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1 **Fibre degradation of wheat straw by *Pleurotus eryngii* under low moisture conditions during solid state**
2 **fermentation**

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12

13 **Running Title:** Fungal decomposing wheat straw under low moisture

14

15 **Significance and Impact of the Study**

16 In this study, a white rot fungus, *P. eryngii*, effectively decomposed fibre under low moisture conditions when
17 grown on wheat straw at similar levels under higher moisture conditions. However, the addition of wheat bran
18 to wheat straw created a heterogenous system that appeared to allow *P. eryngii* to thrive under much lower
19 moisture conditions although lower levels of fibre decomposition was obtained. These factors could influence
20 the preparation of solid state fermentation.

21

22 **Abstract**

23 The application of solid state fermentation offers an alternative to conventional, submerged approaches for a
24 variety of bioconversion processes, including animal feeds, biofuels and fungal bioproducts. Optimising solid
25 state fermentation under low moisture conditions could significantly impact the proportion of dry biomass that

26 could be processed and improve the commercial viability of this approach, because of reduced input costs and
27 higher yields of final products. *Pleurotus eryngii* that appeared to show tolerance to low moisture conditions
28 was grown on saturated and desaturated wheat straw. *P. eryngii* showed insignificant fibre degradation although
29 showed significantly lower biomass decomposition on desaturated wheat straw. Fibre decomposition by the
30 fungus on wheat straw containing wheat bran showed marginally higher decomposition when saturated although
31 there was no difference in biomass decomposition. The levels of delignification achieved were similar under
32 different saturation conditions. It would appear that the fungus effectively decomposed fibre under low
33 moisture conditions often resulting in lower biomass losses.

34

35 **Keywords**

36 moisture content · wheat straw · *Pleurotus* · evaporation · solid state fermentation

37

38 **Introduction**

39 Wood rot fungi that have a high tolerance to low moisture conditions with the ability to grow at much higher
40 solid to liquid ratios could have useful applications in solid state fermentation of agricultural waste products
41 (Wan and Li 2012). Some of the benefits would be lower energy requirements to regulate the temperature of the
42 fermentation, which could significantly increase with larger quantities of waste and the opportunity to extract
43 desirable products using organic solvents (Vamanu 2014). Most studies using agricultural waste substrates
44 reveal that higher moisture contents support optimal growth of wood rot fungi (Asgher *et al.* 2006; Shi *et al.*
45 2008), leading to significantly higher levels of lignin degradation (Shi *et al.* 2008). Many of these studies have
46 focused on the Basidiomycetes, *Phanerochaete chrysosporium*, *Lentinus edodes* and *Cereporiopsis*
47 *subvermispora*, which showed a direct correlation between growth rate and water potential (Badham 1989;
48 Boddy 1983; Wan and Li 2012). An evaluation of a wider range of Basidiomycetes has shown that each has a
49 specific and different set of moisture conditions, which support optimum growth between 65-85% moisture
50 condition and only one fungus grew optimally at a low moisture condition of 50-65% (Zadražil and Brunnert
51 1981). It has been suggested that fungi associated with dried fallen stems are most likely to exhibit higher
52 tolerance to water stress conditions (Boddy 1983). Surprisingly, natural isolates of *Trametes* sp. and *Collybia*
53 *sierraleonis* recovered from tropical regions did not show any increased level of tolerance to water stress

54 conditions compared with fungal strains recovered from temporal regions (Mswaka and Magan 1999; Singh
55 1989).

56 Wheat straw that has been pre-processed using some form of milling process contains vessels which,
57 through capillary action, may draw water inside the plant matrix and the non-homogenous structure of this
58 substrate (haphazardly arranged and different sized straw fragments) will ensure that there are spaces allowing
59 gaseous exchange. However, wheat bran does not have the vessel structures but fine granular structure requires
60 lower moisture conditions for higher enzymatic production as shown by the Ascomycete, *Trichoderma*
61 *longibrachiatum* in producing xylanase (Azin *et al.* 2007), by the Ascomycete, *Aspergillus* sp. in producing
62 cellulase (Vu *et al.* 2011) and by white rot fungus, *Pleurotus pulmonarius* in producing manganese peroxidase
63 (Farani de Souza *et al.* 2006). Presumably, the granular composition of wheat bran causes the particles to
64 become more closely associated, thus limiting the volume of space being formed between the bran particles that
65 would allow gaseous exchange. Such high saturation conditions would have a negative influence on gas
66 diffusion and perhaps other parameters such as enzyme activities, temperature regulation, and substrate
67 inhibition (Gervais and Molin 2003). This is supported in a previous study showing that steady low levels of
68 aeration are required in order to achieve significant rates of delignification (López *et al.* 2002).

69 The aim of this study was to determine the degradation characteristics of saturated and desaturated
70 wheat straw by fungi, which showed quite high tolerance to low moisture conditions. The saturated wheat straw
71 contains freely available water within the plants vessels allowing optimal fungal growth. In contrast, it is
72 presumed that the desaturated wheat straw contains little free water except in the narrowest plant vessels. The
73 availability of free water and bound water will have an impact on microbial utilization (Magan 2007). Fungi
74 were selected based on a prior experiment revealing that *P. erygnii* showed quite high tolerance to low moisture
75 conditions compared with other white rot fungi, while *P. pycnoporus* was somewhat tolerant (Baker,
76 unpublished).

77

78 **Results and discussion**

79 The initial trial showed that *P. erygnii* and *G. tsugae* completely colonized the wheat straw, although *P. erygnii*
80 showed greater changes in the fibre composition. Three fungi showed only partial colonization but showed fibre
81 decomposition in the following decreasing order: *G. resinaceum*, *L. edodes*, and *P. sanguineus*. In a previous

82 study, it was shown that *L. edodes* was quite tolerant to water stress conditions when grown on wood rather than
83 glycerol amended medium (Badham 1989). In our study, *G. australe*, *P. ostreatus* and *C. subvermispora* failed
84 to colonize the wheat straw. This study was performed using smaller Microsacs with filter seams in closer
85 proximity to the substrate may have resulted in the significant decrease in moisture content of the wheat straw.
86 Therefore, only fungi which rapidly grew may have completely colonized the wheat straw.

87 The saturated and desaturated wheat straw substrate, containing mineral nutrients, was inoculated with similar
88 quantities at 41.2 ± 1.5 g and 48.8 ± 11.2 g of *P. eryngii* infected grain, respectively. It can be assumed that the
89 wheat straw that now has a lower moisture content because some of the water previously associated with wheat
90 straw was absorbed by wheat bran. It is possible that the addition of moist infected grain may allow moisture to
91 be translocated by the mycelium to as shown in a previous study in the fungal degradation of stacked wood
92 blocks (Stienen *et al.* 2014). The moisture contents of the saturated and the desaturated wheat straw at the start
93 of the experiment were significantly different ($P = 0.0004$) and the moisture content decreased significantly by
94 the end of the experiment in the saturated wheat straw ($P = 0.009$), whereas the moisture content in the
95 desaturated wheat straw remained unchanged (Table 1). The expectation would be that the moisture content
96 would increase as fungal degradation proceeds, but unlike wood, the fragments of wheat straw would allow for
97 evaporation that would offset the possible increase in moisture content. A direct comparison with the data in
98 another study revealed that these moisture contents lie within the optimal range (Zadrazil and Brunnert 1981),
99 where the optimum may be midway (Saha *et al.* 2017). Fungal degradation significantly decreased the total dry
100 weight by $18.0 \pm 3.0\%$ in saturated wheat straw and by $11.0 \pm 2.0\%$ in desaturated wheat straw ($P = 0.03$). As
101 expected, the fibre composition in the saturated and desaturated experiments at the start was similar (Table 2)
102 and there were no significant differences in fibre composition between saturated and desaturated wheat straw at
103 the end of the experiment after fungal degradation. The fibre composition showed some significant changes
104 after fungal degradation, which differed under both saturation conditions compared with the undegraded wheat
105 straw at the start. It would be expected that after such a long incubation period where the fungus had
106 completely colonized the substrate that differences in fibre composition would be found, if there were any,
107 under the different treatments. It is uncertain whether a longer incubation would have shown differences.
108 Similar levels of delignification occurred under saturated and desaturated conditions and the total percentage of
109 lignin degraded was slightly higher (Table 3).

110 Similar quantities of *P. eryngii* infected grain were inoculated into wheat straw containing wheat bran
111 with 17.8 ± 2.1 g under saturated conditions and with 23.0 ± 4.5 g under desaturated conditions. The moisture

112 contents of saturated and desaturated wheat straw at the start of the experiment were significantly different ($P =$
113 0.007) and the moisture contents remained unchanged until the end of the experiment (Table 1). A direct
114 comparison with a previous study showed that the moisture contents of the desaturated wheat straw was just
115 below the lowest limit examined where degradation and delignification was restricted for most white rot fungi
116 (Zadražil and Brunnert 1981). The moisture contents of wheat straw containing wheat bran were significantly
117 lower compared with wheat straw containing mineral salts ($P < 0.01$) under both saturation conditions and
118 presented much lower moisture conditions. However, the optimal moisture conditions for fungal growth on
119 wheat bran are lower (Farani de Souza *et al.* 2006) compared with wheat straw and wheat bran may support the
120 initial stages of fungal growth. The addition of wheat bran to wheat straw at the start of the experiment
121 revealed that the proportion of non-fibre had significantly increased, whereas the proportion of cellulose had
122 decreased in comparison to the experiments containing only wheat straw (Table 2). The degradation in terms of
123 dry weights by *P. eryngii* in saturated and desaturated wheat straw were $14.1 \pm 5.1\%$ and $6.7 \pm 5.6\%$, but these
124 were not significant different. Fungal degradation resulted in significant compositional changes in the straw,
125 causing an increase in the non fibre content ($P = 0.046$) and a decrease in the hemicellulose content ($P = 0.003$)
126 under saturated conditions compared with desaturated conditions (Table 2). Although a constant amount of
127 wheat bran (72 g) was added to 250 g of wet wheat straw (saturated or desaturated), wheat bran formed $50.9 \pm$
128 2.8% of the total dry weight under saturated conditions and $37.4 \pm 0.4\%$ of the total dry weight under
129 desaturated conditions. Therefore, the proportion of wheat bran associated with saturated wheat straw was
130 significantly greater ($P = 1 \times 10^{-6}$) and growth of *P. eryngii* appeared to be more favourable under saturated
131 conditions. The addition of wheat bran appeared to have lowered the extent of delignification but this was not
132 significantly different under saturated or desaturated conditions (Table 3). It is uncertain whether the lower
133 levels of delignification were caused by using an easily accessible substrate, wheat bran, or by using lower
134 quantities of grain inoculums. The total percentage of lignin degraded was higher in comparison to the
135 percentage of delignification due to the higher biomass losses. *P. eryngii* appeared to cause similar levels of
136 degradation under these water stress conditions compared with more saturated conditions, which is in contrast
137 with most white rot fungi grown under slightly higher moisture conditions of 50% (Zadražil and Brunnert
138 1981).

139 The results confirmed those obtained from an initial trail showing that *P. eryngii* caused similar
140 levels of fibre decomposition under both saturation conditions. The addition of dry wheat bran reduced the
141 moisture content further but this did not affect fibre decomposition by either fungi even under the much lower

142 moisture conditions. The growth of fungi under lower moisture conditions would facilitate the extraction of
143 compounds that require organic solvents and improve the operating conditions for solid-state fermentation.

144

145 **Materials and methods**

146 **Preparation of fungal inoculum**

147 A variety of fungi: *Ganoderma resinaceum* GR, *Ganoderma tsugae* XHMF, *Ganoderma australe* GA1,
148 *Lentinus edodes* CYN, *Pleurotus erygnii*, *Pycnoporus sanguineus* V and *Pleurotus ostreatus* Pox K from the
149 Bangor University culture collection and *Ceriporiopsis subvermispora* D98698 from the VTT (Finland) culture
150 collection were grown on desaturated wheat containing mineral solution in an environmentally controlled room
151 during an initial trial. An initial trial was performed to determine which fungi grew under low moisture
152 conditions using Microsacs (49 cm x 22 cm with 5 filter seams – 4 on one side and 1 on the other). This
153 experiment was repeated in triplicate based on the trial using *P. erygnii* which grew well under low moisture
154 conditions. These fungus was first grown on 2% malt extract agar. A 5 mm² square was excised from the edge
155 of the fungal colony that had been growing on 2% malt extract agar for one week at 22 °C and inoculated into
156 autoclave sterilised (121 °C, 15 min) mushroom spawn bags (Microsac, SacO₂, Belgium). Each Microsac (49
157 cm x 22 cm with 5 aeration filter seams) containing 200 g wheat grain, 1.2 g calcium sulphate and 220 ml
158 distilled water, were heat sealed and incubated for 3 weeks at 22 °C.

159

160 **Preparation of Microsacs containing wheat straw and mineral nutrients**

161 The substrate was prepared by soaking air dried hammermilled wheat straw that was collected through 5-10 mm
162 screens (2 kg), in mineral solution [10 L, containing 2 g L⁻¹ ammonium chloride, 0.5 g L⁻¹, magnesium
163 sulphate, 0.2 g L⁻¹ potassium dihydrogen phosphate, 0.2 g L⁻¹ disodium hydrogen phosphate, 0.35 g L⁻¹
164 manganese sulphate, 0.007 g L⁻¹ ferrous sulphate, and 0.007 g L⁻¹ copper sulphate] at 80 °C for 30 min. After
165 soaking, the excess liquid was allowed to freely drain through a sieve. Half of this wheat straw was used to
166 prepare six Microsacs (40 cm x 51 cm with 4 filter seams) each containing saturated wheat straw (250 g) and the
167 remaining wheat straw was used to determine moisture content.

168 The other half of the wheat straw was placed into a large cloth bag and centrifuged at 2800 rpm for 5 min
169 (White Knight spin dryer) to obtain desaturated wheat straw. This wheat straw was distributed as 250 g into

170 each of six Microsacs and moisture content was determined using the remaining wheat straw. All of the
171 Microsacs containing the wheat straw were autoclaved at 121 °C for 60 min which was demonstrated to have no
172 effect on the moisture content. Once the substrate had equilibrated to room temperature, each Microsac was
173 inoculated with 45.0 ± 8.3 g of fungus infected grain to roughly equal proportions as judged by eye with the aim
174 of preparing the substrate bags relatively quickly to avoid potential contamination issues with an open spawn
175 bag. Then the bags were heat sealed, thoroughly mixed and weighed again to determine the weight of grain
176 added. The Microsacs were incubated at 22 °C, 65% relative humidity for 42 days for *P. eryngii* which
177 reflected the time required for the fungus to completely colonize the wheat straw.

178

179 **Preparation of Microsacs containing wheat straw and organic nutrients**

180 Substrate was prepared by soaking air dried milled wheat straw (2 kg) in hot water (10 L) at 80 °C for 30 min
181 and removing the excess by sieving. Half of this wheat straw was used to prepare six Microsacs each containing
182 250 g wet wheat straw, 73 g dry wheat bran (purchased from a health store and <2 mm diameter) and 11 g dry
183 calcium sulphate which was thoroughly mixed. The other half of wheat straw was centrifuged at 2800 rpm for 5
184 min and 250 g of this desaturated wheat straw was placed into each of six Microsacs along with 73 g dry wheat
185 bran and 11 g dry calcium sulphate which was thoroughly mixed. Each of the Microsacs were thoroughly
186 mixed, autoclaved and once cooled, were inoculated with 20.4 ± 4.6 fungus infected wheat grain and weighed
187 again to determine the weight of grain added. The Microsacs incubated at 22 °C for 21 days in a humidity
188 controlled room at 65%. The addition of wheat bran to wheat straw enabled fungal colonization by both fungi
189 to occur more rapidly compared to the previous experiments where only wheat straw was used.

190

191 **Analysis of samples**

192 The contents of each Microsac was thoroughly mixed and samples were collected at the start and at the end of
193 the experiment to determine mass loss, moisture content and fibre composition. Fibre analysis was determined
194 as previously described (Baker *et al.* 2015) although the lignin content was determined by incubating each of the
195 Ankom Microfiber bags after ADF extraction in 72% (v/v) H₂SO₄ (5 ml) for 2 h at 22 °C, then adding distilled
196 water (140 ml) and autoclaving for 1 h at 121 °C 15 p.s.i. Finally, the bags were dried at 103 °C for 24 h and
197 weighed. The ash content was determined in the Microfiber bags by furnace drying the bags after NDF, ADF
198 and lignin extraction at 600 °C for 4 h. The actual hemicellulose, cellulose and lignin contents were determined
199 by subtracting the ash content after NDF, ADF and lignin extraction, respectively.

200

201 **Determination of moisture content**

202 The moisture content was determined on triplicate samples by oven drying at 70 °C for 24 h to a constant dry
203 weight and calculating the difference of wet weight and dry weight divided by the wet weight which was then
204 expressed as a percentage.

205

206 **Statistical analysis**

207 Statistical analysis of the calculated data was performed using Student's *t*-test and values with $P < 0.05$ are
208 described as statistically significant as described in the results and discussion. The data presented in the tables
209 showing statistical differences were analysed using SPSS Statistics 22 by analysis of variance.

210

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214 **Conflicts of interest**

215 None of the authors has any conflict of interest in publishing this study.

216

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Table 1 The moisture percentage of wheat straw inoculated with *P. eryngii* at the start and at the end **when supplemented** with mineral salts or with wheat bran.

	WS	WS + WB
saturated (at start)	78.7 ± 0.8 ^a	59.4 ± 0.8 ^c
saturated (at end)	75.5 ± 0.9 ^{ab}	63.4 ± 2.6 ^c
desaturated (at start)	67.0 ± 0.4 ^{bc}	44.6 ± 6.9 ^d
desaturated (at end)	67.4 ± 0.5 ^{bc}	46.7 ± 2.9 ^d

Means within a row lacking a common superscript differ (P < 0.05).

Table 2 Comparison of fungal degradation by *P. eryngii* for each of the fibre components under saturated (wet) and desaturated (dry) conditions at the start and end for wheat straw only (WS) or wheat straw with wheat bran (WS+WB). Samples were analysed on days 0 (D0), 21 (D21) and 42 (D42).

		non fibre	hemicellulose	cellulose	lignin	ash
WS	wet D0	19.6 ± 0.2 ^a	29.5 ± 0.5 ^a	41.2 ± 0.8 ^a	6.8 ± 0.5 ^a	4.1 ± 0.1 ^a
	wet D42	32.3 ± 3.2 ^b	22.2 ± 0.5 ^b	37.8 ± 2.7 ^a	4.7 ± 0.8 ^a	4.5 ± 0.1 ^a
	dry D0	19.0 ± 0.5 ^a	30.1 ± 1.0 ^a	41.3 ± 0.8 ^a	5.0 ± 2.6 ^a	3.9 ± 0.1 ^a
	dry D42	31.0 ± 0.1 ^b	21.3 ± 2.3 ^b	36.3 ± 4.1 ^{ab}	4.7 ± 1.5 ^a	4.4 ± 0.3 ^a
WS + WB	wet D0	38.4 ± 0.9 ^b	27.4 ± 0.5 ^a	21.3 ± 0.5 ^c	5.7 ± 0.8 ^a	7.1 ± 0.8 ^b
	wet D21	40.1 ± 1.8 ^b	21.5 ± 0.7 ^b	22.5 ± 1.8 ^c	5.4 ± 0.3 ^a	10.4 ± 1.0 ^c
	dry D0	31.6 ± 7.9 ^b	27.8 ± 1.6 ^a	24.7 ± 6.5 ^c	7.8 ± 1.8 ^a	8.1 ± 1.6 ^b
	dry D21	30.4 ± 3.6 ^b	28.3 ± 4.7 ^a	34.2 ± 14.7 ^{bc}	6.6 ± 0.8 ^a	8.2 ± 1.3 ^b

Means within a row lacking a common superscript differ (P < 0.05).

Table 3 Percentage of delignification and total lignin degradation by *P. eryngii* with respect to different fungal inocula contained within grain into wheat straw immersed in mineral salts (WS) and into wheat straw supplemented with wheat bran (WS+WB) under saturated and desaturated conditions.

		Inoculum weight (g)	Delignification (%)	Total lignin degraded (%)
WS	Saturated	41.2 ± 1.5 ^a	32.3 ± 11.5 ^a	33.3 ± 10.9 ^a
	Desaturated	48.8 ± 11.2 ^a	19.5 ± 14.4 ^a	29.4 ± 10.2 ^a
WS+WB	Saturated	16.9 ± 1.4 ^b	5.9 ± 6.1 ^a	17.9 ± 8.9 ^a
	Desaturated	23.0 ± 4.5 ^b	15.8 ± 10.5 ^a	19.8 ± 12.6 ^a

Means within a row lacking a common superscript differ ($P < 0.05$).