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anantias@ubiobio.cl

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Wei, Dongsheng; Schmidt, Olaf; Liese, Walter
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METHOD TO TEST FUNGAL DEGRADATION OF BAMBOO AND WOOD USING VERMICULITE AS RESERVOIR FOR MOISTURE AND NUTRIENTS

Dongsheng Wei ^{1,*}, Olaf Schmidt¹, Walter Liese¹

ABSTRACT

Proposed is a method to investigate degradation of lignocelluloses by pure cultures of basidiomycetes using preserving jars with vermiculite as reservoir for water and nutrients. Bamboo samples of *Gigantochloa atroviolacea* and *Phyllostachys pubescens* and wood samples of *Fagus sylvatica* and *Pinus sylvestris* were inoculated with the brown-rot fungus *Coniophora puteana* and the white-rot fungus *Schizophyllum commune*. The fungi were cultured on vermiculite containing different amounts of tap water or malt extract solution. Mass loss of the bamboos after 32 weeks was low and did not show a remarkable influence of moisture content and nutrient addition. However, considerable degradation of *Pinus sylvestris* sapwood occurred by *C. puteana* whereby moisture and nutrients influenced aggressiveness.

Keywords: Bamboo degradation, vermiculite, moisture content, nutrient addition.

INTRODUCTION

Bamboo is a fast growing woody grass with increasing importance for sustainable production of materials with many applications for structures and industrial utilization. However, bamboo has a low natural durability and is attacked by fungi during storage, transport, processing and final use (Liese and Kumar 2003). There are several reports on its degradation by fungi (Liese 1959, 1985; Abdurachim 1964, Mohanan 1997, Kim *et al.* 2011, Suprapti 2010, Ma *et al.* 2010, Schmidt *et al.* 2011). The behaviour of bamboo against decay fungi is an important parameter in bamboo establishment and use. Most investigations revealed that bamboo degradation is due to white and soft rot fungi, whereas brown-rot species were less aggressive. Among the investigated fungi, *Coniophora puteana* and *Schizophyllum commune* varied with respect to the test method used: Low maximum mass loss was measured on agar under pure culture condition, whereas considerable degradation occurred in the 'fungus cellar test' where the samples are placed in large metal containers on unsterile garden soil with different moisture accessibility (Schmidt *et al.* 2011). However, *C. puteana* and *S. commune* differed in decay activity: *C. puteana* produced maximum mass loss at low moisture content of 57%, whereas decay by *S. commune* was highest at 182% moisture content (Schmidt *et al.* 2011).

Mixed microbial decay in soils with various water holding capacities was studied by Becker and Kaune (1966). Kaune (1970) and Worrall and Wang (1991) used for wood a vermiculite system with pure cultures of soft-rot fungi and proved its suitability for wood degradation tests. Adaskaveg *et al.* (1991) showed decay of palm wood by basidiomycetes with the vermiculite-block assay. Curling *et al.* (2002) exposed basidiomycetes to wood in a vermiculite-soil system. The American standard soil-block test ASTM D1413 was not used for our study because it is principally similar to our former fungus cellar test (Schmidt *et al.* 2011).

The aim of this study was to test bamboo and wood degradation by pure cultures of basidiomycetes with vermiculite as reservoir of moisture and nutrients.

¹ Section Wood Biology, Centre for Wood Science, University of Hamburg, Leuschnerstrasse 91d, 21031 Hamburg, Germany

*Corresponding author: dongsheng.wei@uni-hamburg.de

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MATERIALS AND METHODS

Fungi

The brown-rot fungus *Coniophora puteana* strain 167 from the laboratory collection and the white-rot fungus *Schizophyllum commune* strain 87 isolated from bamboo (Schmidt *et al.* 2011) were held on agar plates of 2% malt extract (Oxoid) and 1.5% agar (Oxoid).

Bamboo and wood samples

Bamboo species *Gigantochloa atrovioleacea* and *Phyllostachys pubescens* were used. Culm sections of *G. atrovioleacea* (diameter 5.5-7.0 cm) and *P. pubescens* (diameter 5.5-6.5 cm) were obtained from CONBAM, Geilenkirchen, Germany and the Bamboo Centre, Baden-Baden, Germany, respectively. Culms were proven with the curcuma-test (Peylo 2001) to ensure that no boron was present which is commonly used against moulding. The samples were cut to 5 cm length, 2.5 cm width and 0.5–3.5 cm wall thickness. Wood samples of *Fagus sylvatica* and *Pinus sylvestris* were cut to 5 cm length, 2.5 cm width and 1.5 cm height. Samples were put into a climate room at 20±2°C and 65±5% relative humidity (RH) for 4 weeks, weighed, wrapped in plastic (Sengewald Flexopeel, Germany) and send for gamma radiation to BBF sterilization service GmbH (Kernen, Germany). To calculate the initial weight, moisture content at 20°C and 65 % was adjusted downward to the oven-dry value.

Vermiculite test

The decay experiment was performed in 500 ml preserving jars (Fig. 1). Vermiculite of 2-3 mm particle size was obtained from vermiculite-shop (thinex new media, Dortmund, Germany, www.vermiculit-shop.de) and sieved through a 2 mm sieve. Each preserving jar was filled with 200 ml vermiculite. Tap water or 2% malt extract solution were added in 80, 100, 120, 140 and 160 ml amounts per jar. The jars covered with glass lids were sterilized in the autoclave at 121°C and 210 kPa for 30 min. Each two bamboo or wood samples were put aseptically in one jar. Two jars were used for one fungus. Vermiculite and sample were separated by a metal ring to avoid liquid diffusion in the sample. A plug of the test fungus from agar plates was then placed on the samples top. After 32 weeks of incubation at 20±2°C and 65±5% RH, the mycelium was removed from the sample surface, the samples were weighed for final wet weight and oven dried at 103°C for 3 days. Mass loss was calculated by weight comparison before and after incubation. Standard deviation for each 4 specimens per treatment group was calculated according to ANOVA, SPSS software 2002.



(a)



(b)

Figure 1. Vermiculite test setup.

(a) bottle with vermiculite and bamboo samples. (b) incubation room.

Assessment of fungal growth and vitality test after incubation

The development of fungal growth on the samples was assessed according to the scheme in table 1, which values hyphal density and hyphal coverage of sample. After 32 weeks of incubation, portions of overgrown vermiculite were transferred from the jars to malt agar plates to prove the vitality of the mycelium after the vermiculite test.

Table 1. Evaluation scheme for hyphal growth on bamboo and wood samples.

Hyphal density	Description	Hyphal coverage of sample	Description
0	No growth	0	No coverage
1	Sparse mycelium	1	1 - 33% coverage
2	Normal mycelium	2	34 - 66% coverage
3	Thick mycelium	3	67 - 99% coverage
		4	Total coverage

RESULTS AND DISCUSSION

Figure 2 shows details of fungal growth on bamboo samples and on vermiculite particles.

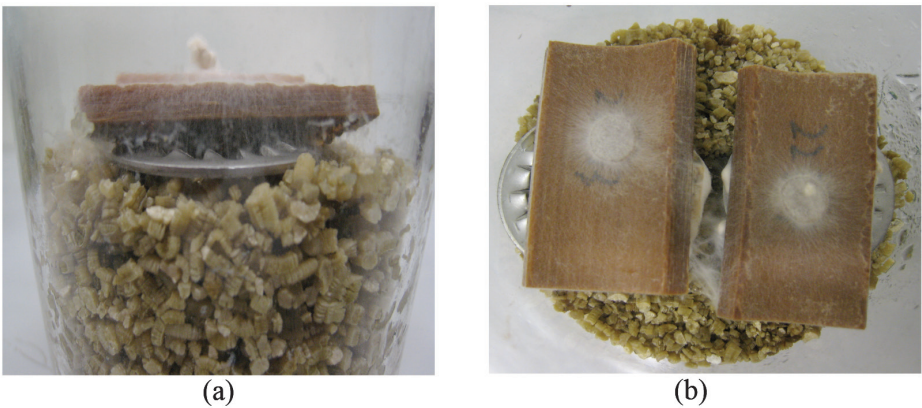


Figure 2. Details of a preserving jar with mycelium of *Schizophyllum commune* covering *Phyllostachys pubescens* samples.
(a) side view. (b) top view.

All bamboo and wood samples were totally covered by mycelium after 32 weeks (Table 2). Hyphal density with tap water was less than with malt extract. However, the density of mycelium is not necessarily related to mass loss in test specimens (Schmidt 2006).

Table 2. Fungal growth on bamboo and wood samples after 32 weeks incubation.

Bamboo	Fungus	Liquid	Liquid (ml) per preserving jar									
			80		100		120		140		160	
			D	C	D	C	D	C	D	C	D	C
<i>Gigantochloa atrovioleacea</i>	<i>Coniophora puteana</i>	Tap water	1	4	1	4	1	4	1	4	1	4
		Malt extract	2	4	2	4	2	4	2	4	2	4
	<i>Schizophyllum commune</i>	Tap water	1	4	1	4	1	4	1	4	1	4
		Malt extract	2	4	2	4	2	4	2	4	2	4
<i>Phyllostachys pubescens</i>	<i>Coniophora puteana</i>	Tap water	1	4	1	4	1	4	1	4	1	4
		Malt extract	2	4	2	4	2	4	2	4	2	4
	<i>Schizophyllum commune</i>	Tap water	1	4	1	4	1	4	1	4	1	4
		Malt extract	2	4	2	4	2	4	2	4	2	4
<i>Fagus sylvatica</i>	<i>Schizophyllum commune</i>	Tap water	1	4	1	4	1	4	1	4	1	4
		Malt extract	2	4	2	4	2	4	2	4	2	4
<i>Pinus sylvestris</i>	<i>Coniophora puteana</i>	Tap water	1	4	1	4	1	4	1	4	1	4
		Malt extract	3	4	3	4	3	4	2	4	2	4

D = hyphal density, C = hyphal coverage of sample

Results of the degradation tests are summarized in figures 3 and 4. Standard deviation is not shown for the less mass loss by *S. commune* (Fig. 3), but is shown for *C. puteana* (Fig. 4) due to its greater decay capacity. Both bamboo species *Gigantochloa atrovioleacea* and *Phyllostachys pubescens* were rather resistant to degradation by the white-rot fungus *S. commune* and the brown-rot fungus *C. puteana* over 32 weeks of incubation, the first bamboo being more resistant than the latter one. Adding different amounts (80 to 160 ml) of water or nutrient solution to vermiculite had only small effect. The final bamboo moisture contents were 61 to 84 %, which is a suitable range for decay fungi (Schmidt 2006).

Figure 3 shows the results for *S. commune*. Maximum mass loss of bamboo was only 4.6%. Thus, possible influences among the parameters, bamboo species, moisture content and nutrient addition, were rather indistinct for this fungus. Low decay was also measured on agar in preserving jars and in Kolle flasks (Schmidt *et al.* 2011) which was already reported by Suprapti (2010) and Kim *et al.* (2011). Abdurachim (1964) obtained 15% mass loss in *Gigantochloa apus*. Also beech wood showed neglectable decay (0.7-2 %) by *S. commune* (Fig. 3), which corresponds to previous results on low degradation of wood samples (Schmidt and Liese 1978). However, this fungus is common on bamboo culms during storage and use (Liese 1985, Mohanan 1997, Liese and Kumar 2003).

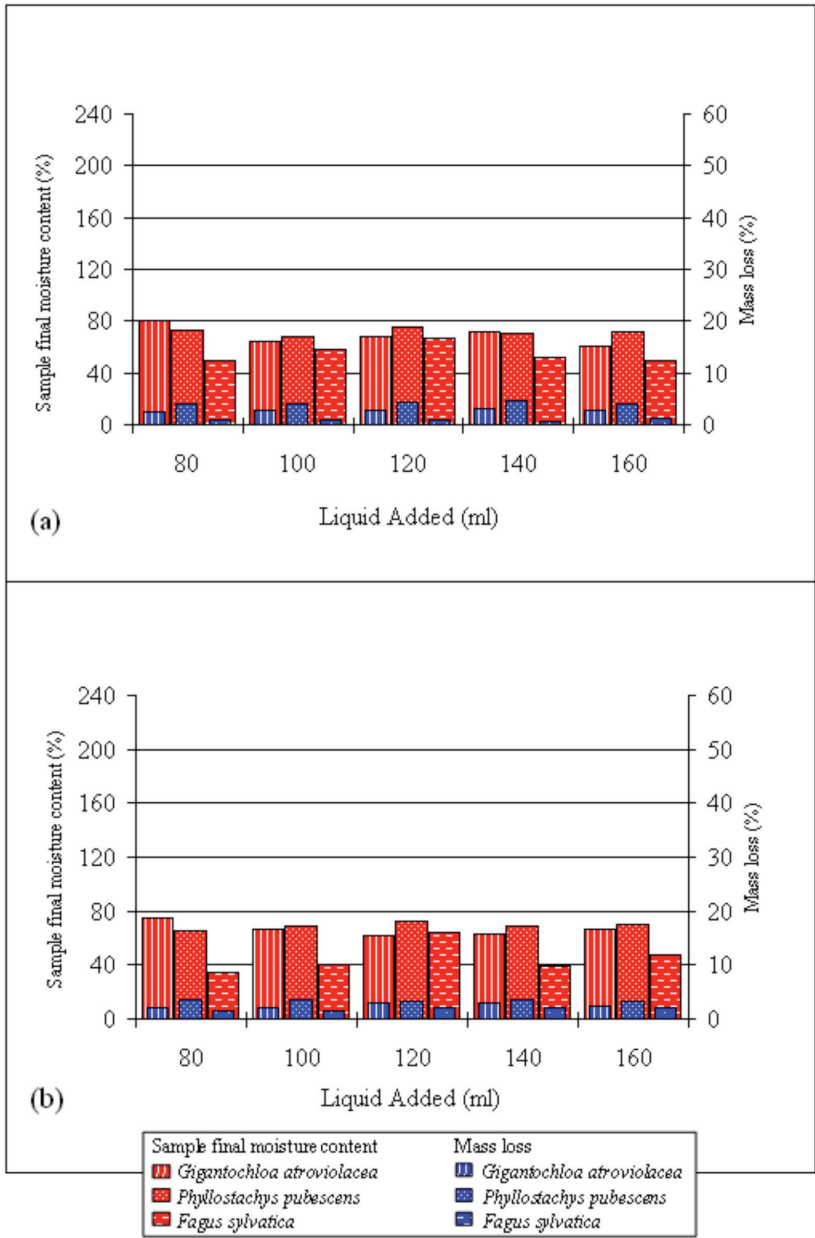


Figure 3. Influence of moisture content and nutrients on mass loss by *Schizophyllum commune*.
(a) incubation with tap water. (b) incubation with malt extract solution.

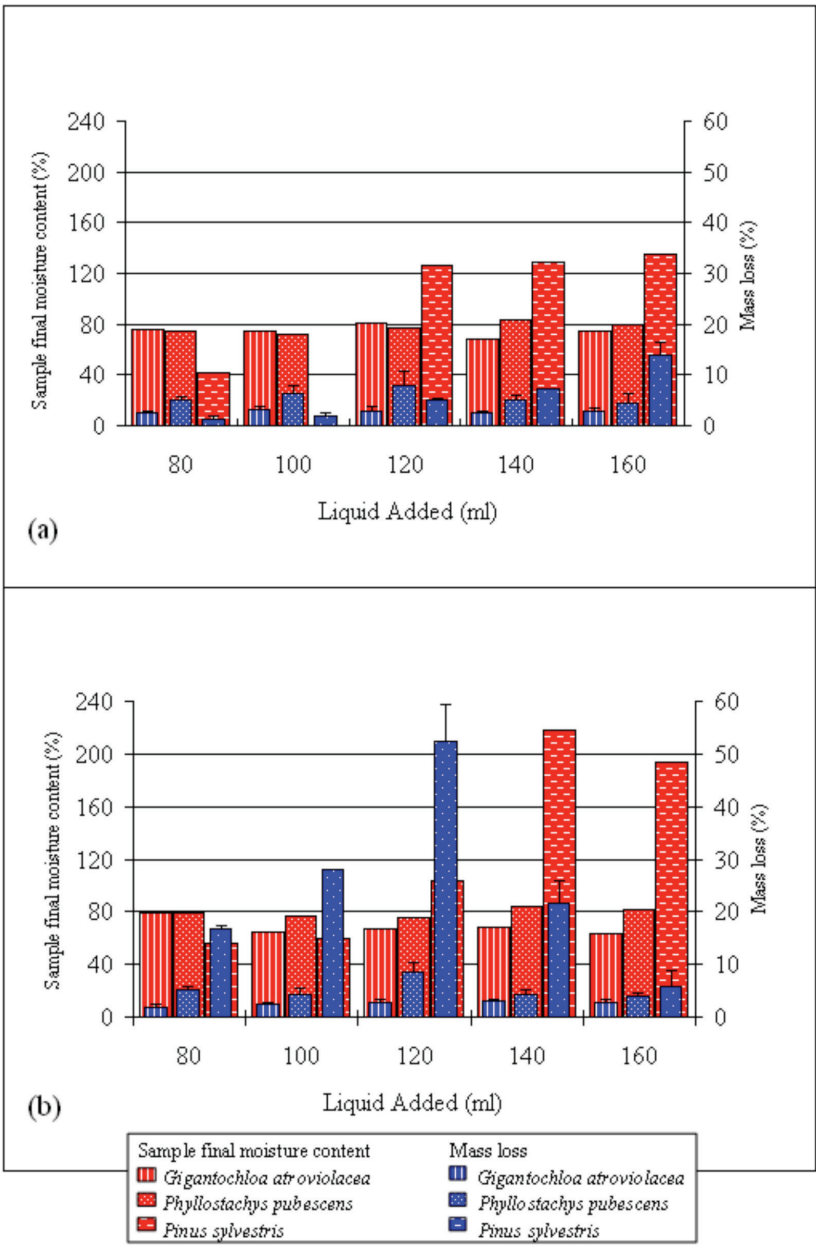


Figure 4. Influence of moisture content and nutrients on mass loss by *Coniophora puteana*. (a) incubation with tap water. (b) incubation with malt extract solution.

Figure 4 shows the results for *C. puteana*. Maximum mass loss of bamboo was 8.4%, whereby *P. pubescens* was more decayed than *G. gigantochloa*. Both liquid additions, tap water and malt extract, increased mass loss from 80- to 120 ml-addition followed by decrease to 160 ml. Scots pine wood revealed significant differences among the liquid additions: With tap water, decay increased stepwise to 14.0 % maximum at 160 ml water addition. Most decay with 52.5% maximum at 120 ml (104 % moisture content) occurred with the malt extract culture. The subsequent decrease of mass loss may be caused by too high moisture content.

Fungi are able to transport water by their mycelia from a moisture source to neighbouring wood (Schmidt 2006). Figures 3 and 4 show that the final moisture content of the bamboo samples was not influenced by the different initial liquid additions. Obviously, both fungi did not transport water from vermiculite to the samples. Presence of the metal ring between vermiculite and sample could not be the reason because *C. puteana* could moisten the Scots pine wood. Figure 4 (bottom) shows that moisture content increased up to 218 %u parallel to water addition. Subsequently, mass loss decreased because of too much water in the wood.

The vitality test after 32 weeks of incubation proved living mycelium in all culture vessels (Fig. 5). Because fruit bodies were not grown in the jars, survival was not due to resistant spores. Thus, it can be deduced that mycelia were active over the whole culture period.

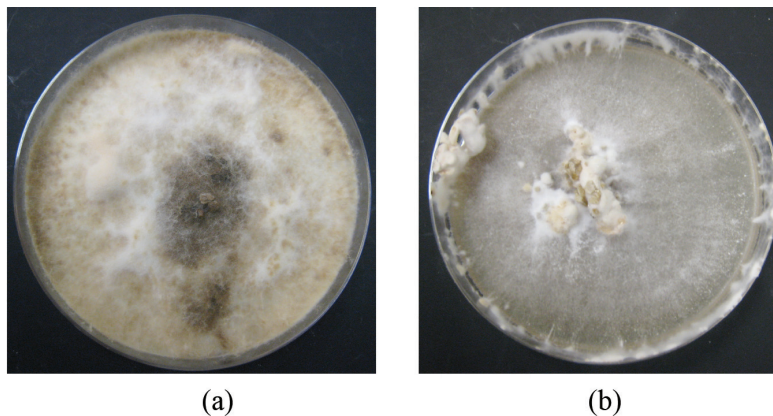


Figure 5. Vitality test on agar after vermiculite incubation.
(a) *Coniophora puteana*. (b) *Schizophyllum commune*.

Schmidt *et al.* (2011) assumed that high bamboo mass loss by *C. puteana* and *S. commune* in the fungus cellar test was mainly caused by the moisture conditions and lesser by components from the unsterile soil. The vermiculite test as pure-culture experiment in closed vessels did not show a significant effect of moisture content and nutrient addition on bamboo degradation. Thus, bamboo degradation in nature may be also influenced by better air conditions, soil minerals or vitamins from soil bacteria. Already Leutritz (1946) demonstrated that soil serves as water holding substrate and provides substances from the soil. The influence of minerals and vitamins on soft rot was reported by Worrall and Wang (1991) and Worrall *et al.* (1991). However, the small differences of mass loss among the bamboos, fungi and test parameters make further discussion problematic. The fungi had been selected due to three reasons: *S. commune* is very common on bamboo and the experimental strain was isolated by us from bamboo; *C. puteana* is an obligatory test fungus in the European standard EN 113; third, it was hoped to explain the influence of moisture content and nutrients in the fungus cellar test. However, with regard to wood, the decay results with *C. puteana* and *P. sylvestris* samples (Fig. 4) show the suitability of the proposed technique for degradation studies. Assumably, our vermiculite method will also show greater fungal activity against bamboo if more aggressive fungi such as *Pleurotus ostreatus* and *Trametes versicolor* (Schmidt *et al.* 2011) are used.

CONCLUSION

The preserving jar/vermiculite technique is suitable for fungal degradation studies of lignocelluloses as shown by the mass loss of *Pinus sylvestris* wood by *Coniophora puteana*. Adding 2% malt extract solution till wood moisture content around 100% provided high mass loss within 32 weeks of incubation. However, with regard to bamboo, interpretations are problematic due to the low activity of both fungi on bamboo.

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