash from the ADF extracts. The percentages of cellulose and lignin were calculated by subtracting the percentages of ash.

The wet mass of wood in the microcosms and the moisture content were determined with samples collected at the start and end of the experiment. The change in moisture content during autoclaving was also taken into account, to calculate the moisture content of the microcosms at the start of the experiment. At the end of the experiment, each microcosm was weighed and a sample was removed after thoroughly mixing the contents with a spatula in order to determine enzyme activities. The residual wood chips were oven dried to determine moisture content which was calculated by subtracting the dry mass from the total wet mass and dividing by the total dry mass. The oven dried wood chips were ball milled in order to determine fibre analysis and ash content. The ash content was determined by heating 1 g in a muffle furnace for 4 h. The total quantities of hemicellulose, cellulose and lignin that were degraded by each fungus were calculated by factoring in mass losses.

2.3 *Enzyme activities*

Enzyme activities were determined from fungal cultures growing in fungal degraded wood (5g) that was suspended in 1 mM sodium acetate buffer (100 mL) at pH 5 and vigorously blended for 1 min in a Waring Blender. The macerated wood extract was centrifuged (11,337 xg 1 min), 1 ml supernatant was removed, filtered through a 0.2 µm membrane filter (Millipore) and serially diluted 10 fold in 1 mM sodium acetate buffer, pH 5. Laccase activities were determined in 1 M sodium acetate buffer, 2.4 pH 5 with 0.5 M 2,29-azinobis (3-ethylbenzthiazoline-6-sulphonate) (ABTS) and manganese peroxidase activities were determined in 50 mM sodium succinate (pH 4.5), 50 mM sodium lactate (pH 4.5) using 0.1 mM $MnSO_4$, 0.1 mM phenol red and 50 µM hydrogen peroxide and measured in a microplate reader at 610 nm as previously described [14]. Cellulase activity was determined using cellulose azure (0.2g) that had been pre-washed repeatedly in deionised water and centrifuged until no colour appeared in the supernatant, and as previously described [15]. The washed cellulose azure pellet was resuspended in 0.05 M sodium acetate buffer (50 mL), pH 5. The assay was performed using 100 µl enzyme extract and 300 µl cellulose azure (Sigma) suspension. The samples were incubated with shaking at 500 rpm (30 °C for 24 h), although 100 µl was removed initially to determine the baseline at time zero and absorbance was measured in a microplate at 595 nm. Xylanase activity was determined using Remazol Brilliant Blue R−D-Xylan (0.1 g) (Sigma) that was dissolved in 0.05 M sodium acetate buffer (50mL), pH 5. The assay was performed as described for cellulose azure, except two volumes of ethanol were added to 100 µl xylanase assay extract and

centrifuged at 9,000 xg [16]. The supernatants (100 µl) were placed into a microplate and the absorbance measured at 595 nm. The absorbances were compared with cellulase activity from *Aspergillus niger* (Sigma) and xylanase activity from *Thermomyces lanuginosus* (Sigma). In most cases, each fungus had completely colonized all of the wood present in the jar within a period of one month. It was assumed that this colonisation is not homogeneous, with the oldest hyphae at the centre of the jar closest to the inoculation point and younger hyphae at the edge of the jar. Therefore, enzyme activities measured represent an average, not truly reflecting localised fluctuations within the microcosm. Previous studies have shown the enzyme activities by wood rot fungi constantly fluctuate [17] and this is probably caused by the availability of nutrients and feedback regulation.

3.4 *Statistical Analyses*

Statistical and correlation analyses were performed using IBM SPSS Statistics version 20. Each value obtained from duplicate microcosms was used to determine significant differences by ANOVA with Tukey's posthoc test. Significant correlations were determined by two tailed bivariate correlation analysis using Spearman's correlation coefficient.

3. Results

3.1 Composition of woods

The moisture contents of the fresh wood chips were determined at the start of the experiment for *F. excelsior* (63.4 ± 0.2%), *A. pseudoplatanus* (67.5 ± 0.2%) and *Q. robur* (60.5 ± 0.8%). Statistical analysis revealed that these were significantly different to each other.

Mechanical dry sieving revealed that wood fragments larger than 3.15 mm constituted 87.5% and 82.5% of the dry mass of *F. excelsior* and *A. pseudoplatanus*, respectively. Only 53.4% of wood fragments from *Q. robur* were larger than 3.15 mm. Cross sectional analysis of the three tree species indicated that the largest vessels in *F. excelsior*, *A. pseudoplatanus* and *Q. robur* were 68.3 ± 15.9 µm, 28.0 ± 10.3 µm and 76.3 ± 25.0 µm, respectively. It was also evident that there were much smaller vessels present in *F. excelsior* and *Q. robur*, whereas the vessels in *A. pseudoplatanus* were homogeneous.

The wood chips from each tree species varied in their chemical composition. The percentage of water soluble compounds in *A. pseudoplatanus* (22.5 ± 0.6%) and *Q. robur* (21.2 ± 1.1%) was significantly higher than in *F. excelsior* (17.1 ± 0.6%) (Fig. 1). The hemicellulose contents of *Q. robur* (25.3 ± 0.2%) and *A. pseudoplatanus* (24.4 ± 0.3%) were similar but significantly higher than in *F. excelsior* (22.5 ± 0.3%). The cellulose contents of each wood species was significantly different to one another where *F. excelsior* (43.4 ± 0.1%) contained the highest followed by *Q. robur* (40.0 ± 0.1) and *A. pseudoplatanus* (38.0 ± 0.4%). The acid detergent lignin content in *Q. robur* (12.0 ± 0.9%) was significantly lower compared with *F. excelsior* (16.3 ± 0.9%) whereas the acid detergent lignin content in *A. pseudoplatanus* (14.0%) revealed no significant difference with either *F. excelsior* of *Q. robur*. The Klason lignin content was higher than the acid detergent lignin with each of the different types of wood and was significantly higher in *F. excelsior* (28.1 ± 0.1%) and *A. pseudoplatanus* (28.7 ± 0.1%) compared with *Q. robur* (25.2 ± 0.8%).

3.2 Decay of F. excelsior

All of the fungi effectively colonized the *F. excelsior* chips as shown by the presence of white mycelia. The moisture contents in the fungal degraded microcosms showed a slight decrease which was not significantly different but only the microcosms containing *P. radiata* and *L. edodes* were significantly different compared with the moisture content at the start. These fungi happened to

show the least mass loss and a similar trend between significant moisture loss and low mass loss was observed with the other wood species. Treatment of the wood with *T. versicolor* 2 and *P. radiata* resulted in the highest levels of degradation, as indicated by the percentage mass losses of 31.8 ± 1.4% and 31.5 ± 0.8% (Fig. 2). These fungal strains degraded significantly more compared to *L. edodes*, *P. gigantea*, *P. chrysosporium* and *G. tsugae*. *T. versicolor* 1 and *C. subvermispora* also showed significantly different higher percentage mass losses compared with those fungi.

T. versicolor 1, *T. versicolor* 2 and *L. edodes* showed that the extent of hemicellulose, cellulose and acid detergent lignin degradation were all significantly different to each other. It was apparent that both strains of *T. versicolor* degraded more cellulose compared to hemicellulose, while *L. edodes* degraded more hemicellulose compared with cellulose. In contrast, *C. subvermispora*, *G. tsugae* and *P. gigantea* showed no significant differences in degrading any of the fibre components. *P. chrysosporium* degraded significantly more hemicellulose and cellulose compared with lignin, whereas *P. radiata* degraded significantly more cellulose compared with acid detergent lignin.

Correlation analysis revealed that mass loss of *F. excelsior* showed a positive correlation with cellulose degradation and cellulase activities (Table 2). The percentage of acid detergent lignin remaining in the decomposed *F. excelsior* appeared to be lower with *C. subvermispora,* compared with the original starting material (Fig. 3), although statistical analysis revealed this was not significantly different. All of the fungi showed a significant decrease in the percentage of Klason lignin remaining in decomposed *F. excelsior* compared with the undecomposed *F. excelsior*. The final percentage of Klason lignin remaining in decomposed *F. excelsior* was considerably lower with *C. subvermispora* compared with all the other fungi.

3.3 Decay of A. pseudoplatanus

Seven of the fungi formed white mycelia that completely colonized *A. pseudoplatanus* chips but *C. subvermispora* did not appear to grow on *A. pseudoplatanus*. The highest mass loss of 31.0 ± 3.7% was caused by the fungal strain *T. versicolor* 2. Both strains of *T. versicolor* and *P. radiata* showed significantly higher mass losses compared with the other fungi. *L. edodes* only gave minimal mass losses.

G. tsugae and *P. radiata* revealed that each of the different fibre components were degraded significantly differently to each other. *G. tsugae* degraded more hemicellulose whereas *P. radiata* degraded more cellulose. *C. subvermispora* and *P. gigantea* revealed no significant differences in degradation between each of the fibre components. Both strains of *T. versicolor* showed significantly higher cellulose degradation compared with either hemicellulose or lignin degradation. *P. chrysosporium* showed significantly higher hemicellulose and cellulose degradation compared with lignin degradation. Finally, *L. edodes* showed higher hemicellulose degradation compared with either cellulose or lignin degradation.

Mass loss appeared to correlate with the residual moisture content and degradation of hemicellulose, cellulose and lignin (Table 2). Most of the fungi especially *C. subvermispora* and *P. gigantea* appeared to show a decrease in the percentage of acid detergent lignin remaining in the decomposed wood chips of *A. pseudoplatanus* compared with the undecomposed wood chips (Fig. 3), although statistical analysis revealed none of these were significantly different. Similarly, none of the fungi showed any significant decrease in the percentage of Klason lignin remaining in the decomposed wood chips compared with the undecomposed wood chips.

3.4 Decay of Q. robur

Only five of the fungi formed white mycelia that colonized wood chips of *Q. robur* and the highest percentage mass loss of 30.8 ± 1.6% was caused by *C. subvermispora.* This is significantly higher compared to *G. tsugae*, *P. chrysosporium* and *L. edodes*. Three fungal strains, *P. gigantea*, *P.*

radiata and *T. versicolor* 1 did not appear to successfully grow on wood chips of *Q. robur* and were not included in the analysis.

P. chrysosporium revealed significant differences in the degradation of each of the components with more hemicellulose degradation and little or no lignin degradation. *C. subvermispora* revealed no significant differences in degradation between any of the fibre components. *T. versicolor* 2 degraded significantly more cellulose and hemicellulose compared with lignin, whereas *G. tsugae* and *L. edodes* degraded significantly more hemicellulose compared with cellulose and lignin.

Correlation analysis revealed that the mass loss in the wood chips of *Q. robur* decreased in proportion with the residual moisture content, hemicellulose degradation, cellulose degradation, laccase activity and manganese peroxidase activity (Table 2). This indicated that lignin degrading enzymes were important in the fungal degradation of *Q. robur*. The percentage of acid detergent lignin remaining in the decomposed wood chips was significantly lower when degraded by *C. subvermispora* and *L. edodes* compared with other fungi which appeared to show a slight increase (Fig. 3). However, they were not significantly different compared with the undecomposed wood chips. In contrast, none of the fungi showed any significant decrease in the percentage of Klason lignin remaining in the decomposed wood chips, when compared with the undecomposed wood chips.

3.5 Comparison of specific fungi between wood samples

T. versicolor 2 consistently showed higher degradation across all of the samples from the three different tree species, compared with other fungi, resulting in significantly higher mass loss in *F. excelsior* and *A. pseudoplatanus* compared with *Q. robur*. This strain appeared to be well adapted in degrading *Q. robur* compared with the other strain, *T. versicolor* 1 which caused no biomass loss. This strain appeared to show higher cellulose degradation in *F. excelsior* and *A. pseudoplatanus* compared with *Q. robur*, but these results were not significantly different. No other significant differences were found with hemicellulose and lignin degradation, nor with enzyme activities relating to xylanase, cellulase, laccase, manganese peroxidase and lignin peroxidase.

G. tsugae showed no differences in mass loss in the wood chips of any of the tree species, yet showed higher cellulose degradation on *F. excelsior* compared with *Q. robur*. No other differences were found.

L. edodes resulted in significantly higher mass loss in the wood chips of *F. excelsior* and *Q. robur* compared with the wood chips of *A. pseudoplatanus*, and even though hemicellulose and cellulose degradation appeared to be greater on the wood chips of *F. excelsior* and *Q. robur* compared with wood chips of *A. pseudoplatanus*, there were no significant differences. Cellulase activity was higher on the wood chips of *Q. robur* compared with the wood chips of *F. excelsior* or *A. pseudoplatanus*. Manganese peroxidase activities were higher on the wood chips of *F. excelsior* and *A. pseudoplatanus* compared with wood chips of *Q. robur*. No other differences were found.

Discussion

Fibre analysis of the undecomposed wood chips revealed distinct differences between each of the three species. The non-fibre (water soluble compounds) content in *A. pseudoplatanus* was lower compared with *F. excelsior* or *Q. robur,* whereas the cellulose content in *F. excelsior* was higher compared with *A. pseudoplatanus* or *Q. robur*. It appears that only one report describes the fibre composition between each of these wood species, although the values cited were from independent studies [3]. The cellulose content in *A. pseudoplatanus* and *Q. robur* were similar compared to the values described in that study, whereas the cellulose content in *F. excelsior* was about 5% higher. One reason for the discrepancy between both studies in determining the cellulose content in *F. excelsior* may be attributed to the use of different protocols, whereas similar results

were obtained when identical protocols were used. A comparison of the data with another study revealed that the cellulose content in *Q. robur* was similar to the values obtained from heartwood compared with sapwood from *Q. robur* [7]. However, it must be noted that a different protocol using GC analysis of silylated sugar monomers after acid hydrolysis was used to determine the cellulose content in this study. The fibre composition data can be compared with only one other study [18] revealing the proportions of hemicellulose and cellulose in *Q. robur* were highly similar. In addition to hemicellulose and cellulose, two different forms of lignin were evaluated; Klason lignin and acid detergent lignin. The acid detergent lignin values were lower than the Klason lignin contents and were similar for each of the wood species. Although both methods quantify insoluble lignin, it would appear that a considerable proportion of lignin (about 10%) is lost when an aggressive acid detergent pretreatment is used before the traditional Klason method. Different lignin extraction methods have been shown to yield dissimilar results [19], perhaps each reflecting deconstructed parts of the lignin superstructure. It appears that only one study has described the acid detergent lignin content of a wood species, reporting a similarly low value using the same procedure on *Populus tremula* (aspen) [20]. In general, the Klason lignin method is considered to reflect the actual lignin content of plants [19]. It was found that the Klason lignin content were higher with *A. pseudoplatanus* compared with *Q. robur*, which is in agreement with the values previously reported [5, 3, 6]. However, the reported lignin contents in this study were slightly higher (1-2%), compared with previous studies and may be due to tree to tree variations [5, 17] or slight differences in laboratory measurements.

The diameter of largest vessels were found in *Q. robur* and the smallest found in *A. pseudoplatanus* which was as previously reported [10, 11]. The actual diameter dimensions were generally lower compared with vessels found in tree trunks [10] but larger than those found in the stems [11]. However, wood is composed of vessels with many different sizes and it is perhaps difficult to make an accurate correlation with degradation characteristics. Nevertheless, the data did reveal a clear correlation between vessel sizes and moisture loss, where high moisture losses were determined with *Q. robur*. It is possible that only fungi showing rapid colonization of *Q. robur* would cause significant degradation before the sudden decline in moisture conditions.

Fungi degrading *F. excelsior* that showed higher levels of cellulose degradation compared with hemicellulose degradation appeared to cause the most mass loss of wood chips, compared with other fungi, which showed similar levels of hemicellulose and cellulose degradation. These fungi may be described as non-selective lignin degrading fungi which produce significant amounts of enzymes close to the hyphae, thereby creating troughs in the cell wall vessels [21~~21~~]. In contrast, selective lignin degrading fungi create boreholes through lignin barriers to allow fungal growth and lower enzyme activities may result in lower mass losses. Correlation analysis also demonstrated a positive relation between mass loss of *F. excelsior* with cellulose degradation and cellulase activity. Fungi resulting in higher mass losses of *A. pseudoplatanus* also showed much higher levels of cellulose degradation, and correlations were found between mass loss with not only cellulose degradation but also hemicellulose and lignin degradation. However, there was no correlation between mass loss and cellulase activity. It would appear that the direct relationship between cellulose degradation and cellulase activity has become more complicated, as other enzymes are more important in degrading these wood chips. Similarly, even though a correlation was found between mass loss and cellulose degradation with *Q. robur*, lignin degrading enzymes rather than cellulase became a more important factor in mass loss. This is supported by microscopy studies revealing fungi degrading the resilient S3 lignin layer of *Q. robur* [22]. During these stages as shown by molecular studies, lignin degrading enzymes are expressed before other enzymes [23] and that laccases may be important in degrading wood extractives which could inhibit growth [24]. It was also evident that fungi causing higher mass losses of *Q. robur* did not necessarily show higher cellulose degradation.

In this study, two independent methods of analysis yielded the same conclusion and the enzyme activities obtained with *P. chrysosporium* growing on *Q. robur* seemed to concur with the proteomic results of a previous study [25]. Cellulase activities appeared to be an important factor leading to higher degradation of *F. excelsior* wood chips, which is not surprising considering that cellulose forms a significant proportion of the total biomass. However, distinct enzyme correlations were found between each of the wood species. A previous study has shown that the growth of *Phanerochaete chrysosporium* grown on milled wood of Poplar after growth on glucose medium caused the upregulation of more than 100 different genes of which many expressed cellulases [26]. The authors conclude that this is evidence that the chemical composition of the tree species has an influence on which enzymes are being expressed by the wood rot fungi.

L. edodes consistently showed higher levels of hemicellulose degradation on all different types of wood compared with cellulose, in contrast to other fungi which showed some variation. A previous study using different fungi revealed that *L. edodes* was unaffected by the presence of a higher cellulose content associated with one Miscanthus species compared with another species [27]. Other studies have also shown that hemicellulose was degraded in preference to cellulose, especially in the earlier stages, when grown on wheat straw, mixed wood chip substrate or mixed lignocellulose substrates probably due to low cellulase activities [28, 29]. It was also revealed that this fungus unravelled the fibrous network enabling a higher proportion of cellulose remaining in spent wood blocks to be converted into methane during anaerobic digestion [30]. Hemicellulose and lignin are important in covering the cellulose microfibrils [31] and the significant fungal degradation of hemicellulose perhaps in combination with other physical or chemical methods, would allow easier access of industrial cellulases to recover glucose that could be used in the biofuel industry. When wood chips of *Quercus acutissima* were degraded by *L. edodes* and then fermented by *Saccharomyces cerevisiae*, most of the available sugars were converted into ethanol [32] demonstrating that conversion of spent mushroom blocks to ethanol is possible. Furthermore, *L. edodes* showed much lower mass losses compared to other fungi which would result in a much higher product recovery. Finally, *L. edodes* had little impact in lignin degradation as compared to *C. subvermispora* which has been considered to be an important factor in improving the digestibility of lignocellulose material as a ruminant feed. However, an enriched source of lignin may find important industrial applications when suitable depolymerisation methods has been developed [33].

The isolate, *T. versicolor* 2, recovered from *Q. robur* caused the highest mass losses in *F. excelsior* and *A. pseudoplatanus* and second highest mass loss in *Q. robur* compared with other fungi. This strain showed similar degradation characteristics to another strain used in the study, *T. versicolor* 2, although *T. versicolor* 2 was unable to effectively degrade *Q. robur*. This fungal variant, *T. versicolor* 1, was highly effective in degrading *F. excelsior* and *A. pseudoplatanus* compared with *T. versicolor* 2. The high degrading characteristics of *T. versicolor*, albeit a different strain, have also been shown in another previous study on birch showing considerably higher wood mass losses compared with 29 other fungal species in the same study [34]. The fungi showing the highest mass losses in *F. excelsior* also showed the highest levels of cellulose degradation. This was also confirmed by correlation analysis showing that cellulose degradation and cellulase activity in *F. excelsior* were important factors leading to mass loss. *T. versicolor* was among the fungi that facilitated the highest levels of cellulose degradation. A previous study reported that this fungus degraded more cellulose than hemicellulose in wheat straw during the initial stages of degradation [35], although similar proportions of cellulose and hemicellulose were degraded in the later stages of degradation.

One of the underlining problems in this study was that a greater proportion of smaller particles were found with *Q. robur* compared with *F. excelsior* or *A. pseudoplatanus*. It is uncertain whether the formation of irregular sized wood chips of *Q. robur* compared with *F. excelsior* or *A.*

pseudoplatanus may be an inherent trait that would continuously occur during the chipping process. Nevertheless, it is important to evaluate how these different sized particles may affect the analysis of the results. A previous study has shown that particle sizes around 250 µm resulted in higher laccase activities [36] and therefore the smaller particle sizes of the *Q. robur* chips used in this study may show higher laccase activities than would be expected if the particle distribution had been similar to the other wood species. However, another study reported a more complex relationship, where higher enzyme activities were associated with particular particle sizes that were dependent on the *Pleurotus* species being grown [37]. With particular focus on *L. edodes*, it was shown that the cellulase activities were significantly higher on *Q. robur* compared with either *F. excelsior* or *A. pseudoplatanus*. Therefore, it is possible that the broader distribution of particle sizes of *Q. robur* may have contributed to a higher level of fungal decay by *L. edodes* which was similar to decay of *F. excelsior*.

4. Conclusions

This study revealed differences in the chemical composition of wood chips from three commonly found tree species. In general, some common trends were observed where those fungi involved in degrading similar proportions of cellulose and hemicellulose such as *T. versicolor* caused higher mass losses. It would appear that the activities of particular extracellular enzymes by wood rot fungi were important in the degradation of wood chips from each type of tree species. The activities of these enzymes may show a more direct relationship with the chemical composition of the wood chips of one species, as in the case of *F. excelsior*, due to the higher cellulose component. However, the chemical composition appeared to play a minor role during fungal degradation of *Q. robur* because the lignin barrier presented a major obstacle. The results seem to suggest that degradation of *F. excelsior* by *C. subvermispora* resulted in higher levels of delignification compared with fungal degradation of wood chips from other tree species. It would appear that the lower hemicellulose content and higher cellulose content in *F. excelsior* are factors driving this fungus towards expression of higher lignin degrading enzymes. Physical attributes such as the width of the largest vessels that would allow fungi to easily penetrate into the wood structure did not influence the extent of fungal degradation, but did appear to be an important factor in moisture loss. It is possible that only fungi which could rapidly grow in *Q. robur* resulted in significant hemicellulose degradation.

Although these are preliminary results, further work is underway to further understand and refine the approaches which could be used in the degradation of forestry residues, using different wood rot fungi. This could have an impact on future biological pre-treatment strategies for forest biomass and the valorisation of the ~~hemicellulose,~~ cellulose and lignin components in these materials, as part of an integrated biorefinery approach. The use of *L. edodes* appears to be promising in retaining a significant proportion of cellulose and lignin which could be converted to biofuel and decomposed into valuable bioproducts, respectively.

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List of Figures

Figure 1 Percentage distribution of soluble compounds ☐, hemicellulose ☐, cellulose ☐, acid detergent lignin ☐ and Klason lignin ☒. The soluble compounds, hemicellulose, cellulose and acid detergent lignin are extracted sequentially using fibre digestion. Klason lignin is determined in a separate sample by acid hydrolysis. Error bars indicate standard deviation.

Figure 2 Fungal degradation after 28 days degradation of *F. excelsior* (top), *A. pseudoplatanus* (middle) and *Q. robur* (bottom) shown in percentage of dry mass losses ☒ by each fungus from highest to lowest. Fungi that showed no degradation in *A. pseudoplatanus* or *Q. robur* are not shown. The fungi used in this study are: fungal strain CM13 (CM), fungal strain RM22b (RM), *Trametes versicolor* (TV), *Ganoderma tsugae* (GT), *Ceriporiopsis subvermispora* (CS), *Phanerochaete chrysosporium* (PC), *Phlebiopsis gigantea* (PG) *Lentinula edodes* (LE) and averages of all fungi that showed degradation (AV). The total percentages of hemicellulose ☐, cellulose ☐ and lignin ☐ degraded by each fungus and standard deviations are shown by error bars.

Figure 3 Percentage of acid detergent lignin and Klason lignin remaining in ash ☐, sycamore ☐ and oak ☐ after fungal degraded material. Error bars indicate standard deviation and where there are no values for *Q. robur* indicates that lignin analysis was not attempted on these samples.

Table 1 Fractions obtained from each dry wood by mechanical sieving.

	F. excelsior	*A. pseudoplatanus*	*Q. robur*
>3.15 mm	87.5%	82.5%	53.4%
>1.4 mm	5.6%	9.2%	30.0%
>600 µm	4.8%	5.8%	12.6%
>250 µm	1.7%	1.8%	2.9%
<250 µm	0.4%	0.8%	1.2%
total sum	99.8%	99.9%	99.8%

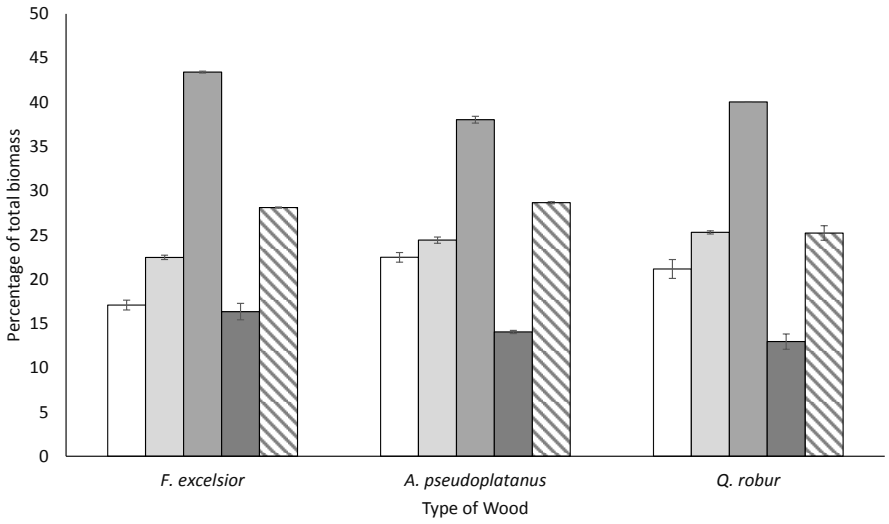
50
45
40
35
30
25
20
15
10
5
0
Percentage of total biomass
F. excelsior
A. pseudoplatanus
Q. robur
Type of Wood

Fig. 1

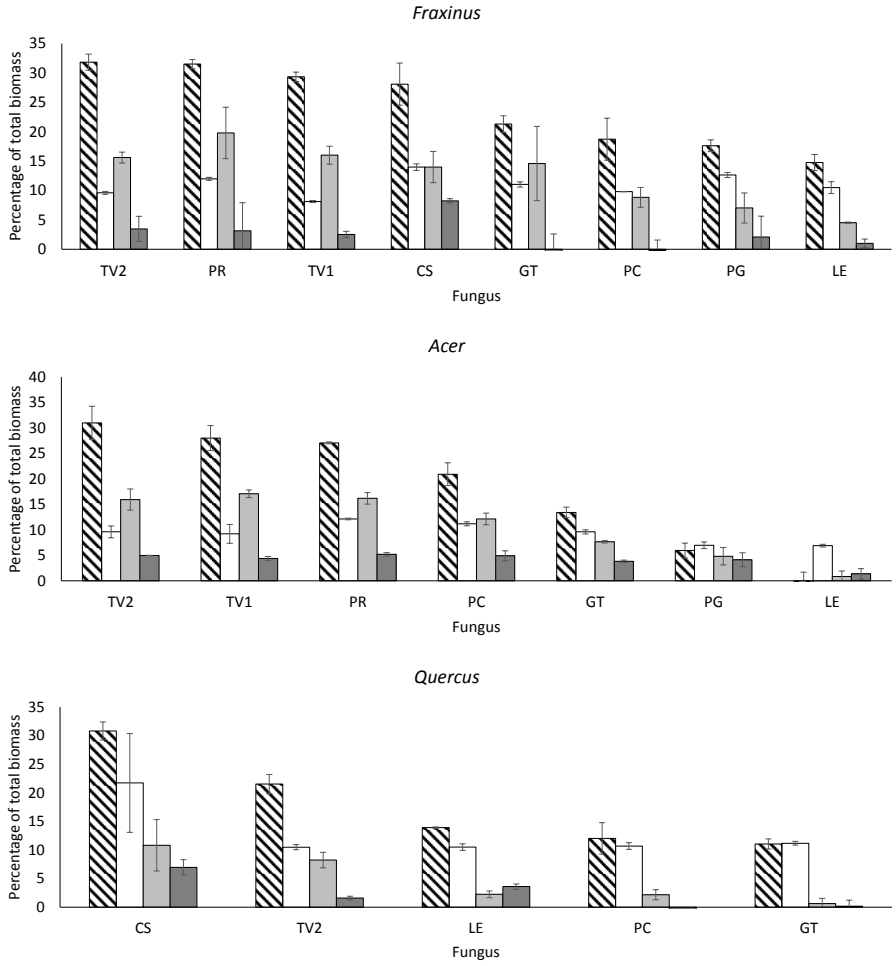
Fraxinus
Percentage of total biomass
35
30
25
20
15
10
5
0
TV2
PR
TV1
CS
GT
PC
PG
LE
Fungus
Acer
Percentage of total biomass
40
35
30
25
20
15
10
5
0
TV2
TV1
PR
PC
GT
PG
LE
Fungus
Quercus
Percentage of total biomass
35
30
25
20
15
10
5
0
CS
TV2
LE
PC
GT
Fungus

Fig. 2

Table 2 Correlations between mass loss of each wood and each of the parameters measured in this study.

	MASS LOSS						DIAMETER	
	Fraxinus		*Acer*		*Quercus*		ALL WOODS	
	SRV	*P*	SRV	*P*	SRV	*P*	SRV	*P*
Mass	-	-	-	-	-	-	0.100	0.612
Moisture	0.368	0.161	0.956**	0.000	0.867**	0.001	-0.403*	0.033
Hemicellulose	-0.068	0.803	0.644*	0.013	0.297	0.405	0.328	0.089
Cellulose	0.838**	0.000	0.952**	0.000	0.903**	0.000	-0.008	0.969
Lignin	0.491	0.053	0.644*	0.013	0.770**	0.009	-0.236	0.227
Cellulase	0.538*	0.031	-0.429	0.126	0.588	0.074	0.143	0.467
Xylanase	0.262	0.327	-0.007	0.982	0.624	0.054	0.318	0.099
Laccase	0.465	0.070	0.521	0.056	0.673**	0.003	-0.161	0.412
MnP	-0497	0.050	0.020	0.946	0.842**	0.002	-0.056	0.776
LiP	0.297	0.263	-0.187	0.523	0.127	0.726	0.035	0.861

SRV and *P* denotes Spearman's Rho value and probability, respectively. Correlations represented by * and ** are significant at 0.05 and 0.01, respectively. Each of the correlations shows mass loss or diameter of the wood vessels in relation to each of the measured factors for *T. versicolor* 2, *C. subvermispora*, *G. lucidum*, *L. edodes* and *P. chrysosporium*.

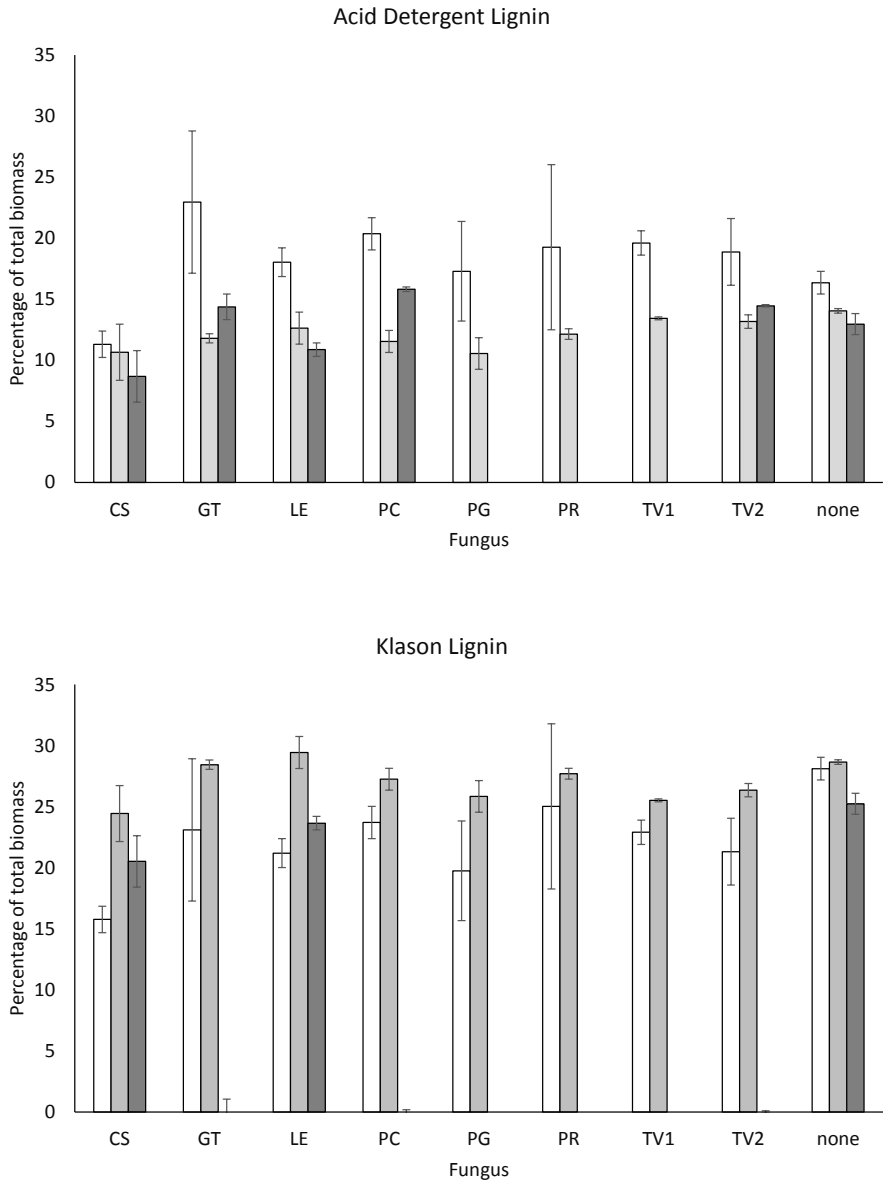
Acid Detergent Lignin
Percentage of total biomass
35
30
25
20
15
10
5
0
CS
GT
LE
PC
PG
PR
TV1
TV2
none
Fungus
Klason Lignin
Percentage of total biomass
35
30
25
20
15
10
5
0
CS
GT
LE
PC
PG
PR
TV1
TV2
none
Fungus

Fig. 3