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Detection of outdoor mould staining as biofinish on oil treated wood

Elke J. van Nieuwenhuijzen [a, *], Michael F. Sailer [b, c], Lone R. Gobakken [d], Olaf C.G. Adan [e], Peter J. Punt [f], Robert A. Samson [a]

[a] CBS KNAW Fungal Biodiversity Centre, Applied and Industrial Mycology, Uppsalalaan 8, P.O. Box 85167, 3508 AD Utrecht, The Netherlands
[b] Saxion, University of Applied Sciences, Centre for Urban and Environmental Development, Tromplaan 28, P.O. Box 70.000, 7500 KB Enschede, The Netherlands
[c] Regge Hout B.V., Breukersweg 9, 7471 ST Goor, The Netherlands
[d] NIBIO, Norwegian Institute of Bioeconomy Research, P.O. Box 115, NO-1431 Ås, Norway
[e] Eindhoven University of Technology, Department of Applied Physics, Den Dolech 2, P.O. Box 513, 5600 MB Eindhoven, The Netherlands
[f] TNO, Microbiology & Systems Biology, Utrechtseweg 48, P.O. Box 360, 3700 AJ Zeist, The Netherlands

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ABSTRACT

Stains on wood are often unwanted in outdoor applications, dark stain formation however is essential to the development of a new protective, self-healing and decorative biotreatment for wood. The biotreatment is based on the formation of surface covering mould staining on linseed oil treated pine sapwood during outdoor exposure. This specific stain formation is called biofinish and an assessment method is proposed in this study. Analysis of the visual stain coverage and quantification of the darkness generated useful input for the detection of biofinishes. Analysis of the microbial composition of a biofinish by PCR amplification and (Sanger) sequencing of fungal DNA sequences was appropriate for identification of *Aureobasidium*, as the dominant genus in several biofinishes, however our results indicate, that a more in depth, next generation sequencing method is preferred for a more elaborate biofinish assessment method. With the basic assessment method, biofinish formation was determined for oil treated wood specimens exposed outdoors in the Netherlands and some selected sites outside the Netherlands. Biofinish formation was demonstrated to be reproducible for pine sapwood, which was treated with raw linseed oil and exposed in the Netherlands. Furthermore olive oil is discovered in this study as a supportive factor for biofinish formation, regardless of the wood type, whereas biofinish formation was not detected on wood treated with stand linseed oil.

1. Introduction

Timber is a common building material for above ground outdoor applications, such as facades cladding, partitions and noise barriers. It is often selected for its (organic) appearance, and can be used either as coated or uncoated. However an uneven discolouration in uncoated and coated wooden surfaces occurs frequently and it is generally attributed to mould growth (Williams et al., 1999; Ray et al., 2004; Kelley et al., 2006; Gobakken and Westin, 2008 and Gobakken et al., 2010a). Microbial growth causing discolouration on building materials is commonly seen as a visual disfigurement and it is associated with a reduced aesthetical service life (Dickinson, 1972; Saad et al., 2004; Chedgy et al., 2007; Gobakken and Vestøl, 2012). In contrast, microbial growth has positive functionalities in dark biofinishes as shown for linseed oil treated wood (Sailer et al., 2010). In this case the natural process of abundant dark staining on wood is embraced due to its homogenous colouring and wood protecting abilities. Although the growth and protection mechanism of this biofinish has yet to be understood, the formation of a surface covering layer of microorganisms on oil treated wood is recognized as a sustainable solution for a biocide free and self-healing finish system.

Sailer et al. (2010) detected biofinishes on pine sapwood specimens impregnated with a chemically refined pure linseed oil dissolved in acetone. The biofinish was identified as a homogenous dark film. However methods for determining the characteristics of the biofinish such as homogenous colouration and darkness of the biofinish were still lacking. Microscopic analysis of the biofinish showed a layer of dark stained, presumably microbial, structures.

* Corresponding author.
E-mail addresses: E.Nieuwenhuijzen@cbs.knaw.nl (E.J. van Nieuwenhuijzen), M.F.Sailer@saxion.nl (M.F. Sailer), Lone.Ross.Gobakken@nibio.no (L.R. Gobakken), O.C.G.Adan@tue.nl (O.C.G. Adan), peter.punt@tno.nl (P.J. Punt), R.Samson@cbs.knaw.nl (R.A. Samson).

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Cultivation and molecular detection confirmed the presence of microorganisms in this biofinish and mainly revealed the presence of the fungal species *Aureobasidium pullulans*. A homogenous surface covering dark finish was also recognized on outdoor exposed (TNO, Delft, The Netherlands) pine sapwood samples, which have been impregnated with raw linseed oil (unpublished data). Screening of the surface with a naked eye was the used method for this assessment. The linseed oil treated samples of this unpublished study and the samples of the study of Sailer et al. (2010) both showed the presence of biofinishes, while no biofinish was observed on the untreated control samples. Although a coherent biofinish assessment has not yet been used, the results indicate the need for impregnation with linseed oil as base for biofinish formation during outdoor exposure.

New studies were initiated to determine if the formation of a dark pigmented biofinish on linseed oil treated pine sapwood is restricted to refined and raw linseed oil, pine sapwood and/or the environmental conditions of a test site. Although it has been demonstrated that several factors affect mould growth on coatings or finishes, for example the type of coating, the type and concentration of fungicide, the pigment-volume content, the type of substrate, exposure time and climate conditions (Creemers et al., 2002; Gobakken and Lebow, 2009; Gaylarde et al., 2011; Shirakawa et al., 2011; Viitanen and Ritschkoff, 2011; Gobakken and Vestøl, 2012; Windt et al., 2014), the interaction and dominance of each factor has not been consistently unravelled. Also it should be noted that mould growth on a surface is not identical to a biofinish, and that conversion of mould growth data to a biofinish assessment method is questionable since a reliable conversion method is missing. This makes it difficult to predict or control the biofinish formation on wood if changes are made to the substrate or geographical location.

The first aim of this study is to develop an assessment method to detect biofinishes on wood. The following definition of a biofinish was used: (1) a dark pigmented layer which (2) covers a wood surface almost entirely without exposing underlying wood structures (3) contains abundant microbial mass and (4) is irreversibly attached to the surface. The first three sections of the definition were transformed into measurable criteria using a set of evaluation methods: (1) colour measurements to characterize the colour of a biofinish (2) macroscopic and microscopic observations to characterize the coverage (the visibility of the underlying wood substrates), (3) microscopic observation, cultivability of fungi and bacteria, DNA isolation and sequence analysis to characterize the microbial presence. The fourth criterion, (4) irreversibly attachment, was not explicitly measured but will be commented on in the discussion.

The second aim of this study was to evaluate the biofinish formation on different combinations of wood species and vegetable oil types following outdoor exposure in and outside the Netherlands. The impact on biofinish formation of wood species, the oil type, the wood–oil combination, the oil uptake were analysed and discussed in relation to the impact of the environmental conditions at the exposure sites.

2. Methods

2.1. Specimen preparation

Six sample sets were used in this study, each with different wood species, (oil) treatments, and varying number of replicates (Table 1). The different woods tested were pine (*Pinus sylvestris* L.) sapwood, spruce (*Picea abies*) or ilomba (*Pycnanthus angolensis*). The wood samples each had a length of 50 mm (longitudinal axis), width of 25 mm and height of 15 mm, except the pine specimens, mainly contained heartwood, which had a dimension of 150 mm (longitudinal axis) x 145 mm × 20 mm (noted 'Pine heartw.' in Table 1). The surfaces of the wood specimens were planed to ensure a smooth surface.

The small oil treated specimens (50 mm × 25 mm × 15 mm) were impregnated with raw linseed oil (Vereenigde Oliefabrieken, iodine value 183 and 0.81% free fatty acids), olive oil (Carbonel, iodine value 82 and 0.34%free fatty acids) or stand linseed oil (Vliegenthart, viscosity P45). The impregnation was carried out in an impregnation vessel (Scholz) using a vacuum time of 30 min at -1×10^5 Pa followed by 1 h pressure of 8×10^5 Pa. A vacuum time of 1 h at -1×10^5 Pa followed by 2 h pressure of 8×10^5 Pa was used for the larger pine specimens (150 mm × 145 mm × 20 mm).

The oil retention of the wood was determined by comparison of the mass of the specimens before and after impregnation. To determine the average moisture content, additional test pieces of wood were dried at 105 °C. The moisture content of these specimens was up to 10% for the small wood samples (50 mm × 25 mm × 15 mm) and 13% for the larger pine samples. The spruce samples impregnated with stand oil (sample set 1) had the lowest mean oil retention: 109 kg/m^3 with a standard deviation (SD) of 20 kg/m^3 (Table 2). The pine sapwood samples impregnated with olive oil had the highest mean oil retention: 550 kg/m^3 with a SD of 7 kg/m^3 (Table 2). The spruce samples, which are known for their restricted accessibility during impregnation (Liese and Bauch, 1967; Ulvcrona et. al, l 2006), had an oil retention approximately half of that of pine sapwood or ilomba. The use of stand linseed oil, the most viscous of the selected oils, resulted in a lower oil uptake with spruce and pine sapwood than with other oils. In the case of the stand oil treated specimens, the mean oil retention of the ilomba samples was lower compared to others (Table 2). The low oil retention of ilomba can be attributed to a treatment error during the impregnation of one sample. The mean raw linseed oil retention of the pine sapwood specimens of set 2 was 545 kg/m^3, which is a slightly higher compared to the same type of samples of set 1 and set 3–6 (Table 2). The difference between the mean oil retention of samples from set 3–6 and the samples of the same type from set 1 was maximal 51 kg/m^3. The large pine specimens had an oil retention of 361 kg/m^3 with a SD of 8 kg/m^3. The oil retention of large pine was lower than the small pine sapwood samples. This can be attributed to presence of heartwood and the relative lower impact of the highly accessible sawn edges of the sample.

The impregnated specimens of sample set 2 were sterilized twice on two consecutive days in water vapour without pressure (European Standard, 1996; Fritsche and Laplace, 1999) before exposure outdoors.

2.2. Outdoor exposure and handling procedures

Five sites, located in different countries (Table 1), were used for outdoor exposure in this study. All specimens were placed horizontally, 10–40 cm above the ground or a flat roof (in case of the Netherlands), in an outdoor test field fully exposed to wind, sun and rain. The samples were exposed for 1.5–2 years.

At the end of the test the samples exposed in the Netherlands were put in sterile petri dishes and transported to the laboratory for analysis. The light and electron microscopy analysis were performed after 3 days of storage at 10 °C, a temperature similar to the outdoor temperature. Sample sets 3–6, which were exposed in Argentina, Australia, South Africa and Norway respectively, were sent by mail in an uncontrolled climate condition and analysed at