

Biodegradation of Different Genotypes of *Miscanthus* by Wood Rot Fungi

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Miscanthus, which is comprised of several different genotypes, is an important high-biomass crop with applications in the biofuel industry and in the formation of biocomposite materials. The overall composition of *Miscanthus* can be altered *via* degradation with wood rot fungi. The starting composition revealed that the cellulose content of *Miscanthus x giganteus* was higher than that in *Miscanthus sacchariflorus* and that the lignin contents were similar in both genotypes. Of the wood rot fungi, only *Lentinus edodes* appeared to have completely colonized *M. sacchariflorus* and showed significant degradation. In contrast, all of the brown rot fungi showed partial colonization of both *Miscanthus* genotypes and had little effect on the fibrous composition. Cellulose degradation by some white rot fungi increased with cellulose content whereas cellulose degradation by other fungi was independent of cellulose content. All of the white rot fungi showed similar rates of lignin degradation, except for *Pleurotus ostreatus*, which was higher on *M. sacchariflorus*. The effect of the moisture contents of *Miscanthus* on cellulose and lignin decomposition by *Phlebiopsis gigantea* SPLog6 and *Coniophora puteana* 11E was also investigated. These results revealed subtle differences in the growth of white rot fungi on different *Miscanthus* genotypes.

Keywords: *Miscanthus sacchariflorus*; *Miscanthus x giganteus*; *Lentinus edodes*; *Pleurotus ostreatus*

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INTRODUCTION

Recently, there has been an interest in growing fibrous plants in the grassland areas of Wales for the production of bioethanol and biocomposite materials (Charlton *et al.* 2009). *Miscanthus* is one such high-biomass crop, whose fiber length is sufficient to form paper (Ververis *et al.* 2004), and which has been incorporated already as a component within mixed biocomposite boards (Park *et al.* 2012). The total lignin content of *Miscanthus* is generally much higher than in other agricultural crops, *e.g.*, wheat straw, falling between 23.7% and 28.5%, which resembles the lignin content in soft and hard woods (Donaldson *et al.* 2001; Ververis *et al.* 2004; Goff *et al.* 2012). The majority of the lignin occurs in the outer ring of the *M. x giganteus* stem and decreases exponentially towards the pith (Kaack *et al.* 2003). In comparison to the stems, the leaves contain similar proportions of lignin but differ by having lower fiber content (Kim *et al.* 2012).

Most research has investigated the fungal biodeterioration of commercial wood, such as the discoloration caused by *Trichoderma* sp. (Huh *et al.* 2011) or the fungal degradation of timber (*e.g.* Nicolotti *et al.* 2010), but more recently the emphasis has shifted towards bioindustrial applications. The exposure of potential forestry waste to

Coniophora puteana led to a decline in its total mass, resulting in an increased release of glucose (Ray *et al.* 2010), although scale-up with the prospect of biopulping using either the white rot fungus, *Ceriporiopsis subvermispora*, or the brown rot fungus, *Postia placenta*, showed little effect on lignin degradation (Giles *et al.* 2012). Another study identified *Trametes velutina* in being effective in delignifying *Populus tomentosa* after 12 weeks incubation (Wang *et al.* 2014). In addition to wood, white rot fungi grows on a wide range of lignocellulose materials originating from agricultural waste that would otherwise present problems in disposal, and by means of the fungi these materials can be turned into useful products such as biofuels, animal feeds, or paper, or further biorefined into potentially valuable products (Sánchez 2009; Menon and Rao 2012). The growth of different types of white rot fungi has been examined on corn stover (Shrestha *et al.* 2008), wheat straw (Hatakka 1983; Moyson and Verachtert 1991; Wan and Li 2010; Arora *et al.* 2011; Shrivastava *et al.* 2011), rice straw (El-Din *et al.* 2013), ula grass (*Carex meyeriana*) (Mao *et al.* 2013), and Bermuda grass (Gamble *et al.* 1994).

Few studies have examined the growth of wood rot fungi on *Miscanthus*. In Osono's study, *Trametes versicolor* exhibited the most active degradation of leaves, at 43% of the total mass, when the leaves were laid onto 2% agar for 12 weeks. However, the leaves contain easily biodegradable components (Kim *et al.* 2012) that are perhaps readily accessible, whereas the composition of *Miscanthus* stems is quite different. It was calculated that *T. versicolor* had degraded 46% of the lignin, but the amount of lignin remaining in the degraded material showed little change from the original starting material. In another study, fungi associated with *M. x giganteus* were isolated, and studies of its degradation showed that some Ascomycota were principally involved, although it was concluded that they were unlikely to be as effective as white rot fungi under suitable conditions (Shrestha *et al.* 2011). A recent study using three white rot fungi revealed that growth occurred causing changes in the fibrous composition that varied depending on the fungus (Baker *et al.* 2015).

The aim of this study was to examine the biodegradation of *M. sacchariflorus* and *M. x giganteus* by different wood rot fungi. The effects would be assessed based on dry weight loss, degradation of cellulose, and degradation of lignin. The fungi used in the study were comprised of six white rot fungi, three brown rot fungi, and a fungus involved typically in the discoloration of wood, *Trichoderma* sp.

EXPERIMENTAL

Preparation of Microcosms

Three types of *Miscanthus* genotypes – *M. sacchariflorus* with low fibrous content (LF); *M. giganteus* x *M. sacchariflorus* x *M. sinensis*, sterile triploid with high fibrous content (HF); and *M. sacchariflorus* x *M. sinensis*, tetraploid were provided by the Institute of Biological, Environmental, and Rural Sciences (IBERS), Aberystwyth University. The plants were established from rhizome material in late spring of 2005 and planted in replicate plots of 25 m² with 49 plants per plot. The plant material was harvested in March 2011 and roughly chipped, air-dried, and stored in a dry environment. The material (10% w/v) was thoroughly mixed in hot distilled water at 80 °C, left for 30 min, and sieved. Then, 20 g of wet plant material containing 3.851 ± 0.008 g of oven-dry weight *Miscanthus* was placed into each glass jar and sealed with a metal lid containing a 10-mm hole that was plugged with cotton wool to allow for gaseous exchange. The glass jars were

autoclaved for 60 min at 121 °C and 103.4 kPa. When the glass jars had cooled, they were inoculated in triplicate with agar squares excised from fungal cultures that had grown for one week on malt extract agar plates. Three jars remained un-inoculated to act as the control. All fungi used in this study were kept at the Bangor University culture collection. The fungi used in this study were white rot fungi (*Lentinus edodes* CYN, *Pleurotus ostreatus* Pox K, *Phlebiopsis gigantea* SPLog6 (Forest Research), *Ganoderma australe* GA1, and *Ganoderma lucidum* GL1), brown rot fungi (*Fibroporia vaillantii* (FPRL14A), *Coniophora puteana* 11E, and *Gloeophyllum trabeum* (MUCL 11353; BAM 109; FPRL 108N; MUCL 11663), and an Ascomycete (*Trichoderma* CP021). Each of the glass jars was weighed empty and then weighed again when it was filled with the autoclaved *Miscanthus* and the inoculant. The glass jars were incubated in the dark, in an environmentally controlled room at 22 °C, and at 65% humidity for four weeks. On the final day, the glass jars were weighed, and the samples were removed to determine their moisture content. The remainder was placed into a blender for 1 to 2 min until fine particles were apparent, then oven-dried at 105 °C for 24 h and used for subsequent analyses.

To determine the effects of moisture content on the growth of fungi, another experiment was performed in which increasing amounts of water were added to the different microcosms. A sample of air-dried *M. sacchariflorus* x *M. sinensis* (5.22 ± 0.013 g) was placed into each glass jar, and 5.75 (omitted for *C. puteana* 11E), 10.45, 15.17, 19.91, 24.59, and 29.39 g of distilled water was added to each in triplicate. These microcosms were autoclaved and inoculated as previously described, except that they were either inoculated with either *P. gigantea* SPLog6 or *C. puteana* 11E. A yeast extract (1% w/w) was included in the microcosms that were inoculated with *C. puteana* 11E in order to encourage mycelial growth throughout the jars. The microcosms were incubated and destructively analyzed as previously described.

Analysis of Microcosms and Original Plant Material

The wet weight of the *Miscanthus* in the microcosms was measured, and samples were collected for a determination of the moisture content before autoclaving. The moisture lost during autoclaving was taken into account to accurately calculate the moisture content of the microcosms at the start of the experiment. At the end of the experiment, a sample was removed from each microcosm in the region where the fungus had colonized the *Miscanthus*. The sample was oven-dried at 105 °C for 24 h to determine the moisture content, as previously described for wood (Knight 1931). Once the water content was calculated, it became possible to determine the dry mass remaining in each microcosm after weighing the total wet mass. The fiber content was determined by means of acid detergent fiber extraction using an Ankom 2000 fiber analyzer (Ankom Technology, New York, USA). Briefly, 0.5 g of oven-dried *Miscanthus* was placed into each filter bag that was then heat-sealed, and a filter bag without *Miscanthus* was included as a blank control. The filter bags were washed in a solution of 2 g/L cetyl trimethylammonium bromide and 1 N sulphuric acid (Lowry *et al.* 1994) and processed as described in the manufacturer's instructions (Ankom). Lignin was extracted from a 0.3 g oven-dried *Miscanthus* using the Klason method (Hatfield *et al.* 1994) with a minor modification, as 3 h of incubation at 20 °C with 5 mL of 72% (v/v) sulphuric acid was found to result in higher digestion than 2 h of incubation. The ash content was determined by heating 1 g of dry *Miscanthus* (undegraded and degraded by each of the fungi) in a muffle furnace for 4 h. The cellulose percentages were calculated by subtracting the lignin and ash percentages from the fiber percentages. The percentages of lignin were calculated by subtracting the ash percentages.

Finally, the percentages of cellulose and lignin were obtained by normalizing to the microcosms that remained uninoculated and showed no changes in fibrous content.

Statistics

Using IBM SPSS Statistics version 20, statistical analyses in the form of ANOVA followed by Tukey's *post hoc* test were performed on the values of composition of each *Miscanthus* genotype and on data obtained using different moisture contents. Statistical analysis of the mass loss percentages was determined using *t*-Test for each fungus degrading both *Miscanthus* genotypes.

RESULTS AND DISCUSSION

Composition of *Miscanthus* Genotypes before Degradation

The cellulose content of *M. x giganteus* was significantly different from the cellulose contents of the other two genotypes, *M. sacchariflorus* and *M. sacchariflorus x M. sinensis* (Fig. 1). Lignin extracted from both *Miscanthus* genotypes showed that there were no significant differences between them. These results correlated well with those in previous studies (Hodgson *et al.* 2010). The percentages of lignin recovered were perhaps a little higher than those previously described using the Klason method (Ververis *et al.* 2004; Goff *et al.* 2012). The temporary storage of *M. giganteus* may have marginally increased the percentages of cellulose and lignin caused by the losses in volatiles or by degradation by *Ascomycetes* (Graham *et al.* 2012). However, once the *Miscanthus* was harvested, dried, and chipped, it was used within a few months, so it was unlikely that *Ascomycetes* caused much degradation.

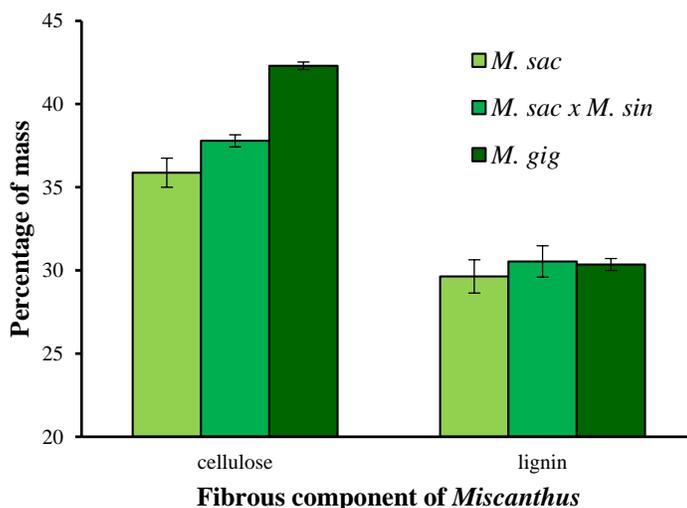


Fig. 1. The percentages of cellulose and direct Klason lignin extraction were determined in the autoclaved *M. sacchariflorus* LF (*M. sac*), the *M. sacchariflorus x M. sinensis* (*M. sac x M. sin*), and the *Miscanthus x giganteus* (*M. x gig*).

Effects of Fungi on Dry Mass

The fungal degradation of *M. sacchariflorus* and *M. x giganteus* in terms of mass loss from each microcosm can be found in the Appendix (Table 1). *L. edodes* CYN, *P.*

ostreatus Pox K, *P. gigantea* SPLog6, *G. lucidum* GL1, and *Trichoderma* CP021 formed visible mycelia that clearly colonized the entire jar containing both genotypes of *Miscanthus*, whereas *G. australe* GA1, *F. vaillantii* FPRL 14A, *C. puteana* 11E, and *G. trabeum* FPRL 108N only colonized *Miscanthus* around the point of inoculation. Some fungi, such as *L. edodes* CYN in *M. sacchariflorus* and *C. puteana* 11E in *M. x giganteus*, appeared to show greater degradation of the plant material compared to other fungi (Fig. 2).

Statistical analyses revealed that the dry weight associated with *L. edodes* CYN was significantly lower than that of *G. trabeum* 108N, and that the dry weight associated with *C. puteana* 11E was significantly lower than that associated with *G. australe* GA1 and *F. vaillantii* 14A. The dry weight losses showed large errors, and it was speculated that due to the heterogeneity of the grass material, the small portion sampled might have only crudely reflected the total amount degraded in each microcosm. Consequently, smaller weight losses that might have occurred would not have been detected, and a previous study with *P. gigantea* grown on wheat straw revealed a low weight loss (Salvachúa *et al.* 2011).

Effect of Fungi on Cellulose

Assuming there is an inverse correlation between cellulose and hemicellulose in the various *Miscanthus* genotypes, it would be expected that these hemicelluloses would be degraded more quickly in *M. x giganteus* than in *M. sacchariflorus*. None of the cellulose data showed any significant differences between the different fungi (Fig. 2 and Table 1). When the percentages of cellulose degradation were calculated, *P. gigantea* SPLog6, *G. australe* GA1, and *G. lucidum* GL1 showed higher degrees of cellulose degradation on *M. x giganteus* than on *M. sacchariflorus*, and this was found to be significant using the t-test (Fig. 2). Therefore, cellulose degradation appeared to be directly correlated with the cellulose content of each genotype of *Miscanthus*.

These results obtained with *P. gigantea* compared well with the results of another strain grown on wheat straw that resulted in high cellulose degradation (Salvachúa *et al.* 2011). *P. gigantea* is considered effective at degrading all components of lignocellulosic materials (Hori *et al.* 2014), whereas *Ceriporiopsis subvermispora* is considered a more selective degrader of lignin (Wan and Li 2010). However, especially large differences in the proportion of cellulose that was degraded were observed between *G. australe* GA1 and *G. lucidum* GL1, perhaps reflecting high cellulase activities occurring in *M. x giganteus* in response to the higher cellulose contents. It would appear that these fungi are not selective lignin degraders. However, not all fungi showed the same response to the higher content of cellulose in *Miscanthus*. In contrast, the extent of cellulose degradation by *L. edodes* CYN was similar in both genotypes, which would seem to indicate that the presence of more cellulose in *M. x giganteus* did not dramatically increase the cellulase activity. Consequently, it would appear that as the cellulose content increased within *Miscanthus*, the fungi such as *L. edodes* CYN that exhibited lower levels of cellulase activity may have produced a degraded product that had lower lignin content than that of other fungi.

Therefore, it would appear that among the fungi used in this study, *L. edodes* CYN showed the most selective lignin degradation when grown on *Miscanthus*. Its growth on other lignocellulosic materials may have resulted in the expression of other isoenzymes involved in cellulose degradation.

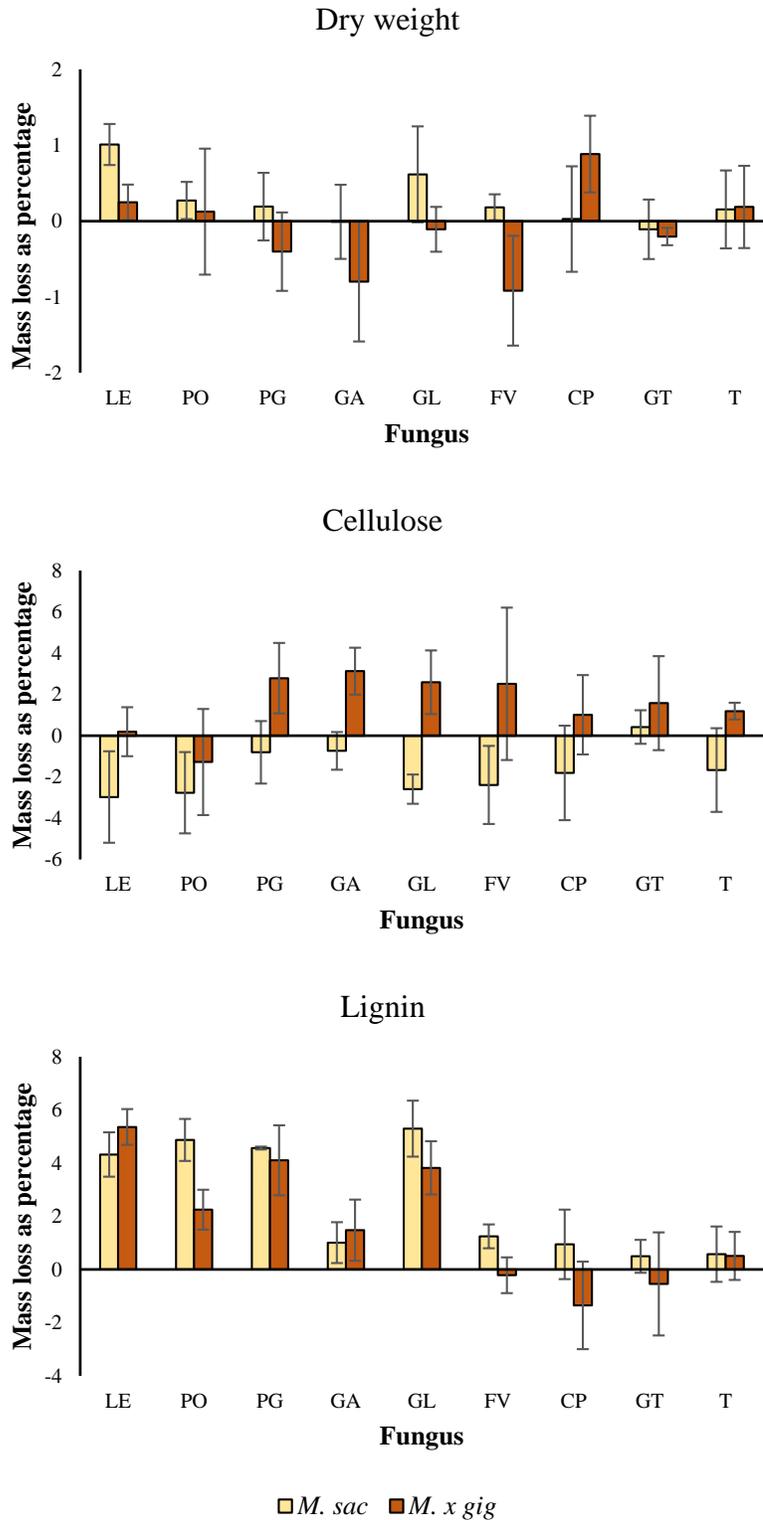


Fig. 2. Changes in the percentages of dry weight cellulose, and lignin from the original proportions present in the *M. sacchariflorus* LF genotype (yellow bars) and *M. x giganteus* HF genotype (orange bars) after degradation by different fungi: (LE) *Lentinus edodes* CYN, (PO) *Pleurotus ostreatus* Pox K, (PG) *Phlebiopsis gigantea* SPLog6, (GA) *Ganoderma australe* GA1, (GL) *Ganoderma lucidum* GL1, (FV) *Fibroporia vaillantii* 14A, (CP) *Coniophora puteana* 11E, (GT) *Gleophyllum trabeum* FRRL 108N, and (T) *Trichoderma* sp.

Effects of Fungi on Lignin

An analysis of the lignin contents remaining in the fungi-degraded material revealed that the fungal degradation of the *M. sacchariflorus* LF genotype could be divided into two distinct groups (Fig. 2 and Table 1). Significantly higher proportions of lignin were degraded in the microcosms inoculated with *L. edodes* CYN, *P. ostreatus* Pox K, *P. gigantea*, and *G. lucidum* GL1 compared with the microcosms inoculated with *G. australe* GA1, *F. vaillantii* 14A, *C. puteana* 11E, *G. trabeum* 108N, and *Trichoderma* sp. CP021. The fungal degradation of the *M. x giganteus* HF genotype showed that *L. edodes* CYN, *P. ostreatus* Pox K, *P. gigantea* SPLog6, *G. australe* GA1, *G. lucidum* GL1, and *Trichoderma* sp. CP021 all achieved higher percentages of lignin degradation than did *C. puteana* 11E. However, *L. edodes* CYN and *P. gigantea* also showed higher percentages of lignin degradation compared to *P. ostreatus* Pox K, *G. australe* GA1, *F. vaillantii* 14A, *C. puteana* 11E, *G. trabeum* 108N, and the uninoculated control. The percentages of lignin degradation achieved by *P. ostreatus* Pox K and *F. vaillantii* 14A grown on *M. sacchariflorus* were significantly higher than those grown on *M. x giganteus*. In contrast, *L. edodes* CYN, *P. gigantea* SPLog6, and *G. lucidum* GL1 showed no significant differences in lignin degradation when grown on *M. sacchariflorus* versus *M. x giganteus*.

In contrast to the indigestible lignin that is obtained in other studies when lignin is extracted after the recovery of fiber, in this study lignin was extracted directly from *Miscanthus*. The same direct procedure was used to recover lignin from *Miscanthus* leaves that had been degraded by white rot fungi, and although *T. versicolor* had degraded a significant proportion of lignin, the proportion of lignin that remained appeared to show little change (Osono 2010). Other, less-studied fungi, such as *Marasmius* sp. and *Coccomyces* sp., appeared to be far more effective in removing up to 38% of the lignin from the remaining partially degraded *Miscanthus* leaves. One possible reason for this was the ready availability of nutrients in the *Miscanthus* leaves, in contrast to the nutrients present in chopped *Miscanthus* stems, which are inaccessible until delignification occurs. Previous studies have suggested that up to 30% of the indigestible lignin in wheat straw can be degraded by different white rot fungi (Singh *et al.* 2011; Knežević *et al.* 2013), and it would appear that considerably less delignification occurs in *Miscanthus*. In these studies, a variety of modifications were used that involved either inoculation using spores or the use of a mineral medium that could promote fungal colonization. It was found that the protein content in another genotype of *Miscanthus* was about 2.4% (Huyen *et al.* 2010), which is quite high compared to the protein content of other agricultural materials, *e.g.*, corn stover and hardwoods (Duncan and Schilling 2010). High nitrogen contents are known to have a negative effect on lignin-degrading activity (Reid 1983). It may be possible to increase delignification by incubating fungi such as *L. edodes* CYN and *C. subvermispora*, which appear to show more selective lignin degradation, on *Miscanthus* for longer periods.

Effects of Moisture Content on Fungal Growth

The mass of the *Miscanthus* degraded by *P. gigantea* SPLog6 appeared to increase with increases in moisture content. However, statistical analyses revealed that although there were no significant differences between the smaller microcosms (6 g dry weight), in the larger microcosms (30 g dry weight), significantly higher decomposition occurred among those with moisture contents of 227% and 406% compared with the microcosms with moisture contents of 43%. Cellulose degradation was significantly higher in the microcosms with moisture contents of 78% compared with those with moisture contents of 309% and 406%.

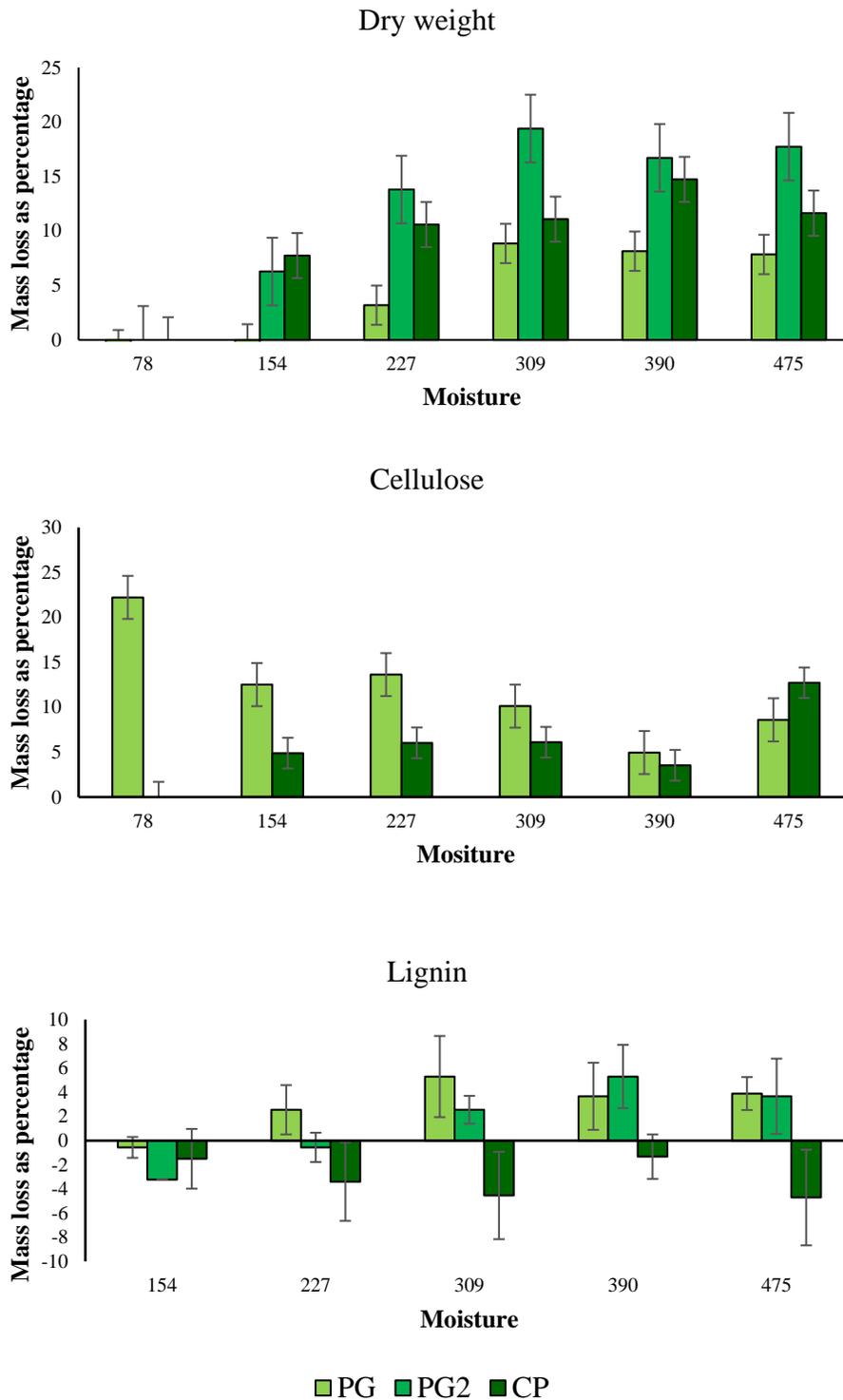


Fig. 3. The effect of different moisture contents on the percentages of dry-weight cellulose and lignin remaining in *M. sacchariflorus* x *M. sinensis* degraded by (PG) (*P. gigantea* SPLog6) and CP (*C. puteana* 11E). (PG2) are microcosms containing 100 g wet *Miscanthus*; (PG) and (CP) contain 30 g *Miscanthus*.

In the microcosms that had moisture contents of 227%, 309%, and 390%, respectively, the amount of lignin degraded was $8.3 \pm 1.1\%$, $6.7 \pm 0.5\%$, and $6.8 \pm 1.9\%$. Significantly more lignin was degraded in these microcosms than in the microcosms with moisture contents of 78%. Although some white rot fungi can affect degradation using radical generation, biomass is generally degraded using a combination of lignin-modifying peroxidases (manganese peroxidase, lignin peroxidase, and versatile peroxidase), laccases, oxalic acid, and hydrogen peroxide (Lundell *et al.* 2010), and lower moisture contents would ensure that enzymes were in close proximity to the substrates. To retain similar levels of enzyme activity, higher concentrations of enzymes may need to be produced when moisture conditions increase.

C. puteana 11E appeared to show increases in decomposition with increases in moisture content, although statistical analyses revealed no significant differences. Although more cellulose appeared to be degraded in the microcosms with moisture contents of 475%, neither cellulose nor lignin showed any significant differences in degradation within microcosms of any particular moisture content compared to others. However, statistical analyses of the fiber contents in the microcosms with moisture contents of 475% showed that significantly more fiber had been degraded than in microcosms with moisture contents of 154%, 309%, and 390% (data not shown). Brown rot fungi uses oxidative systems to degrade biomass during the initial stages of decomposition (Baldrian and Valášková 2008), which may show optimal activity when the biomass particles are in close proximity to each other. In fact, milled corn stover has been the only non-wood material where the growth of brown rot fungi was investigated (Duncan and Schilling 2010).

CONCLUSIONS

1. There were clear differences between white rot fungi which completely colonized *M. sacchariflorus* causing changes in the fibrous composition, whereas brown rot fungi (*Fibroporia vaillantii* 14A, *Coniophora puteana* 11E, *Gleophyllum trabeum* FRRL 108N) only partially colonized *M. sacchariflorus*. Among the white rot fungi, *L. edodes* CYN was found to cause the most degradation of *M. sacchariflorus*.
2. *P. gigantea* SPLog6 and both *Ganoderma* sp. (*G. australe* GA1 and *G. lucidum* GL1) appeared to degrade more cellulose as the quantity of cellulose present in the *Miscanthus* genotypes increased. In contrast, the other fungi analyzed in this study (*L. edodes* CYN and *P. ostreatus* Pox K) showed no significant differences in the extent of cellulose degradation between the two different *Miscanthus* genotypes.
3. *P. ostreatus* Pox K showed higher delignification when grown on the *M. sacchariflorus* than on *M. giganteus*, which was accounted for by the lower cellulose content. Other white rot fungi did not differ in the extent of delignification between the two *Miscanthus* genotypes.
4. Generally white rot fungi showed low levels of delignification in *Miscanthus* compared with other lignocellulosic substrates, although small improvements could be made such as moisture content optimization. Other physiological parameters may have further impacts on delignification and conditions could be strictly controlled in future studies using a solid state bioreactor.

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APPENDIX

Table 1. Percentages of fiber, cellulose, and lignin remaining in the *M. sacchariflorus* LF genotype (LF) and *M. x giganteus* HF genotype (HF) after degradation by different fungi*

	Dry mass			Fibre			Cellulose			Lignin		
	LM	HM	t-Test	LM	HM	t-Test	LM	HM	t-Test	LM	HM	t-Test
LE	1.07	0.26	0.02	-0.31	5.81	0.02	-5.51	-0.30	0.10	5.20	6.11	0.17
	0.72	0.47		2.13	6.71		-1.40	1.54		3.54	5.17	
	1.25	0.01		2.21	4.12		-2.02	-0.68		4.23	4.80	
PO	0.26	0.00	0.78	1.50	-1.13	0.47	-4.28	-4.20	0.47	5.78	3.07	0.01
	0.52	-0.64		0.86	2.25		-3.48	0.66		4.34	1.59	
	0.03	1.01		3.97	1.79		-0.54	-0.30		4.50	2.09	
PG	0.00	0.11	0.20	2.65	7.24	0.03	-1.88	1.63	0.10	4.53	5.61	0.58
	0.70	-0.93		1.76	7.90			4.74		4.57	3.16	
	-0.13	-0.38		4.87	5.53		0.26	1.98		4.61	3.54	
GA	0.12	-0.74	0.21	0.81	4.64	0.00	-1.09	1.84	0.01	1.90	2.80	0.59
	0.40	-1.61		-0.82	4.44		-1.42	3.54		0.61	0.90	
	-0.55	-0.04		0.83	4.73		0.30	4.00		0.53	0.73	
GL	1.05	-0.24	0.14	2.99	5.62	0.00	-3.33	0.81	0.01	6.32	4.82	0.15
	0.90	-0.32		2.81	7.22		-2.54	3.39		5.35	3.83	
	-0.11	0.23		2.31	6.38		-1.91	3.57		4.22	2.81	
FV	0.06	-0.98	0.06	0.27	4.05	0.26	-0.56	3.75	0.11	0.84	0.30	0.04
	0.38	-1.60		-2.62	5.46		-4.34	5.42		1.73	0.04	
	0.11	-0.16		-1.09	-2.62		-2.27	-1.64		1.18	-0.98	
CP	0.14	0.77	0.15	-0.40	-0.48	0.56	-2.43	-0.95	0.18	2.03	0.00	0.27
	0.66	0.44		-2.40	-0.70		-3.72	1.08		1.32	-0.02	
	-0.71	1.44		0.22	0.16		0.73	2.90		-0.51	-0.03	
GT	-0.53	-0.18	0.70	1.90	0.94	0.88	0.70	-0.69	0.45	1.20	1.62	0.43
	0.24	-0.10		-0.26	0.40		-0.50	1.54		0.24	-1.14	
	-0.04	-0.33		1.09	1.76		1.05	3.87		0.04	-2.11	
T	-0.07	-0.36	0.94	-2.19	2.51	0.03	-3.93	1.01	0.08	1.73	1.49	0.94
	0.74	0.20		-0.80	1.97		-1.09	1.65		0.29	0.32	
	-0.21	0.73		-0.28	0.61		0.01	0.89		-0.29	-0.28	

* (LE) *Lentinus edodes* CYN, (PO) *Pleurotus ostreatus* Pox K, (PG) *Phlebiopsis gigantea* SPLog6, (GA) *Ganoderma australe* GA1, (GL) *Ganoderma lucidum* GL1, (FV) *Fibroporia vaillantii* 14A, (CP) *Coniophora puteana* 11E, (GT) *Gleophyllum trabeum* FRRL 108N, and (T) *Trichoderma* sp. The columns showing t-test are statistical analysis between LF and HF samples for each fungus and those in bold are significantly different.

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