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Running title: Incipient brown rot decay in modified wood

Incipient brown rot decay in modified wood: Mass loss, structural integrity, moisture and acetyl content monitored in high resolution

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Abstract

No coherent explanation for why wood degrading fungi does not cause mass loss in modified wood has as yet been presented. Understanding the mode of action of these materials is important for efficient development and improvement. Fungi growing in liquid culture undergoes three growth phases; the lag, logarithmic and stationary phase. Similar growth phases for filamentous fungi have been reported in solid food and modelled in solid wood. The aim of this study was to find out whether brown rot fungi undergoes the same growth phases in solid wood as in liquid culture and study the effect of acetylation and furfurylation on the growth pattern. Monitoring of mass loss and structural integrity over 300 days of exposure of acetylated and furfurylated wood to the brown-rot fungi *Postia placenta* was performed. Mass loss results of untreated wood indicated that fungi in solid wood go through phases similar to the growth phases seen in liquid cultures. Low mass loss and maintained structural integrity suggest that the fungi in the modified wood materials were still in the lag phase, while the fact that mass was lost at all suggests that degradation did occur and that the fungi were in the logarithmic phase.

Keywords: Acetylated wood, basidiomycetes, furfurylated wood, mode of action, *Postia placenta*, wood degradation

1. Introduction

When decomposition of wood takes place in wooden structures it leads to great economic losses. Schmidt (2006) estimated the costs of refurbishment to €3000 per square metre of living space. In the US, it has been estimated that every year as much as 10% of the harvested roundwood is used to replace timber in service that has been decayed, resulting in extra costs of hundreds of millions of dollars (Zabel and Morell 1992). Brown rot attack is a particular challenge because it causes more loss of strength at low mass loss than white rot, resulting in great damage within a short period of time (Eaton and Hale 1993; Witomski et al. 2016). Brown rot fungi preferably attack coniferous wood, which is used in the majority of constructions in the Northern hemisphere (Schmidt 2006). New alternatives to traditional, toxic wood protection methods are being developed, such as modified wood (Hill 2006). In order to efficiently develop and improve modified wood materials, understanding the mode of action of wood modification is utterly important.

Modified wood is defined as chemically or physically altered wood materials with increased decay resistance and which are non-toxic under service conditions and at the end of service life (Hill 2006). Acetylation of wood is commonly achieved by reacting wood with acetic anhydride, which causes acetyl groups to bind to the OH-groups of the wood constituents (Rowell et al. 1994; Larsson Brelid et al. 2000; Hill et al. 2005). Furfurylation of wood involves impregnation of the wood with furfuryl alcohol and subsequent curing during which polymerised furfuryl alcohol (poly(furfuryl alcohol)) is formed (Goldstein 1960). Decrease in equilibrium moisture content (EMC) and increase in decay resistance in acetylated wood has been ascribed primarily to the volume of added modification agent, i.e. bulking (Papadopoulos and Hill 2003; Papadopoulos et al. 2004).

Microorganisms in a liquid culture go through three different stages: i) the lag phase in which the microorganisms adapt to the new environment, ii) the logarithmic phase where the microorganisms are actively degrading the nutrient in the medium and grow exponentially, and iii) the stationary phase where the growth is impaired by nutrient deficiency, a change in pH or an accumulation of toxic compounds (Baranyi and Roberts 2000; Madigan et al. 2000; Rolfe et al. 2012). *Penicillum chrysosporum* was shown to go through lag, logarithmic and stationary phase like phases when grown on a solid food substrate, while *Physisporinus vitreus* has been modelled to go through similar phases when growing in wood (Fuhr et al. 2011; Arquiza and Hunter 2014). If the growth phases of microorganisms in liquid culture are applicable on filamentous fungi growing in wood, the lag phase might be equivalent to the time it takes for the fungi to adapt to the environment provided by the wood material. For example, the absence of glucose will lead to an up-regulation of genes involved in wood degradation (Aro et al. 2005; Martinez et al. 2009). At the end of the lag phase, the fungi will start the chelator mediated Fenton (CMF) degradation. No change in composition will be noticed, but there will be a change in structure (Fackler et al. 2010). Using CMF degradation, the fungi will depolymerise cellulose chains and hemicelluloses and modify lignin through induction of the Fenton reaction in which hydroxyl radicals are formed (Fenton 1894; Goodell et al. 1997; Arantes et al. 2012). The depolymerisation of the wood cell wall polysaccharides will lead to a loss in strength, which is noticeable before mass loss can be detected (Wilcox 1978; Winandy and Morrell 1993; Curling et al. 2002; Brischke et al. 2008; Fackler et al. 2010; Maeda et al. 2014). Once the CMF degradation has opened up the wood structure sufficiently, the enzymatic degradation machinery will start degrading the wood constituents and thus the fungi are now in the logarithmic phase. Enzymatic degradation will further reduce the strength of the wood while also causing mass loss (Curling et al. 2002; Brischke et al. 2008; Fackler et al. 2010). When the fungi have degraded the wood material to such an

extent that all available nutrients are depleted, the fungi has reached the stationary phase in which the degradation rate will flatten out.

In a liquid culture, fungal growth is measured as the change in fungal biomass. In wood, where the mycelium cannot be extracted, fungal biomass is determined by assays of cell constituents such as ergosterol, total extractable liquid phosphates, nucleic acids and chitin as well as indicators of biological activity such as GTP, enzyme and respitory activities (Lena et al. 1994). To be reliable, an indicator of fungal biomass must correlate to mycelium increase and be independent of growth conditions. In a solid substrate, measuring the loss of mass of the substrate is an indirect measurement of the growth of the fungi (Mohebby et al. 2003; Verma et al. 2008). Since mass loss only occurs once the enzymatic degradation have begun, measuring mass loss will not show the incipient degradation in which only CMF degradation occurs (Fackler et al. 2010). Chelator mediated Fenton degradation instead causes structural changes in the wood cell wall, such as modification of lignin and depolymerisation of celluloses (Fackler et al. 2010). Measurements of CMF degradation may therefore include strength loss analyse, especially of specimens exposed to fungi for such a short time that mass loss cannot be detected. In recent years, an alternative to measuring strength loss in decayed wood has been developed, called the High-energy multiple impact (HEMI) test, which instead addressed the structural integrity of the wood (Brischke et al. 2006; Rapp et al. 2006). This method detects both, changes in fibre strength (in the fibre direction) and strength between fibres (against the fibre direction). In the early stages of decay, both should mainly be affected by CMF degradation because radicals randomly attack the wood polymer. The advantages of HEMI tests are small variances, high reproducibility of results, short time for specimen preparation, and a small number of specimens needed (Rapp et al. 2006). Furthermore, in this study the possibility to measure structural integrity in miniblock samples was important. In modified wood materials, CMF degradation may also affect the amount of modification chemicals in the wood material, depending on whether the modification chemical can be degraded by hydroxyl radicals.

In the research on decay resistance and mode of action of modified wood, the majority of studies have measured the mass loss after a fixed time of exposure, similarly to durability standards such as EN 113 and AWPA E 10, or only at a few different time points (E10-91 1991; European Committee for Standardization (CEN) Belgium 1996a; Papadopoulos and Hill 2002; Rapp et al. 2008; Verma et al. 2009; Esteves et al. 2010). Therefore, from the current literature it is difficult to determine the effect of wood modification on the growth dynamics of wood degrading fungi (Papadopoulos and Hill 2002; Rapp et al. 2008; Verma et al. 2009; Esteves et al. 2010). Looking at multiple samples harvested continuously over a long period of time, would possibly provide better insights into the dynamics of the decay of modified wood and potentially reveal whether the growth phases of microorganisms in liquid culture can be applied also on wood degrading fungi growing in solid untreated and modified wood. Measuring the loss of strength or structural integrity during fungal exposure of modified wood may show if there is a depolymerisation or loss of components that influence the strength of wood, such as cellulose. Detection of loss of structural integrity before mass loss can be detected will indicate the occurrence of CMF degradation before the enzymatic degradation has begun and thus the onset of the logarithmic phase. Measuring the amount of modification chemicals in the modified wood materials before mass loss can be detected may also indicate occurrence of CMF degradation.

The aim of this study was to investigate whether brown rot fungi growing on acetylated and furfurylated wood undergoes the same growth phases as are seen for fungi in liquid cultures;

144 the lag, logarithmic and stationary phase. This was done through high-frequency monitoring
145 of mass loss and structural integrity during exposure of acetylated and furfurylated wood to *P.*
146 *placenta* for more than 300 days. Acetyl content was measured in acetylated wood under the
147 same conditions.
148

149 # 2. Materials and methods

150 ## *2.1 Wood material and sample preparation*

151 Miniblock samples (10 x 5 x 30 mm^3) (Bravery 1979) of Southern yellow pine sapwood were
152 acetylated or furfurylated as previously described (Larsson-Brelid 1998; Lande et al. 2004;
153 Ringman et al. 2015). The samples were selected based on weight percent gain (WPG) and
154 EMC (20ºC, 65% relative humidity (RH)), resulting in i) for acetylated wood samples, an
155 average WPG of 22.6 % (ranging from 19.0 % to 25.9 %) and an average EMC of 3.54%
156 (ranging from 2.80% to 7.23%), and ii) for furfurylated wood samples, an average WPG of
157 69 % (ranging from 45.2 % to 98.3 %) and an average EMC of 4.53% (ranging from 3.38% to
158 5.56%). The selection was made based on the decay protection threshold levels proposed by
159 Thybring (2013). The samples were leached according to EN 84 (European Committee for
160 Standardization (CEN) Belgium 1996b), conditioned in 20ºC and 65% RH for two weeks and
161 the EMC after conditioning was recorded. The samples were sterilised with gamma radiation
162 (>30kGy, all samples sterilised at the same time) and placed two by two in Petri dishes
163 containing sterile soil and subsequently inoculated with 1 ml *P. placenta* (strain FPRL 280)
164 liquid culture. In total 576 acetylated, 596 furfurylated and 216 untreated specimens were
165 inoculated and 24 samples of each treatment were placed in Petri dishes without fungi (non-
166 inoculated controls).

167 ## *2.2 Mass loss and moisture content*

168 Samples (n = 8) were harvested continuously during the decay test, approximately every week
169 for untreated and every four weeks for treated samples. At harvest, mycelium covering the
170 samples was removed and each sample was weighed wet and dried (103 °C, 18 h). The decay
171 test was terminated after 60 days (untreated), 357 days (acetylated) and 396 days
172 (furfurylated).

173 ## *2.3 Structural integrity*

174 Four dried samples from each set of mass loss samples were selected for HEMI tests. From
175 each sample, 3 specimens of 10 × 5 × 10 mm^3 were cut out with a clipper. The development
176 and optimisation of the HEMI test have been described by Rapp et al. (2006). The following
177 procedure was used: 12 oven-dried specimens were placed in the bowl of a heavy-impact ball
178 mill, together with one steel ball of 40 mm, three of 12 mm, and three of 6 mm diameter. The
179 bowl was shaken for 60 s at a rotary frequency of 23.3 s^{-1} and a stroke of 12 mm. The
180 fragments of the 12 specimens were fractionated on a slit screen (ISO 5223, 1996), slit width
181 of 1 mm). The following values were calculated: i) the degree of integrity (I), which is the
182 ratio of the mass of the 12 biggest fragments to the mass of all fragments after crushing, ii) the
183 fine fraction (F), which is the ratio of the mass of fragments under 1 mm to the mass of all
184 fragments, multiplied by 100, and iii) the resistance to impact milling (RIM), which is
185 calculated from I and F as follows:
186

187 $$RIM = (I - 3 \cdot F + 300)/4 \ [\%]$$

188

189 The threefold weighing of the fine fraction was performed according to earlier studies (Rapp
190 et al. 2006) and can finely distinguish between different intensities of fungal decay. To ensure
191 that resistance to impact milling varies between 0 and 100 %, the constant, 300, was added.

192 2.4 Acetyl content

193 Prior to chemical analyses, dried samples were cut into small pieces and then ground with a
194 vibration mill (MM 400, Retsch) under cooling with liquid nitrogen and finally air-dried
195 overnight. The moisture content of the air-dried wood samples was determined separately by
196 drying at 105 °C. The determination of acetyl groups was carried out according to Månsson
197 and Samuelsson (1981) by means of an aminolysis with pyrrolidine and subsequent GC
198 analysis on a GC 2010 (Shimadzu) equipped with a BP5 (SGE) or a HP-5 (Agilent-
199 Technologies) capillary column. Temperatures: Inj.: 300°C; Det.: 310 °C; column 115°C.
200 Columns: BP5 (30 m, 0.25 μm film, 0.25 mm ID) and HP-5 (30 m, 0.25 μm film, 0.32 mm
201 ID).

202 3. Results

203 3.1 Mass loss

204 Untreated wood had 2% mass loss after 14 days, 19% mass loss after 28 days and reached
205 41% mass loss after 55 days (Fig. 2A). During the first 7 days the mass loss was negative.
206 The acetylated and furfurylated wood materials reached a maximum of 4% and 7% mass loss,
207 respectively (single samples) during the 300 days of the test period (Fig. 2A). Just as for
208 untreated wood, the mass loss was negative one week after exposure for both modified wood
209 materials. The mass loss increased for 55 days in acetylated wood and 98 days in furfurylated
210 wood with a rate that was 100 times lower than in the logarithmic phase of the untreated
211 wood. After these time points the decay rate flattened out and the average mass loss for
212 acetylated wood was 1.44% and for furfurylated wood 1.95% during the remaining part of the
213 decay test. Even though the WPG of the furfurylated wood samples varied considerably, mass
214 loss did not vary accordingly. This is probably due to that all samples were treated to a level
215 above the decay threshold of 35% WPG proposed by Thybring (2013). The acetylated
216 samples were on average above the proposed decay threshold of 20% WPG but a few single
217 samples had a WPG slightly lower.

218 3.2 Moisture content

219 In untreated wood, moisture content was ranging from 22-72% below 5% mass loss (one
220 sample had a moisture content below 25% and less than 3% mass loss, which EN 133 states
221 as abnormal (European Committee for Standardization (CEN) Belgium 1996a)), after which
222 moisture content increased with mass loss (Fig. 2B). The moisture content of the acetylated
223 and furfurylated wood samples varied between 13-52% and 20-43% respective, with no clear
224 trend except a possible decrease during the last 100 days (Fig. 2B). However, the moisture
225 content of the modified wood samples did never decrease below the moisture content of that
226 of the acclimatised (22°C, 65% RH) samples before the decay test.

227 3.3 Growth phases

228 The mass loss data of the present study was plotted logarithmically (Fig. 3). For untreated
229 wood, it was possible to detect three different stages in the mass loss curve similar to the
230 phases seen in liquid fungal cultures; the lag phase where the fungi adapt to the new
231 environment, the logarithmic phase where the growth rate of the fungi increases
232 logarithmically, and the stationary phase where the growth rate of the fungi flattens out.

Unfortunately, many of the samples in the lag phase had negative mass loss and did therefore not show in the logarithmic graph. For acetylated and furfurylated wood, there seems to be an increase in degradation rate up to approximately 150 days, however the rate is 100 times lower than in the logarithmic phase of fungi in the untreated wood.

3.4 Structural integrity after exposure to brown rot fungi

In untreated wood, structural integrity decreased with exposure time throughout the experiment (Fig. 4A). Loss of structural integrity was 10% after one week of exposure and negative mass loss, 21% at 1% mass loss, 50% at 7% mass loss, and 98% at 34% mass loss, compared to the zero-time sample. The non-inoculated control sample, incubated under the same conditions as the inoculated samples for 14 and 29 days, had a loss of structural integrity of 6%. Structural integrity showed good correlation to mass loss (R^2=0.89) (Fig. 4B).

The modification treatments caused loss of structural integrity in the samples (Fig. 4A). Acetylation decreased structural integrity by approximately 15% and furfurylation with approximately 50% compared to untreated wood. However, it has to be noticed that the variation in WPG for the furfurylated samples were large. Average loss of structural integrity over the whole decay test in acetylated wood was 9% compared to the zero-sample (Fig. 4A). In acetylated wood, samples with negative mass loss had 4% loss of structural integrity (fig 4B). In furfurylated wood, samples with negative mass loss had higher structural integrity than the zero-time sample (Fig. 4B). The average loss of structural integrity over the whole decay test was 0.5% in furfurylated wood (2% without the two samples with increased structural integrity). Looking at the time frame when mass is lost in the modified wood materials there is no correlation between loss of structural integrity and loss of mass (R^2=0.056 and 0.098 for acetylated and furfurylated wood respectively), while untreated wood in the same range of mass loss had a high positive correlation between loss of structural integrity and mass loss (R^2=0.859) (Fig. 4B).

3.5 Acetyl content

Measurements of acetyl content indicated that acetyl in acetylated wood was not degraded during the time course of this experiment (Fig. 5). Since measuring acetyl is a destructive method, acetyl before exposure to fungi was calculated from WPG using a standard curve of five samples. The point of intersection was 0.01, corresponding to the natural acetyl content in pine (Rowell 2005). Figure 5 shows the loss of acetyl during fungal exposure over time and the non-inoculated control (n=3). No significant difference between any of the samples could be detected. Since the non-inoculated control samples showed on average 3% loss of acetyl, it is possible that the calculation of original acetyl resulted in values a little low, in which case the loss of acetyl in all samples would be even less. However, the lack of a significant difference between exposed samples over time indicates that during the 120 days when bound acetyl was measured, no fungal degradation of acetyl occurred.

4. Discussion

4.1 Mass loss

The mass loss of the modified wood samples in the present study (Fig. 2A) is in accordance with previous studies, in which mass loss in furfurylated wood after 16 weeks of exposure to P. placenta was reported to be 1.1-2.4% at >120% WPG, 4.3% at 75% WPG and 1.11% at 38.9% WPG, although the samples dimensions were bigger than in the present study and dimensions as well as treatment methods varied between the previously reported studies

(Lande et al. 2004; Esteves et al. 2010). In previous durability studies on acetylated wood, no or little mass loss was seen in acetylated wood with approx. 20% WPG up to 36 weeks (Hill 2009; Pilgård et al. 2012; Alfredsen and Pilgård 2014).

Negative mass loss, as seen in Figure 2A, could be explained by that mass loss will not show until the decrease in mass due to degradation becomes larger than the increase in mass due to increasing fungal mass. Negative mass loss has been previously reported during brown rot decay, e.g. in Brischke et al. (2006; 2008) and Meyer and Brischke (2015), who suggested that, besides ingrown mycelium, nutrients could have been transported from the agar to the wood samples by the fungus. In the present study, soil plates were used, why ingrown mycelium probably constituted the major part of the negative mass loss. The fact that the mass of the ingrown mycelia cannot be distinguished from the wood mass leads to that it is impossible to see from mass loss data when the wood starts to be degraded.

4.2 Moisture content

In this study, fungi were added as liquid culture, which may explain the difference in moisture content between acclimatised samples before the decay test and the inoculated samples harvested in the beginning of the decay test. This is supported by the comparatively low moisture content values of the non-inoculated control samples, which were not inoculated with any liquid (Fig. 2B). The variation in initial moisture content of the samples may be due to that the liquid culture droplet sometimes stayed on top of the sample but sometimes ran into the soil.

In a previous study, moisture content in acetylated wood with approximately 20% WPG was 10-50% after four weeks and 5-70% after 28 weeks (Alfredsen and Pilgård 2014). Although the variation was higher, these results are in general in accordance with the results reported here. Schmöllerl et al. (2011) reported the moisture content in acetylated wood (23% WPG) to be approximately 45% after 2 weeks, 20% after 14 weeks and 15% after 26 weeks. During the same time period the moisture content in furfurylated wood (37% WPG) varied between 25-35%. However, in Schmöllerl et al. (2011) no water was added to the soil which may have caused the samples to dry out during the test.

4.3 Growth phases

The results from the untreated wood supports the proposed model that depicts that filamentous fungi growing in wood go through a lag, logarithmic and a stationary phase (Fig. 3) (Fuhr et al. 2011). It is, however, important to note that all measurements were done on the substrate and not on the fungi and therefore, even if it is unlikely, it is possible that the increase in fungal biomass does not show the same pattern as the decrease in the substrate mass.

Since the fungi seem to undergo the lag, logarithmic and stationary phases in untreated wood, they probably undergo these phases also in modified wood. The question is in which growth phase the fungi were in this experiment. The degradation rate in the modified wood materials were 100 times lower during the time period when mass loss increased than during logarithmic phase in untreated wood, which may suggest that the fungi in the modified samples were in the lag phase (Fig. 3). However, if the fungi in the modified wood samples in this experiment were in the log phase throughout the experiment, the question remains why the increase in mass loss eventually flattens out and, of course, what kind of mass is lost. The samples are leached before inoculation and therefore there should be only little nutrients and non-polymerised modification chemicals in the lumen. The untreated as well as modified non-

inoculated control samples had a mass loss of 0.5-1% up to 23 weeks of incubation (Fig. 3). It is possible that this is at least partly due to evaporation of volatile substances. The mass loss seen in the modified samples is however 2-3 times higher than in the non-inoculated controls.

If the fungi in the modified wood samples are in the logarithmic phase instead, the flattening out of the mass loss curve may be due to that the fungus is beginning to die, due to e.g. starvation. Furthermore, if the fungi are in the logarithmic phase, the mass loss seen is probably wood cell wall hemicelluloses and celluloses degraded by CMF and enzymatic degradation. If the degradation takes place mainly in areas where the modification level is locally very low, the fungi may go into starvation when the low treatment areas have been mainly degraded. As mentioned above, the moisture content is lower in the modified wood samples than in the untreated samples in which the fungi are in the logarithmic phase. However, if degradation occurs in areas with locally lower treatments levels, it is possible that the moisture content also is higher in these areas. It is also possible that the sample have dried a little between when the degradation occurred and when the sample was harvested.

In a complex solid material, such as wood, it is not unlikely that the fungi alternate between restricted and unrestricted growth. This may be due to stepwise invasion in the longitudinal direction (Fuhr et al. 2011). In modified wood, it may also be due to that the fungi will degrade areas with locally lower treatment levels faster than ones with higher treatment levels. Maybe, *P. placenta* in the acetylated and furfurylated samples in this experiment degraded the low treated areas exponentially during the first part of the decay experiment, but then went into a new lag phase. In that case it would be possible that the fungi would have started exponential degradation of areas with higher treatment levels once it had overcome the inhibition by the modification and if the test had been run longer.

4.4 Structural integrity after exposure to brown rot fungi

In a previous study by Brischke et al (2006), the correlation between structural integrity and mass loss during *Coniophora puteana* degradation of untreated wood was estimated to R^2=0.99, compared to R^2=0.9 in the present study (Fig. 4B). On the other hand, the correlation between compression strength and mass loss caused by the brown rot *Fomitopsis palustris* was again lower than the results in this study, R^2=0.76 (Maeda et al. 2014). The differences in results between the studies may be due to the differences in degradation patterns between different species of brown rot fungi. Brischke et al. (2012) showed that furfurylation of pine sapwood decreased structural integrity with 16%, but the WPG was only 15.6%. Assuming linear relationships, extrapolation of the results also with lower WPG in Brischke et al (2012) gives approximately 75% loss of resistance to impact milling at 70% WPG, which is in accordance with the results in Figure 4A.

In untreated wood subjected to brown rot, it is widely accepted that considerable strength loss occurs before mass loss can be detected (Winandy and Morrell 1993; Curling et al. 2002). The authors have not found any previous recordings of structural integrity in acetylated and furfurylated wood after brown rot exposure, but the results in this study indicate that the correlation between strength loss and mass loss may be different in modified wood materials than in untreated wood

For untreated wood, structural integrity of the samples with negative mass loss was lower than in the zero-time sample, which indicates changes in the chemical structure such as depolymerisation of cellulose caused by CMF degradation (Fig. 4B). However, it is also possible that a smaller mass than the fungal mass in the samples was degraded. Unfortunately,

375 due to that all four samples were run in a single test run, the structural integrity values are
376 mean values and standard deviation and significance could not be calculated. The large
377 variation in both WPG and mass loss in the modified wood samples makes it impossible to
378 find significant differences in structural integrity for these materials. However, the samples
379 are randomly distributed and hence it is unlikely that a considerable loss of structural integrity
380 over time would be masked by an increasing mean WPG. Therefore, we conclude that the
381 acetylated and furfurylated samples probably lose no or little structural integrity during
382 exposure to *P. placenta*, which would suggest that CMF degradation may not have occurred.
383 Furthermore, these results support the theory that the fungi in both acetylated and furfurylated
384 wood were in the lag phase. The fact that the non-inoculated control samples (incubated for
385 56 and 154 days) had similar structural integrity as the inoculated samples further supports
386 this theory.
387
388 In future studies, it is important to run samples with similar mass loss together when
389 performing the HEMI test. Replicates with more even mass loss, may be achieved by using
390 samples with identical WPG. Since four miniblock samples are needed for a single run of the
391 HEMI test, more replicates would also allow for replicate runs and hence provide the
392 possibility to calculate mean values and standard deviation.

393 4.4 Acetyl content

394 Figure 4 shows the loss of acetyl in per cent of original acetyl content over time in acetylated
395 wood. The results indicates that no or little acetyl was degraded in the acetylated samples in
396 this experiment, since no significant change in acetyl content is seen between 6 and 120 days
397 of exposure. All of the samples show approximately 3% lower levels of acetyl after exposure
398 to fungi, but it is unlikely that this is due to that 3% acetyl was degraded during the first week
399 after which no further degradation of acetyl occurred, at least if the cause of degradation in
400 supposed to be CMF degradation. The apparent loss of 3% acetyl in all samples is probably
401 rather connected to the conversion of WPG to acetyl content. The absence of degradation of
402 acetyl in the acetylated wood, may suggest that CMF degradation did not occur in these
403 samples or that the hydroxyl radicals were not able to degrade the ester bond connecting the
404 acetyl groups to the wood polymers. On the other hand, the results show that the acetyl
405 content of the acetylated wood remained intact during the 300 day decay test which means
406 that the treatment level remained intact.
407

408 5. Conclusions

409 Our results indicate that *P. placenta* growing on solid untreated pine, undergoes the same
410 growth phases as fungi in a liquid culture and thus support the model in which the filamentous
411 fungi *P. vitreus* was predicted to go through a lag, logarithmic and stationary phase while
412 growing on solid wood (Fuhr et al. 2011). Furthermore, our results show that degradation in
413 acetylated and furfurylated pine miniblock samples with the treatment levels used in this
414 study is inhibited or kept at a slow rate for more than 300 days of exposure to *P. placenta*.
415 The question remains whether this was i) due to that the fungi were unable to adapt to the
416 environment provided by the modified wood materials and thus were still in the lag phase, or
417 ii) if CMF and enzymatic degradation occurred during the first 200 days, during which the
418 fungi in this case were in the logarithmic phase, but not to a sufficiently high degree to
419 maintain fungal growth. No or little loss of structural integrity throughout the decay test
420 supports the theory that the fungi were still in the lag phase and unable to degrade or
421 depolymerise the wood cell wall polymers. Maintained structural integrity also indicates that
422 CMF degradation did not occur in either of the modified materials. Lack of degradation of

acetyl in acetylated wood may also support this theory or be a result of that the hydroxyl radicals were not able to degrade the ester bond linking the acetyl group to the wood polymer. In any case, it shows that the treatment level in the acetylated wood was not affected by 300 days of exposure to *P. placenta*.

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