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Penetration capacity of the wood-decay fungus *Physisporinus vitreus*

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Abstract

Purpose: Bioincising is a biotechnological process for improving the permeability of refractory wood such as Norway spruce heartwood using the wood-decay fungus *Physisporinus vitreus*. The degradation of the bordered pit membranes by *P. vitreus* in its first stage of wood colonization enhances the uptake of preservatives and wood modification substances, whereas the strength of the material is not significantly reduced.

Methods: We propose to study bioincising by means of a mathematical model, because many factors affect the growth and effects of *P. vitreus* in Norway spruce in such a complex way that an evaluation of the optimal incubation conditions (i.e. water activity, temperature or pH) is very expensive or even not possible solely using laboratory experiments.

Results: Using a hyphal growth model we demonstrate here for the first time how to optimize bioincising by linking the microscopic growth behavior of *P. vitreus* with macroscopic system properties of the wood. Moreover, we propose universal measures of wood-decay fungi, i.e., penetration velocity, penetration work and penetration capacity, which may figure as measures for the efficiency of wood colonization. For example, our simulation shows that an increase of the hyphal growth rate (i.e. changing the incubation conditions) from 1 to 2 $\mu m \cdot d^{-1}$ results in an increase of the mycelium's growth velocity from 0.8 to 1.75 $\mu m \cdot d^{-1}$ and an increase of the penetration capacity from 0.5 to 0.6 $10^{-3} \cdot mm^2 \cdot d^{-1}$ using a pit degradation rate of 2 $\mu m \cdot d^{-1}$.

Conclusions: Information about the penetration velocity, penetration work and penetration capacity is of significance for both its biotechnological use and the study of the colonization strategy of wood-decay fungi in general.

Keywords: Filamentous fungus, Fungal colony, Mycelia modeling, Bioincising, Picea abies, Bordered pit

Background

The primary goal of a modeling framework for the optimization of bioincising is to analyze the influence of biotic and abiotic factors on the wood's permeability, which is in combination with the wood's strength, the quality criterion of bioincised wood (i.e. a higher permeability results in a higher treatability of the wood). The chemical composition (including environmental factors such as the water activity, temperature and type of medium) and the physical structure (i.e. the distribution of tracheids and pits) of the resource determine the penetration behavior of a wood-decay fungus as shown in Figure 1. Thereby, wood-decay fungi of the class basidiomycetes and ascomycetes

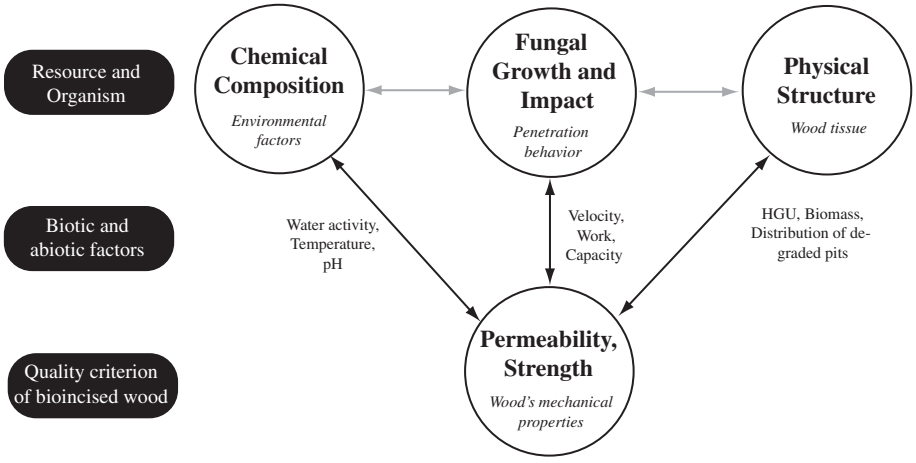

Figure 1 ***Background*****: Resource, Organism, biotic and abiotic factors influence the quality of bioincised wood.** We propose to study the optimization of the biotechnological process bioincising by means of mathematical models, because of the complexity of the system (fungus - wood).

colonize wood by forming a root-like network called mycelium. This highly interconnected structure consists of elongated tubular cells called hyphae that explore and colonize a substrate by branching. Characteristics for such filamentous organisms are their highly dynamic and adaptive response to their environment (Rayner & Boddy 1988). Thereby, the processes governing the growing fungus take place on multiple scales from the nanoscopic (e.g., uptake of nutrients) to the macroscopic level (e.g., transport of nutrients) (Fuhr et al. 2011a). It is this complexity that is a challenge for the biotechnological use of fungi such as *P. vitreus*.

Using a discrete modeling approach called the fungal growth model (FGM), in previous studies the metabolism of *P. vitreus* (Fuhr et al. 2011b) and the effect of the wood tissue on its growth (Fuhr et al. 2011a) have been analyzed, whereas the penetration behavior was not studied in detail until now (Figure 1). Thus, the focus of this work is to study the rule of the bordered pits to obtain a complete modeling framework covering all relevant effects for the optimization of the permeability of bioincised wood sample.

The degradation of the bordered pit membranes in its first stage of Norway spruce (*Picea abies* (L.) H. Karst.) wood colonization might be the main reason for the significant increase of the permeability of treated wood (Schwarze et al. 2006). This biotechnological process, which is termed bioincising, can be used to improve the uptake of wood preservatives and wood modification substances (Schwarze & Schubert 2009; Schwarze & Schubert 2011). In this context several studies concerning *P. vitreus* addressed the anatomy of treated Norway spruce wood (Fuhr et al. 2011a; Lehringer et al. 2010; Stührk et al. 2010; Fuhr et al. 2012), the influence of environmental factors to its radial growth rate (Fuhr et al. 2011b; Schubert & Schwarze 2009; Schubert et al. 2010) and the alteration of wood properties (Spycher et al. 2008; Schwarze et al. 2008; Lehringer et al. 2011a; Lehringer et al. 2011b). Despite their potential, the methods mentioned are unable to illustrate the influence of microscopic effects such as the pit degradation rate on the macroscopic behavior of the fungus, because wood is an opaque material and the *in vivo* observation of processes inside a wood sample has not been possible until now. Therefore, we propose the use of mathematical models to study and optimize the penetration

behavior of *P. vitreus*, since its growth behavior was successfully simulated by FGM, even in complex physical and chemical structured environments (Fuhr et al. 2011a).

The pits are valve-like structures between the voids within the wood (i.e. pores or lumens) and regulate the transport of nutrients in the tree. For example, in the living wood (i.e. sapwood) of Norway spruce the bordered pits are permeable for liquids. Whereas, in the dead part of the tree (i.e. heartwood) they are closed and lignified (Liese & Bauch 1967). It is this closing, called aspiration, of the bordered pits that makes efficient treatment of this wood species impossible without energy- and cost-intensive technical processing.

The degradation of the bordered pit membranes in their first stage of growth appears to be a common strategy of wood-decay fungi to colonize softwoods (Schwarze et al. 2006; Liese & Schmid 1961; Daniel 1994; Green et al. 1995; Green & Highley 1997). The size, structure and distribution of bordered pits is a significant factor for the accessibility of wood tissue both for fluids and fungal hyphae (Rayner & Boddy 1988). The time required by a hypha to penetrate through a bordered pit either by the support of pressure or enzymes (i.e. pit penetration time) obviously influences the ability of the mycelium to colonize a refractory wood such as Norway spruce heartwood.

The material is presented in three parts. In the first step, we discuss the growth pattern of *P. vitreus* using the FGM and laboratory experiments (Figures 2 and 3). In the second step, we use an analytical model to analyze the penetration velocity of *P. vitreus* to quantify the key factors determining the growth and expansion of the mycelium in wood modeled by the FGM (Figure 4). Moreover, such an analytical model enables us to discuss various modes of pit penetration by *P. vitreus*, i.e. pit penetration by pressure or enzymes (Table 1). However, the analytical model does not provide information about the effects of *P. vitreus* on wood tissue, e.g. the distribution of degraded pits. Therefore, we use the FGM to discuss the penetration work and the penetration capacity of *P. vitreus*

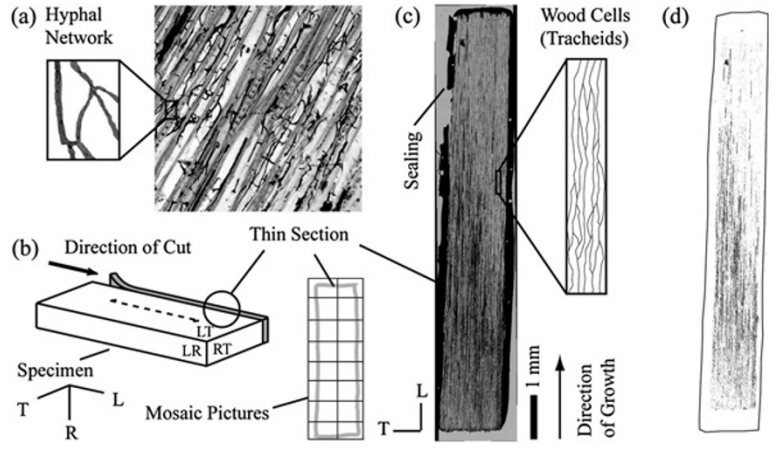

Figure 2 ***Light Microcopy*: (a) Wood-decay fungi such as the white-rot *Physisporinus vitreus* exploit a wood tissue by forming a root-like network (mycelium) consisting of filaments (hyphae) with a diameter of approximately 2 µm.** This hyphal network is visible under the light microscope. Specimens are usually cut into thin section as shown in (**b**), in order to analyze the distribution of mycelium in the wood by a sequence of light microscopy pictures. **(c)** These single pictures are stitched together and form a mosaic picture covering a whole thin section. **(d)** Using digital image processing one can identify the fungus and analyze mycelium's morphology in a whole sample. The principal directions of wood are usually termed as longitudinal, radial and tangential, denoted by the letters L, R and T.

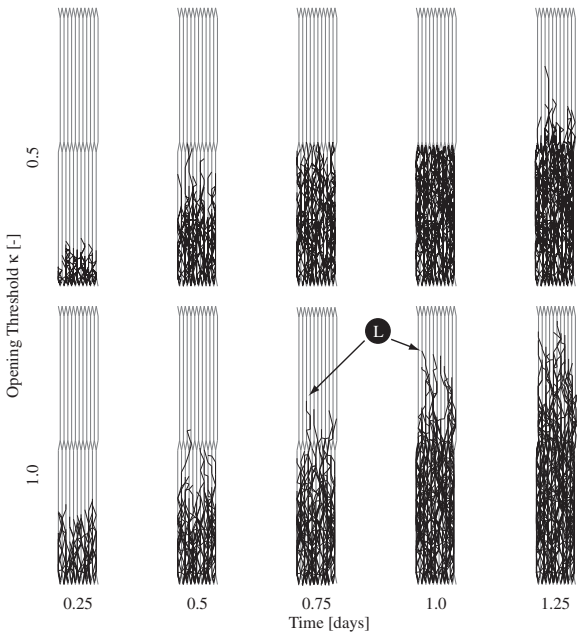

Figure 3 ***Growth pattern.*** The evolution of the mycelium begins from the left longitudinal face by using the model parameters given in Table 2. Fixed boundaries are imposed on all faces. The fungus reaches the right longitudinal face after approximately 32 and 42 hours for $\kappa = 1.0$ and 0.5 (Eq. 1) respectively. *L* denotes leading hyphae.

(Figures 5 and 6). We propose these universal measures for wood-decay fungi, because common network measures such as the hyphal growth unit (Plomley 1958) do not provide information about the effects of a fungus on its resource.

Methods

Specimen material

The white-rot basidiomycete *Physisporinus vitreus* EMPA 642 was cultivated in 9 cm Petri dishes on 2% malt extract agar (MEA) at 23°C and 70% relative humidity (RH). After 2 weeks we placed sterilized (121°C, 20 min and 2 bar vapor pressure) Norway spruce (*Picea abies* (L.) H. Karst.) samples with a size of approximately 10 mm (longitudinal) × 2 mm (radial) and 10 mm (tangential) on the mycelium. The faces of the samples, with the exception of one radial tangential face, were sealed with a topcoat (Nuvovern ACR Emaillack, Walter Mäder AG, Killwangen, Switzerland) by brushing, so that the *P. vitreus* colonized the wood via the unsealed side in a longitudinal direction (Figure 2a).

Light microscopy

After 10 days of incubation at 23°C and 70% RH we cut thin sections of approximately 30 μm thickness from the wood samples using a microtome as shown in Figure 2b. We stained the fungus with lactophenol blue, took mosaic images with a pixel size of approximately 0.65 μm × 0.65 μm using a Zeiss LSM 510-NLO (Figure 2c) and analyzed the images by digital image processing (Figure 2d).

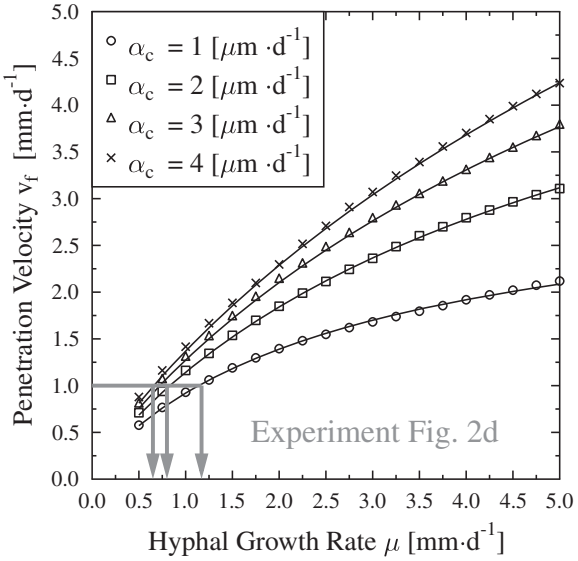

Figure 4 ***Penetration velocity.*** The hyphal growth rate μ plotted versus the growth velocity (Eq. 8). The symbols represent simulations for α_c = 1, 2, 3 and 4 μm·d^{-1} using the parameters of Table 2 and the tracheid framework of Figure 7a. The solid lines are the corresponding analytical models (Eq. 12a) using Eq. 12a – 12d and N_t = 2.65, p = 0.5 and q = 3. Each symbol represents the average over 1000 realizations and the uncertainty of the data points is within the range of the symbols.

Hyphal growth model

To analyze the penetration of the wood-decay fungus *P. vitreus* we use a two-dimensional discrete modeling approach in which both the mycelium and the nutrients are considered discrete structures. The present FGM is a two-dimensional version of the simulation scheme developed by Fuhr et al. (2011a). Typical model parameters used throughout this work are given in Table 2.

Inspired by the approach of Comstock (1970; Siau 1984) we model the Norway spruce tracheids as elongated rectangles with tapered ends. The shape of the tracheids is determined by their length l_L, width l_T and the length of the overlapping zone l_O (Figure 7a) where most of the bordered pits are located (Sirviö & Kärenlampi 1998). The diameter of a bordered pit is described by the parameter D. These pits play an important role in the growth of many wood-decay fungi (Schwarze et al. 2006; Liese & Schmid 1961; Daniel 1994; Green et al. 1995; Green & Highley 1997), because they are the shortest path for a wood-decay fungus in a longitudinal direction as shown by the dotted line in Figure 7a. The nutrient points are randomly uniformly distributed along the cell walls of the tracheids with the density ρ mm^{-1}.

Table 1 ***Pit degradation*****: Pit degradation rates α_c (enzymatic, Eq. 3) and α_p (pressure, Eq. 7) evaluated for the pit penetration times $\tau = \tau_c = \tau_p$ = 0.5, 1, 1.5 and 2 days**

Pit penetration time [d]τ	Pit degradation rate [μm/d]	
	α_c (κ = 0.2)	α_p (β_1 = 0.175, β_2 = −1.5)
0.5	4	2.77
1	3	0.87
1.5	2	0.44
2	1	0.27

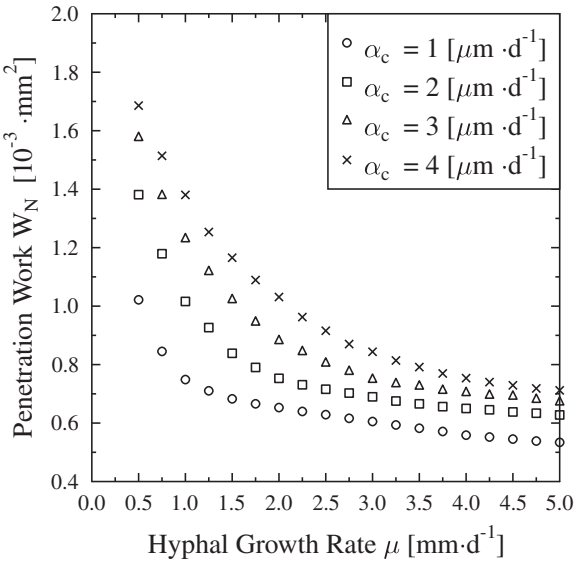

Figure 5 ***Penetration work.*** The hyphal growth rate μ plotted versus the penetration work (Eq.9) of *P. vitreus*. The symbols represent simulations for α_c = 1, 2, 3 and 4 μm·d^{-1} using the same simulation setup as in Figure 4. Each symbol represents on average over 1000 realizations and the uncertainty of the data points is within the size of the symbols.

Edges, nodes and tips form the mycelium of the fungus, whereby the position of the nodes is restricted to the position of the nutrient points as shown in Figure 7b. The characteristic growth of the fungus *P. vitreus*, which degrades the initially closed bordered pit membranes in its first stage of wood colonization, is simulated by a pit-to-pit growth of the hyphae. Thereby the mean growth rate of a hypha is given by μ. The evolution of the mycelium is driven by key processes such as the uptake and transport of nutrients, branching and polarization of the hyphae.

At each iteration step m of the algorithm the mycelium may grow by one edge. Thereby the length of the new edge and the angle measured to its predecessor is restricted by the growth cut-off length ξ and the growth cut-off angle θ. The mean edge length is λ. Each extension of the mycelium costs the fungus a specific amount of nutrients depending on the length of the edge. The fungus accumulates these nutrients in the nodes by degrading the pits at each time step initially for $m = m_0$ by α_I and for $m > m_0$ by α_c, where m_0 is the time when a new node is created at a pit. Branching occurs if the amount of nutrients at a node exceeds a certain level β_t (at a tip node) and β_s (at an interior node). A tip node is a node with one edge, whereas an interior node has more than one edge.

The cell walls limit the available set of nutrient points within a tracheid, in contrast to the unrestricted growth of the mycelium in Petri-dishes (Fuhr et al. 2011b). In the present model, an active hypha can only reach all pits within the same tracheid, but according to Fuhr et al. (2011a), we assume that a hypha is able to penetrate through the degraded torus into an adjacent tracheid if

$$F_j^{(m))} < \kappa, \tag{1}$$

where F_j, the amount of nutrients at point *(j)* and $\kappa \in [0,\nu]$, denotes a specific pit opening threshold. Details about the model construction are given in (Fuhr et al. 2011a).

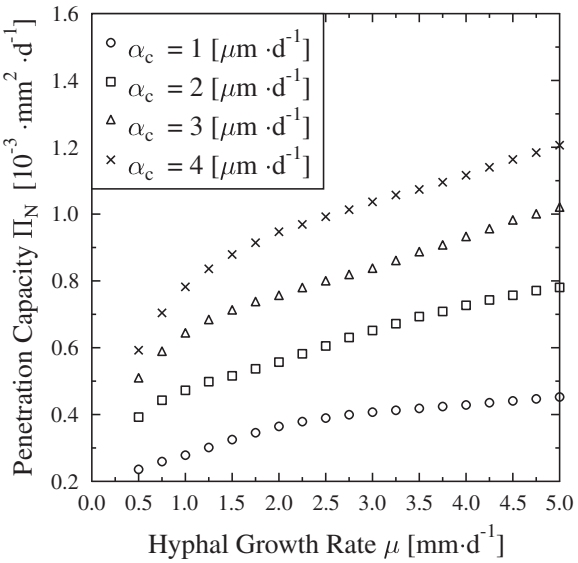

Figure 6 ***Penetration capacity.*** The hyphal growth rate μ plotted versus the penetration capacity (Eq. 10) of *P. vitreus*. The symbols represent simulations for α_c = 1, 2, 3 and 4 μm·d^{-1} using the same simulation setup as in Figure 4. Each symbol represents on average over 1000 realizations and the uncertainty of the data points is within the range of the symbols.

The simulation begins by placing n_k^0 starting nodes, called pellets, with an initial nutrient concentration n_n^0 on the longitudinal face of the wood specimen (Figure 7a). All pits are initially closed (i.e. $F_j > \kappa$) and their initial amount of nutients is ν. The unit of the resource that sustains fungal growth, called pit nutrient, is given in micrometers (μm), because we prefer to express the fungal activity (Eq. 1) with a measure, which is observable in laboratory experiments, e.g. holes in the torus of the bordered pits (directly) or a permeability (indirectly). However, the pit nutrient, given in micrometers, can be converted into other quantities of interest. For example, the mass of degraded lignin may be calculated by multiplying the pit nutrient by the degraded area given in μm^2 times the density of the lignin given in microgram per volume [μg/μm^3].

Analytical growth model

The shortest time T_s of a hypha to grow in a longitudinal direction from a tracheid into an adjacent one, indicated in Figure 7a by the dotted line connecting the points 1 and 3 (by the super indices of T) may be given by

$$T_S^{(1-3)} = \frac{l_S}{2 \cdot \mu_{12}} + \frac{l_S}{2 \cdot \mu_{23}} + \tau, \tag{2}$$

where μ_{ij} is the growth velocity of a hyphae (i.e. hyphal growth rate) within a tracheid measured between the points *(i)* and *(j)* (see Figure 7a), l_S is the length of the shortest path and τ is the pit penetration time, i.e. the time required for a hypha to grow through a bordered pit. Generally, there are two modes of pit penetration by a hypha.

Table 2 *Model parameters*: typical model parameters used throughout this work

Wood:	Symbol	Value	Unit
Tracheids	$[l_L, l_T, l_0]$	[2.5, 0.08, 0.04]	mm
Density of nutrient point	ρ	40	1*/^d-1
Diameter of bordered pit	D	10	μm
Fungus:			
Mean hyphal growth rate	μ	1	l*d^-1
Mean edge length	λ	2/3*ξ	l
Growth cut-off length	ξ	0.125	l
Growth cut-off angle	θ	90	°
Growth costs	$[a,b]$	[0, 0]	-
Pit initial nutrient	ν	D	μm
Pit initial degradation rate	a_I	0	-
Pit degradation rate	a_C	4	μm*d^-1
Pit opening	κ	0.1	-
Apical branching threshold	β_t	0.6*ν	-
Lateral branching threshold	β_s	0.35*ν	-
Simulation:			
Initial number of pellet	n_k^0	60	-
Initial number of tips	n_s^0	1	-
Initial nutrient concentration	n_n^0	3/2*β_t	-

We use the same notation as in Fuhr et al. (2011a).

In the first case the torus is eroded by enzymes, e.g. *P. vitreus* (Schwarze et al. 2006). Whereas, in the second case a hypha breaks through the torus by mechanical pressure, e.g. blue stain fungi (Liese & Schmid 1961), which is typically indicated by cracks in the lignified torus. We indicate the mode of penetration by lower indices, i.e. enzymatic erosion (c) and pressure (p).

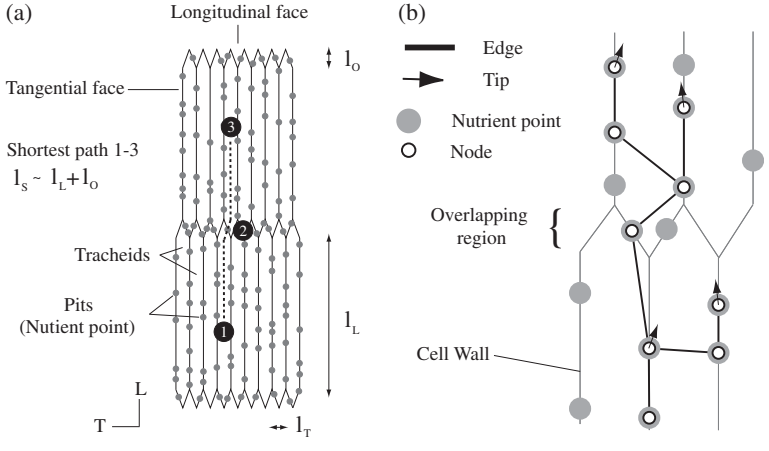

Figure 7 *Model description*. (a) A longitudinal-tangential section of Norway spruce wood is characterized by the cellular structure of the tracheids. The principal directions of wood are usually termed as longitudinal, radial and tangential, denoted by the letters L, R and T. The pits are the substrate for the fungus (i.e. nutrient points) and distributed with a density ρ on the substratum of the fungus (i.e. tracheid cell walls) by using uniform random numbers. **(b)** The mycelium is modeled as an assemblage of edges, nodes and tips. The evolution of the mycelium is characterized by a pit-to-pit growth of the fungus. Details about the model construction can be found in Fuhr et al. (Fuhr et al. 2011a).

For the first case we may write for the pit penetration time

$$\tau_c = \frac{D \cdot \kappa}{\alpha_c}, \tag{3}$$

where D is the diameter of a bordered pit and $\kappa \in [0, 1]$ is an opening threshold. Thus, $D \cdot \kappa$ is the diameter of a hole, which is prerequisite for a vegetative hypha to penetrate through the torus. α_c is the pit degradation rate, given in $\mu m \cdot d^{-1}$, to dissolve the torus.

For the second case, the pit penetration by pressure, we assume that the fungus bores a hole in the torus with a constant rate α_p, i.e. the diameter d of a hole in the torus after t days is given by

$$d(t) = \alpha_p \cdot t. \tag{4}$$

Bardage and Daniel (1998) studied the penetration of seven fungal strains through two different types of membranes with various pore sizes. They found that the ability of fungi to penetrate micropores depends on time, i.e. smaller pores require longer penetration times. They reported penetration times between 1 and 10 days for micropores of size 0.6 – 0.2 μm. Whereby, no fungus was able to penetrate micropores smaller than 0.2 μm within 15 days. Based on these experiments, we assume that the relation between the diameter of a hole d and the penetration time t is roughly given by the power law

$$\frac{t}{t_{max}} = \beta_1 \left(\frac{d}{d_{max}} \right)^{\beta_2}, \tag{5}$$

using t_{max} = 10 d, d_{max} = 0.6 μm, β_1 = 0.175 and β_2 = −1.5 as shown in Figure 8. Therefore, the diameter of a hole after τ_p days (Eq. 4) is equal to a hole that is prerequisite for penetration after τ_p days (inserting Eq. 4 into Eq. 5), i.e.

$$\frac{\tau_p}{t_{max}} = \beta_1 \left(\frac{\alpha_p \cdot \tau_p}{d_{max}} \right)^{\beta_2}. \tag{6}$$

Solving Eq. (6) for the penetration time τ_p leads to

$$\tau_p = \left[t_{max} \cdot \beta_1 \cdot \left(\frac{\alpha_p}{d_{max}} \right)^{\beta_2} \right]^{\frac{1}{1-\beta_2}} \tag{7}$$

For example, a hypha bores a hole into a cell wall by α_p = 0.25 μm per day. After approximately 2.12 days the diameter of the hole is approximately 0.53 μm (Eq. 4). This hole size corresponds to a penetration time of 2.12 days (inserting d = 0.53 μm into Eq. 5). Thus, the time of fungal activity at the cell wall is equal to the penetration time and therefore the hypha is able to grow through the bored hole. We directly obtain the penetration time τ_p of approximately 2.12 days by inserting α_p = 0.25 into Eq. 7.

Penetration velocity, work and capacity

For biotechnological applications of wood-decay fungi, it is important to understand their capability to penetrate into a specific direction of a wood tissue in a certain amount of time. Therefore, we define the penetration velocity of a fungus (or hypha) as

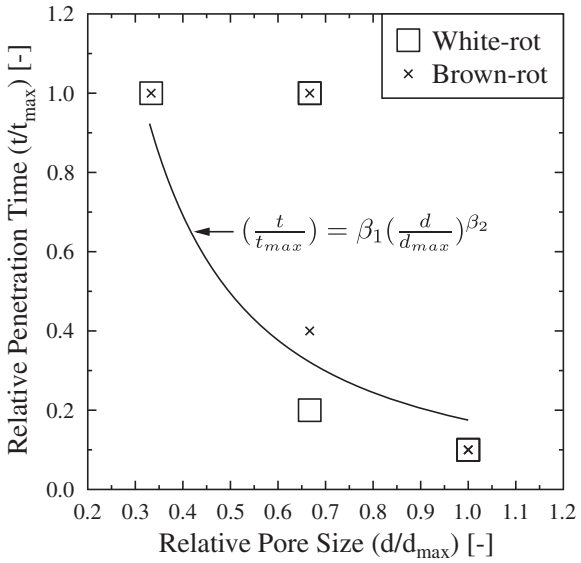

Figure 8 ***Penetration Time*****: Pore size plotted versus penetration time of wood-decay fungi using d_{max} = 0.6 μm and t_{max} = 10 days.** The solid line represents $\beta_1 = 0.175$ and $\beta_2 = -1.5$. Experimental data reproduced after Bardage and Daniel (Bardage & Daniel 1998).

$$v_f = \frac{Penetration\ depth}{Time}. \tag{8}$$

Furthermore, we define the penetration work of a fungus as

$$W_Q = Quantity \cdot Penetration\ depth, \tag{9}$$

where the subscript letter Q denotes a quantity of interest, e.g. the total hyphal length of the mycelium, the total number of open pits or the mass loss of the wood. In addition, we propose the penetration capacity of a fungus as

$$\Pi_Q = \frac{Penetration\ Work}{Time}. \tag{10}$$

Obviously, the penetration capacity of a fungus strongly depends on factors such as temperature, water activity and pH, the microclimate, the presence of wood preservatives and other factors, since wood decay-fungi are sensitive to their environment. The scalar Π_Q may figure as a measure for the efficiency of a specific fungus to colonize a certain wood tissue.

For simplicity, throughout this work, we use the terms 'penetration work' and 'penetration capacity' for the specific penetration work and penetration capacity using the total amount of degraded pit nutrients as the quantity of interest.

Results

Growth pattern

After 10 days of incubation, we observe that the fungus starting from the unsealed longitudinal face penetrates approximately 10 mm into the wood sample (Figure 2d). This corresponds to a penetration velocity of approximately 1 mm·d^{-1}. The morphology

of the growth front is characterized by hyphae growing at different rates (i.e. leading hyphae). The density of the mycelium at the unsealed end is much higher than on the growth front. We measure that a hypha crosses 3–4 tapered ends from the unsealed to the opposite face.

Figure 3 shows the evolution of the mycelium over a time span of 36 hours for $\kappa = 0.5$ and 1.0 (see Eq. 1) using the model parameters of Table 2. The system consists of 20 tracheids with 1070 pits in total. The fungus begins growing from the left longitudinal face and fixed boundaries are imposed to all faces. For $\kappa = 1.0$ the fungus grows without any resistance by the pit membranes and after approximately 32 hours the first hypha reaches the longitudinal face on the right side, whereas the fungus requires in the second case (i.e. $\kappa = 0.5$) approximately 42 hours for the same distance. The total hyphal length of the mycelium is approximately 130 mm for both conditions, whereas the number of tips is approximately 350 and 500 for $\kappa = 1.0$ and 0.5 respectively. In addition, the model shows that for $\kappa = 0.5$ *P. vitreus* requires approximately 15 per cent more nutrients to reach the right longitudinal face.

Penetration velocity

Figure 4 shows the velocity of the mycelium v_f (Eq. 8) evaluated for hyphal growth rates between 0.5 and 5 mm·d^{-1} and pit degradation rates between 1 and 4 μm·d^{-1} (Eq. 3) using the model parameters of Table 2 and the tracheid framework of Figure 7a. The system consists of 2 (longitudinal) × 10 (tangential) tracheids and fixed boundaries are imposed to all faces. The fungus begins growing from the lower longitudinal face and the velocity is evaluated by measuring the time of the mycelium between points 1 and 3. The numerical results of these experiments are shown in Figure 4 by the symbols ○ (α_c =1 μm·d^{-1}), □ (α_c = 2 μm·d^{-1}), Δ (α_c = 3 μm·d^{-1}) and × (α_c = 4 μm·d^{-1}).

We observe that the penetration velocity v_f increases with increasing values of μ and α_c. For example, at a hyphal growth rate of $\mu = 0.5$ the relative difference between the penetration velocity at $\alpha_c = 1$ and $\alpha_c = 4$ is smaller than 1, whereas at $\mu = 5$ the difference is larger than 2. The influence of the pit degradation rate on the relationship between the hyphal growth rate and the penetration velocity seems to be nonlinear. It would be interesting to know more about this relationship, because the hyphal growth rate depends on environmental factors and may allow us to optimize the process of bioincising. Thus, the next two paragraphs develop such a relationship.

In a first step, we assume that the penetration velocity between the points 1 and 2 differs from the penetration velocity between the points 2 and 3 (see Figure 7a), i.e. before and after the overlapping area. The overlapping area consists of the tapered ends of the tracheids (see Figure 7b), where most of the pit are located. Thus, according to Eq. 2 and Eq. 8 we write for the velocity of the mycelium between the points 1 and 3

$$v_f = \frac{l_S}{T_s^{1-3}} = \frac{l_S}{\frac{l_S}{2}\left(\frac{1}{\mu_{12}} + \frac{1}{\mu_{23}}\right) + \tau}. \tag{11}$$

Second, we assume that the hyphal growth rate of the leading hypha (Figure 7) before (μ_{12}) and after (μ_{23}) the overlapping area is faster than the rest of the mycelium by a factor μ^* (before) and $q{\cdot}\mu^*$ (after) respectively. In addition, the penetration time depends on the number of nodes per pits N_t, i.e. the more nodes per pit the higher the

pit degradation. For the pit penetration time τ, we may write the hyphal growth rate μ_{12} and μ_{23} and the factor μ^*

$$\tau = \frac{\tau_c}{N_t} = \frac{D \cdot \kappa}{N_t \cdot \alpha_c}, \tag{12a}$$

$$\mu_{12=\mu+\mu^*}, \tag{12b}$$

$$\mu_{23=\mu+q\cdot\mu^*}, \tag{12c}$$

$$\mu^* = N_t \cdot \alpha_c \cdot \left(\mu \cdot \frac{\tau}{L_S}\right)^p. \tag{12d}$$

The solid lines in Figure 4 show Eq. 11 using Eq. 12a, 12b, 12c, 12d and N_t = 2.65, q=3 and p=0.5. The velocity of 1 mm·d^{-1} measured from the experiment of Figure 2d corresponds approximately to hyphal growth rates μ (α_c) = 0.66 (4), 0.73 (3), 0.84 (2) and 1.14 (1) mm·d^{-1}. The pit degradation rates α_c = 1, 2, 3 and 4 μm·d^{-1} correspond to pit penetration times τ_c = 2, 1, 0.67 and 0.5 days (Eq. 12a) and pit degradation rates by pressure α_p = 2.77, 0.87, 0.44 and 0.27 μm·d^{-1} as shown in Table 1.

Penetration work and penetration capacity

Figure 5 and Figure 6 show the penetration work W_N (Eq. 9) and the penetration capacity Π_N (Eq. 10) of *P. vitreus* measuring as quantity Q the amount of degraded nutrients given in micrometer. Since we measure the penetration depth in millimeters, the unit of the penetration work and the penetration capacity are 10^{-3}·mm^2 and 10^{-3}·mm^2·d^{-1} respectively. A penetration capacity of 1.0 10^{-3}·mm^2·d^{-1} means that the growth front of the mycelium penetrating 1 mm·d^{-1} into the wood degrades 1 micrometer pit membranes in total. We use the same simulation setup as in Figure 4. We observe that the penetration work decreases with increasing hyphal growth rates, whereas the penetration capacity increases with increasing hyphal growth rates. In addition, higher pit degradation rates result in a higher penetration work and penetration capacity.

Discussion

The growth of physisporinus vitreus

The penetration behavior of *P. vitreus* into Norway spruce heartwood is supposed to be characterized by a stepwise capture of wood tissue (Fuhr et al. 2011a), because the aspirated and lignified bordered pits hinder the expansion of the mycelium (Figure 2). Only the degrading of either the bordered pits or the cell wall enables the fungus to grow from one tracheid to another. Thereby the ratio between the velocity of the hypha within the tracheids (i.e. hyphal growth rate) and the pit degradation rate is of interest, because this ratio influences the density of the mycelium, the number of tips in the system and the penetration velocity of the growth front as shown by our simulations (Figures 3 and 4).

Rays are not considered in the model, because they affect fungal growth in radial direction. The present study focuses on the penetration of the mycelium in longitudinal direction as shown in Figure 3.

Penetration velocity

The FGM assumes that the hyphal growth rate (μ) and the pit degradation rate (α_c) are the key factors for the colonization of Norway spruce wood in its first stage of growth. Using the analytical model (Eq. 11), we are able to quantify the influence of both factors on the penetration velocity of *P. vitreus*. The results suggest that a doubling of the hyphal growth rate enables *P. vitreus* to reduce the pit degradation rate by a factor 4 (Figure 4), to reach a penetration velocity of 1 mm·d^{-1} (Figure 2d). The hyphal growth rate is mainly influenced by the water activity, temperature and pH (Schubert et al. 2010), whereas the effect of environmental factors on the ability of *P. vitreus* to penetrate the bordered pits is unknown. Thus, changing the incubation conditions offers an optimization of the bioincising process (see next section 'Optimization of bioincising').

The measured hyphal growth rates between approximately 0.5 and 1.5 mm d^{-1} (Figure 4) are in good agreement with *in vivo* experiment of *P. vitreus* at standard conditions (Stührk 2011) and therefore confirm our model assumption. We are able to estimate for the first time the pit degradation rate of *P. vitreus*. Our simulations in combination with laboratory experiments show that the pit degradation rates (α_c and α_p) are approximately 1 to 4 and 0.3 – 8 μm d^{-1} for a penetration either by enzymes or by pressure (Table 1).

The penetration time τ_p is based on the of the experiments of Bardage and Daniel (1998). However, the influence of environmental effects such as temperature and pH on the penetration behavior of hyphae of white-rot fungi such as *P. vitreus* in wood may not covered by the experiments of Bardage and Daniel (Bardage & Daniel 1998) and may limit the extension of our model besides standard conditions. Thus, more quantitative and qualitative experimental studies about the penetration of hyphae through pores are needed.

Optimization of bioincising

We use a discrete modeling approach to study the biotechnological process of bioincising, because such a model provides information about the effects of *P. vitreus*, e.g. the amount of degraded pits, and therefore enables an optimization of the bioincising process. For example, our simulation shows that an increase of the hyphal growth rate from 1 to 2 μm·d^{-1} results in an increase of the growth velocity of the mycelium from 0.8 to 1.75 μm·d^{-1}, a decrease of the penetration work from 1 to 0.75 10^{-3}·mm^{-2} and an increase of the penetration capacity from 0.5 to 0.6 10^{-3}·mm^2·d^{-1} using a pit degradation rate of 2 μm·d^{-1} (Figures 4, 5 and 6). A penetration work of 1 to 0.75 shows that, for the tracheid framework given in Figure 7a, the growth front of the mycelium penetrating 1 mm into the wood degrades approximately 1 to 0.75 micrometer pit membranes in total (see Figure 5). Thereby, a penetration capacity of 0.5 to 0.6 10^{-3}·mm^2·d^{-1} indicates that the growth front of the mycelium penetrating 1 mm·d^{-1} into the wood degrades approximately 0.5 to 0.6 micrometer pit membranes in total, which is a measure for the permeability of the wood (Fuhr et al. 2011a). Moreover, the penetration capacity may figure as a measure for the efficiency of wood-decay fungi to colonize wood, since a high pit degradation rate may facilitate the capture of their resource. Thus, it would be interesting to measure and compare various penetration capacities from several wood-decay fungi, e.g. choosing as quantity of interest the biomass, amount of degraded pits or permeability.

Conclusions

The last step would be an optimization of bioincising on a larger scale using the modeling framework as described above. On a macroscopic scale, the influence of the distribution of pellets, which are the inocula on the surface of wood blocks, is of interest because the penetration velocity of *P. vitreus* in radial (i.e. rays) and longitudinal (i.e. tracheids) direction is much higher than in tangential direction. Thus, combining environmental factors with the amount and distribution of the inocula on the wood surface will help assist designing incubation conditions that are required to induce a certain degree of wood permeability by *P. vitreus*.

Abbreviations

EMPA: Swiss federal laboratories for materials and technology; FGM: Fungal growth model; MEA: Malt extract agar; pH: Potential hydrogenium; RH: Relative humidity.

Competing interests

The authors declare no conflict of interest.

Authors' contributions

MF carried out the analytical model and the simulations using the FGM, and participated in the LM experiments. MF and MS drafted the manuscript. CS carried out the LM experiments and helped to draft the manuscript's experimental section. MF, MS, FS and HS conceived of the study, and participated in its design and coordination. HH and FS helped to draft the manuscript. All authors read approved the final manuscript.

Authors' information

There are no relevant information about the authors for the interpretation of the article.

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