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Dissertation

Valorization of wood processing effluent for biotechnological reduction of volatile organic compounds in pinewood

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Abstract

This study focused on using medium density fiberboard (MDF) effluent as a culture medium for *Pseudomonas putida* PX1 (*P. Putida*) for reducing volatile organic compounds (VOC) in pinewood. This process effluent stream is rich in wood extractives and primarily composed of various carbohydrates, polyphenols (lignans and stilbenes), and organic acids, particularly fatty and resin acids. In a first step, a general workup procedure for MDF effluent was developed by removing and recovering growth inhibiting substances, such as resin acids by centrifugation and membrane filtration, followed by recovery of valuable bioactive compounds, particularly lignans and stilbenes, by adsorption. Using this approach, a scalable, fixed-bed adsorption system packed with polyvinylpolypyrrolidone (PVPP) was developed to remove and selectively recover polyphenols (lignans and stilbenes) from MDF effluent. The capacity of the PVPP for 7-hydroxymatairesinol (HMR) as a model lignan was determined as 37.4 mg g^{-1} at 1% breakthrough. However, highly polar substances, such as sugars, sugar alcohols, small organic acids, and salts, were not retained on the column and remained in the flow-through for further use as a cultivation medium for *P. putida*, a VOC degrading soil bacterium. *P. putida* was successfully cultivated in this residual carbohydrate rich effluent stream. It was shown that a wide range of substrates in the effluent stream in addition to C5- and C6-sugars, such as glycerol, acetate, succinate, and citrate, were efficiently metabolized without adding other nutrients. In a further step, *P. putida* cultures were applied to pinewood strands, markedly reducing VOC. To address requirements for industrial fiberboard production, the potential for seasonally varying MDF effluent in a year-round cultivation process of *P. putida* to reduce VOC in pinewood strands was investigated Seasonal variations with abundant fermentable carbon sources during colder periods and few carbon sources during warmer periods were observed across four years. VOC reduction in pinewood strands with *P. putida* after mixed substrate fermentation under controlled conditions showed very promising results for industrial application. Total VOC (TVOC) emissions decreased by more than 55% in only 3 h. Most aldehydes and terpenes were effectively reduced by 67%–100%, except for Δ-3-carene and α-terpinolene, which decreased by 20%–22%. Combined, our results identify a promising approach to reuse the currently unused MDF effluent stream in a year-round biotechnological process to produce VOC-reduced wood products, such as oriented strand boards (OSB).

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, 17.05. 2022 Dipl.-Ing. Martin Lindemann

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Contents

Abbreviations

AgBB	Ausschuss zur gesundheitlichen Bewertung von Bauprodukten
Approx.	Approximately
BOD	Biological oxygen demand
CFU	Colony-forming units
COD	Chemical oxygen demand
e.g.	Exempli gratia – for example
END	Enterodiol
ENL	Enterolactone
EU	European Union
GHS/LPC	Globally harmonized system of classification, labeling and packaging of chemicals
h	Hour
HMR	7-hydroxymatairesinol
HPAEC/PAD	High-performance anion-exchange chromatography with pulsed amperometric detection
HPLC/DAD	High-performance liquid chromatography with diode-array detection
HPSEC	High-pressure size exclusion chromatography
i.e.	Id est – that is
IC	Ion chromatography
ISt	Internal standard
LCI	Lowest concentration of interest
MDF	Medium density fiberboard
MPLC	Medium pressure liquid chromatography
MWD	Molecular weight distribution
n	sample size (natural number)
OD 600	Optical densities measured at a wavelength of λ=600 nm
OSB	Oriented strand boards
P. Putida	*Pseudomonas putida* PX1 (NCIMB 10684)
PHA	Polyhydroxyalkanoates
pO_2	Partial pressure of oxygen
PVPP-RS	Polyvinyl polypyrrolidone - Regenerable
SEM	Scanning electron microscopy
sp.	Species

SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SSE	Stuffing screw effluent
SVOC	Semi volatile organic compounds
TD	Thermo desorption
TMP	Thermomechanical pulp
TOC	Total organic carbon
TVOC	Total volatile organic compounds
UV	Ultra violet
VFA	Volatile fatty acids
VOC	Volatile organic compounds
VVOC	Very volatile organic compounds
WHO	World health organization
λ	Wavelength

1. Introduction

Wood processing industries must deal with various raw material streams, particularly in wood-based panel production. Producing medium density fiberboard (MDF) is very water-intensive with approx. 1,400 L/m³ fiberboard according to Wilson (2010), leading to large amounts of process effluent originating primarily from pretreatment by squeezing out steam-pretreated softwood chips. Raw material variability and subsequent effluent streams require flexible solutions with constant monitoring to ensure process stability and meet regulatory limits for disposal or reuse. Biological wastewater treatment (active sludge treatment) provides a flexible and continuously adaptable microbial consortium to lower chemical/biological oxygen demand (COD/BOD), as well as volatile organic compounds (VOC) from waste air streams in MDF production (Portenkirchner et al., 2003). Following additional processing steps, particularly membrane filtration and reverse osmosis, the effluent stream can be reused in the process, but is laborious and costly without exploiting effluent for additional benefits. MDF process effluent is mainly composed of various carbohydrates, polyphenols (lignans and stilbenes), and organic acids, particularly fatty and resin acids. Recovering valuable compounds from unused process streams can reduce the organic carbon load, resulting in a lower oxygen demand during biological treatment and thus lowering treatment costs. Polyphenols are of great interest due to their antioxidant effects, such as scavenging reactive oxygen species and thus have potential as cancer-protecting substances (Deyama and Nishibe, 2016). Lignans are present in different softwood species, such as spruce, fir, pine, and larch, where they are mainly concentrated in the knots (Holmbom et al., 2003) and are considered important phytoestrogens as they exhibit a variety of biological activities when metabolized to the mammalian lignans enterolactone (ENL) and enterodiol (END) by intestinal bacteria (Yoder et al., 2014). Recovery of polyphenols from MDF effluent is challenging due to the complex matrix and low concentrations of valuable target substances, such as lignans and stilbenes. Traditionally, the primary goal when handling wastewater from pulping and biomass processing industries was solely to decrease chemical/biological oxygen demand (COD/BOD) and eliminate toxic substances with minimal cost. A key aspect of improving overall sustainability for the industry is direct reuse of effluent (Ashrafi et al., 2015). The current literature focuses more on utilizing effluent streams, for instance, by fermentation. Specific microorganisms can use multiple carbon sources to

produce a desired product, while simultaneously reducing COD/BOD. For example, acidogenic fermentation of pulp mill effluents can be the initial step for generating polyhydroxyalkanoates (PHA) by producing volatile fatty acids (VFA) (Bengtsson et al., 2008). A vast number of *Pseudomonas* species are ubiquitous in soil and water and can adapt to challenging environments, including adverse conditions, such as high and low temperatures and poor nutrient availability. Further, pseudomonads are known for their ability to metabolize a wide range of substrates, such as hydrocarbons, aromatic compounds, and terpenes (Mirpuri et al., 1997, Poblete-Castro et al., 2012, Yoo et al., 2001). Some *Pseudomonas* species can metabolize aldehydes and terpenes in liquid culture (Bicas et al., 2008; Cheng et al., 2013; Kleinheinz et al., 1999) and pinewood strands (Widhalm et al., 2016). α-Pinene, the major terpene in pinewood (Kleinheinz et al., 1999), is classified as an irritant and hazardous to the environment, according to the Globally Harmonized System of Classification, Labeling, and Packaging of Chemicals (GHS/LPC). Large amounts of wood products made from pinewood in buildings, such as oriented strand board (OSB), can deteriorate indoor air quality due to elevated concentrations of aldehydes and terpenes, whereby chronic exposure to these VOC may lead to "sick-building-syndrome" (Makowski and Ohlmeyer, 2006; Wilke et al., 2013). While terpenes are naturally occurring in softwoods originating from wood resin, most aldehydes, such as hexanal, octanal, nonanal, and their related unsaturated derivatives are emitted as secondary VOC, stemming mainly from the autoxidative cleavage of unsaturated fatty acids. Therefore, these secondary emissions show a delayed release, which is important for storage conditions and further use (Makowski et al., 2005). For unsaturated aldehydes, starting from 2-butenal up to 2-undecenal, very low EU-LCI (lowest concentration of interest) values were derived in 2015 and remain under discussion in 2021 for incorporation in the German AgBB scheme (AgBB, 2015; AgBB, 2021). Developing and implementing innovative VOC reduction strategies is crucial for complying with these requirements.

In this thesis, a sustainable process reusing MDF effluent as a cultivation medium for VOC degrading bacteria and subsequent application of these bacteria to reduce emissions in pinewood was developed. Valuable fractions, such as lignans and stilbenes, were recovered by adsorption before cultivation. Industrial applicability of the process was emphasized by optimizing cultivation conditions in mixed substrate fermentation and reducing treatment times of pinewood strands while aiming to effectively reduce VOC emissions.

2. State of the art

2.1. MDF effluent treatment and reuse

MDF is widely used in furniture, laminate flooring, door panels, and drawer fronts and provides excellent substrate for paintings, foils, or wood veneers. In 2020, MDF accounted for approximately 21% (12 million m^3) of the total wood-based panel production in EU27, UK, and EFTA, according to the annual report of the European Panel Federation (2021).

In general, MDF is created by breaking down debarked wood logs into wood chips that are further refined into fibers. These wooden fibers are mixed with resin and undergo pressure and high temperatures to form panels. Wood chips are pretreated with steam to facilitate refining by softening lignin in the middle lamella while avoiding degradation of structural lignin and cellulose. McDonald et al. (2000) found by analyzing screw press effluent from an MDF plant that effluent mainly contained low molecular sugars and low amounts of degraded lignin. During thermomechanical pretreatment, a large effluent stream is generated by squeezing water out of the steam-pretreated wood chips that is rich in total organic carbon (TOC) and contains various carbohydrates, polyphenols, and organic acids. The latter include colloidal fatty and resin acids stabilized by hemicelluloses and various cations (Otero et al., 2000). After removing particles, resin, and fatty acids by sedimentation and flocculation or flotation, biological wastewater treatment (active sludge treatment) provides a flexible and continuously adaptable microbial consortium to lower chemical/biological oxygen demand (COD/BOD), as well as VOC from waste air streams in MDF production (Portenkirchner et al., 2003; Wagner, 2000). After additional processing steps, particularly membrane filtration and reverse osmosis, the effluent stream can be reused in the process, but is laborious and costly without exploiting the effluent for additional benefits. Different approaches to valorize MDF effluents or similar effluents from pulp mills or thermomechanical pulp (TMP) processes have been investigated, but have not yet been established at scale. For example, methane production by anaerobic digestion of TMP wastewaters was recently demonstrated by Gao et al. (2016) in a hollow fiber-submerged anaerobic membrane bioreactor and was successfully applied to MDF, according to Arias et al. (2020). Dessì et al. (2018) revealed hydrogen production from TMP wastewaters by dark fermentation preferably at high temperatures is possible. An innovative approach for converting carbohydrates to electricity using pulping

industry wastewater in a microbial fuel cell using *Pseudomonas fluorescens* as the primary energy-producing bacterium was published by Kaushik and Jadhav (2017). Furthermore, VFA, a substrate for polyhydroxyalkanoates (PHA) production (Queiros et al., 2014), can be produced from pulp mill effluents by acidogenic fermentation (Ben et al., 2011; Bengtsson et al., 2008).

2.2. Polyphenols

Polyphenols are of great interest due to their antioxidant effects, such as scavenging reactive oxygen species, and thus offer potential cancer-protecting substances (Deyama and Nishibe, 2016). Lignans are considered among phytoestrogens, exhibiting a variety of biological activities when metabolized into mammalian lignans enterolactone (ENL) and enterodiol (END) by intestinal bacteria (Yoder et al., 2014). Phytoestrogens are weak estrogens found in plants, which are structurally similar to endogenous estrogens, but show both estrogenic and anti-estrogenic effects (Adlercreutz et al., 1992). Currently, lignans and their promoted health benefits are attracting significant research interest. Yeung et al. (2020) used quantitative analysis of approximately 10,700 lignan-based publications starting from 1970 to reveal 80% were published after the year 2000 and 50% after 2010. Lignans are used in different research fields concerning human health, such as colon, breast, prostate, and intestinal cancers, as well as menopausal syndromes and cardiovascular disease (Landete, 2012). For example, Qu et al. (2005) showed lignans contribute to antitumor activity of wheat bran in colon cancer SW480 cells and treatment with END or ENL, as well as their combined treatment, led to reduced cell numbers. Moreover, a meta-analysis of 21 studies (Buck et al., 2010) exploring the effects of lignans and enterolignans on breast cancer risk found high lignan intake of postmenopausal women was associated with significantly lower risk of breast cancer. This was, however, not applicable for all women participating in these studies. Given the mechanisms behind the anticarcinogenic effects of END and ENL are not yet fully understood, more research is needed to develop methods for treating and preventing cancer using lignans. Bylund et al. (2005) showed that HMR, the main lignan from purified Norway spruce (*Picea abies*) knot extracts, significantly inhibits growth of lymph node carcinoma in prostate cancer xenografts in mice. These results corroborate with a previous study using lignan-rich diets (Bylund et al., 2000), confirming the biological activity of lignans. The lignan nortrachelogenin can provoke prostate

cancer cells to produce tumor necrosis factors, related to apoptosis-inducing ligands, while not triggering the non-malignant prostate cell line (Peuhu et al., 2013). More information on structure, source, isolation, and bioactivity of lignans and their glucosides can be found in the review published by Teponno et al. (2016) containing information from over 200 peer-reviewed articles from 2009–2015. Stilbenes, such as resveratrol and pinosylvin and their derivatives have also received increasing interest due to their antioxidant (Stojanović and Brede, 2002), antibacterial, and antifungal properties (Lee et al., 2005). Cancer chemopreventive/anti-inflammatory (Park et al., 2004; Park et al., 2013) and antioxidant (Fang et al., 2002) effects of pinosylvin in cancer cells, as well as the protective effects of pinosylvin in human retinal pigment epithelial cells against oxidative stress (Koskela et al., 2014), have also been reported.

Polyphenols are typically obtained by extracting different plant materials, such as fruits, herbs, vegetables, bark, or knot wood with a polar solvent (Rajbhar et al., 2015). Flaxseed, sesame seed, sunflower seeds, and cereals, such as rye and wheat, are commonly used due to their high polyphenol content and availability. Recovering polyphenols from effluents has the advantage of using target substances already extracted, but usually the concentration is quite low and dealing with large amounts of diluted effluent streams with complex composition is challenging. Valorization of wood hydrolysate from TMP, fractionating hemicelluloses, aromatic compounds, and extractives has previously been investigated in several research papers together with filed patents (Al Manasrah et al., 2012; Persson et al., 2010; Sundberg et al., 2002). Recently, a membrane process with consecutive ultra- and nano-filtration steps to obtain lignin- and lignan-enriched fractions from TMP-effluent were developed (Villain-Gambier et al., 2020). Lignans are present in different softwood species such as fir, pine, and larch, where they are mainly concentrated in the knots (Holmbom et al. 2003). HMR is the predominant lignan (70%–85%) in Norway spruce (*Picea abies*) knots with lignan content between 6% and 24% (Willför et al., 2003a) and various lignans up to 3% have been found in pine knots (*Pinus spp*.) (Willför et al., 2003b). Additionally, pinosylvin stilbenes were found in pine heartwood/living knots (0.2%–2% and 2%–8%) (Hovelstad et al., 2006). Eckerman and Holmbom (2004) developed and patented a process called ChipSep to separate knots from normal wood, enabling the industrial production of HMR from knots. The Swiss company Linnea SA (Riazzino, Switzerland) has been producing HMR as potassium acetate

adduct from Norway spruce (*Picea abies*) knots at large scale as a dietary supplement (Korte et al., 2014). Dietary supplements comprising HMR, flaxseed, or sesame lignans are currently marketed in the US by retailers including Products Development LLC (Chandler, AZ, USA).

2.3. VOC

VOC, in general, are defined by a low boiling point and high vapor pressure. VOC are divided into subgroups starting from very volatile organic compounds (VVOC) to VOC and up to semi volatile organic compounds (SVOC), according to the WHO (1989). While the subgroups appointed by the WHO (1989) are defined within specific boiling point ranges, the European Collaborative Action (ECA 1997) defined retention time (RT) ranges following separation on a non-polar GC-column limited to specific substances: VVOC:RT < C6 (n-hexane); VOC:RT range between C6 and C16 (n-hexadecane); and SVOC: RT range between C16 and C22 (n-docosane). The definition by the ECA (1997) is used by the Committee for Health-related Evaluation of Building Products (AgBB) in the actual German AgBB (2021) scheme and in VOC testing methods, such as DIN ISO 16000-6 (2010). Emission limits for single substances are sourced from LCI values and, for the actual AgBB (2021), most LCI values are adopted from LCI values published by the European Commission subgroup on EU-LCI values (agreed EU-LCI values (December 2021)). In particular, for unsaturated aldehydes, starting from 2-butenal up to 2-undecenal, very low EU-LCI values were derived in 2015 and in 2021 are still considered for incorporating into the German AgBB scheme (AgBB, 2015; AgBB, 2021).

2.4. VOC emissions from wood

The major VOC emitted from softwoods are aldehydes and terpenes, while hardwood emissions consist mainly of aldehydes and organic acids (Roffael 2006, Risholm-Sundman et al. 1998, Paczkowski et al. 2013). Terpenes are comprised of approximately 85%–98% of TVOC emissions, depending on the wood species, with spruce (*Picea abies*) and larch (*Larix decidua*) on the lower and scots pine (*Pinus sylvestris*) on the higher end (Englund and Nussbaum, 2000; Steckel et al., 2010; Wajs et al., 2007). The mayor terpenes in softwood, particularly pine, are α-pinene and Δ3-carene and, to a lesser extent, β-pinene, terpinolene, camphene, and limonene (Harman-Ware et al., 2016; Risholm-Sundman et al., 1998). Terpenes were found as primary emissions that are naturally occurring substances originating

from resin, whereas aldehydes are secondary VOC emissions formed mainly from autoxidation of unsaturated fatty acids. Aldehyde emissions initially increase and decrease over a long period, while terpene emissions continuously decrease from day 0 (Butter and Ohlmeyer, 2021; Makowski and Ohlmeyer, 2006). Engineered wood products, especially with thermal treatment during production, e.g., hot pressing, differ in their emission behavior compared with unprocessed wood. Multiple studies investigating VOC emissions from OSB found increased shares of aldehydes several weeks after production. Shortly after production, terpenes were the dominant substance group, but increasing amounts of aldehydes may be due to oxidation of unsaturated fatty acids, accelerated during manufacturing (Makowski et al., 2005; Salthammer et al. 2003; Wilke et al., 2013).

2.5. Reduction approaches for VOC

Numerous strategies have been developed to lower total VOC emission levels and reduce the negative impacts on indoor air quality and consequently human health. In addition to microbial reduction, several other approaches to reduce VOC emissions have been reported, including thermal reduction strategies such as heat treatment of pinewood (Manninen et al., 2002) and particleboards (Jiang et al., 2017), thermal degradation of terpenes (McGraw et al., 1999), and elevated storage temperature within wood chips for pellet production (Gabriel et al., 2015). Chemical degradation of terpenes from pinewood with sodium carbonate, sodium sulfite, sodium hydroxide, and mixtures of these substances has also been investigated by Roffael et al. (2015). Qin et al. (2020) compared VOC emissions from pine (*Pinus radiata*) following treatment with either sodium bicarbonate or aqueous ozone solution. Furthermore, studies have explored biodegradation and biotransformation with different fungi in liquid culture (Lee et al., 2015; Qi et al., 2002) and reduced VOC from waste gases with biofilters containing fungi (Jin et al., 2006; Van Groenestijn and Liu, 2002). Furthermore, Agrawal and Joseph (2000) described fungal bioconversion of α-pinene by *Aspergillus niger* and Stratev et al. (2011) successfully treated pinewood (*Pinus sylvestris*) with *Ophiostoma piliferum*, reducing aldehyde emissions and precursors. Kallioinen et al. (2003) and Burnes et al. (2000) further showed bacteria, mostly different *Pseudomonas* species, isolated from spruce (*Picea abies*) and pine (*Pinus sylvestris*) wood chips can degrade fatty acids and resin acids, which are aldehyde precursor substances, in liquid solution and on wood chips. Based on preliminary

work to this thesis, Stratev et al. (2015) used effluents, including a natural microbial consortium, from wood processing industries to reduce aldehydes within pinewood and found complete removal of aldehydes after five weeks. *Pseudomonas* was part of this microbial consortium, as well as *Klebsiella* and *Bacillus*. Recently, Widhalm et al. (2018) showed pretreating pinewood strands with a mixed culture of *Pseudomonas* and the ascomycete *Penicillium nigricans* significantly reduced α-pinene, β-pinene, and Δ3-carene emissions from OSB.

3. Research issues and goals

MDF is widely used in furniture, laminate flooring, door panels, and drawer fronts. During thermomechanical pretreatment, a large effluent stream rich in TOC containing various carbohydrates, polyphenols, and organic acids is generated. This effluent stream requires expensive treatment before disposal, the potential of which remains unused. Therefore, the main goal of this thesis was to develop a reuse scenario for MDF effluent and leverage its full potential as a sustainable, environmentally benign process. At the production site of the MDF plant, OSB are also manufactured and are frequently used as indoor construction elements. Here VOC reduction is explored, as exposure limits concerning VOC emissions (TVOC, LCI) are continuously lowered. Therefore, this research focused on valorization of MDF effluent by recovering valuable substances and reusing the stream in a biotechnological VOC reduction approach for pinewood strands, which can be used in OSB manufacturing.

After thoroughly characterizing MDF effluents, polyphenols, namely lignans and stilbenes, were identified as valuable substances due to their antioxidant and potential cancer-protecting properties. The first challenge was to address the large amounts of diluted process effluents that contain only small amounts of polyphenols (50–100 mg l^{-1}). Using technical implementation, a scalable fixed-bed adsorber for recovering lignans and stilbenes, which can be regenerated, was successfully developed. Carbohydrate rich flow-through after adsorption served as a starting point for biotechnological VOC reduction (Lindemann et al., 2020a, **Publication 1**).

The next step involved cultivating VOC reducing bacteria, particularly *P. putida*, in MDF effluents after polyphenol adsorption to subsequently reduce VOC in pinewood strands. *P. putida* can metabolize a wide range of substances in MDF effluents, yielding sufficient biomass for distinct VOC reduction when applied to pinewood strands following a short adaption step with a model terpene. Pre-cultivation of *P. putida* in carbohydrate rich MDF effluents and subsequent VOC reduction showed comparable results to pre-cultivation in synthetic M9 minimal medium (Lindemann et al., 2020b, **Publication 2**).

Monitoring MDF effluents for four years revealed seasonal variability, in particular sugar quantity, that required adjusted amounts of N-source for optimized biomass production. For industrial application, year-round, stable cultivation processes and short treatment times of wood strands are essential. Cultivation conditions for *P. putida* in MDF effluents were thus optimized for nitrogen demand in shake flask experiments and cultivation conditions in a batch bioreactor with controlled pH, stirring, aeration, and off-gas analytics. These optimizations drastically reduced treatment times of pinewood strands and the adaption phase (Lindemann et al., 2022, **Publication 3**).

4. Materials and Methods

All experiments conducted and methods used in this thesis are detailed in the following materials and methods section. Further detail is available in the associated published articles, which are referenced at the end of each paragraph [P1, P2, P3].

4.1. Analysis and work-up of MDF effluent

Origin of MDF effluents

MDF effluents were sampled monthly from an MDF plant in Wismar, Germany (Egger Holzwerkstoffe Wismar GmbH & Co. KG) for four years (04_2017-04_2021). Briefly, the MDF process involves steaming of wood chips at approximately 120°C at elevated pressure, followed by squeezing water out of the steamed wood chips using a stuffing screw. This stuffing screw effluent (SSE) was then diluted by a steam condensate, yielding the MDF effluent. Spruce (*Picea Abies*) and pine (*Pinus Silvestris*) are primarily used for MDF production at the Wismar plant. Softwood comprises more than 90% of the wood mix, but a precise composition cannot be assigned to sampled batches because only rough daily averages are available [P1, P2, P3].

Work-up procedure for MDF effluent

Fibers and non-colloidal particles were removed from MDF effluents via centrifugation. Tangential flow membrane filtration, using a 30-kDa cut-off membrane cassette, was then performed to remove colloidal particles, resin, and fatty acids. Centrifuged and filtrated effluents were then loaded onto a medium pressure liquid chromatography (MPLC) column filled with regenerable PVPP-RS. Flow-through containing unbound substances was collected and the loading procedure was stopped prior to polyphenol breakthrough that were then eluted with methanol (described later), followed by column recalibration. The complete production scheme and product streams are shown in Figure 1 [P1, P2, P3].

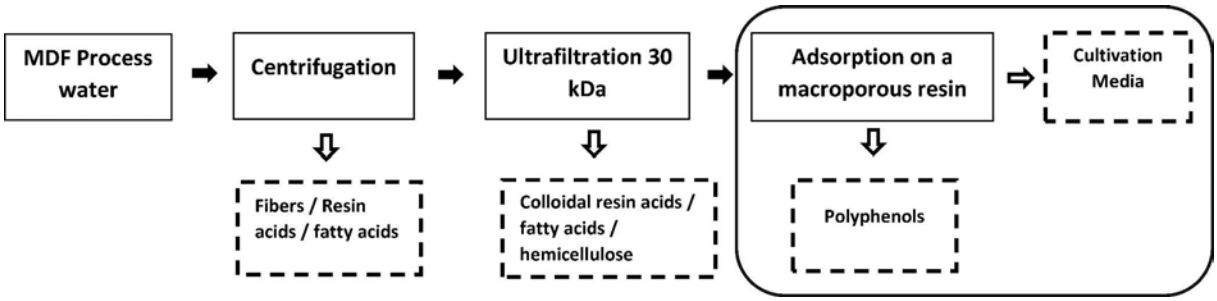

Figure 1: Process scheme of integrated biorefinery concept for MDF process waters (Lindemann et al., 2020a).

Analysis of carbohydrates in MDF effluent

Sugars and sugar alcohols in different process effluent samples were determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) Separation: Isocratic elution in water with post-column addition of base for PAD. Sugars were quantified by calibrating peak areas with authentic standards. Sugar alcohols were not adequately separated with this method. A sum parameter was thus, defined by quantifying sugar alcohols as an equivalent to pinitol (the predominant sugar alcohol identified in process effluents). This fraction primarily represented sugar alcohols as confirmed by gas chromatography coupled with mass spectrometry (GC/MS) as an orthogonal method. Column eluates of five consecutive injections were pooled, freeze-dried, derivatized (silylation), and analyzed by GC/MS. Sugar alcohols were identified by mass spectra using commercial mass spectral libraries and quantified as percentages of total peak area [P2, P3].

Anion, cation, and organic acid analysis

Inorganic ions and organic acids in process effluent samples were determined by ion chromatography equipped with conductivity detectors and an autosampler for simultaneous injection into both anion and cation columns via two sample loops. All ions were quantified by peak area calibration using authentic standards for chloride, nitrate, sulfate, phosphate; acetate, propionate, formate, succinate, citrate; sodium, ammonium, potassium, magnesium, and calcium [P2, P3].

COD of MDF effluent analysis

According to DIN ISO 15705 (2002), COD of MDF effluents was performed directly at the MDF plant in Wismar, Germany (Egger Holzwerkstoffe Wismar GmbH & Co. KG). To facilitate comparison with other substances, COD is represented in g/L rather than conventional mg/L [P3].

Scatterplots, histograms, and correlation coefficients

OriginPro was used to plot scatterplots and histograms. For Spearman's correlation coefficient, data were ranked (average rank) and calculated according to the following equation (Dodge, Y. 2008b):

$$\rho_s = 1 - \frac{6\sum_{t=1}^{n} d_i^2}{n(n^2-1)},$$

Where d_i^2 denotes the difference between the two ranks of two different observations and *n* represents the number of observations. The partial Spearman's correlation coefficient was calculated using the following equation (Dodge, Y. 2008a):

$$\rho_{s,par} = \frac{\rho_{s:x,y} - (\rho_{s:x,z} \times \rho_{s:y,z})}{\sqrt{1-\rho_{s:x,z}^2} \times \sqrt{1-\rho_{s:y,z}^2}},$$

where ρ_s:(x,y; x,z; y,z) represents the Spearman's correlation coefficient between two variables (x,y; x,z; y,z) whereby x,y denote variables to correlate and z represents the control variable [P3].

4.2. Adsorption and recovery of polyphenols

PVPP-RS particle size and shape analysis

Particle size measurements of PVPP-RS were conducted using laser diffraction in liquid suspension. The analyzed light energy data referred to a sphere with the same volume as the measured particle, yielding a diameter of an equivalent sphere. The derived distributions were volume/mass distributions and calculated means D[4,3] were volume/mass moment means (De Brouckere mean diameter). Particle shape and size were also examined by scanning electron microscopy. Samples were sputter-coated before analysis [P1].

pH optimization of polyphenol adsorption

HMR was dissolved in pH 3, 5, 7, and 9 buffer solutions and loaded onto self-packed solid-phase extraction (SPE) cartridges filled with PVPP-RS. Equilibration, loading, washing, and elution of SPE cartridges were performed at ambient temperature. Equilibration and washing were performed with respective buffers and elution was performed with methanol. HMR concentration in the collected fractions was determined by UV absorption at a $\lambda = 280$ nm wavelength in a spectrophotometer. For calibration, HMR solutions were separately prepared for each pH in their respective buffer [P1].

PVPP-RS polyphenol capacity

A Ø 50-mm MPLC was filled with PVPP-RS after swelling in H_2O and removing fine material. The slurry was transferred into the column and the column bed was packed using an MPLC-system. To plot a breakthrough curve, column flow-through was monitored using a preparative UV detector. HMR was used as a lignan model substance and column breakthrough was monitored at $\lambda = 280$ nm. An HMR solution with neutral pH was loaded onto the column until complete breakthrough was achieved. Adsorbed HMR was eluted with methanol. Column capacity was calculated based on the area under the breakthrough curve using numeric integration [P1].

Polyphenol adsorption and recovery

Two columns of different sizes, Ø 25 mm and Ø 80 mm, were used for MDF effluent adsorption. Flow rates up to 15 ml min^{-1} for the smaller column and 60 ml min^{-1} for the larger

column were applied. An MPLC setup with two pumps, a preparative UV detector (λ = 280 nm), and a fraction collector was used for loading, elution, and monitoring adsorption. After loading the column with MDF effluent, a washing step with dilute acid was performed until UV absorption at 280 nm decreased to starting values. Gradient elution of polyphenols was performed with methanol and dilute acid. The collected polyphenol fractions were analyzed by GC/MS and high-performance liquid chromatography with diode-array detection (HPLC/DAD). After elution, columns were washed with base and acid, then recalibrated with dilute acid. To ensure polyphenols were completely retained in the column, some of the collected fractions during the adsorption process were extracted using a non-polar solvent and extracts were analyzed by GC/MS after derivatization (silylation). The collected polyphenol fractions were also characterized in the same manner by GC/MS after derivatization. For sugars and sugar alcohols, fractions were freeze-dried rather than extracted before derivatization (silylation). Substances were identified using commercial mass spectral libraries. Peak areas in total ion chromatograms were quantified as equivalents of internal standard (ISt) heneicosanoic acid for substances with a RT of <21 min, while betulin was used for substances with a RT of >21 min, including lignans. Pinosylvin monomethyl ether was additionally confirmed via HPLC RT and UV (DAD) spectrum compared with an authentic standard using a reversed-phase column [P1, P2, P3].

4.3. *P. Putida* Cultivation

Microorganism, medium preparation, growth conditions

Pseudomonas putida NCIMB 10684 (*P. putida*), was obtained from the National Collection of Industrial, Food, and Marine Bacteria (Aberdeen, Scotland, UK) and maintained in Petri dishes on Standard Nutrient Agar No. 1. This strain was selected due to its superior ability to degrade α-pinene at a relatively low optimal temperature according to public databases (Widhalm et al., 2016). *P. putida* was grown at an optimal temperature of 25°C in sterilized M9 minimal salts medium in sealed Erlenmeyer flasks (Widhalm et al., 2018) to obtain a liquid phase culture for further tests. Each flask was also supplemented with $MgSO_4$, trace elements solution, and glucose and the resulting medium was used for subsequent tests. For all experiments, 1 mL of pre-culture was inoculated into additional flasks for cultivating various media.

By measuring optical density (OD600) on a spectrophotometer, bacterial growth was determined according to Cheng et al. (2013). The number of colony-forming units (CFU) was determined per mL of suspension (CFU mL^{-1}). A serial dilution of bacterial suspension was made with 0.9% aqueous NaCl for this purpose, streaked out on agar plates, incubated at 25°C, and counted after 24–48 h [P2, P3].

P. putida growth experiments

The presence of inhibiting substances and bioavailable carbon sources [P2] other than monosaccharides in MDF effluents were examined with *P. putida* liquid cultures in original MDF effluent after removing fibers and colloids, before polyphenol adsorption, and during flow-through of the polyphenol adsorption column without adding other nutrients. The original MDF effluent or column flow-through were sterilized before inoculation, but pH remained unadjusted and varied from 5.6 to 7.0.

Growth of *P. putida* on individual carbon sources present in MDF effluent [P2]

Myo-inositol, citrate, acetate, succinate, glycerol, and xylitol were tested as individual carbon sources. $MgSO_4$ and trace element solution were added to all flasks and three out of six flasks were further supplemented with glucose. Bacterial growth was determined by measuring optical densities at a wavelength of λ = 600 nm (OD 600) using a spectrophotometer.

Optimized biomass production for availability of nitrogen sources [P3]

Carbohydrate rich MDF effluent was used in either its native form or supplemented with ammonium chloride as a nitrogen source. Additionally, MDF effluent with low amounts of available carbon sources was used in its native form or supplemented with either glucose or both glucose and ammonium chloride. Samples were taken at the beginning of cultivation and after 24 h to determine biomass (OD 600) and media composition [P2, P3].

Batch fermentation

Batch fermentation was performed in a glass bioreactor with a total volume of 6 L together with a combined carbon dioxide/molecular oxygen (CO_2/O_2) analyzer. A carbohydrate rich MDF effluent was used for batch fermentation. Airflow rate remained constant (high) until

the conditioning phase. Stirrer speed, temperature, and pH also remained constant throughout batch fermentation and conditioning phases. Partial oxygen pressure (pO2) was measured online using a sensor based on oxygen-dependent luminescence quenching. A conditioning phase with a model terpene (α-pinene) was applied after 24 h batch fermentation. During this conditioning phase, airflow rate decreased and the exhaust gas cooler cooled down to minimize α-pinene losses from evaporation. [P3]

4.4. VOC reduction within pinewood

Pinewood treatment with *P. putida* and VOC sampling

Pinewood strands were obtained from Fritz Egger GmbH (Wismar, Germany) and moisture content was unadjusted (100% ± 10% [$w_{water}/w_{dry\ wood}$]). For pinewood treatment with *P. putida*, pre-cultures grown in M9 medium or various MDF effluents were first conditioned for terpene degradation by adding small amounts of model terpene (α-pinene) and further incubated for 24 h. Conditioned bacterial cultures were then used to inoculate crushed fresh pinewood strands. Emission tests of pinewood strands under laboratory conditions were initially performed in headspace vials, then in µCTE™ microchambers. In general, crushed pinewood strands were inoculated with conditioned *P. putida* pre-culture by applying liquid cultures to strands with a 1 ml/g ratio and incubating at 25°C ± 2°C (Widhalm et al., 2018). Unsterile strands moistened with tap water served as controls. For detailed evaluation of volatile substances, further experiments were performed in microchambers. A microchamber consisted of six parallel cells each with a volume of 48 cm^3 arranged in a heating block with a constant flow of approximately 50 ml/min. Crushed pinewood strands were weighed into Erlenmeyer flasks, inoculated with bacterial solution, and incubated for defined treatment times. Strands were then heat treated overnight, cooled in a desiccator, and put into µCTE™ microchambers. Emissions were adsorbed on Tenax®Ta tubes and measured via thermodesorption (TD) connected to GC/MS [P2, P3].

Analysis of VOC emissions

Wood samples in headspace vials were analyzed for volatilized compounds by solid-phase microextraction and subsequent gas chromatography–mass spectrometry (SPME/GC–MS) (Stratev et al., 2015). Cyclodecane, dissolved in methanol, served as an ISt and added to

headspace vials immediately before analysis. VOC was adsorbed on a SPME fiber inserted through the septum into the vial at an elevated temperature. Temperature program and other separation parameters were set according to Stratev et al. (2015). Tenax^{Ta} tubes were analyzed following DIN ISO 16000-6 (2010) using a TD-unit connected to a GC/MS system [P2, P3].

5. Summary of scientific publications

In the first publication: "Selective recovery of polyphenols from MDF process waters by adsorption on a macroporous, cross-linked pyrrolidone-based resin" (**Publication 1**), a work-up procedure for MDF effluent to remove fibers and growth inhibiting substances as well as to fractionate and recover valuable substances was developed. The focus in this publication was laid on the development of a scalable, fixed-bed adsorption system for the removal and selective recovery of polyphenols (lignans and stilbenes) from MDF effluent. Before adsorption, fibers and non-colloidal particles were removed from the effluent by centrifugation and a tangential flow membrane (30-kDa cut-off) filtration was carried out to remove colloidal particles, resin and fatty acids. Polyphenols were then isothermally adsorbed on a MPLC column packed with PVPP-RS, a regenerable macroporous, cross-linked pyrrolidone-based resin. The capacity of PVPP-RS for HMR as a model lignan was determined to be 37.4 mg g^{-1} at 1% breakthrough. pH-optimization of the adsorption process, conducted on a smaller scale using self-packed SPE cartridges, showed that the fraction of HMR bound to PVPP is decreasing with increasing pH. Best results were thus obtained at the lowest tested pH (3). However, at pH 5 and 7 still more than 90% of HMR were adsorbed. Loading at slightly acidic pH and subsequent gradient elution of polyphenols with methanol were monitored at λ =280 nm, and elution conditions for selective polyphenol recovery from MDF effluent were optimized based on GC/MS analyses of the obtained fractions. Lignans were eluted in successive fractions containing the individual lignans in different proportions, followed by a pinosylvin fraction. Highly polar substances such as sugars and sugar alcohols, however, were not retained on the column and remained in the flow-through. Brown deposits accumulating at the top of the column over time were largely but not completely removed by regeneration of the column with NaOH, followed by formic acid. The majority of brown deposits was recovered in the NaOH eluate and most likely represented polymerized aromatics such as

lignin oligomers as supported by molecular weight distribution (MWD) analyses by means of alkaline high-pressure size exclusion chromatography (HPSEC). The potential of crosslinked pyrrolidone-based (PVPP) resins for the selective adsorption and recovery of valuable polyphenols, particularly lignans from MDF process effluents using a fixed bed adsorber, was successfully demonstrated.

The second publication:" An integrated process for combined microbial VOC reduction and effluent valorization in the wood processing industry" (**Publication 2**) focused on the utilization of pre-treated MDF effluent as a culture medium for *P. putida* for reduction of volatile organic compounds (VOC) in pinewood. All MDF effluents contained glucose, fructose, mannose, arabinose, and galactose in varying proportions. The most obvious differences among effluent samples were high concentrations of glucose, mannose and particularly fructose in two process effluents sampled in April and May compared to effluents sampled in August and September indicating a seasonal effect (see **Publication 3**). The inhibiting effect of polyphenols on the cultivation of *P. putida* was demonstrated with simple growth experiments in shaking flasks, conducted with two different MDF effluents, before and after adsorption of polyphenols onto PVPP as described in **Publication 1**. In a second step, we monitored the growth of *P. putida* with single carbon sources, apart from monosaccharides, that naturally occur in process effluents, particularly organic acids and sugar alcohols, either alone or in combination with glucose. *P. putida* metabolized acetate, citrate, succinate, and glycerol as sole carbon sources but not myo-inositol and xylitol. No substance was completely inhibitory for growth of *P. putida*, because a distinct increase in biomass (OD 600) occurred in all media supplemented with glucose as additional carbon source, but for some an extended lag phase was observed. *P. putida*, cultivated in MDF effluents was applied to pinewood strands for VOC reduction after a 24h adaption phase with small amounts of a model terpene (α-pinene) added to the culture. This *P. putida* culture applied to pinewood reduced emissions of α-pinene and Δ3-carene by 80% and 50%, respectively, while β-pinene and α-terpinolene were reduced by 100% within 4 days. During work-up of larger volumes of MDF effluent to remove polyphenols, a partial breakthrough of UV absorbing substances (λ=280nm) was observed, but no polyphenols (i.e., lignans and pinosylvin) were detected as determined by GC/MS. Potential candidates could be glycosylated polyphenols or low molecular weight (MW) lignin- carbohydrate complexes. The latter assumption is supported by comparison of UV spectra and MW distributions (HPSEC)

of original effluent and different fractions obtained during the adsorption process. Changes in the chemical composition of the flow-through over time may affect the VOC reducing potential of *P. putida* on pinewood. Therefore, the flow-through of two independently processed effluents was fractionated and the first three fractions were used for pre-cultivation of *P. putida*, followed by application of cultures onto pinewood strands. A distinct reduction in TVOC emissions was always achieved irrespective of fraction, but fraction 1 from both effluents provided the best conditions for pre-cultivation and subsequent VOC degradation. It was shown, that MDF effluent after removal of inhibiting substances, as presented in **Publication 1**, is suitable for cultivation of *P. putida* and subsequent reduction of aldehydes and terpenes in pinewood.

In the third publication: " Potential of a year-round, closed-loop process for volatile organic compounds reduction in pinewood strands by *Pseudomonas putida* PX1 cultivated in seasonally varying process effluents" (**Publication 3**)the focus was laid on the analytical assessment of the seasonal variability of MDF effluent and its impact on the cultivation of *P. putida* and subsequent VOC reduction in pinewood. Furthermore, fermentation characteristics were determined and optimized for industrial application. Parameters important for cultivating microorganisms such as carbohydrates, organic acids, anions, and cations were monitored monthly for 4 years to determine the crucial factors for establishing a robust year-round continuous industrial process. Sugar concentrations and, to a lesser extent, the concentration of sugar alcohols underlie great seasonal variations with high quantities during the colder months of the year and low quantities during the warmer months. However, another reason for the fluctuations is the varying proportion of stuffing screw effluent (SSE) in the total process effluent, because, in the current process, SSE originating from the wood chips is diluted with the condensate from a steam generator cycle. The proportion of SSE in MDF effluent is generally higher during the colder months (50%–90%) than during the warmer months (10%–50%) of the year. This may be explained by higher amounts of rainfall, and as a consequence, moister wood logs during these months. To assess the relationships between relevant variables, scatterplots, histograms and correlation coefficients (Spearman's rank) were compared. A significant correlation between sugars and mean temperature (seasonal effect) was found when conducting partial correlations controlled for the dilution (proportion of SSE in MDF effluent) confirming the seasonal variability with low sugar concentrations in summer and fall and high concentrations in winter

and spring. For optimal growth of *P. putida* on the fermentable carbon sources available in MDF effluent, further essential nutrients such as nitrogen and minerals must be present in sufficient quantities. Thus, a theoretical nitrogen demand was calculated based on literature data for *P. Putida* KT2440. According to this demand, nitrogen sources present in MDF effluent required for optimal biomass production are insufficient when abundant carbon sources are available and should be supplemented. In shake flask experiments *P. putida* was grown in two different MDF effluent batches, representing MDF effluent with high and low nutrient (carbon source) content, respectively, either in their original form or supplemented with an additional carbon or nitrogen source. Nitrogen supplementation proved crucial for optimal biomass production. Interestingly, while almost no biomass was produced under nitrogen-limitation, still all carbon sources were depleted, eventually transformed to carbon storage polymers. In a batch bioreactor with controlled pH, stirring, aeration and off-gas analytics, growth kinetics of *P. Putida* cultivated in MDF effluent were monitored. Glucose and succinate were metabolized at high rates from the start, whereas most of the other substrates showed a lag phase from 2 to 4 h. Upon depletion of organic acids and all sugars, except fructose, a sharp decline of both oxygen uptake and off-gas CO2 occurred. A metabolic switch to fructose was apparent at approximately 4.5 h when oxygen uptake resumed. Ammonium was the preferred nitrogen source but also nitrate was used after depletion of the easier source. The conditioning step with α-pinene was successfully reduced in this setting to 2 hours compared to 24 hours before (see publication 2). This time reduction could be attributed to superior mixing and aeration in the bioreactor compared to shaking flask cultures. Additionally, treatment time of pinewood strands was drastically reduced to 3 h, to fulfill requirements for industrial application, while still reducing total VOC emissions by more than 55% and most aldehydes and terpenes by 67-100%. The time saved for conditioning, and particularly for the treatment of pinewood strands, is crucial for industrial applications and shows the potential of microbial VOC reduction and MDF effluent recycling.

6. Conclusions

The potential of a fixed-bed adsorber with a crosslinked pyrrolidone-based (PVPP) resin to selectively adsorb and recover polyphenols, particularly lignans and stilbenes from MDF effluents was demonstrated. Polyphenols often inhibit microorganisms, and although their removal would increase the efficiency of a downstream biological clarification plant, a reuse scenario of the carbohydrate-rich stream after adsorption seems more promising. To cultivate *P. putida* in MDF effluent for subsequent VOC reduction in pinewood, the removal of polyphenols was found necessary. Additional carbon sources and minerals were not required to produce substantial amounts of biomass. Apart from sugars, several other carbon sources in MDF effluents are metabolized as a single carbon source as well as in combination with glucose.

Further, application of *P. putida* biomass obtained from pre-cultivation in MDF effluent efficiently reduced VOC emissions from pinewood. To deal with seasonal variations of MDF effluents, results of 4 years monitoring suggest that online or at-line measurements of major carbon and nitrogen sources in the MDF effluent feed and nutrient supplementation are necessary to ensure a robust process for optimized biomass production. Cheap carbon sources, for instance, mixed glucose and fructose molasses, may be used because *P. putida* can metabolize various carbon sources. In future research, even a continuous fermentation process may be envisioned that can be paced according to the needs of the production line of, for example, OSB. A key challenge in this context, however, will be the implementation of the adaptation phase with either a subsequent adaption step or continuous dosage of α-pinene. The economic feasibility of the process is strongly dependent on the price of the value-added product, in this case, the VOC-reduced OSB, and on international regulatory limits (EU-LCI). Furthermore, VOC-reduced OSB has great potential for the Asian market, as Asian customers are not accustomed to odorous terpenes emitting from European softwood species.

7. References

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8. Scientific publications

Publication 1

Lindemann, M., Rieder-Gradinger, C., Kuncinger, T., Srebotnik, E., 2020a. Selective recovery of polyphenols from MDF process waters by adsorption on a macroporous, cross-linked pyrrolidone-based resin. Holzforschung 74, 217–225.

Contribution Martin Lindemann:

Conception of the study, literature research, materials and methods, experimental procedure, analysis, data interpretation, and writing and editing of manuscript.

Publication 2

Lindemann, M., Widhalm, B., Kuncinger, T., Srebotnik, E., 2020b. An integrated process for combined microbial VOC reduction and effluent valorization in the wood processing industry. Bioresour. Technol. Rep. 11, 100471.

Contribution Martin Lindemann:

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Publication 3

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Selective recovery of polyphenols from MDF process waters by adsorption on a macroporous, cross-linked pyrrolidone-based resin

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An integrated process for combined microbial VOC reduction and effluent valorization in the wood processing industry

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ABSTRACT

This study focused on the utilization of medium-density fiberboard (MDF) effluent as a culture medium for *Pseudomonas putida* for reduction of volatile organic compounds (VOC) in pinewood. *P. putida* was successfully cultivated in the residual carbohydrate-rich effluent after purification by centrifugation, 30 kDa membrane filtration and recovery of bioactive compounds via adsorption onto polyvinyl polypyrrolidone (PVPP). Besides monosaccharides, *P. putida* can metabolize glycerol, acetate, succinate, and citrate present in MDF effluent without addition of other nutrients. *P. putida* cultures applied to pinewood reduced emissions of α-pinene and Δ3-carene by 80% and 50%, respectively, and β-pinene and α-terpinolene were reduced by 100% within 4 days. The valorization of industrial process effluents for VOC reduction could assist the wood processing industry in sustainably lowering VOC emissions of their products.

1. Introduction

Wood processing industries must handle large amounts of process effluent, especially effluent from pretreatment of woody biomass. During the production of medium density fiberboards (MDF), a large effluent stream is generated by squeezing water out of steam pretreated softwood chips. This water is rich in total organic carbon (TOC) and contains various carbohydrates, polyphenols, and organic acids. The latter include colloidal fatty and resin acids stabilized by hemicelluloses and various cations (Otero et al., 2000). Effluent treatment is complex and costly, requiring multiple steps such as sedimentation, flocculation, biological treatment, membrane filtration and reverse osmosis. Utilization of currently unused process effluent stream would be beneficial via reducing carbon load and thus decreasing oxygen demand during biological treatment. In our previous work (Lindemann et al., 2020), we presented a method for recovery of valuable polyphenols (lignans and stilbenes) from MDF effluent by adsorption onto a commercial polyvinyl polypyrrolidone (PVPP) resin. However, after adsorptive removal of polyphenols, a residual complex matrix of carbohydrates, small organic acids, and salts is left that would still need treatment before disposal or reuse.

Traditionally, the main goal when handling wastewaters from pulping and biomass processing industries was solely to decrease chemical/biological oxygen demand (COD/BOD) and eliminate toxic substances at minimum cost. A key aspect of improving overall sustainability for the industry is direct reuse of the effluent (Ashrafi et al., 2015). Current literature now focuses more on utilizing effluent streams, for instance, by fermentation. Specific microorganisms can use multiple carbon sources to produce a desired product, while simultaneously reducing COD/BOD. For example, acidogenic fermentation of pulp mill effluents can be the initial step for production of polyhydroxyalkanoates (PHA) by producing volatile fatty acids (VFA) (Bengtsson et al., 2008). Queirós et al. (2014) showed that VFA produced by acidogenic fermentation from hardwood spent sulfite liquor is a substrate for PHA production by a mixed microbial culture under aerobic conditions. Kaushik and Jadhav (2017) published an interesting approach, utilizing pulping industry wastewater to produce electricity in a microbial fuel cell, with *Pseudomonas fluorescens* being the main energy producing bacterium.

A vast number of *Pseudomonas* species are ubiquitous in soil and water and can adapt to challenging environments, including adverse conditions, such as high and low temperatures and poor nutrient availability. Further, pseudomonads are known for their ability to metabolize a wide range of substrates, such as hydrocarbons, aromatic compounds and terpenes (Mirpuri et al., 1997, Poblete Castro et al., 2012, Yoo et al., 2001). For example, Bicas et al. (2008) described the

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biotransformation of monoterpenes in two *Pseudomonas* species, and Widhalm et al. (2016) showed that an adapted mixed culture of *Pseudomonas* metabolized aldehydes and terpenes in pinewood. Greatly reduced emissions of these compounds were observed after only 3 days. α Pinene, the major terpene in pinewood (Kleinheinz et al., 1999), is classified as an irritant and hazardous to the environment according to the Globally Harmonized System of Classification, Labeling and Packaging of Chemicals (GHS/LPC). Large amounts of wood products made from pinewood in buildings may thus negatively affect indoor air quality due to elevated concentrations of aldehydes and terpenes. Chronic exposure to these VOC may be a cause of "sick building syndrome" (Makowski and Ohlmeyer, 2006; Wilke et al., 2013). To avoid a negative impact on indoor air quality and consequently on human health, various strategies to lower total VOC emission levels have been pursued. Manninen et al. (2002) and McGraw et al. (1999) found that terpene emissions from pinewood are less after heat treatment than after air drying. Chemical degradation of terpenes from pinewood with sodium carbonate, sodium sulfite, sodium hydroxide and mixtures of these substances has also been investigated by Roffael et al. (2015). Furthermore, biodegradation and biotransformation with different fungi in liquid culture (Lee et al., 2015; Qi et al., 2002) and reduction of VOC from waste gases with biofilters containing fungi (Van Groenestijn and Liu, 2002) has been reported. Recently, Widhalm et al. (2018) showed that pretreatment of pine wood strands with a mixed culture of *Pseudomonas* and the ascomycete *Penicillium nigricans* resulted in a significant decrease of α pinene, β pinene and Δ3 carene emissions from oriented strand boards (OSB).

In this study, we demonstrate the suitability of MDF process effluent originating from steam pretreatment of wood chips as a medium to cultivate *P. putida*, after recovering resin, fatty acids, and polyphenols. Cultures of *P. putida* can reduce aldehydes and terpenes in pinewood strands. No additional carbon source or other nutrients are required. Reusing MDF effluent for the cultivation of VOC reducing bacteria would allow the manufacture of wood products with reduced emissions from treated pine wood strands sustainably and at low cost.

2. Material and methods

2.1. Origin of MDF process effluents and general preparation procedures

Process effluents were obtained from a MDF plant in Wismar, Germany (Egger Holzwerkstoffe Wismar GmbH & Co. KG) between April and September 2017 (PE A: April 2017; PE B: May 2017; PE C: August 2017; PE D: September 2017). TOC values of the sampled effluents varied between 1 and 2 g/L (data not shown). Briefly, the MDF process involves steaming of wood chips at about 120 °C at elevated pressure, followed by squeezing water out of the steamed wood chips using a stuffing screw. The material is then fed into a defibrator where it is mechanically broken down into fibers, while the water is discharged as process effluent exhibiting TOC values between 1 and 2 g/L (data not shown). Each effluent sample was sampled into a sterile container at around 80 °C directly behind the stuffing screw. The container was immediately sealed to avoid contamination. Spruce and pine are the primary woods used for MDF production at the Wismar plant, but small percentages of fir and beech are also used. The amount of softwood comprises > 90% of the wood mix, although a precise composition cannot be assigned because only rough daily averages are available.

The work up procedure for the process effluent involved several steps. Briefly, fibers and non colloidal particles were removed from MDF process effluent by centrifugation. Tangential flow membrane filtration using a 30 kDa cut off membrane cassette was then performed to remove colloidal particles, resins, and fatty acids. Adsorption and removal of polyphenols was performed using a medium pressure liquid chromatography (MPLC) column (25 mm diameter) filled with PVPP RS (5.8 g). The flow through of the column was collected and loading stopped before polyphenol breakthrough. Polyphenols were then recovered by elution with methanol. Full details on preparation and the development of the adsorption system can be found in Lindemann et al. (2020).

2.2. Chemicals

Chemicals for medium preparation, monoterpenes and toluene D8 standard and sugar/sugar alcohol standards, (D (+) glucose, D (+) xylose, D (+) arabinose, D (+) galactose, D (+) mannose, D (−) fructose, D (+) pinitol), trimethylchlorosilane (TMCS, ≥99.0%), heneicosanoic acid (> 99%) and betulin (> 98%) were obtained from Sigma Aldrich (St. Louis, MO, USA). Anion multi element standards (Certipur® Anionen Multielement Standardlösung I (F^-, PO_4^{3-}, Br^-) and II (Cl^-, NO_3^-, SO_4^{2-})) and cation multi element standard (Certipur® Kationen Multielement Standardlösung VI) (NH_4^+, K^+, Na^+, Ca^{2+}, Mg^{2+}) were purchased from Merck KGaA (Darmstadt, Germany). Organic acid standards (acetate, propionate, formate, succinate, and citrate) were purchased from Carl Roth GmbH & Co.KG (Karlsruhe, Germany). PVPP RS (Divergan® RS, cross linked poly 1 (2 oxo 1pyrrolidinyl) ethylene for use in regenerable processes, average particle size 80 100 μm) was kindly provided by BASF SE (Ludwigshafen, Germany). Milli Q Water was prepared according to DIN ISO 3696 (1991). All other chemicals used were of analytical grade.

2.3. Microorganism, medium preparation, and growth conditions

Pseudomonas putida NCIMB 10684 was obtained from the National Collection of Industrial, Food and Marine Bacteria (Aberdeen, Scotland, UK) and maintained in Petri dishes on Standard Nutrient Agar No.1 (Carl Roth GmbH, Karlsruhe, Germany). This strain was selected due to its superior ability to degrade α pinene at a relatively low temperature optimum as revealed by a search of public databases (Widhalm et al., 2016).

P. putida was grown at an optimal temperature of 25 °C in 50 mL of sterilized M9 minimal salts medium in sealed 250 mL Erlenmeyer flasks on a rotary shaker at 200 rpm for 72 h (Widhalm et al., 2018) to obtain a liquid phase culture for further tests. One liter of M9 contained 6 g Na_2HPO_4, 3 g KH_2PO_4, 0.5 g NaCl, and 1 g NH_4Cl. Each flask was also supplemented with 0.1 mL of 1 M aqueous $MgSO_4$, 0.125 mL trace elements solution, and 0.25 mL of a 20% (w/v) glucose solution. This medium was used for several subsequent tests. For the trace element solution, 2.7 g $FeCl_3$, 0.2 g $ZnCl_2$, 0.2 g $CoCl_2$, 0.2 g Na_2MoO_4, 0.1 g $CaCl_2$, 0.13 g $CuCl_2$, 0,05 g H_3BO_3 and 10 mL (37%) HCl were diluted with sterile water to 100 mL. For all experiments, 1 mL portions of this pre culture were inoculated into additional flasks for cultivation in various media.

2.4. Growth experiments of P. putida in liquid culture

The presence of inhibiting substances and bioavailable carbon sources other than monosaccharides in MDF process effluents were examined with *P. putida* liquid cultures in original effluent after removal of fibers and colloids and before adsorption of polyphenols, and in the flow through of the polyphenol adsorption column without the addition of other nutrients. Original effluent or column flow through were sterilized before inoculation but pH was not adjusted and varied from 5.6 to 7.0. Subsequently, *myo* inositol, citrate, acetate, succinate, glycerol, and xylitol were tested as individual carbon sources at final concentrations of 0.01 M in M9 medium. For each carbon source, six 100 mL sterilized Erlenmeyer flasks containing 40 mL medium were prepared, followed by the addition of 0.1 mL $MgSO_4$ (1 M) and 0.125 μl trace elements solution. Three of the six flasks were further supplemented with 250 μl of a 20% (w/v) glucose solution. Each flask was inoculated with 2 mL of M9 grown *P. putida* pre culture, capped with a

cotton plug and incubated at 200 rpm and 25 °C.

Bacterial growth was determined by measuring optical densities of bacterial cultures at a wavelength of 600 nm (OD 600) on a Shimadzu UV 1800 spectrophotometer (Shimadzu Corp., Kyoto, Japan) (Cheng et al., 2013). When effluents exhibited variable background absorption at 600 nm, the OD 600 value immediately after inoculation was subtracted from OD 600 values at later time points. Data thus represent newly formed biomass.

2.5. Pinewood treatment with P. putida and VOC sampling

Pinewood strands with approx. dimensions 2.5 cm × 12 cm length and 0.5 cm thickness were obtained from Fritz Egger GmbH (Wismar, Germany). Moisture content was 100% ± 10% ($w_{water}/w_{dry\ wood}$) and was determined for 10 random samples with a Sartorius MA 150 moisture analyzer (Sartorius AG, Göttingen, Germany).

For pinewood treatment with *P. putida*, pre cultures grown for 72 h in M9 medium or various effluents were first conditioned for terpene degradation by adding 0.05 mL α pinene and further incubated for 24 h. Conditioned bacterial cultures were then used to inoculate crushed fresh pinewood strands.

Emission tests of pinewood strands under laboratory conditions were initially performed in headspace vials and later on also in µCTE™ micro chambers/thermal extractors (Markes International Ltd., Llantrisant, UK). Specifically, 2 g of crushed pinewood strands were inoculated with 2 mL conditioned *P. putida* pre culture and incubated in 20 mL headspace vials (Thermo Scientific, Vienna, Austria) at 25 °C ± 2 °C (Widhalm et al., 2018). Unsterile strands moistened with 2 mL of tap water served as controls. For a detailed evaluation of volatile substances, further experiments were performed in µCTE™ micro chambers. A micro chamber consists of six parallel cells with a volume of 48 cm^3 each, arranged in a heating block. Two grams of crushed pinewood strands were weighed into Erlenmeyer flasks, inoculated with 2 mL of bacterial solution, and incubated for defined treatment times. Thereafter, strands were heat treated at 103 °C ± 2 °C overnight, cooled in a desiccator and then put into micro chambers. After a conditioning phase of 1 h at 23 °C, VOC were sampled on Markes C1 AAXX 5003 Tenax TA tubes (Markes International Ltd., Llantrisant, UK). Sampling time was 30 min at an airflow rate of 50 mL/min. A one µl toluene D8 standard was added to each tube before sampling.

2.6. Analysis of VOC emissions with GC MS

Wood samples in headspace vials were analyzed for volatilized compounds by solid phase microextraction and subsequent gas chromatography mass spectrometry (SPME/GC MS) (Stratev et al., 2015). A solution of cyclodecane dissolved in methanol (1:100) served as an internal standard. Five µl of this solution was added through the septum to each headspace vial immediately before measurement. VOC were adsorbed on a Supelco divinylbenzene/carboxene/polydimethylsiloxane SPME fiber inserted through the septum into the vial for 25 min at 50 °C. Fiber desorption was performed on an Agilent 7890A/5975C GC MS system with a CTC Combi PAL autosampler and splitless injection of the analytes into an Agilent 19091S 433 column with dimensions 30 m × 0.25 mm × 0.25 µm. Temperature program and other separation parameters were set according to Stratev et al. (2015).

Tenax tubes were analyzed following DIN ISO 16000 6 (2010) using a TD 100 Thermodesorption Unit (Markes), connected to an Agilent 7890A/5975C GC MS system. Analytes were separated on a HP PONA column (Agilent) with the dimensions 50 m × 0.2 mm × 0.5 µm. The following temperature program was used: 3 min at 35 °C, 35 °C → 160 °C at 10 °C min^{-1}, 160 °C → 260 °C at 20 °C min^{-1}, hold time at 260 °C was 10 min.

2.7. Analysis of carbohydrates in process effluent with HPAEC/PAD

Sugars and sugar alcohols in different process effluent samples were determined by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC PAD) on a Dionex ICS 5000+ system (Thermo Scientific) with Dionex CarboPac PA1 guard and analytical (2 × 250 mm) columns (Thermo Scientific) at 0.26 mL/min as follows. Preconditioning: 200 mM NaOH/65 mM sodium acetate for 10 min, followed by water for 7 min. Separation: Isocratic elution in water with post column addition of 67 mM NaOH for 18 min. Injection volume: 10 µl. Sugars were quantified by calibration of peak areas with authentic standards (D arabinose, D galactose, D glucose, D mannose, D xylose, D fructose) run along with samples in each sequence.

Sugar alcohols were not adequately separated with this method. A sum parameter was therefore defined by quantifying sugar alcohols as pinitol (the predominant sugar alcohol identified in process effluents) equivalents of total peak area eluting between 1.5 min and 2.2 min. This fraction represents mainly sugar alcohols as confirmed by GC MS as an orthogonal method. Column eluates from 1.5 min to 2.2 min of 5 consecutive injections were pooled, freeze dried, derivatized, and analyzed by GC MS according to Lindemann et al. (2020). The sugar alcohols were identified by mass spectra using Wiley and NIST mass spectral libraries and quantified as percentages of total peak area.

2.8. Analysis of anions, cations, and organic acids in process effluent with IC

Inorganic ions and organic acids in process effluent samples were determined by ion chromatography (IC) on a Dionex ICS 5000+ system (Thermo Scientific) equipped with conductivity detectors and an autosampler (Dionex AS DV) for simultaneous injection onto both anion and cation columns via two 25 µl sample loops.

Inorganic anions and organic acids were separated on a Dionex IonPac AS11 HC analytical column (4 × 250 mm) with a Dionex ATV HC trap pre column (9 × 75 mm) at 1.5 mL/min as follows. Gradient elution: 1.5 mM NaOH from 0 to 5 min, 1.5 mM 5 mM NaOH from 5 to10 min, 5 mM 23 mM NaOH from 10 to 18 min, 23 mM 50 mM NaOH from 18 to 20 min, 50 mM from 20 to 25 min, 50 mM 1.5 mM from 25 to 27 min. Suppressor: Dionex AERS 500 (4 mm); suppressor current 19 mA from 0 to 5 min, 83 mA from 5 to 18 min, 186 mA from 18 to 27 min.

Cations were separated on a Dionex CS12A analytical column (4 × 250 mm) with a CG12A guard column (4 × 50 mm) at 1 mL/min. Separation: Isocratic elution in 20 mM methanesulfonic acid for 28 min. Suppressor: Dionex CERS 500 (4 mm); Suppressor current 59 mA.

All ions were quantified by calibration of peak areas with authentic standards of chloride, nitrate, sulfate, phosphate; acetate, propionate, formate, succinate, citrate; sodium, ammonium, potassium, magnesium, calcium.

3. Results and discussion

3.1. Process effluent composition

The chemical composition of the soluble fraction of four process effluent samples PE A, PE B, PE C, and PE D, collected over 6 months and after removing fibers, resin acids, fatty acids, and colloidal particles (Lindemann et al., 2020), was characterized by determining the presence of essential nutrients for the growth of *P. putida*, particularly inorganic anions and cations, monosaccharides, sugars alcohols, and organic acids (Fig. 1). All samples contained glucose, fructose, mannose, arabinose, and galactose in varying proportions. The most obvious differences among effluent samples were high concentrations of glucose, mannose and particularly fructose in PE A and PE B, compared to PE C and PE D. Data obtained from 26 effluent samples collected regularly over 3 years indicated seasonal variations with elevated

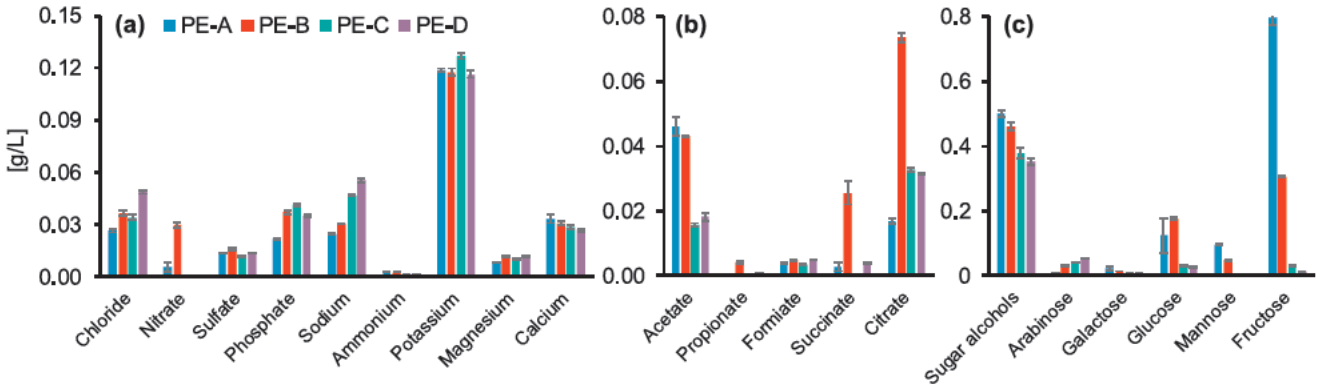

Fig. 1. Inorganic ions (a), organic acids (b), and sugars/sugar alcohols (c) identified in PE-A, -B, -C and -D.

monosaccharide and sugar alcohol levels during winter and spring (November May), and reduced levels during summer and autumn (June October). Hou (1985) observed similar seasonal fluctuations of sugars in spruce trunk wood. PE A and PE B were collected in April and May, while samples PE C and PE D were collected in August and Sep tember. Thus, and thus the observation likely reflects seasonal varia tion.

As another possible carbon source for *P. putida* relatively large amounts of sugar alcohols were found in all samples. Sugar alcohols are shown in Fig. 1 as a sum parameter because they eluted as a group of unresolved peaks by the routine HPAEC method employed (data not shown). However, GC MS analysis of the HPAEC eluate comprising these peaks confirmed that 75.6% of the peaks present in the total ion chromatogram were sugar alcohols, particularly D pinitol (34.8%), glycerol (19.7%), D arabitol (8.1%), D mannitol (5.1%), myo inositol (4.2%), isopinitol (1.7%), inositol (0.8%), D threitol (0.8%), and 2 deoxyribitol (0.4%).

Inorganic anions and cations that are required for microbial growth, particularly sodium, ammonium, magnesium, calcium, and phosphate, were present in all process effluent samples. Concentrations were si milar in all samples, except for nitrate. Nitrate was only found in PE B and, at lower concentrations, also PE A, but not in PE C and PE D. Acetate and citrate were major organic acids found in all process ef fluent samples. PE A and PE B contained twice the amount of acetate as compared to PE C and PE D. Strikingly high concentrations of citrate and particularly succinate were detected in PE B. Succinate was absent in PE C and present only in minor concentrations in PE A and PE D. Data collectively suggest that utilization of process effluents as a nu trient source for *P. putida* may require effluent composition monitoring to support proper adjustment of cultivation conditions to ensure con sistent results.

3.2. Cultivation of *P. putida* in process effluents and synthetic media

Hourly measurements of OD600 were used as an indicator of bio mass during growth of *P. putida* in two carbohydrate rich process ef fluents PE A and PE B (Fig. 2). Data demonstrate for both effluent samples that polyphenols strongly inhibited the growth of *P. putida*. Removal of polyphenols by adsorption onto PVPP abolished inhibition resulting in growth rates similar to those obtained with synthetic media. Adsorption onto PVPP was employed for preparing all process effluents utilized for subsequent *P. putida* fermentation.

In a second step, we monitored the growth of *P. putida* with single carbon sources, apart from monosaccharides, that naturally occur in process effluents, particularly organic acids and sugar alcohols, either alone (Fig. 3a) or in combination with glucose (Fig. 3b).

P. putida metabolized acetate, citrate, succinate, and glycerol as sole carbon sources. However, no growth was observed with myo inositol and xylitol. Glycerol and acetate both exhibited lower OD 600 values

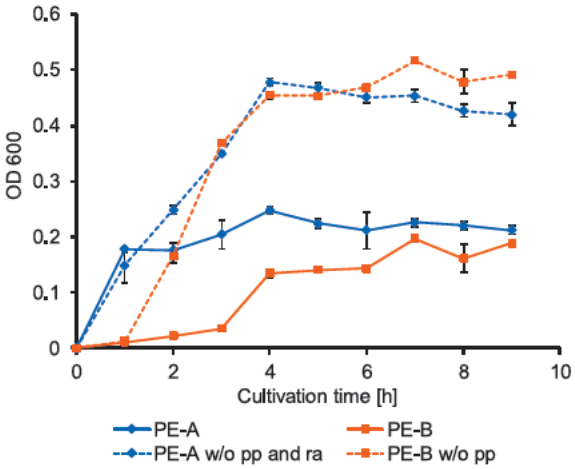

Fig. 2. Growth curves of *P. putida* in shake flasks in two different process ef fluent samples PE-A and PE-B with and without (w/o) polyphenols (pp).

after 10 h of incubation compared to citrate, succinate, and the control (glucose). For acetate, its lower C/mol ratio may explain slower growth since no further increase in OD 600 occurred after 24 h. For glycerol, we observed an extended lag phase and slower growth rate during the first 8 h that could be due to time needed for elaborate upregulation of glycerol catabolic genes and downregulation of other possible routes for carbon consumption (Nikel et al., 2014). These results are in good agreement with Hintermayer and Weuster Botz (2017) who in vestigated batch growth with organic acids and glycerol as single carbon sources. No investigated substance was completely inhibitory for growth of *P. putida*, because a distinct increase in OD 600 occurred in all media supplemented with glucose after 24 h. However, the pre sence of several substances such as acetate and particularly xylitol ex tended the lag phase and thus the onset of bacterial growth in liquid culture. In contrast, mixtures of glucose with succinate or citrate ap peared to accelerate microbial growth in comparison to glucose as single carbon source, which indicates simultaneous consumption of both carbon sources. These results provide an initial basis for predicting the impact of carbon source variability and complexity in process ef fluents on the growth rate of *P. putida* and allow compensation for nutrient deficiencies or growth inhibition by supplementing with sti mulating nutrients or extending fermentation time.

3.3. Effect of pre cultivation on subsequent terpene degradation in pinewood by *P. putida*

P. putida was pre cultivated in M9 medium under standard condi tions as previously described (Widhalm et al., 2017), as well as in un treated (PE B crude) and purified (PE B) process effluent where

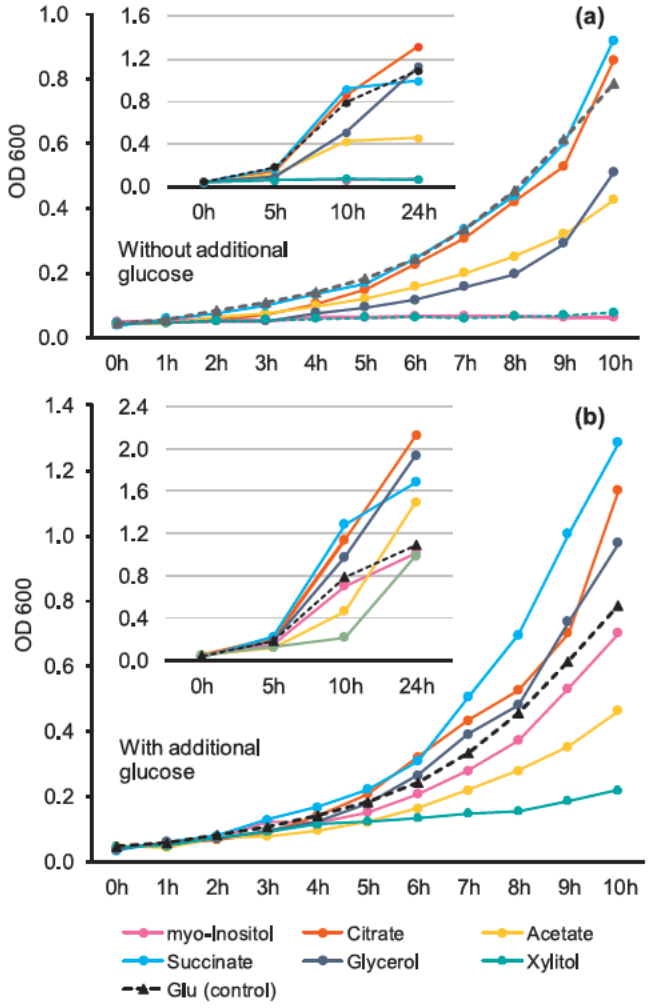

Fig. 3. Growth curves of *P. putida* in shake flasks in M9 with various carbon sources, with (a) and w/o (b) glucose as additional carbon source. (SD of 3 replicates for each data point is provided in the supplementary materials.)

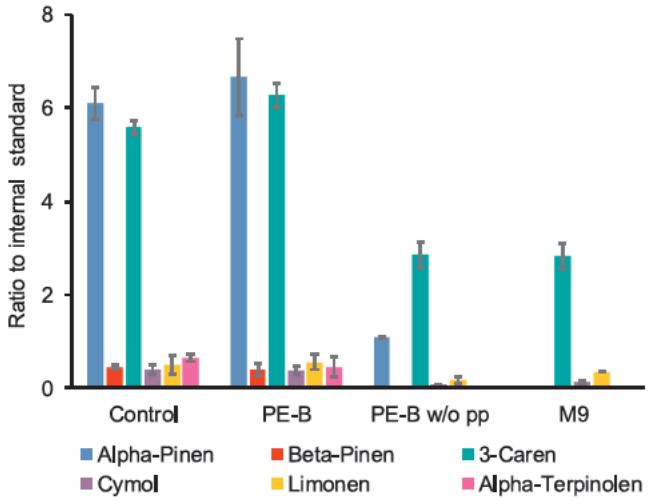

Fig. 4. VOC emissions of pinewood strands treated with *P. putida* pre-cultivated in process effluent PE-B with and without (w/o) polyphenols (pp) compared to M9 and an uninoculated control.

polyphenols have been removed. Pre cultures were applied onto pine wood strands to judge the effects of pre cultivation on terpene de gradation. After 4 days of incubation, degradation was detectable by SPME/GC MS as reduced emissions of major terpenes (Fig. 4). As ex pected from the cultivation study described in Section 3.2, process ef fluent without prior removal of polyphenols (PE B crude) did not show any reduction of terpenes compared to the uninoculated control. The pre cultivation of *P. putida* in purified effluent lacking polyphenols (PE B), however, was successful and resulted in a drastic reduction of ter pene emission comparable to that observed with M9 medium alone. Specifically, α pinene, Δ3 carene, p cymene, and limonene emissions were reduced by 80%, 50%, 80%, and 65%, respectively, while β pinene and α terpinolene were completely absent after treatment bac teria were pre cultivated in purified PE B effluent. The incomplete (80%) reduction of α pinene emission was the only obvious difference compared to standard pre cultivation in synthetic M9 medium. Al though pre cultivation in M9 medium and process effluent PE B both included a conditioning step with α pinene before application onto pinewood, the plurality of other available carbon sources in PE B may have hindered the full adaption of the terpene degrading enzyme system of *P. putida*. Thus, simultaneous metabolism of terpenes and residual carbon sources in PE B may have retarded α pinene degrada tion, whereas *P. putida* pre cultivated in M9 was probably more efficient due to the absence of other carbon sources during the adaption phase as shown by Widhalm et al. (2017).

3.4. Fractionation of process effluents by adsorption onto PVPP

Adsorptive removal of polyphenols (mainly lignans and stilbenes) has proved essential for successful utilization of process effluents as growth media for *P. putida*. Therefore, the adsorption process was stu died in more detail using a fixed bed column packed with PVPP. Highly polar substances present in process effluents, particularly carbohy drates, sugar alcohols, salts, and organic acids were not retained by PVPP and were thus fully recovered in the flow through as previously shown (Lindemann et al., 2020). However, monitoring the flow through at 280 nm during continuous loading with process effluent also revealed a partial breakthrough of UV absorbing substances as shown in Fig. 5a for the loading cycle of effluent batch PE C. Although UV ab sorption increased over time, no polyphenols (i.e., lignans and pino sylvin) were detected in the flow through as determined by GC MS (data not shown). Polyphenols strongly bound to PVPP and were fully recovered at the end of the loading cycle by elution with a small volume of methanol followed by a washing step with NaOH (Lindemann et al., 2020). Thus, the UV increase during loading may be ascribed to highly polar substances that would not or only weakly bind to PVPP and cannot properly be identified with GC MS (data not shown) due to their irregular structures. Potential candidates are glycosylated polyphenols or low molecular weight (MW) lignin carbohydrate complexes (LCC; Tarasov et al., 2018). The latter assumption is supported by comparison of UV spectra (Fig. 5b) and MW distributions (Fig. 5c) of original ef fluent, column eluate, methanol eluate, and NaOH wash. UV spectra of crude effluent and methanol eluate both showed a distinct maximum at 280 nm consistent with authentic polyphenol (7 Hydroxymatairesinol) standard, while distinct maxima were virtually absent. MW distribution analysis (Fig. 5b) of the methanol eluate revealed two distinct peaks at 443 Da and 743 Da. The major peak *co* eluted with the polyphenol (7 hydroxymatairesinol) standard. In contrast, crude effluent, flow through fractions, and particularly NaOH wash showed a polydisperse (PD > 1.4) MW distribution comprising a large share of higher MW material > 1.000 Da. Judged by its strong binding to PVPP, higher MW material in the NaOH wash may represent relatively non polar lignin oligomers rather than polar LCC. To determine the effect of these un defined UV absorbing substances and other unidentified substances possibly present in the flow through, *P. putida* was pre cultivated in consecutive fractions of the column flow through, followed by appli cation onto pinewood strands for comparison of VOC reduction.

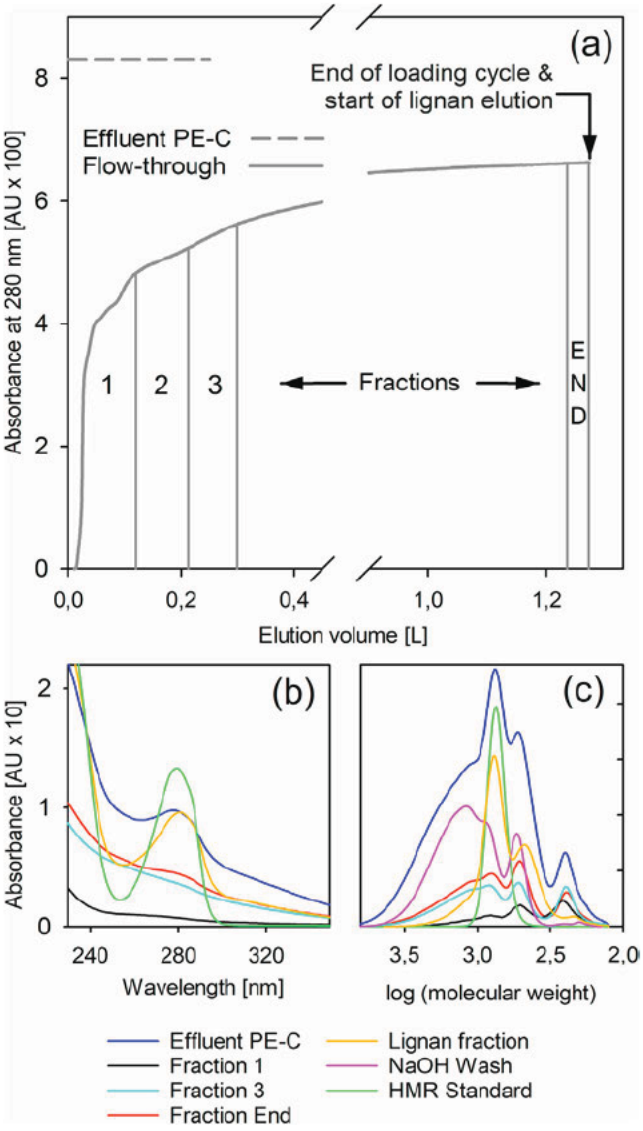

Fig. 5. UV absorption of the flow-through during a loading cycle of MDF process effluent PE-C on an MPLC column packed with PVPP (a). Absorbance spectra (b) molecular weight distributions (c) of effluent and various effluent fractions.

3.5. Effect of pre cultivation in consecutive process effluent flow through fractions on VOC reduction

Changes in the chemical composition of the flow through overtime during adsorption of polyphenols onto PVPP may affect VOC reducing potential of *P. putida* on pinewood. Therefore, the flow through of two independently processed effluents, PE C and PE D, was fractionated and the first three fractions were used for pre cultivation of *P. putida*, fol lowed by application of cultures onto pinewood strands (Fig. 5 for PE C). After two days of incubation, VOC emissions from the strands were measured under controlled conditions in test chambers by collecting released VOC onto sorbent tubes followed by GC MS. The complete GC MS dataset of individual VOC showing extensive reduction for all detected volatile aldehydes and terpenes, including the most re calcitrant, Δ3 carene (Widhalm et al., 2018), is provided as supple mentary material. In Fig. 6, all these emissions from pinewood strands, treated with *P. putida* conditioned to single eluate fractions, are sum marized and represented as total VOC (TVOC). A distinct reduction in TVOC was always achieved irrespective of fraction, but fraction 1 from both effluents provided the best conditions for pre cultivation and

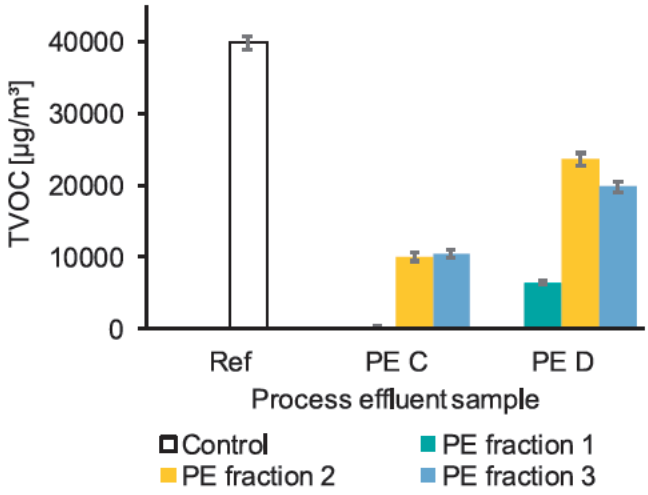

Fig. 6. TVOC emissions of pine wood strands treated with *P. putida* pre-cultivated in three consecutive flow-through fractions obtained during adsorption of polyphenols onto PVPP. Two different process effluent samples, PE-C and PE-D, are compared with an uninoculated control.

subsequent VOC degradation. Reduced performance of fraction 2 that did not further deteriorate with fraction 3, indicates growth inhibition by weakly binding substances breaking through the column. If phenolic lignin oligomers or LCC are indeed involved in the observed inhibition, their removal or inactivation might further enhance the performance of process effluent for pre cultivation of *P. putida*. One technical possibility would be pretreatment of process effluent with lignin polymerizing enzymes such as fungal laccase (Srebotnik and Hammel, 2000).

4. Conclusions

We successfully demonstrate the suitability of MDF process effluent as a cultivation medium for *P. putida* after adsorptive removal of in hibitory polyphenols. Additional carbon sources and minerals are not required. Apart from sugars, several other carbon sources in MDF ef fluents are metabolized as a single carbon source as well as in combi nation with glucose. Further, application of *P. putida* biomass obtained from pre cultivation in process effluent efficiently reduced VOC emis sions from pinewood. Results collectively contribute to the develop ment of an environmentally benign biotechnological process for the wood industry.

CRediT authorship contribution statement

Martin Lindemann:Conceptualization, Investigation, Methodology, Data curation, Visualization, Writing original draft.**Bernhard Widhalm**:Conceptualization, Investigation, Methodology, Data curation, Visualization, Writing original draft.**Thomas Kunciger**:Resources, Conceptualization, Project administration.**Ewald Srebotnik**:Conceptualization, Visualization, Writing review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influ ence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2020.100471.

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Potential of a year-round, closed-loop process for volatile organic compounds reduction in pinewood strands by *Pseudomonas putida* PX1 cultivated in seasonally varying process effluents

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ABSTRACT

Sustainable utilization of waste streams from bioresources is a challenging opportunity for the future. This study investigated the potential of a seasonally varying process effluent stream from medium-density fiberboard production for a year-round cultivation process of *Pseudomonas putida* PX1 for reduction of volatile organic compounds (VOC) in pinewood strands. For four years, seasonal variations with abundant fermentable carbon sources during colder periods and few carbon sources during warmer periods were observed. Nitrogen sources present in process effluent required for optimal biomass production are insufficient when abundant carbon sources are available and should be supplemented. VOC reduction in pinewood strands with *P. putida* after mixed substrate fermentation showed very promising results for industrial applications. Total VOC emissions were reduced by more than 55% in only 3 h. Most aldehydes and terpenes were effectively reduced by 67%–100%, except for Δ-3-carene and α-terpinolene, which were reduced by 20%–22%.

1. Introduction

Wood processing industries must deal with various raw material streams, particularly in wood-based panel production. Apart from different wood species, seasonal parameters such as moisture content and varying chemical compositions influence processing parameters and, in particular, the resulting extractive-rich effluent stream during pretreatment (Widsten et al., 2003). This effluent stream is generated during medium-density fiberboard (MDF) production by squeezing out steam-pretreated softwood chips and requires complex and costly effluent treatment before reuse or disposal. Raw material variability and subsequent effluent streams require flexible solutions with constant monitoring to ensure stable processes to meet regulatory limits. Biological wastewater treatment (active sludge treatment) does provide a flexible and continuously adapting microbial consortium to lower chemical/biological oxygen demand (COD/BOD) as well as volatile organic compounds (VOC) from waste air streams in MDF production (Portenkirchner et al., 2003). After additional processing steps, particularly membrane filtration and reverse osmosis, the effluent stream can be reused in the process, but it is laborious and costly without exploiting the potential of the effluent for additional benefits. Thus, direct reuse of the effluent would be preferable to improve overall sustainability (Ashrafi et al., 2015).

Current research focuses more on using effluent streams, for instance, by fermentation, to use the potential of wastewater to convert a broad range of substances to a desired product such as methane, hydrogen, or bioplastics (polyhydroxyalkanoates (PHA)). Methane production by anaerobic digestion of thermomechanical pulp (TMP) wastewaters was recently demonstrated by Gao et al. (2016) in a hollow fiber-submerged anaerobic membrane bioreactor. Dessì et al. (2018) found that hydrogen production from TMP wastewaters by dark fermentation preferably at high temperatures is possible. An innovative approach to converting carbohydrates to electricity using pulping industry wastewater in a microbial fuel cell, with *Pseudomonas fluorescens* being the main energy-producing bacterium, was published by Kaushik and Jadhav (2017). Furthermore, volatile fatty acids, a substrate for PHA production (Queirós et al., 2014), can be produced from pulp mill effluents by acidogenic fermentation (Ben et al., 2011; Bengtsson et al.,

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2008). Pseudomonads are well known for their ability to withstand challenging environments and are used in bioremediation of several recalcitrant substances such as various phenolic compounds (Wasi et al., 2013; Shourian et al., 2009; Kumar et al., 2005). Some *Pseudomonas* species have also been used to metabolize aldehydes and terpenes in liquid culture (Cheng et al., 2013; Bicas et al., 2008; Kleinheinz et al., 1999) and pinewood strands (Widhalm et al., 2016). In previous work (Lindemann et al., 2020a, 2020b), the authors presented a method for recovering valuable polyphenols from MDF process effluent (PE) via adsorption and a process for microbial VOC reduction in pinewood strands by *Pseudomonas putida* PX1 cultivated on the residual carbohydrate-rich effluent stream.

While terpenes are naturally occurring in softwoods originating from wood resin, most aldehydes such as hexanal, octanal, nonanal, and their related unsaturated derivatives are emitted as secondary VOC, resulting mainly from the autoxidative cleavage of unsaturated fatty acids. Thus, these secondary emissions have a delayed release, which is important for storage conditions and further use (Makowski et al., 2005). The Globally Harmonized System of Classification, Labelling, and Packaging of Chemicals classifies aldehydes and terpenes as irritants and hazardous to the environment. Several pinewood products in buildings, especially particleboards promptly used after production because of rapid drying and short storage times, may be affecting indoor air quality negatively due to higher aldehyde and terpene emissions. To avoid chronic exposure to these VOC, which have a negative impact on indoor air quality and ultimately on human health, known as “sick-building-syndrome” (Wilke et al., 2013; Makowski and Ohlmeyer, 2006), total VOC emissions should be reduced. For unsaturated aldehydes, starting from 2-butenal up to 2-undecenal, very low EU-LCI (lowest concentration of interest) values were derived in 2015 and are still under discussion in 2021 for incorporation in the German AgBB scheme (AgBB, 2015; AgBB, 2021). To comply with these requirements in the future, innovative VOC reduction strategies will be inevitable. Apart from microbial reduction, several other approaches to reduce VOC emissions have been reported. Thermal reduction strategies, including heat treatment of pinewood (Manninen et al., 2002), thermal degradation of terpenes (McGraw et al., 1999), and elevated storage temperature within wood chips for pellet production (Gabriel et al., 2015), have been reported. Chemical degradation of terpenes in pinewood using sodium sulfite and sodium hydroxide was performed by Roffael et al. (2015). The use of biofilters for VOC reduction from waste gases and contaminated air was reported by Van Groenestijn and Liu (2002) using fungi and by Natarajan et al. (2017) using a mixed microbial culture. Biotransformation of terpenes in liquid culture with different fungi was demonstrated by Lee et al. (2015) and Qi et al. (2002).

The primary objective of this study was to show that the carbohydrate-rich effluent stream discharged from MDF production can be used year-round as a nutrient medium for continuous production of *P. putida* biomass, which, in turn, is applied to pinewood strands for VOC reduction. When combined with drastically reduced treatment times for pinewood strands using *P. putida*, the results show that the process is technically feasible, paving the way for zero-waste industrial production of VOC-reduced pinewood strand boards.

2. Materials and methods

2.1. Chemicals

Sigma Aldrich (St. Louis, MO, USA) provided chemicals for media preparation, monoterpenes, toluene D8 standards, and sugar/sugar alcohol standards, i.e., D-(+)-glucose, D-(+)-xylose, D-(+)-arabinose, D-(+)-galactose, D-(+)-mannose, D-( )-fructose, and D-(+)-pinitol. Anion multi-element standards (Certipur® Anionen-Multielement-Standardlosung I (F^-, PO_4^{3-}, Br^-) and II (Cl^-, NO_3^-, SO_4^{2-})) and cation multi-element standard (Certipur® Kationen-Multielement-Standardlosung VI (NH_4^+, K^+, Na^+, Ca^{2+}, Mg^{2+})) were purchased from Merck KGaA (Darmstadt, Germany). Carl Roth GmbH & Co. KG (Karlsruhe, Germany) provided organic acid standards (acetate, propionate, formate, succinate, and citrate) were purchased from. Milli-Q-Water was prepared according to DIN ISO 3696 (1991). Other chemicals used were of analytical grade and purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Origin of PE and general workup procedure

PE batches were sampled monthly from an MDF plant in Wismar, Germany (Egger Holzwerkstoffe Wismar GmbH & Co. KG) for 4 years (04_2017-04_2021). Briefly, the MDF process involves steaming of wood chips at approximately 120 °C at elevated pressure, followed by squeezing water out of the steamed wood chips using a stuffing screw. This stuffing screw effluent (SSE) is diluted by a steam condensate, yielding the PE used in this study. Primarily, spruce and pine are used for MDF production at the Wismar plant. The number of softwood comprises more than 90% of the wood mix, but a precise composition cannot be assigned to the sampled batches because only rough daily averages are available. The workup procedure for PE involved several steps, including centrifugation, filtration, and adsorption, to remove fibers, colloidal and non-colloidal resin, and fatty acids, as well as polyphenols. Additional information about the PE workup for subsequent use as a nutrient medium for cultivating bacteria can be found in Lindemann et al. (2020a).

2.3. Analysis of anions, cations, and organic acids in PE by ion chromatography (IC)

According to Lindemann et al. (2020b), inorganic ions and organic acids in PE samples were determined by IC on a Dionex ICS 5000+ system (Thermo Scientific) equipped with conductivity detectors and an autosampler (Dionex AS-DV) for simultaneous injection onto both anion and cation columns. All ions were quantified by calibrating peak areas with authentic standards of chloride, nitrate, sulfate, phosphate, acetate, propionate, formate, succinate, citrate, sodium, ammonium, potassium, magnesium, and calcium. Citrate and succinate data were available only from 10/2019 onward, as they were not analyzed before that date.

2.4. Analysis of carbohydrates in PE by HPAEC/PAD

Sugars and sugar alcohols in PE samples were determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC/PAD) on a Dionex ICS 5000+ system (Thermo Scientific). Sugars were quantified by calibrating peak areas with authentic standards of D-arabinose, D-galactose, D-glucose, D-mannose, D-xylose, and D-fructose. Sugar alcohols were inadequately separated using this method. Therefore, a sum parameter was defined by quantifying sugar alcohols as pinitol (the predominant sugar alcohol identified in PE equivalents. This fraction mainly represented sugar alcohols, as confirmed by gas chromatography–mass spectrometry (GC–MS) as an orthogonal method. Detailed information about gradients and IC systems can be found in Lindemann et al. (2020b).

2.5. Analysis of COD of PE

According to DIN ISO 15705 (2002), COD of PE was performed directly at the MDF plant in Wismar, Germany (Egger Holzwerkstoffe Wismar GmbH & Co. KG). Spectroquant® COD Cell Tests (Merck, Darmstadt, Germany) were used to measure COD. To facilitate comparison with other substances, COD is represented in g/L, rather than of the conventional mg/L.

2.6. Cultivation of microorganisms

By measuring the optical density (OD600) on a Shimadzu UV-1800

spectrophotometer (Shimadzu), bacterial growth was determined according to Cheng et al. (2013). The number of colony-forming units (CFU) was determined per mL of suspension (CFU mL^{-1}). A serial dilution of the bacterial suspension was made with 0.9% aqueous NaCl for this purpose. From each dilution, 0.1 mL was then streaked out on agar plates and incubated at 25C for 24 h.

2.6.1. Pre-culture

Pseudomonas putida NCIMB 10684 (PX1) was obtained from the National Collection of Industrial, Food, and Marine Bacteria (Aberdeen, Scotland, UK) and kept in Petri dishes on Standard Nutrient Agar No.1 (Carl Roth GmbH, Karlsruhe, Germany). According to Widhalm et al. (2018), *P. putida* was pre-grown in 100 mL Erlenmeyer flasks containing 50 mL sterile M9 minimal salts medium, and for pre-cultivation of the fermentation inoculum, 500 mL flasks were used, containing 200 mL M9. In addition, 0.2% (*v*/v) 1 M aqueous $MgSO_4$ solution, 0.25% (v/v) trace element solution according to Widhalm et al. (2018), and 0.1% (w/v) glucose were added. Cultivation was performed in a rotary shaker (Infors AG, Bottmingen, Switzerland) at 25C and 200 rpm.

2.6.2. Shake flask experiments

100 mL Erlenmeyer flasks containing 50 mL sterile filtered PE were used. PE A was used in either its native form or supplemented with 5.4 g/L ammonium chloride. PE B was used in its native form or supplemented with either 0.5 g/L glucose or both 0.5 g/L glucose and 5.4 g/L ammonium chloride. All flasks were inoculated with 2% (*v*/v) pre-culture and grown on a rotary shaker at 25 °C and 200 rpm. As previously stated, samples were taken at the beginning of the cultivation and after 24 h to determine biomass (OD600) and media composition.

2.6.3. Batch fermentation

Batch fermentation was performed in the "Minifors 2" glass bioreactor (Infors AG, Bottmingen, Switzerland) with a total volume of 6 L, along with a BlueInOne Ferm combined CO_2/O_2 analyzer (BlueSens GmbH, Herten, Germany). Native PE A was inoculated with 10% (v/v) pre-culture to a final volume of 4 L. The airflow rate was kept constant at 0.5 L/min until the conditioning phase. Stirrer speed, temperature, and pH were kept constant throughout the batch fermentation at 800 rpm, 25 °C, and pH 7.0, respectively. 4 M NaOH and 20% (v/v) H_3PO_4 were used to adjust and maintain the pH at 7.0 ± 0.1. pO_2 was measured online using a VisiFerm DO Arc 325 H0 –Sensor (Hamilton, Reno, Nevada, USA), based on oxygen-dependent luminescence quenching.

A conditioning phase of 2 h with 0.12% (v/v of the remaining working volume, which decreased from 4 L to approx. 2 L because of continuous sampling) α-pinene was applied after 24 h batch fermentation. During this conditioning phase, the airflow rate was reduced to 0.1 L/min, and the exhaust gas cooler cooled down to 2 °C to minimize α-pinene losses due to evaporation.

2.7. Pinewood treatment and analysis of VOC emissions

VOC reduction tests were conducted on pinewood strands in µCTE™ microchambers (Markes International Ltd., Llantrisant, UK). Fritz Egger GmbH (Wismar, Germany) provided pinewood strands with approximate dimensions of 2.5 cm × 12 cm length and 0.5 cm thickness. Moisture content was 100% ± 10% ($w_{water}/w_{dry\ wood}$) and was determined for 10 random samples using a Sartorius MA 150 moisture analyzer (Sartorius AG, Gottingen, Germany). A microchamber system is made up of six parallel cells, each with a volume of 48 cm^3 each, arranged in a heating block. Particles were weighed into a 50 mL Erlenmeyer flask and were then treated with pre-conditioned bacterial suspension (1 mL per g of wood). Untreated wood strands samples and wood strands treated with the same amount of water were used as controls. After the defined treatment time, the treatments were terminated by heat at 103 °C ± 2 °C overnight. After cooling down, VOC emissions from wood strands were sampled on Markes C1-AAXX-5003 Tenax TA tubes (Markes, UK). The sampling time was 30 min at an airflow rate of 50 mL/min. 1 µL toluene D8 standard was added to each tube before sampling. Tenax tubes were analyzed according to DIN ISO 16000-6 (2011) using a TD-100 thermodesorber (Markes, UK), which was connected to an Agilent 7890A/5975C GC–MS system. Analytes were separated on an HP-PONA (19091S-001) column (Agilent) with the dimensions 50 m × 0.2 mm × 0.5 µm. The following temperature program was used: 3 min at 35 °C, 35 °C → 160 °C at 10 °C min^{-1}, 160 °C → 260 °C at 20 °C min^{-1}, and hold time at 260 °C was 10 min (Stratev et al., 2015).

2.8. Scatterplots, histograms, and correlation coefficients

OriginPro (Version, 2021, OriginLab Corporation, Northampton, MA, USA) was used to plot scatterplots and histograms.

For the Spearman's correlation coefficient, data were ranked (average rank) and calculated according to the following equation (Dodge, Y. 2008b):

$$\rho_s \quad 1 \quad \frac{6\sum_{i \ 1}^{n} d_i^2}{n(n^2 \quad 1)} \tag{1}$$

where d_i denotes the difference between the two ranks of two different observations, and n represents the number of observations.

The partial Spearman's correlation coefficient was calculated using the following equation (Dodge, Y. 2008a):

$$\rho_{s,par} \quad \frac{\rho_{s:x,y} \quad (\rho_{s:x,z} * \rho_{s:y,z})}{\sqrt{1 \quad \rho_{s:x,z}^2} * \sqrt{1 \quad \rho_{s:y,z}^2}} \tag{2}$$

where ρ_s:(x,y; x,z; y,z) represent Spearman's correlation coefficient between two variables (x,y; x,z; y,z); x,y denote variables to correlate; z represents control variable.

To calculate correlation and partial correlation coefficients, the data point 02_2021 was removed for all variables, as there was an obvious outlier (very untypical volume flows, probably due to a documentation mistake) in SSE in PE, and SSE in PE was used in many correlations and as the control variable in partial correlations.

3. Results and discussion

In previous work (Lindemann et al., 2020a, 2020b) the authors showed that after removal of growth-inhibiting substances and selective recovery of polyphenols, cultivation of *P. putida* PX1 as well as subsequent microbial reduction of VOC in pinewood strands is possible. However, fluctuating composition and quantities of PE from wood processing complicate substantial reuse in terms of circular economy approaches. A first step to overcome these challenges is a comprehensive analytical assessment over a prolonged period to evaluate the overall potential of the process stream. Parameters important for cultivating microorganisms such as carbohydrates, organic acids, anions, and cations were monitored monthly for 4 years to determine the crucial factors for establishing a robust year-round continuous industrial process.

In Fig. 1, the concentrations of different monosaccharides and sugar alcohols present in PE from fiberboard production for four years (04/2017–04/2021) are shown (numerical data, including standard deviations, are provided in the supplemental material). The main sugars found in PE are fructose and glucose, whereas galactose and arabinose are typically found in lower concentrations. Xylose is not shown because, in most samples, its concentration was below 1 mg/L. Sugar concentrations and, to a lesser extent, the concentration of sugar alcohols underlie great seasonal variations with high quantities during the colder months of the year and low quantities during the warmer months. Seasonal fluctuations in monosaccharides content have already been reported by Hou (1985) for sugars in spruce trunk wood. Terziev et al. (1997) found more low-molecular-weight sugars (mainly glucose and

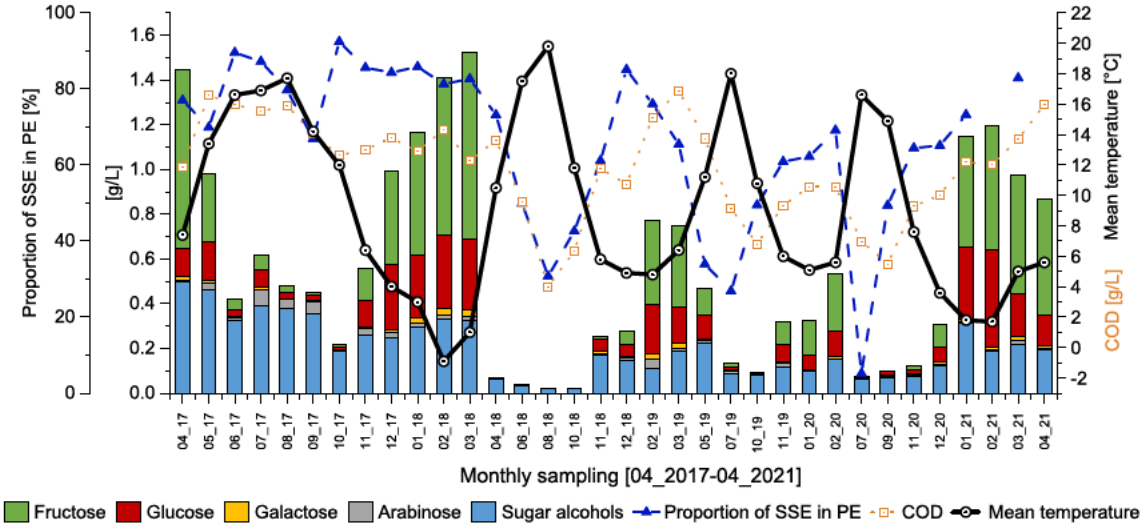

Fig. 1. Stacked bars represent sugar and sugar alcohol concentrations in process effluent (PE) sampled monthly for four years from 04/2017 to 04/2021. Line plots show the proportion of stuffing screw effluent (SSE) in PE in [%], the monthly mean temperature (island Poel, close to the fiberboard plant), and the chemical oxygen demand (COD) of PE (in g/L for better comparability).

fructose) in pine sapwood during autumn and winter than during spring and summer. These findings agree well with the data, considering that there is a variable time gap between felling the tree and actual use in the production of approximately 1–3 months. However, another reason for the fluctuations is the varying proportion of SSE in PE because, in the current process, SSE originating from the wood chips is diluted with the condensate from a steam generator cycle, containing ammonia and small amounts of amines (FINEAMIN 06, FINEAMINE, Switzerland) as corrosion inhibitors. Thus, the observed fluctuations most probably are a combination of these two factors. As shown in Fig. 1, the proportion of SSE in PE is generally higher during the colder months (50%–90%) than during the warmer months (10%–50%) of the year. However, distinct fluctuations were also observed yearly: during the warmer period in 2017 (05–10/2017), the dilution of SSE in PE was distinctively less than the following years from 2018 to 2020. This may be explained by higher amounts of rainfall, and as a consequence, moister wood logs during these months (2017: 544; 2018: 161, 2019: 315, 2020: 342 L/m^2; total rainfall between May and October). Extreme conditions prevailed from 04/2018 to 10/2018 with a very dry (see total rainfall above) and hot spring and summer, decreasing the moisture content of wood logs. Consequently, only a drastically reduced amount of SSE could be squeezed out of the wood chips using the stuffing screw after hot steam pretreatment, resulting in a very low proportion (10%–20%) of SSE in relation to the steam condensate. This dilution, along with the generally decreased wood sugar content during the warm season may, to a large extent, explain the extremely low concentrations of sugars and sugar alcohols in PE observed during this period.

Based on data in Fig. 1, scatterplots, histograms, and correlation coefficients are shown in Fig. 2 to assess the relationship between sugar concentration (as a sum parameter, including sugar alcohols), mean

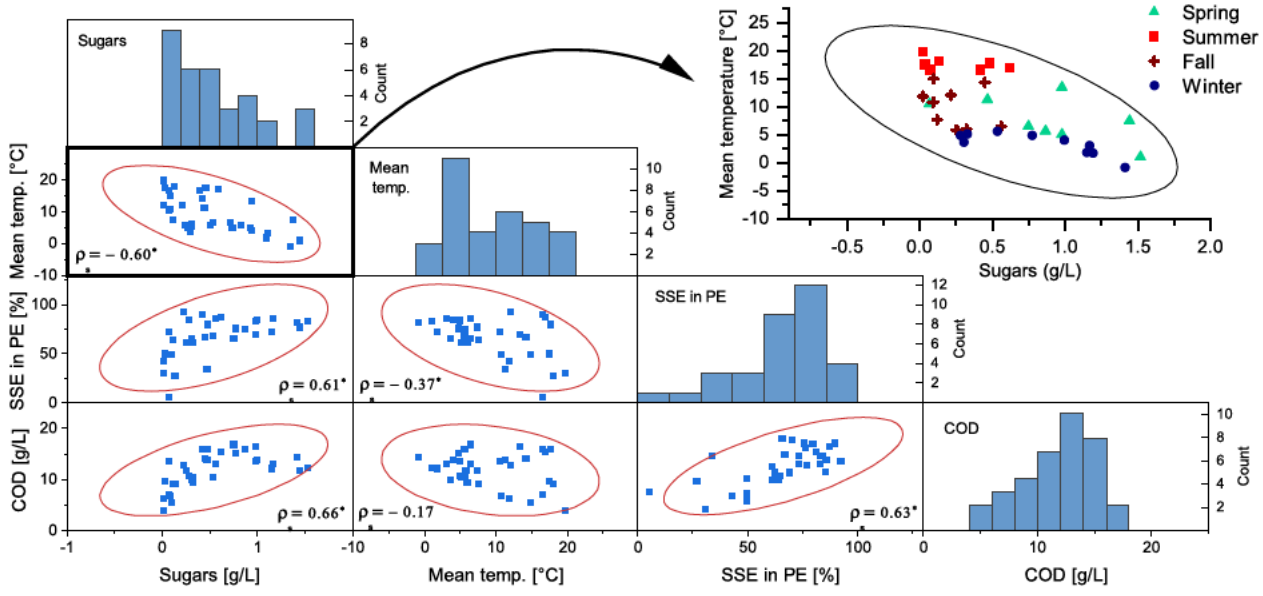

Fig. 2. Scatterplots, correlation coefficients (Spearman), and histograms of 4 selected variables from monthly sampling for 4 years: sugars (sum parameter including sugar alcohols), mean temperature, SSE in PE, and COD. Significant correlations with a critical value according to Ramsey (1989) of ρs0.345 > | (nondirectional $\alpha = 0.05$, $N = 33$), are flagged with an *. The confidence ellipses displayed in the scatterplots are calculated for a 95% confidence interval.
The scatterplot mean temperature/sugars is further detailed in the upper right corner with color-coded seasons to highlight the variability over the year.

ambient temperature (measured close to the plant), SSE in PE, and COD. Spearman's rank correlation coefficient was used because the four relevant variables did not meet the criteria (normality and linearity) for the more commonly used Pearson correlation coefficient. According to the minimal critical value by Ramsey (1989), significant correlations, (critical values of $\rho_s > 0.345$: nondirectional α 0.05, N 33), were found between sugars/COD, sugars/SSE in PE, COD/SSE in PE, and mean temperature/sugars and mean temperature/SSE in PE. As SSE in PE influences the amount of sugars and COD in PE to some extent, partial correlations (Spearman) controlled for SSE in PE were performed. When controlled for SSE in PE, sugars and mean temperature still show a significant moderate negative correlation of $\rho_{s,par}$ 0.51 with only little influence of the controlled variable (ρ_s 0.60). These seasonal variations are evident in the color-coded scatterplot, where low sugar concentrations mainly occurred in summer and fall, whereas high sugar concentrations above 0.7 g/L occurred exclusively in winter and spring. The correlation COD/sugars in PE appears to be influenced more by the control variable SSE in PE, as the partial correlation coefficient $\rho_{s,par}$ decreased from 0.66 to 0.44. This seems reasonable, as COD is influenced by several substances—apart from sugars—that might not underlie such seasonal variations and are possibly influenced to a larger extent by dilution (i.e., SSE in PE). No significant correlations were found for the monitored anions, organic acids, and mean rainfall (raw data are provided in the supplemental material).

For optimal growth of *P. putida* PX1 on the fermentable carbon sources available in PE, further essential nutrients such as nitrogen and minerals must be present in sufficient quantities. Thus, the theoretical nitrogen demand was calculated using a C/N ratio of 6.8 for *P. putida* KT2440 based on growth yield data determined by Sun et al. (2006). The following 2 equations were used to estimate the total fermentable carbon and the corresponding theoretical nitrogen demand:

$$\textit{total fermentable carbon}\left[\frac{g}{L}\right] \quad \sum C_{sugars} + C_{organic\ acids} + f^{*}C_{sugar\ alcohols} \tag{3}$$

where C represents the concentration of carbon provided by the substance group specified in the subscript in g/L;

f represents the estimated fraction of fermentable sugar alcohols (0.25 based on the residual concentration of sugar alcohols after 24 h cultivation)

$$\text{theoretical N demand}\left[\frac{g}{L}\right] \quad \text{total fermentable carbon}\left[\frac{g}{L}\right] * \frac{C}{N}\text{Ratio*safety margin} \tag{4}$$

where C/N Ratio represents carbon to nitrogen ratio for *P. putida* KT2440 based on glucose as the sole carbon source (Sun et al., 2006); safety margin denotes 1.1 ➔10% safety margin to compensate for fluctuations.

The result is shown in Fig. 3, where the theoretical nitrogen demand is compared to the concentration of nitrogen sources (sum of ammonium and nitrate) detected in the PE sampled for 4 years. The concentration of nitrogen sources in the samples does not seem to follow a seasonal trend. Although nitrogen sources are present in the steam generator cycle effluent, there is no significant correlation between the nitrogen concentration in PE and the dilution of SSE in PE. As a result, the theoretical nitrogen demand frequently exceeds the nitrogen available in PE, especially during the colder periods of the year when the concentration of sugars in PE is high. Therefore, for the optimal utilization of PE as a fermentation medium throughout the year and ensure a stable process, carbon and nitrogen sources must be monitored to increase the limiting nutrient to the required level when indicated. Other key nutrients, such as magnesium and phosphate, were found to be available in sufficient quantities throughout the year, according to the demand based on growth yield data (approx. C/P ratio 42; C/Mg ratio 236) determined by Sun et al. (2006).

Fig. 4 shows the results of shake flask experiments of *P. putida* PX1 grown in two different PE batches, PE A (01.2020) and PE B (07.2020), representing PE with high and low nutrient content, respectively. PE A and PE B were used either in their original form or supplemented with an additional carbon or nitrogen source. The concentrations of sugars, sugar alcohols (a), organic acids, as well as ammonium, nitrate (b), and OD600 (a and b) as an indicator of biomass, are shown at the start and after 24 h of cultivation. Inoculum volume (2% v/v) was kept low to minimize the impact of excess nutrients from the pre-culture. The native, nutrient-rich PE A showed a distinct increase in biomass after 24 h cultivation, even without carbon or nitrogen supplementation. Biomass further increased when nitrogen (ammonium) was supplemented. In contrast, biomass increases in native PE B was low, as had to be expected from the lack of carbon sources. The addition of 0.5 g/L glucose, roughly matching the carbon available from all carbon sources in PE A, increased biomass, but it was still considerably less than that seen with native PE A even though all added glucose had been consumed after 24 h cultivation. Only with the simultaneous addition of a nitrogen source (ammonium chloride), biomass formation was comparable to that observed with PE A. This is remarkable because even without supplementation, all detectable sugars had already been consumed after 24 h. However, it is characteristic of *P. putida* to convert excess carbon sources into storage polymers, particularly PHAs and glycogen under nutrient limitation. Hervás et al. (2008) found that under nitrogen limitation, upregulation of a set of genes involved in the accumulation of

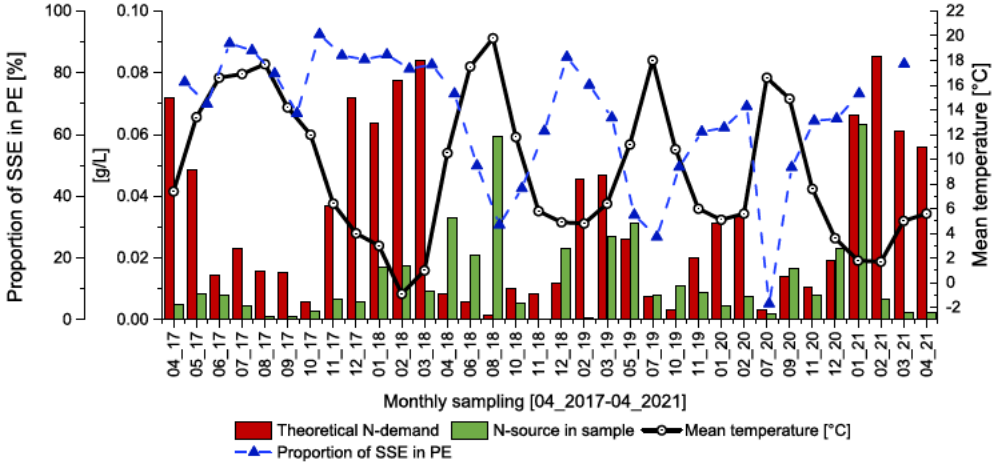

Fig. 3. The bar graph compares theoretical nitrogen demand based on fermentable carbon sources to available nitrogen sources in PE sampled monthly for 4 years from 04/2017 to 04/2021. Line plots show the monthly mean temperature (island Poel, close to the fiberboard plant) and the proportion of SSE in the PE.

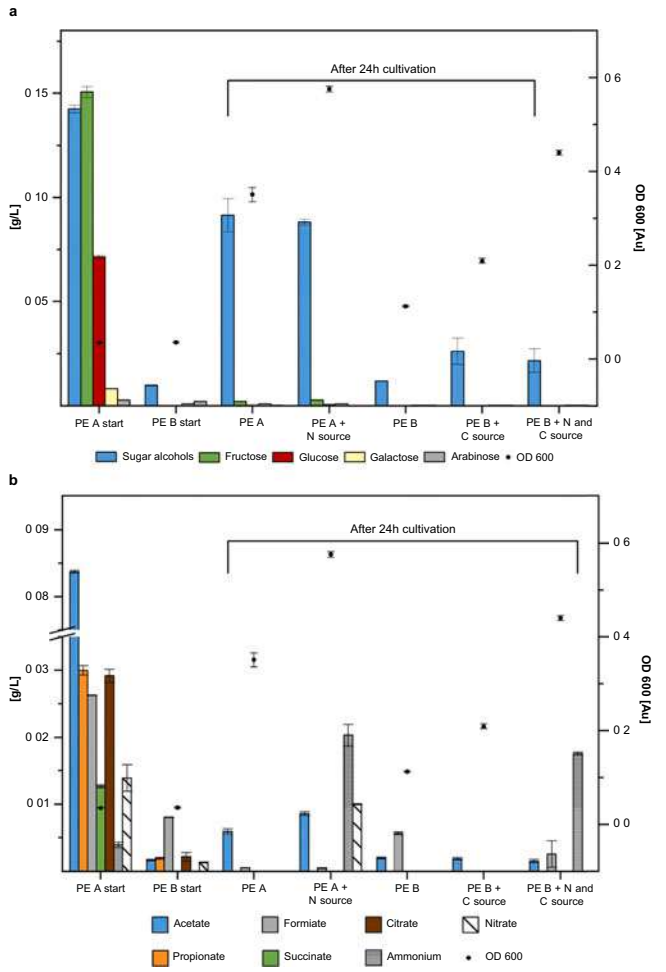

Fig. 4. Concentrations of sugar alcohols, sugars (a), organic acids, ammonium, nitrate (b), and OD 600 (a and b) in native and supplemented PE A and PE B before and after cultivation for 24 h in shake flask experiments.

carbon storage polymers (PHAs and glycogen) occurred in *P. putida*, while carbon catabolism was repressed. These results further support the importance of nutrient supplementation for optimum growth.

Sugar alcohols in PE A with and without nitrogen supplementation were only partly consumed during the 24 h cultivation (Fig. 4a) and even increased to some extent in supplemented PE B. This probably is an artifact caused by bacterial metabolites that may have co-eluted with the sugar alcohol fraction thus overestimating them during HPAEC/PAD analysis. Due to incomplete separation by HPAEC/PAD, sugar alcohols in native PE are represented as a sum parameter and account for approximately 76% of the total peak area, as previously discussed (Lindemann et al., 2020a).

In Fig. 5, fermentation data of a 24 h batch fermentation of *P. putida* PX1 with PE A as a culture medium are shown. OD600, sugar alcohols, sugars, organic acids, ammonium, and nitrate were analyzed hourly for the first 8 h, and 24 h. CO_2 off-gas and pO_2 were recorded online, whereas temperature, pH, stirrer speed, and aeration were kept constant throughout the fermentation process.

Glucose and succinate were metabolized at high rates from the start, whereas most of the other substrates showed a lag phase from 2 to 4 h. Between 3 and 4 h fermentation times, uptake rates for most substrates started to increase and CO_2/pO_2, reaching their maximum/minimum values. Upon depletion of organic acids and all sugars, except fructose, a sharp decline of both oxygen uptake and off-gas CO_2 occurs. A metabolic switch to fructose, the major carbon source in PE A, is apparent at approximately 4.5 h when oxygen uptake resumes and speeds up concomitantly with rapid fructose consumption. At 6 h, oxygen uptake started to drop again probably because of ammonium depletion and switch to nitrate as a nitrogen source. Ammonium was the preferred nitrogen source during the main phase of the fermentation until depletion, while nitrate consumption occurred mainly between 8 and 24 h. After 8 h, fructose was depleted, and oxygen uptake remained constant for a while, probably due to the consumption of residual carbon sources. Because no samples were taken after 8 h, it is unclear whether it is acetate depleting at 10 h with another sudden drop in oxygen uptake. While the shaking flask experiments in Fig. 4 showed that nitrogen availability is a key factor for successful biomass production, the goal of this batch fermentation was to obtain a more detailed picture of interdependencies between carbon and nitrogen sources and to observe the simultaneous consumption of different carbon sources.

After the 24 h fermentation time, a 2 h conditioning step with 0.12% (*v*/v) α-pinene and reduced aeration (0.1 L/min) was applied. Previous work (Widhalm et al., 2016, 2017) has demonstrated that the metabolism of monoterpenes, in general, is significantly accelerated in *P. putida* PX1 by adding α-pinene as the sole carbon source directly to the culture. As a result, the biochemical machinery *of P. putida PX1* is already adapted to the target substances when the culture is added to pinewood later in this study. α-pinene is used in the conditioning step as one of the major monoterpenes released from pinewood and is thus primarily responsible for increased terpene emissions from pinewood products (Yoo and Day, 2002; Stratev et al., 2015). Here, the conditioning step was successfully reduced to only 2 h compared to 24 h in previous work (data not shown; Lindemann et al., 2020b). This successful time reduction could be attributed to the bioreactor's superior mixing and aeration, compared to shake flask cultures, and the resulting higher availability of oxygen and α-pinene during the conditioning phase. As microbial degradation of α-pinene by *P. putida* PX1 requires oxygen, for instance, in the first oxygenation step for α-pinene epoxide formation (Trudgill, 1990), high concentrations of dissolved oxygen may be beneficial for both the conditioning phase and bacterial treatment of pinewood strands. Pinewood strands were treated with the conditioned bacterial suspension for 3 h at 23 °C in airflow-controlled microchambers (μCTE™ microchambers: Markes International Ltd., Llantrisant, UK). The resulting VOC emissions after bacterial and control treatments are listed in Table 1. Results indicate that the bacteria reduced total volatile organic compounds (TVOC) emissions by more than 55% compared to water-only treatment. Most aldehydes and terpenes were effectively reduced (67%–100% reduction), except for Δ-3-carene and α-terpinolene (approximately 20%–22% reduction). The high concentration of α-terpinolene in the water treated sample did not follow the overall trend of a slight reduction in aldehydes and terpenes through water treatment when compared with the control. This deviation can be explained by an inhomogeneous distribution of the precursors —resin and fatty acids—in pinewood strands, or by a measuring error. TVOC was slightly reduced in the control treatment with water compared to the untreated, i.e., dry strands. According to Widhalm et al. (2016), this effect may be due to the activation of microorganisms already present on the non-sterile wood strands by the addition of water. Microbial VOC reduction after batch fermentation of *P. putida* PX1 in PE A and subsequent application onto pinewood strands was very successful even though both the conditioning phase and treatment time of strands were drastically reduced from 24 h to 2 h and 4 days to 3 h, respectively, compared to previously published data (Lindemann et al., 2020b). The time saved here for conditioning, particularly for the treatment of pinewood strands, is crucial for industrial applications and shows the potential of microbial VOC reduction and PE recycling. In future research, even a continuous fermentation process may be envisioned. A key challenge in this context, however, will be the implementation of the adaptation phase with either a subsequent adaption step or continuous dosage of α-pinene.

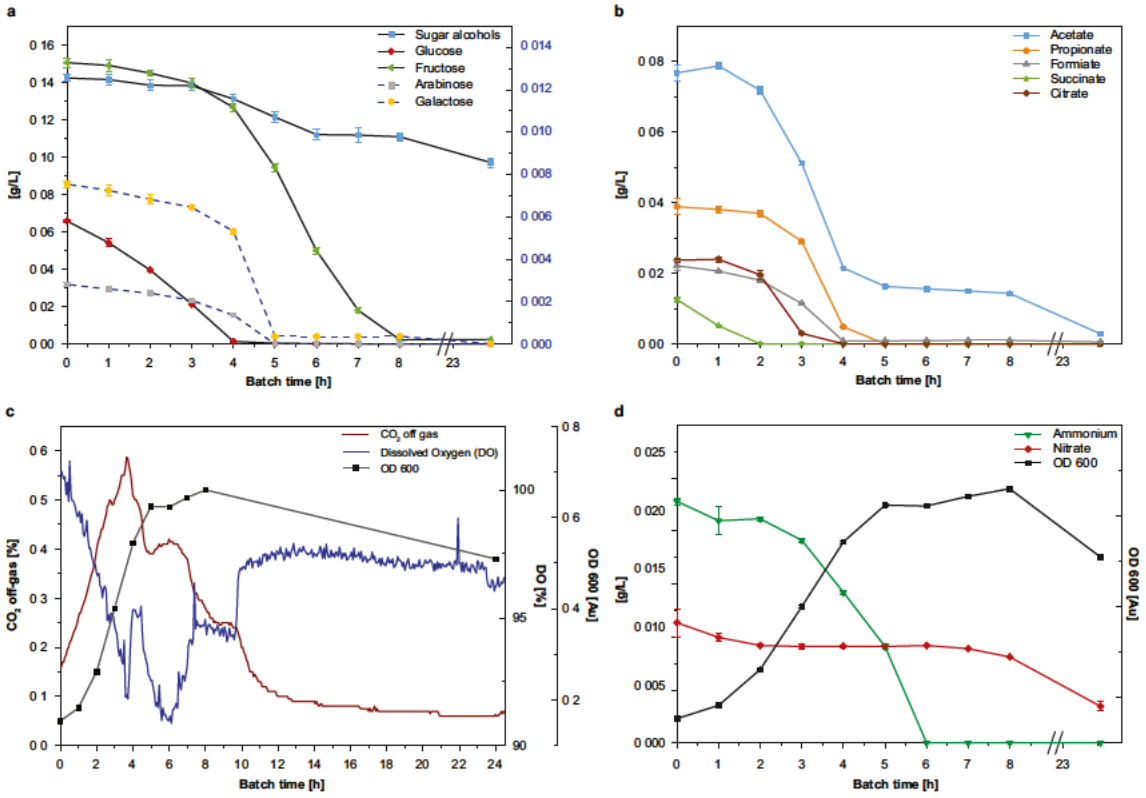

Fig. 5. Composition of PE A during fermentation controlled by hourly sampling for 8 h, and a final sampling after 24 h (a: sugars and sugar alcohols; b: organic acids; d: nitrogen sources and OD600; c: constant monitoring of CO_2 off-gas and dissolved oxygen (DO) and OD600).

Table 1
Pinewood strand emissions after treatment with conditioned bacteria or water, or without treatment, expressed as µg/m^3 of toluene equivalents (TE). Individual compounds, TVOC, and standard deviation (S.D.) of three replicates are shown.

	Treatment		
	None	Water	Bacteria
Pentanal	23.4	19	0.0
Hexanal	44.7	49.8	11.6
α-Pinene	258.3	254.9	84.2
Camphene	20.8	18.2	4.0
Octanal	23.4	23.5	2.7
Δ-3-carene	226.7	213.3	180.0
Xylene	12.0	5.0	2.8
Limonene	6.9	6.8	1.8
Nonanal	84.0	50.7	3.5
α-Terpinolene	7.6	28.6	5.9
TVOC	707.9	669.7	296.3
S.D.	45.7	26.3	34.9

4. Conclusions

The results suggest that online or at-line measurements of major carbon and nitrogen sources in the PE feed and nutrient supplementation are necessary to ensure a robust process. Cheap carbon sources, for instance, mixed glucose and fructose molasses, may be used because *P. putida* can metabolize various carbon sources.

The economic feasibility of the process is strongly dependent on the price of the value-added product, in this case, the VOC-reduced OSB, and on international regulatory limits (EU-LCI). Furthermore, VOC-reduced OSB has great potential for the Asian market, as Asian customers are not accustomed to odorous terpenes emitting from softwood.

CRediT authorship contribution statement

Martin Lindemann: Conceptualization, Investigation, Methodology, Data curation, Visualization, Writing – original draft. **Bernhard Widhalm:** Conceptualization, Investigation, Methodology, Data curation, Visualization. **Thomas Kuncinger:** Resources, Conceptualization, Project administration. **Ewald Srebotnik:** Conceptualization, Visualization, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2022.100995.

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Yoo, S.K., Day, D.F., 2002. Bacterial metabolism of α- and β-pinene and related monoterpenes by *Pseudomonas sp*. Strain PIN. Process Biochem. 37, 739–745.

Curriculum vitae
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PERSONAL DATA

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Date of birth: 29.07.1990

Nationality: Austrian

WORK EXPERIENCE

04/2016 to present — **Wood K plus - Kompetenzzentrum Holz GmbH**
Division Wood Materials Technologies,
Team „Indoor quality & emission control“
Project fields: Chemical Analysis, Biotechnology, Biorefinery, Extraction, Adsorption, Filtration

EDUCATION

05/2016 to present — **Vienna University of Technology**
Doctoral programme in Engineering Sciences
Dissertation field: Technical Chemistry
PhD Thesis: Characterization of process waters from wood processing industries

12/2013 - 05.2016 — **Vienna University of Technology**
Master Curriculum: “Technical Chemistry” passed with distinction.
Specialisation in: “Sustainable technologies and environmental engineering”
Master thesis (Institute of Chemical, Environmental and Bioscience Engineering, Prof. Ewald Srebotnik):”Cellulose depolymerization during organosolv treatment”

08/2014 – 01/2015 — **Universitat Politècnica de Valencia**
Exchange semester in Valencia, Spain (during master studies)

09/2009 – 12/2013 — **Vienna University of Technology**
Bachelor Curriculum “Technical Chemistry”
Bachelor Thesis (Institute of Polymer Chemistry and Technology, Prof Simone Knaus):”Photochemical modification of polypropylene with functional arylazides“

Vienna, May 2022

RESEARCH EXPERIENCE

Institute of Chemical, Environmental and Bioscience Engineering

- Organosolv extractions
- Analysis of Carbohydrates, Lignin, Hemicellulose, Ash in biomass
- Analysis of Degree of polymerisation of wheat straw cellulose

Institute of Polymer Chemistry and Technology

- Synthesis of functional arylazides
- Functionalisation of polypropylene
- Infrared spectrometry (FTIR-ATR) of functionalised polypropylene
- Dynamic contact angle measurements

Industrial Research

- Analysis of industrial effluents
- Crossflow filtration of industrial effluents
- Cultivation of VOC degrading microorganisms
- Extraction of woody biomass
- Analysis of Polyphenols from biological samples

RESEARCH SKILLS

Chemical analysis	Chromatography and Mass spectrometry
	HPLC
	IC (Anions, cations, organic acids)
	HPAEC / PAD (carbohydrates: sugars/sugar alcohols)
	HPSEC (Lignin, Cellulose, Hemicellulose)
	GC / FID; GC / MS (Headspace / SPME, liquid extracts)
	Infrared spectrometry (FTIR-ATR)
Biotechnology	Fermentation, Industrial Biotechnology
	Cultivation of bacteria for VOC reduction (biodegradation)
Chemical engineering	Scale up experiments for adsorption of polyphenols from liquid extracts
	Optimisation of various extraction processes with woody biomass, wheat straw, fungi
	Membrane filtration processes (Crossflow filtration)

PUBLICATIONS / CONFERENCE ARTICLES

Peer-reviewed

Lindemann, M., Widhalm, B., Kunzinger, T., Srebotnik, E. 2022. Potential of a year-round, closed-loop process for volatile organic compounds reduction in pinewood strands by Pseudomonas putida PX1 cultivated in seasonally varying process effluents. Bioresource Technology Reports, 100995.

Vienna, May 2022

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Lindemann, M., Friedl, A., Srebotnik, E. 2017. Enhanced Cellulose Degradation of Wheat Straw during Aqueous Ethanol Organosolv Treatment. *BioResources*, **12**(4), 9407-9419.

Felhofer, M., Bock, P., Xiao, N., Preimesberger, C., Lindemann, M., Hansmann, C., & Gierlinger, N. (2021). Oak wood drying: precipitation of crystalline ellagic acid leads to discoloration. *Holzforschung*, 1(ahead-of-print)

Conferences

ECAB5 (Florence, Italy): Valorization of effluents from wood processing industry by removal of bioactive polyphenols and subsequent fermentation. Conference abstract and oral presentation.

EWLP 2018 (Aveiro, Portugal) Selective recovery of polyphenols from MDF process waters by adsorption on a macroporous, cross-linked pyrrolidone-based resin. Conference abstract and poster presentation.

NWBC 2017 (Stockholm, Sweden): Enhanced cellulose degradation of wheat straw during aqueous ethanol organosolv treatment. Conference abstract and poster presentation.

SKILLS

German:	native speaker
Englisch:	excellent command / highly proficient in spoken and written English
Spanish:	basic communication skills (A2)
Greek:	basic communication skills (A2)

COMPUTER LITERACY

MS Office (Word, Excel, Power Point), OriginLab, Agilent Chemstation, Chromeleon Software for Chemical analysis, OPUS Spectroscopy software

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