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Monitoring of fungal colonization of wood materials using isothermal calorimetry

Lars Wadsö [a, *], Sanne Johansson [a], Stig Bardage [b]

[a] Building Materials, Lund University, Sweden
[b] SP Sustainable Built Environment, Stockholm, Sweden

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ABSTRACT

We have developed a method based on isothermal calorimetry to monitor the colonization of wood materials by decay fungi. The heat produced is a direct and continuous measure of the activity (respiration) of the fungus. This pre-study shows that wood materials treated in different ways give results that reflect the known ranking of the materials regarding resistance to decay. In addition to this, the 40 days measurements show several phases and periods of oscillating respiration. As the calorimeter continuously monitors the colonization and degradation process, this method provides more information than classical/standardized decay tests in which typically only the final mass loss is determined.

1. Introduction

Methods of grading the resistance to attack by decay fungi of natural and treated wood are of importance as they allows for rational choices between different wood materials for outdoor applications (Brischke et al., 2006). The presently used European standard EN 350 (EN350-1 2016) divides wood into five durability classes with pine sapwood as reference material. Most testing to quantify durability of wood is based on natural exposure of wood in ground (for example EN 252 (EN252:2014)) or above ground situations, see for example references (Blom and Bergström, 2006) and (Brischke and Rapp, 2008). Even if these methods can be seen as being accelerated – for instance by water traps that give elevated moisture levels – they are still long term measurements that may require several years of exposure and the methods of assessing decay are in most cases rather crude, e.g. visual inspection and manual sensing of the integrity of the wood. A quicker alternative is laboratory methods like EN 113 (EN113:1997) that use mass loss as indicator of efficacy of wood preservatives. Such methods are destructive as the sample needs to be dried to assess mass loss, so each sample can only be used for one measurement point.

It is of interest to investigate new approaches to complement the traditional methods. The present paper therefore describes the first steps in the development of a rapid method to assess colonization and degradation rates of wood. The method is based on isothermal calorimetry that measures the heat production rate (thermal power) from the fungal metabolism, which is related to the respiration rate of the fungus and the degradation rate of the substrate.

2. Materials and methods

2.1. Wood materials

Three wood materials were used:

2.1.1. M1

Fast grown spruce (*Picea abies*) sapwood from the Swedish province of Halland (the material is described in reference (Fredriksson et al., 2010)).

* Corresponding author. Building Materials, Lund University, Box 118, 221 00 Lund Sweden.
E-mail address: lars.wadso@byggtek.lth.se (L. Wadsö).

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2.1.2. M2

Thermally modified Scots pine (*Pinus sylvestris*, maximal treatment temperature 212 °C; Thermowood class D, Firstwood Premnitz, Germany).

2.1.3. M3

Preservative-treated Scots pine, class NTR AB (treated to use class 3 (above ground) end uses).

2.2. Fungus

The brown rot fungus *Postia placenta* (Fr.) M.J. Larsen & Lombard (strain FPRL 280) was used in all experiments.

2.3. Isothermal calorimetry

Isothermal calorimetry is a method to measure the thermal power (heat production rate) from physical, chemical or biological processes. As all processes produce heat this is a general method that can be used in many different fields, and it was more than 200 years ago that Lavoisier and Laplace were the first to use a calorimeter for biological experiments (Lavoisier and LaPlace, 1783, 1955). More recent biocalorimetric works have been reviewed by (Calvet and Prat, 1963; Beezer, 1980; Bastos, 2016). The main author has used isothermal calorimeters in several studies of microbial growth on agar (Wadsö et al., 2004; Li et al., 2007, 2009; Li and Wadsö, 2011), food-stuffs (Markova and Wadsö, 1998; Alklint et al., 2004, 2005; Wadsö and Gomez, 2009; Wadsö, 2015) and wood (Xie et al., 1997; Bjurman and Wadsö, 2000; Wadsö et al., 2010, 2013; Li and Wadsö, 2013; Wadsö et al., 2013). The latter seems to be an underdeveloped field as – except for the above mentioned references – we have only found two other papers dealing with studies of decay fungi by isothermal calorimetry (Verma et al., 2008; Ghosh et al., 2012).

In an isothermal calorimeter the thermal power produced by a small sample (the sample vial is typically 1–100 mL) is measured. In biological samples with aerobic respiration (like fungi) a substantial amount of heat is produced when oxygen is consumed, and this is often easy to measure even with comparatively simple calorimeters. In this context, isothermal calorimetry can be seen as a continuously measuring respiration rate meter. The results can also be used for biothermodynamical modeling (Wadsö and Hansen, 2014).

The thermal power P (W) measured by an isothermal calorimeter is the product of the rate of a process dn/dt (mol/s) and its enthalpy change ΔH (J/mol):

$$P = \Delta H \cdot \frac{dn}{dt} \quad (1)$$

In the present case the process is respiration (as decay fungi are aerobic organisms) with an enthalpy change of −455 kJ/mol_{oxygen} (Hansen et al., 2004). This enthalpy change is close to constant (Thornton, 1917; Wadsö and Hansen, 2014) (for example for biological substrates like carbohydrates, lipids and proteins) when expressed per mol oxygen consumed, and the negative sign shows that the process produces heat, is exothermal. Calorimetrically measured thermal powers on fungi can therefore be converted to oxygen consumption rate.

The main advantage of isothermal calorimetry in comparison to traditional oxygen consumption measurements is that calorimetry gives a continuous (minute-by-minute) monitoring with a much higher level of detailing than is possible with respirometry, as an isothermal calorimeter directly measures the rate of a process at high precision and accuracy, while a respirometer commonly measures oxygen concentration as a function of time (the integral of the rate).

Two types of processes take place in an aerobic organism: the catabolic degradation of substrate to carbon dioxide and water, and the anabolic remodeling of organic molecules, for example from substrate to biomass. From a basic energetic perspective, the catabolic processes have the enthalpy given above, while, in comparison, the anabolic processes have much lower enthalpies. The thermal power measured in an isothermal calorimeter thus has its main origin in the degradation of substrate to carbon dioxide and water, not the building of biomass. It should also be noted that it is not – by calorimetry alone – possible to differentiate between heat from different processes, for example from combustion of small free molecules, cell wall polymers, or fungal biomass (Wadsö and Hansen, 2014).

2.4. Sample stacks

We here give a general description of how the samples were made; a detailed measurement protocol is given in the appendix.

In the present method we use samples that are made of a combination of the wood material to be tested and wood containing a decay fungus (inoculums). At the start of a measurement the wood in the inoculums is heavily degraded, but the fungi still shows a high activity. Each sample consists of a stack of 8 disks of the test material and 4 inoculum disks, both with a diameter of 15 mm, as is seen in Fig. 1. The stack is held together by a stainless steel thread. The thicknesses of the thin disks (inoculums) and the thick disks (test materials) were 1.5 and 3 mm, respectively. All disks were cut so that the circular top and bottom surfaces are facing in the longitudinal direction so that the samples will quickly absorb liquid water (see appendix).

The thin inoculum disks were prepared by placing wood disks on a Petri dish with malt extract agar (MEA) that was covered with fungal mycelium. After 10–14 days the disks were removed and used as inoculums when the sample stacks were made. In a preexperiment the mass loss of such disks was measured as a function of the time they were placed on the mycelium in a Petri dish.

Two main measurement series of eight samples each are presented in the present paper. In the first series, seven samples were untreated wood (M1 above) and one sample contained only inoculum disks. In the second series two samples were untreated wood (M1), two were thermally modified wood (M2), two were impregnated wood (M3), one contained only inoculum disks, and one contained only sterile untreated wood (M1) disks. In the first series the untreated samples were removed at different times from the calorimeter to determine mass loss; in the second series all

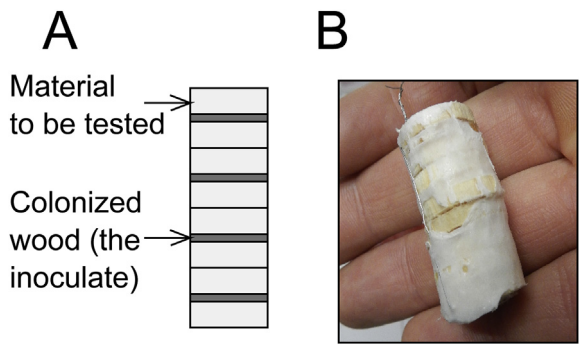

Fig. 1. A. Schematic side view of a sample stack containing 15 mm cylindrical thick disks of both a material to be tested (3 mm disks) and heavily colonized wood thin disks (the inoculum, 1.5 mm disks). B. Untreated sample after measurement.