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Emissions of selected microbial volatile organic compounds released by *Aspergillus niger* growing on meranti wood (*Schorea* sp.)

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Abstract: *Emissions of selected microbial volatile organic compounds released by Aspergillus niger growing on meranti wood (Schorea sp.).* Gas chromatography-mass spectrometry/headspaces solid-phase microextraction (GC-MS/HS-SPME) was applied to identify emissions of Microbial Volatile Organic Compounds (MVOCs) from *Aspergillus niger* growth on meranti wood (*Schorea* sp.). The emission from *A. niger* contained 1-octen-2ol, 3-octanone and decane. GC-MS/HS-SPME is a simple, rapid and reproducible method for MVOC identification from *Aspergillus niger* growth on meranti wood.

Keywords: meranti, , HS-SPME, GC-MS *A. niger,* MVOC

INTRODUCTION

Wood continues to be a highly appreciated construction material. At present it is in fashion to use exotic wood in interior design. One of the problems potentially encountered in contemporary interiors is connected with high humidity. This is the effect, among other things, of airtight windows. This leads to the presence of moulds on wooden interior finishings. During their growth microfungi release microbial volatile organic compounds (MVOCs). Many authors ascribe allergies to the presence of MVOCs in interior air. For this reason it is of great importance to determine compounds produced by moulds, as they frequently cause diseases or impair physiological functioning of patients sensitive to allergens. It is generally known that the qualitative and quantitative MVOC composition is dependent on the substrate on which moulds develop. To date no studies have been conducted on MVOCs released by moulds growing on exotic wood. Meranti is a very common wood species used in the construction industry. This wood has found numerous applications as a construction and joinery material, in furniture making, boatbuilding and even in the production of musical instruments. It was decided to conduct studies aiming at the preliminary assessment of MVOC emissions during growth of a microfungus *A. niger* on meranti wood.

MATERIAL AND METHODS

Meranti (*Schorea* sp.) with a density of 0.58 g/cm^3 was selected for analyses. Meranti wood samples of 30 × 15 × 5 mm were prepared (with the first dimension along the grain, while the last one was tangential). Before analyses wood was conditioned in a chamber at a temperature of 21^{o}C and relative humidity of 75%. The test microfungus was *A. niger* ŁOCK 0439 1973 IHAR strain 201 coming from the collection of the Institute of Fermentation Technology and Microbiology, the Łódź University of Technology. The microfungus was cultured on agar slants with an addition of Czapek-Doxa salt and malt extract (30 g extract per 1 l culture medium). After two weeks from inoculation of agar slants an aqueous spore suspension with a concentration of 10^6 spores per 1 ml was prepared. The number of spores was determined in the Thoma counting chamber and supplemented with physiological saline until the required concentration was reached. In Petri dishes previously sterilised wood samples were placed on the surface of sterile medium and they were inoculated with the aqueous suspension of *A. niger* spores. Inoculation was performed using a cosmetic spray bottle so that approx. 3±1 ml aqueous spore suspension were sprayed onto the sample surface.

There were three samples per one dish. After inoculation Petri dishes were placed in an incubation chamber at 28°C and relative humidity of over 95%. Analysis of MVOCs was performed before inoculation and at days 3 and 6 from inoculation. At 24 h before analysis the inoculated sample was placed in a special 40-ml vial designed for SPME analyses and it was sealed tightly with a silicone septum, which could be pierced with a special holder equipped with an adsorber.

In order to isolate volatile compounds an adsorber was used – fibre coated with 75μm Carboxen–polydimethylsiloxane (Carboxen–PDMS). The method proposed by Cofta and Waliszewska (2013) was applied to assay MVOCs.

RESULTS AND DISCUSSION

Among fibres analysed in this study the fibre with the carboxen/polydimethylsiloxane stationary phase (Cofta and Waliszewska 2013) was decided to be optimal for the isolation of volatile compounds emitted by meranti wood and released by *A. niger*. This was connected first of all with the number of compounds extracted from meranti wood samples and it was also suggested by literature studies (Cavalli et al. 2004, Doleschall et al. 2003 Jeleń et al. 2000). Moreover, another criterion was the area of peaks of 1-octen-2ol, 3-octanone and decane, i.e. compounds considered the most important secondary metabolites released during fungal growth (Kaminski et. al. 1972 and 1974). 1-octen-2ol and 3-octanone are probably formed as oxidation products of linoleic acid (Wurzenberger and Grosch, 1984). Peak areas for selected chemical compounds are presented in Fig. 1. The analyses focused on days 3 and 6 of *A. niger* growth, i.e. the initial stage of microfungus growth. In this growth phase the microorganism released the greatest amounts of MVOCs (Schuchardt and Kruse 2009). In macroscopic analyses conducted with the naked eye up to day 4 of culture no growth of *A. niger* was observed on samples. Only in the successive days of the mycological test hyphae were noticed, followed by spore formation. In the public opinion wood of exotic species is considered to be resistant to biodegradation, which was partly confirmed by macroscopic analyses. However, an increase in the concentrations of secondary metabolites (1-octen-2ol, 3-octanone and decane) from the time of inoculation indicates that meranti wood is not resistant to the attack of *A. niger*. Attempts are being made, aiming at the selection of markers indicating the occurrence of a biological corrosion centre. 8-carbon compounds are believed to play that role. For this reason research should be extended to include determinations of the dependence of dynamics of synthesis of these volatile secondary metabolites in different fungal species.

In the profile of volatile compounds released by *A. niger* no characteristic terpenes were found such as α-pinen and limonene released by the test microfungus (Börjesson et al. 1992, Wilkins and Larsen 1995, Pasanen et al. 1997, Fischer et al. 1999, Demyttenaere et al. 2004, Jeleń and Grabarkiewicz-Szczęsna 2005, Moularat et al. 2008, Schuchardt and Kruse 2009,. Joblin et al. 2010). Results indicate that the analysis of MVOCs in the case of *A. niger* would need to be performed over a longer time in order to verify whether the synthesis of typical secondary compounds such as α-pinene and limonene for *A. niger* is a permanent or only a temporary phenomenon.

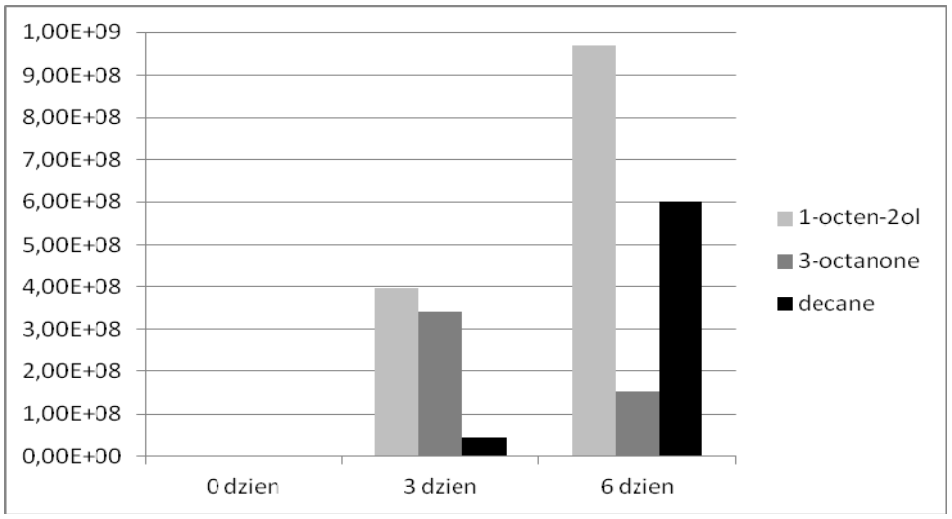

Fig. 1 Amounts of volatile compounds expressed by the total peak area for selected metabolites produced by *A. niger*

CONCLUSION

The application of SPME to assay MVOCs facilitated identification of secondary volatile metabolites (1-octen-2ol, 3-octanone and decane) of *A. niger*. MVOCs were detected as early as at day 3 from inoculation, which indicates susceptibility of meranti wood to infestation by the test microfungus.

A varied profile of volatile compounds was found for *A. niger* growing on meranti wood in comparison to literature data. No terpene hydrocarbons such as α-pinene or limonene were detected.

Studies need to be extended over a longer time of the mycological test and they should include other species of microfungi in order to provide more insight into biodeterioration of meranti wood by moulds.

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Streszczenie: *Emisja wybranych mikrobiologicznych lotnych związków organicznych emitowanych przez Aspergillus niger rosnący na drewnie meranti* (*Schorea sp.*). *A. niger* porastający drewno meranti syntetyzuje lotne mikrobiologiczne związki organiczne (MVOC). Oznaczono MVOC za pomocą techniki SPME. Jako markery wzrostu mikrogrzyba testowego wytypowano następujące związki 1-octen-2ol, 3-octanone i decane. Analiza profilu chromatograficznego *A niger* porastającego próbki wykazała, że drewno meranti nie jest odporne na biodeterioracje. Zauważono brak typowych drugorzędowych metabolitów takich jak α-pinen i limonen dla mikrogrzyba testowego porastającego drewno meranti.

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