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DISSERTATION

Biotechnological approach for the reduction of VOC in Oriented Strand Boards

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Abstract

Aldehydes and especially terpenes are among the most frequently emitted volatile organic compounds (VOC) in the wood processing industry. Wood products made of pine wood might influence human's well-being and the indoor environment because of the classification of VOC as irritants. Therefore, the actual study presents a biotechnological approach for the reduction of aldehydes and particularly the three major terpenes in pine wood, α-pinene, β-pinene and Δ3-carene, to obtain emission-reduced Oriented Strand Boards (OSB). The starting point of the study was the selection of bacterial strains based on their ability to metabolise α-pinene as single carbon source in liquid culture. *Pseudomonas putida* and *Pseudomonas fluorescens* showed the best results and were therefore adapted and applied for further tests on pine wood particles and strands at laboratory scale, in which the wood samples were incubated with bacterial strains for 72 h at room temperature. Residual emissions were analysed by means of GC-MS. The most promising results were obtained with a mixture of both *Pseudomonas* strains, and after various optimization steps the experiments were stepwise enlarged towards technical scale. To address the requirements of industrial OSB production, the focus was laid on the reduction of incubation time at consistent degradation rates. OSB were then manufactured from the microbially treated pine wood strands. The microorganisms caused a distinct reduction of α-pinene and β-pinene in manufactured OSB within 48 h and even 24 h incubation time. A successful degradation of Δ3-carene in both, liquid culture and pine wood, was achieved by an optimised culture of the ascomycete *Penicillium nigricans*. This approach successfully solved the problem that the *Pseudomonas* strains efficiently degraded aldehydes and pinenes, but were unable to metabolise Δ3-carene. By merging fungus and bacteria, all of the three major terpenes were reduced within OSB, indicating that there were no antagonistic effects between the species. In the course of the research, the fungal enzyme laccase in the presence of a redox mediator system turned out to be very efficient in Δ3-carene conversion into non-volatile oxidation products under laboratory conditions. Moreover, a mixture of bacteria and laccase showed good results in terms of Δ3-carene reduction in pine wood particles. Collectively, the results of the present study revealed the potential of biotechnology for reducing potentially harmful terpene emissions from wood products.

Eigenständigkeitserklärung

Ich bestätige mit meiner Unterschrift, dass ich die Dissertation selbstständig verfasst, keine anderen als die angegebenen Hilfsmittel benutzt habe und mich auch sonst keiner unerlaubten Hilfe bedient habe. Weiters bestätige ich, dass ich diese Dissertation weder im Inland noch im Ausland in irgendeiner Form als Prüfungsarbeit vorgelegt habe.

Wien, 22.01.2019 Dipl.-Ing. Bernhard Widhalm

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Ich widme diese Arbeit meiner Mutter (†), die mich immer unterstützt hat, den Abschluss meiner Dissertation aber leider nicht mehr miterleben durfte.

Contents

Abbreviations

µl	microlitre
µm	micrometre
% (v/v)	volume percent
°C	degrees Celsius
ABTS	2,2’azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)
AgBB	Ausschuss zur gesundheitlichen Bewertung von Bauprodukten
approx.	approximately
cm	centimetre
cp.	compare
DDT	Dichlorodiphenyltrichloroethane
ECA	European Collaborative action
e.g.	*exempli gratia* – for example
FID	flame ionisation detector
g	gram
GC	gas-phase chromatograph
GHS/LPC	Globally Harmonized System of Classification, Labelling and Packaging of Chemicals
h	hour
HBT	1-hydroxybenzotriazole
kg	kilograms
kPa	kilo pascal
l	litre
m	metre
MC	moisture content
min	minutes
ml	millilitre
mM	millimolar
MS	mass spectrometry
No.	number
OSB	Oriented Strand Board
PAH	polycyclic aromatic hydrocarbons

PM	particulate matter
PMDI	polymeric methylene di(phenylisocyanate)
sp.	species
SPME	solid phase microextraction
SVOC	semi-volatile organic compounds
TRBA	Technische Regel für Biologische Arbeitsstoffe, Bundesanstalt für Arbeitsschutz und Arbeitsmedizin
TVOC	Total Volatile Organic Compounds
U	enzymatic activity
UV	ultraviolet
VOC	volatile organic compounds
VVOC	very volatile organic compounds
WHO	World Health Organisation

1. Introduction

Pine wood (*Pinus sylvestris* L.) is one of the most frequently used materials for wood products in Europe and thus has a great influence on building and indoor environments. In recent years, there was a high increase of the market for Oriented Strand Boards (OSB) made from Scots Pine, mainly used as construction elements and for flooring and ceiling (Makowski et al. 2008, Wilke et al. 2013). Wood and wood products, especially made from softwood species, are well-known sources of VOC emissions (Risholm-Sundman et al. 1998). Pine wood, which is widely used for furniture and wood composite materials in Europe, is one of the major sources of aldehydes and terpenes, which are responsible for the typical odor of wood (Gminski et al. 2011). Most of the emissions arise during production processes like drying and pressing of wood composites, but a certain amount of VOC is also emitted from the manufactured wood product. The emission rate depends (next to the wood species) on the drying and storing conditions (Kleinheinz et al. 1999; Roffael et al. 2006). Terpene emissions from wood and wood products follow a simple decay curve over time, so the highest emissions are expectable shortly after the production process (Makowski et al. 2005). Primarily in new buildings, the amount of VOC is considerable because of the use of wood products in construction and furnishing (Hodgson et al. 2000). The trend of increasing energy efficiency in modern building industry yielded a continuous settlement of air exchange rates and therefore an easier accumulation of VOC in interior spaces (Makowski et al. 2008).

According to the Globally Harmonized System of Classification, Labelling and Packaging of Chemicals (GHS/LPC), aldehydes and terpenes are classified as irritants. Thus, a large amount of wood products made from pine wood in buildings might impair indoor environment (Makowski and Ohlmeyer 2006). Long-term exposition to highly emitting wood products might be responsible for harmful conditions, might cause health disorders and thus have an influence on human's well-being and working-efficiency (Mersch-Sundermann 2007). Although the sense of many substances in indoor air is subjective, the amount of VOC should not be neglected in terms of air pollution. They might cause irritation of the mucous membranes of eyes, nose and throat. Emissions from building products are often noticed as odour nuisance, for which there certainly was no generally approved

testing method (AgBB 2018). Also the lung function parameters could be affected (Gminski et al. 2011). Furthermore, a mixture of monoterpenes with α-pinene as main component might cause inflammation of the respiratory tract (Sagunski and Heinzow 2003). Exposition test scenarios indicated that continuous VOC emissions might cause the so-called “Sick-Building Syndrome”, although there is no universally accepted clinical definition of this syndrome (Wilke et al. 2013, Redlich et al. 1997). Therefore, a number of studies are dealing with the measurement, control and the reduction of volatile compounds.

The actual work presents a biotechnological approach for the reduction of aldehydes and terpenes within pine wood to lower the total emission level of OSB, to be prepared for future trends concerning the consistently decreasing threshold values for wood products. The reduction of aldehydes was a minor topic because of their known biodegradability in wood as shown in previous studies (Kuncinger et al. 2013, Stratev et al. 2015). Emphasis therefore was laid on the three major and persistent terpenes within pine wood, α-pinene, β-pinene and Δ3-carene as well as the up-scaling process from laboratory scale tests to industrially manufactured boards.

2. State of the Art

2.1 VOC definition

VOC is the common abbreviation for Volatile Organic Compounds, which are defined as compounds with a low boiling point and high vapour pressure. The World Health Organisation (WHO) has classified clusters of volatile compounds according to their boiling point area (Tab. 1) at standard atmospheric pressure (101.3 kPa).

Tab. 1: Classification of volatile organic compounds according to WHO (1988)

	compounds	**Boiling point (°C)**	**example**
VVOC	very volatile organic compounds	<0 – 50/100	propane
VOC	volatile organic compounds	50/100 – 240/260	α-pinene
SVOC	Semi-volatile organic compounds	240/260 - 380	DDT
POM	Particulate organic matter	>380	PAHs

Another definition is given by the European Collaborative action (ECA), where all substances within the retention area between C6 (n-hexane) and C16 (n-hexadecane) after separation on a non-polar GC-column, are counted among the VOC (ECA 1997).

2.2 VOC emissions from wood

Aldehydes and terpenes are among the major groups of VOC emitted by wood and wood products and that is why these substances are of great interest in this work. Aldehydes are secondary VOC emissions, arising from autoxidation of unsaturated fatty acids in the course of the manufacturing processes. On the other hand, terpenes are primary emissions, they are naturally occurring substances originating from resin (Makowski and Ohlmeyer 2006). Therefore, aldehyde emissions initially increase and abate over a long time period, while terpene emissions are continuously decreasing (Ohlmeyer et al. 2008).

α-pinene, β-pinene and Δ3-carene count to the bicyclic monoterpenes and are the major terpenes in softwoods, particularly in pine wood (Harman-Ware et al. 2017). In softwood species, monoterpenes, sesquiterpenes and diterpenes are the main occurring terpenes, which have several protective functions in plants (Granström 2007). α-pinene actually constitutes 20% to 90% of turpentine and is one of the

typical volatile pollutants found in waste gases of the wood-processing industry (Jin et al. 2006). However, the amount of monoterpenes in wood depends on the growing season and the geographical site of the living tree (Mersch-Sundermann 2007).

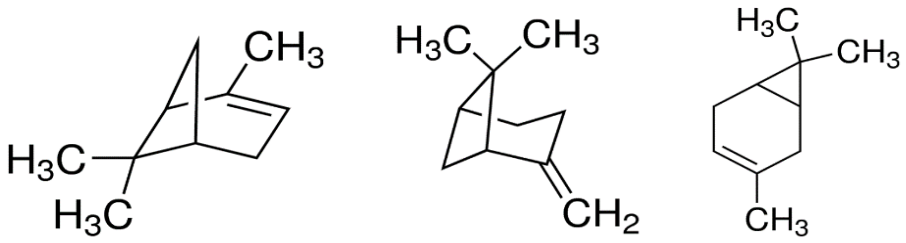

Fig.1: The three major terpenes within pine wood, α-pinene, β-pinene and Δ3-carene (left to right).

2.3 VOC reduction approaches

Since high VOC emissions and high emitting materials are of great concern, there are already many different studies with various approaches to reduce volatile substances. One approach was the thermal degradation of selected terpenes at 120°C to determine the reaction products (McGraw et al. 1999). Roffael et al. (2011) attempted to reduce subsequent aldehyde emissions from thermomechanical wood by treatment with ammonia. Thus, the emitted amount of formaldehyde was reduced by about 90%. Another study of the same author dealt with the application of sodium sulphite and hydrogen peroxide for the reduction of monoterpenes within pine wood strands (Roffael et al. 2015). Gabriel et al. (2015) reflected on the influence of storage temperatures and durations of pine wood on the terpene emissions. They found out that pine wood stored at 80°C for 2 days emitted less terpenes than stored at 40°C for 4 weeks. Another argument for terpene reduction at higher temperatures was given by Manninen et al. (2002), who stated that the terpene emissions of heat-treated wood (24 h at 230°C) were about 8 times lower than emissions from air-dryed wood. On the other hand, aldehyde emissions were much higher in heat-treated wood.

There are also a few biotechnological approaches for reducing VOC. The fungal bioconversion of α-pinene by *Aspergillus niger* was described by Agrawal and Joseph (2000). Another study using microorganisms in a biofilter was presented by

Jin et al. (2006) using *Ophiostoma* species for the filtration of α-pinene polluted air. For purification of the contaminated industrial off-gas, five fungal species were tested for their ability to degrade VOC in gas-phase (Qi et al. 2002). The fungal treatment of pine wood to degrade the volatile substances was performed by Stratev et al. (2011). Savithiry et al. (1998) investigated the degradation of α-pinene and its metabolic products caused by the thermophilic bacterial strain *Bacillus pallidus* at temperatures of 50°C and above. Another terpene degradation study was published by Dhamwichukorn et al. (2001), who investigated the bacterial growth and the utilization of methanol and α-pinene in liquid culture at 55°C.

The reason for using *Pseudomonas* strains for the degradation of terpenes in the present study is their reference in the following studies. Kallioinen et al. (2002) reported the degradation of fatty acids and resin acids, precursor substances of aldehydes, by *Pseudomonas fluorescens* within spruce wood chips. *Pseudomonas* sp. is already known for its degrading potential of substances which are toxic for other microorganisms (e.g. terpenes), not only at bioremediation processes (Villaverde and Fernandez-Polanco 1998, Jecu et al. 2006, Di Martino et al. 2011), but also within bioreactors and biofilters (van Keulen et al. 1997, Kleinheinz 1999, Munoz et al. 2008). *Pseudomonas* strains were also applied for the reduction of extractives, i.e. fatty acids and resin acids, within pine wood (Burnes et al. 2000). Cantwell et al. (1987) reported, that *Pseudomonas* sp. were also able to grow on and thus metabolize other acyclic isoprenoids like geraniol and citronellol, which occur in small amounts in wood.

Preliminary work preceding the actual thesis dealt with the application of effluents from industrial wood processing and thus the microbial consortium contained in them onto pine wood for the reduction of aldehydes. The microbial consortium included strains of the genera *Klebsiella*, *Bacillus*, and *Pseudomonas* as well. Actually, the bacterial strains in the process effluents caused a complete removal of aldehydes within pine wood after 5 weeks of incubation. However, degradation of terpenes was not reported in this study (Stratev et al. 2015).

The specific degradation of Δ3-carene was less investigated so far. Miyazawa and Kano (2010) described the selective oxidation of Δ3-carene by the larvae of *Spodoptera litura*. The biotransformation of Δ3-carene by the fungus *Penicillium nigricans* was described by Muddapur et al. (2015). Moreover, they detected the

neutral and acidic metabolic products, which were of relevance for verification of the degradation tests of the present thesis. Another study discussed the enzymatic bioconversion of Δ3-carene and the reaction scheme up to the final autoxidation products by a dioxygenase (Lehnert et al. 2012).

2.4 Microbial and enzymatic degradation pathways

The pathway of microbial degradation of terpenes was derived from the detected and identified main metabolic products. Yoo and Day (2002) as well as Tudroszen et al. (1977) stated that *Pseudomonas* strains are able to convert α-pinene and β-pinene via a few possible specific degradation pathways into acidic components (such as perillic acid and cumic acid). One of the first metabolic steps is the opening of the bicyclic ring structure. Main metabolic semi-finished products were other terpenes - cymene and limonene as well as α-pinene oxide. Since α-pinene and its isomer β-pinene have a similar structure, also their degradation pathways and metabolic products were very similar. Furthermore, the authors found out that limonene was a very good carbon source for *Pseudomonas* sp., resulting in a comparatively rapid subsequent conversion into acidic products.

The research study of Fontanille and Larroche (2002) showed another metabolic pathway in the degradation of α-pinene via the reaction product α-pinene oxide, which was enzymatically converted until the end product isonovalal was generated. Bicas et al. (2008) similarly reported isonovalal signals by GC-FID analysis of β-pinene degradation tests by *Pseudomonas rhodesiae*. Moreover, the plurality of metabolic pathways of α-pinene and β-pinene was shown in this study. It turned out that the terpene degradation pathway essentially depended on the used substrate (single terpene or a mixture of a few terpenes) and the concentration of terpenes.

An early study regarding the degradation of Δ3-carene was presented by Stumpf et al. (1990). After testing 121 fungi and bacterial strains for their ability to cleave the Δ3-carene, it turned out that *Mycobacterium smegmatis* converted 3-carene into carenones and chaminic acid by oxidation. Since the control flasks also showed a minimal amount of carenones, it was assumed that they were also formed by autoxidation. Dvorakova et al. (2011) mainly determined hydroxylated and oxygenated compounds as metabolic products. However, they also identified the carenones 2-caren-4-one, 3-caren-5-one and 3-caren-2-one as end products of the

oxidation pathway, irrespective of whether the biotransformation was performed within plants (e.g. *Nicotiana tabacum*) or caused by microorganisms. Degradation of Δ3-carene by *Penicillium nigricans* resulted in both, neutral and acidic products. The neutral products carvone, dihydrocarvone, carveol, trans-*p*-mentha-5,8-dien-3-ol and trans-*p*-mentha-5,8-dien-2-one as well as the acidic products perillic acid and 2-hydroxy-*p*-menth-8-ene-oic acid did no longer contain the cyclopropane ring (cp. Δ3-carene in Fig. 1). An interesting finding was that the fungal cells pre-cultivated on Δ3-carene showed higher values of oxygen consumption for generating the acidic products (Muddapur et al. 2015).

The main focus of Lehnert et al. (2012) was the enzymatic bioconversion of Δ3-carene by using a dioxygenase of *Pleurotus sapidus*. The authors found out that the amount of generated carenones was much higher by enzymatic bioconversion than by autoxidation. Thus, the dioxygenase catalysed the introduction of oxygen at allylic positions at the aromatic ring and, furthermore, there could be cleavage of the cyclopropane ring. In summary, also during the enzymatic degradation of 3-carene, there are a number of pathways leading to several end products (see Lehnert et al. 2012).

3. Research issues and research goals

Scots pine wood (*Pinus sylvestris* L.) is one of the major sources of aldehyde and terpene emissions. Since OSB are frequently used as indoor construction elements in buildings and because emissions of volatile organic compounds may cause health damage, VOC-emission levels must be reduced. The present thesis therefore deals with the specific biotechnological degradation of volatile organic compounds in pine wood for the production of Oriented Strand Boards (OSB) made of pine wood strands.

Under the aspect of technical implementation into a routine production process, the focus was on the reduction of VOC emissions by microbial degradation of aldehydes and especially terpenes in pine wood particles and strands with emphasis on the decomposition of the aldehydes pentanal and hexanal as well as on the major terpenes present in pine wood, which are α-pinene, β-pinene and Δ3-carene. The initial approach was to select various microorganisms, test them for their ability to degrade α-pinene in liquid culture, and optimise promising strains step-by-step starting from liquid culture to pine wood particles. The obtained results served as the initial point for a biotechnological approach for obtaining emission-reduced wood composites (see Widhalm et al. 2016).

In the next step the research goal was the reduction of the major monoterpenes in pine wood strands rather than particles as raw material for OSB manufacturing by applying a specific pre-cultivated mixture of *Pseudomonas putida* and *Pseudomonas fluorescens*. These microorganisms were first tested on small amounts of strands under laboratory conditions to define the appropriate growth parameters for the desired VOC reduction. Subsequently, laboratory OSB were produced from strands treated with bacteria on a larger scale. Now the focus was on an adoptable terpene reduction in an incubation time as short as possible. The successful degradation of α-pinene and β-pinene in a short time on technical scale could be a first step for a suitable integration of the biotechnological treatment procedure into the OSB production process (see Widhalm et al. 2017).

Pseudomonas strains efficiently degraded α-pinene and β-pinene but were unable to degrade Δ3-carene to an extent that significantly lowered its emission level in OSB. The resistance of Δ3-carene to biodegradation in previous work is considered a serious drawback for an industrial application of the method to minimise the total

emission level of subsequently manufactured OSB. Therefore, another research issue was the efficient reduction of Δ3-carene. The mould fungus *Penicillium nigricans* was identified as a promising candidate to be tested in liquid culture, followed by the treatment of pine wood strands in combination with the previously proven mixture of two *Pseudomonas* strains. Furthermore, in addition to the fungal treatment, an enzymatic approach for the reduction of Δ3-carene was carried out. Fungal laccase was chosen to test the assumption that this enzyme might be able to oxidise Δ3-carene and thus further accelerate its degradation rates in pine wood (see Widhalm et al. 2018).

4. Materials and Methodology

4.1 Microorganisms

Bacterial and fungal strains were selected respective their ability to degrade terpenes by literature research. A combination of *Pseudomonas putida* and *Pseudomonas fluorescens* (*Pseudomonas* Mix) was finally chosen for further degradation tests. For higher temperatures the thermophilic strain *Bacillus pallidus* was used at 55°C – 65°C at preliminary tests under laboratory conditions, but not for terpene degradation tests in pine wood. *Penicillium nigricans* was used for the reduction of Δ3-carene. Microorganisms were cultivated in Petri-dishes on Standard Nutrient Agar No.1 and on Malt Extract Agar, respectively. Liquid cultures were consequentially prepared by transferring the strains in terms of a liquid suspension into a M9 minimal salts medium with 0.5% (v/v) aqueous glucose solution as carbon source and 0.2% (v/v) $MgSO_4$ and 0.25% (v/v) Trace elements solution as additives. After an adoptable growth in liquid phase specific pre-cultivation was done by transferring a part of the cultures in a next step into the minimal salts medium with 0.1% (v/v) of the respective terpene (α-pinene or Δ3-carene, subsequently designated as “M9-pinene medium” and “M9-carene medium”) in order to focus the enzymatic system of the strain on the target substance. In liquid culture tests, microorganisms were tested both, apart and as a mixed culture. On pine wood, exclusively the mixed culture was applied.

4.2 Enzyme preparation

For a rapid Δ3-carene degradation, the enzyme laccase was used. Laccase was specifically produced by the authors working group (see Widhalm et al. 2018). Enzymatic activity (U) was determined by monitoring laccase induced oxidation of ABTS [2,2’azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] in a UV-1800 spectrophotometer. 100 mM 1-hydroxybenzotriazole (HBT) served as mediator system. The activity was adapted to the desired value by dilution with water.

Mycobacterium smegmatis, whose effective Δ3-carene degradation was attested by Stumpf et al. (1990), was not used for reduction tests in the present study according to its classification into risk group 2 by German TRBA 466 (Technische Regel für Biologische Arbeitsstoffe, Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, 2015).

4.3 Terpene degradation in liquid culture

Liquid culture tests were usually performed at room temperature (23°C ± 2°C) in 250 ml sealable Erlenmeyer flasks with 50 ml M9 medium, in which 2 ml of the respective pre-culture were added. 250 ml flasks were used due to adequate oxygen supply. Flasks without bacteria served as control samples. Reduction of α-pinene or Δ3-carene in liquid culture was determined by liquid-liquid extraction of the culture using cyclohexane as solvent after a predefined incubation time. For comparativeness of the samples, β-pinene was added as internal standard briefly before extraction. Results from terpene degradation tests were considered as a first selection of suitable microorganisms for further degradation tests and the following up-scaling process.

As there was an optimum pH-value for laccase reactions, the bioconversion tests of Δ3-carene in liquid culture was performed in sodium succinate buffer (pH 5). Therefore, 2 µl of Δ3-carene were added into 2 ml buffer solution followed by addition of 50 µl laccase (enzymatic activity 10U-100U depending on the experimental set-up) and 100 µl HBT. 2 µl β-pinene served as internal standard as well.

4.4 Pine Wood samples

Pine wood particles and strands were provided by Fritz Egger GmbH & Co. OG. Strands are industrially fabricated pine wood flakes with a size of approx. 2.5 cm width, 10 – 15 cm length and 0.5 cm thickness. Wood samples had a moisture content (MC) of around 100% (w/w) ± 10%. MC was detected by a moisture analyser (Sartorius). Since fresh pine wood was advantageous for terpene degradation tests and emission determination, not immediately used wood samples were stored deep-frozen.

4.5 Degradation tests within pine wood

Inoculation of pine wood particles and strands basically took place in 20 ml Headspace vials or 100 ml Erlenmeyer flasks under unsterile conditions to adapt the research design to industrial requirements. The samples were inoculated by adding 0.5 ml of the specific pre-cultivated bacterial mixture per g pine wood and incubated at room temperature (23°C ± 2°C). Mixtures of microorganisms and enzymes were prepared by merging the equal amounts of all components. Since

the positive influence of oxygen supply was determined during liquid culture tests, samples were ventilated by means of an aerator pump with an airflow rate of 1.3 l min^{-1}. After the defined incubation time, degradation process was stopped by heat-treatment at 103°C ± 2°C of the samples. Cyclodecane dissolved in methanol (1:100) served as internal standard and was added immediately before chemical analysis.

4.6 Production of emission reduced OSB

During the next up-scaling step, the treatment of strands and the following production of OSB, 2.5-3 kg fresh humid strands (MC ~ 100%) were used per board. Treatment took place in sealed 80 l barrels at room temperature. For a better distribution of the microorganisms, the samples were stirred manually every day of the incubation period. After the incubation time, the strands were dried in a rotating drum dryer at 160°C until the target MC <10%. To align the drying times of reference samples and treated samples, controls were moistened with water instead of microbial solution. Afterwards, the dry strands were blended with polymeric methylene di(phenylisocyanate) (PMDI) and pressed on a laboratory press (Simpelkamp) and cut into dimensions of 500 x 500 x 10 mm.

4.7 Chemical analysis

Analysis of liquid culture extractives was performed on a GC-system with a flame ionization detector (FID). Since it was the goal of the liquid culture tests to determine the degradation of the selected terpene, only a few substances were of interest. Therefore, authentic standards (dilution factor 1:100 in cyclohexane) of α-pinene, β-pinene and Δ3-carene were measured in addition to the samples to identify the respective retention times. The (residual) amount of terpenes within the extract was determined as the ratio of the appropriate peak area to the internal standard.

Chemical analysis of emitted volatiles from pine wood particles and strands as well as the analysis of laccase liquid extracts was done by solid-phase microextraction (SPME)/GC-MS. Thus, the treated pine wood particles or strands were heated (50°C) within the Headspace vials for 25 minutes while a SPME fibre containing of divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS) was adsorbing the emitted substances. In case of the laboratory OSB, small pieces were cut out of the boards and placed into headspace vials for chemical analysis. Desorption was

performed in a GC-MS system with a CTC Combi PAL Autosampler. Splitless separation of the volatiles was done on a HP-5 MS column with the dimensions 30m x 0.25mm x 0.25µm.

5. Summary of scientific publications – main results

The first publication “Reduction of Aldehydes and Terpenes within Pine wood by microbial activity” dealt with the identification and optimization of growth conditions of bacteria, which can degrade both, aldehydes and particularly terpenes within pine wood particles as a raw material for the production of particle boards. The focus was on pentanal and hexanal (aldehydes) as well as α- and β-pinene (terpenes). The mentioned volatiles count to the most frequently emitted volatile organic compounds in wood-processing industry. Production processes of wood composites initiate and increase the formation of VOC emissions. Aldehydes and terpenes are classified as injurious to health and hazardous to the environment.

At first, bacterial strains were selected for their ability to metabolise α-pinene as single carbon source in liquid culture medium. The degradation rate was then determined by GC analysis. Strains belonging to the genus *Pseudomonas* showed the best results (98% degradation of α-pinene after 72h). Comparably good results were also achieved with the thermophilic strain *Bacillus pallidus* (90% degradation) at 55°C. Furthermore, an adapted mixed culture of Pseudomonas species was inoculated onto wood particles and incubated at room temperature for three days. SPME measurements of emitted volatiles and subsequent GC-MS analysis indicated a complete removal (100%) of aldehydes and, even more importantly, α- and β-pinene. Using unsterile pine wood particles, pre-treatment with *Pseudomonas* species was directed in a first step to industrial application.

The second publication “Biodegradation of terpenes for emission-reduced Oriented Strand Boards (OSB)” built on the results of the first publication and broached the issue of applying the bacterial combination of *Pseudomonas* species onto pine wood strands to obtain emission reduced OSB produced in technical scale. In this study, the main focus was on α- and β-pinene as well as Δ3-carene, which are the major terpenes in softwoods, and furthermore, the focus was laid on an adoptable terpene reduction in a short incubation time. Laboratory tests with inoculated strands were carried out to optimize parameters such as aeration and incubation time, chemical analysis of residual terpene emissions was performed again by means of SPME/GC-MS. Daily aeration, specific pre-cultivation, and increased inoculum size eventually resulted in a bacterial reduction of the major softwood terpenes α-pinene, β-pinene, and Δ3-carene by 60%, 70%, and 40%, respectively, after only 2 days of

incubation. Based on these results, OSB were manufactured from strands after bacterial pre-treatment for 2 or 4 days. As expected, terpene emissions from OSB decreased with increased incubation time. However, even after only 2 days of incubation, α-pinene and β-pinene emissions from OSB were appreciable reduced by 40% and 70%, respectively. Other terpenes, which certainly had a comparatively low share in TVOC-value, were also reduced in the course of the microbial treatment.

While both pinenes were efficiently degraded by specifically selected and adapted *Pseudomonas* strains, Δ3-carene appeared to resist degradation. Therefore, the topic of the third publication “Microbial and enzymatic approach for the reduction of terpenes in pinewood” was the application of the fungus *Penicillium nigricans*, which is a selective Δ3-carene degrader, in combination with the bacteria strains. The synergistic action of both microorganisms was required for the first simultaneous degradation of all three major terpenes in pine wood including Δ3-carene. Δ3-carene emissions from laboratory OSB were 60% and 35% lower than that of controls after 4 and 2 days of microbial pre-treatment, respectively. At the same time, α-pinene emissions were reduced by 85% and 45%, while β-pinene emissions were completely absent. In order to boost Δ3-carene degradation to a level that meets industry demands, the author attempted to decompose Δ3-carene by oxidation using the oxidoreductase laccase isolated from the white-rot fungus *Trametes pubescens* as a biocatalyst. It was shown the first time that laccase accomplishes an almost complete oxidation of Δ3-carene in defined liquid medium and a 30% reduction in pinewood particles after 24 h of incubation in the presence of a redox mediator. In liquid culture, carenones (car-3-ene-5-one, car-3-ene-2-one and car-2-ene-4-one) were detected as final non-volatile metabolic products. The results revealed the potential of biotechnology for reducing possibly harmful terpene emissions from wood products.

6. Conclusions

A mixed culture of *Pseudomonas* species suitable for the degradation of terpenes in liquid culture and solid pine wood particles was successfully identified. First optimization steps such as inoculum cultivation in α-pinene as the sole carbon source and sufficient oxygen supply proved to be most essential requirements and resulted in a complete absence of both α- and β-pinene emissions from pine wood particles after only three days. However, the recalcitrance of Δ3-carene prevented at that time the complete removal of terpenes by bacterial treatment. Furthermore, applying the bacterial mixture onto pine wood strands successfully reduced the α- and β-pinene emissions of OSB. The optimization steps from preliminary tests were essential for an adoptable terpene reduction in a short incubation time of only 2 days. However, a major drawback was the recalcitrance of Δ3-carene that prevented the complete removal of terpenes by bacterial treatment at that time. OSB made from pine wood strands treated with *Pseudomonas* did not show a distinct reduction of Δ3-carene emission as observed with unprocessed strands treated with the same microorganism under laboratory conditions. The successful integration of a method for the microbial reduction of terpene emissions from OSB into an industrial production process, however, will require that all of the three major terpenes contained in pine wood, α-pinene, β-pinene and Δ3-carene, are extensively degraded within a very short time. In the present study, this challenge was largely met by combining the specific metabolic capabilities of two different microorganisms, a mixed culture of *Pseudomonas* bacteria and the filamentous ascomycete *Penicillium nigricans*. Laboratory OSB produced from pine wood strands, pre-treated with these microorganisms under appropriate conditions, exhibited significantly reduced emission levels for all relevant terpenes. The overall performance was substantially better than previously reported for pre-treatments with *Pseudomonas* alone. The only limitation was that Δ3-carene degradation required a pre-treatment time of more than 1 day to be effective, most probably due to the low growth rate of *P. nigricans*. The laccase-mediator system demonstrated its potential to overcome this limitation but further research is needed to function effectively on pine wood strands. In summary, the method developed here provides a solid basis for further research and development towards a feasible process for industrial application.

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8. Peer-reviewed scientific publications

The following peer-reviewed scientific publications constitute the present doctoral thesis:

WIDHALM, B., TERS, T., SREBOTNIK, E., RIEDER-GRADINGER, C. 2016. Reduction of aldehydes and terpenes within pine wood by microbial activity. Holzforschung 70: 895 – 900.
Contribution Bernhard Widhalm:
Conception of the study, Literature research, Selection of Materials and Methods, Experimental procedure, Analysis, Data Interpretation, writing and editing of the Manuscript

WIDHALM, B., RIEDER-GRADINGER, C., KUNCINGER, T., SREBOTNIK, E. 2017. Biodegradation of terpenes for emission-reduced Oriented Strand Boards (OSB). Holzforschung 71: 259 – 264.
Contribution Bernhard Widhalm:
Conception of the study, Literature research, Selection of Materials and Methods, Experimental procedure, Analysis, Data Interpretation, writing and editing of the Manuscript

WIDHALM, B., RIEDER-GRADINGER, C., KUNCINGER, T., SREBOTNIK, E. 2018. Biotechnological approach for α-pinene, β-pinene, and Δ3-carene degradation in pine wood for reduced terpene emissions from Oriented Strand Boards. Int. Biodeterior. Biodegrad. 134: 103 – 109.
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