Prospective application of melanized fungi for the biofiltration of indoor air in closed bioregenerative systems

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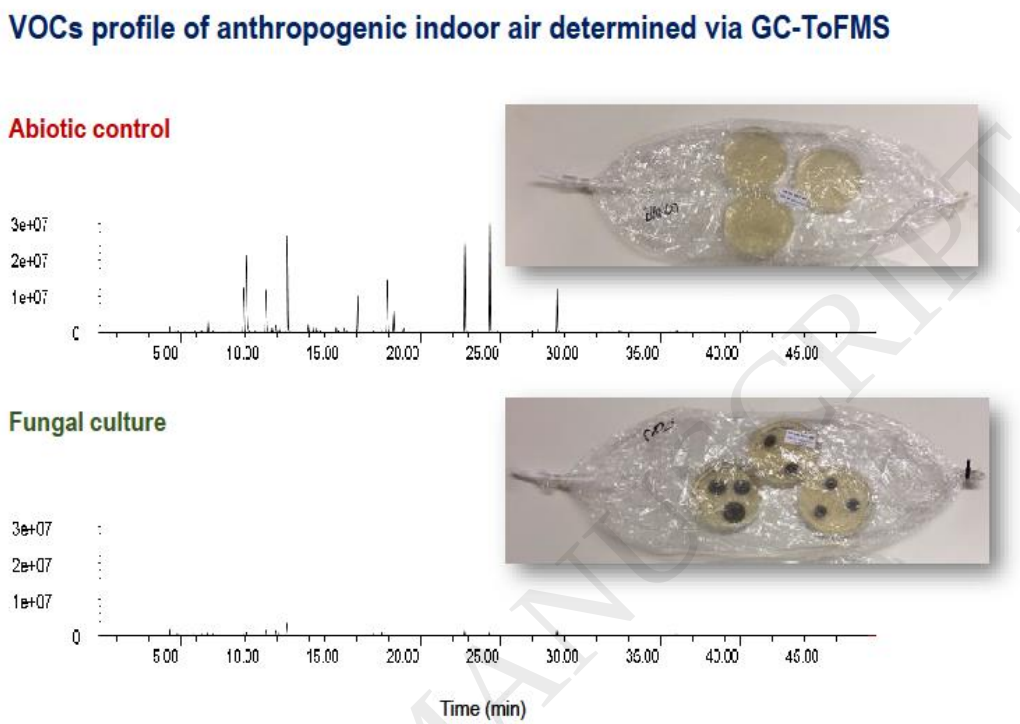
Graphical abstract



Highlights

- The indoor air from agrifood research laboratories comprise a complex COVs mixture.
- Black yeasts reduce the total VOCs concentration from indoor air by more than 95%.
- Fungal hydrophobicity and biodegradation capacity play a major role in VOCs removal.
- The emission of fungal volatile metabolites is rather low and species-specific.
- Black yeasts might be used advantageously in the biofiltration of indoor air.

Abstract

Cultures of melanized fungi representative of the black yeast orders *Capnodiales* (*Cladosporium cladosporioides* and *Neohortaea acidophila*) and *Chaetothyriales* (*Cladophialophora psammophila*) were confined with indoor air from the laboratory during 48 hours. Volatile organic compounds (VOCs) from the headspace were analyzed by thermal desorption gas chromatography time-of-fly mass spectrometry (TD-GC-ToFMS, detection threshold 0.1 $\mu g \cdot m^{-3}$) and compared against an abiotic control. A mixture of 71 VOCs were identified and quantified in the indoor air (total concentration 1.4 $mg \cdot m^{-3}$). Most of these compounds were removed in the presence of fungal biomass, but 42 newly formed putative volatile metabolites were detected though at comparatively low total concentrations (<50 $\mu g \cdot m^{-3}$). The VOCs emission profile of *C. cladosporioides*, a ubiquitous and well-known species often associated to the sick building syndrome, was consistent with previous literature reports. The specialized *C. psammophila* and *N. acidophila*, isolated respectively from gasoline polluted soil and from lignite, displayed rather specific VOCs emission profiles. Mass balances on the fungal uptake and generation of VOCs resulted in overall VOCs removal efficiencies higher than 96% with all tested fungi. Applied aspects and biosafety issues concerning the suitability of black yeasts for the biofiltration of indoor air have been discussed.

Keywords

Air biofiltration; black yeasts; fungal volatile metabolites; gas chromatography time-of-flight mass spectrometry; sick building syndrome.

1. INTRODUCTION

People in Western Europe spend in average more than the 80% of their time in indoor environments, so that they are significantly exposed to the pollutants contained therein [1]. The sick building syndrome (SBS) has been defined by the World Health Organization as a medical condition where people in a building suffer from unspecific symptoms of illness or feel unwell for no apparent reason. SBS has commonly been linked to poor indoor air quality because of failures in heating, ventilation and air conditioning (HVAC) systems. The ultimate causes, though, have been attributed to the accumulation of volatile organic compounds (VOCs) emitted by construction materials and appliances, cleaning products, etc. The buildup of anthropogenic but also biogenic VOCs (e.g. the phytohormone ethylene) compromises the efficiency of closed bioregenerative life support systems (CBLSS) in space missions [2].

Airborne fungi are increasingly gaining importance in view of health hazards caused by aerosolized spores, identified as one of the major indoor allergens [3]. Spores might also carry mycotoxins and even cause infectious diseases. Indoor fungi are also known to emit a wide array of volatile primary and secondary metabolites that might contribute further to the SBS [4]. Hence, most research on mold contamination of building materials has been focusing on highly sporulating and mycotoxin producing species in the genera *Aspergillus*, *Cladosporium*, *Fusarium*, *Paecylomyces*, *Penicillium*, and *Stachybotrys* [3, 5]. These taxa were also reported in the sealed environment of the MIR space station, where fungal colonization became a serious problem after 15 years in orbit [6].

Contrariwise, fungi have also been investigated thoroughly in relation to their capacity to remove volatile contaminants in air biofilers [7]. With this technology, polluted air is flown through a porous matrix that serves as a support for the development of microorganisms. By

controlling the humidity of the support, fungi can be enriched over bacteria and the lack of a free-flowing water layer enhances the direct transfer of hydrophobic VOCs from the gas phase onto the fungal biofilm, where they are eventually biodegraded [8]. Most biofilter applications have been devised for the treatment of industrial off-gases containing rather simple VOCs mixtures in the $mg \cdot m^{-3}$ – $g \cdot m^{-3}$ concentration range [9]. Biofiltration has also been proposed for the treatment of indoor air, which is characterized by having very complex mixtures of VOCs that are present at the $ng \cdot m^{-3}$ – $\mu g \cdot m^{-3}$ range. However, such low concentrations might not support microbial growth and limit biodegradation kinetics in biofilters [10]. Constrains related to poor pollutant transfer from gas phase to biofilm, low biodegradability of recalcitrant substances, and potential biohazard from aerosolized microorganisms have also been highlighted [11].

Several fungal species have been studied concerning their physiological adaptation to biofilter conditions and their metabolic capacity to biodegrade toxic volatile compounds, but the so-called black yeasts appear to be particularly suitable for this purpose [12]. This functional group of fungi owe its name to their strongly melanized thallus and by an ability to grow either as filaments, budding cells, or by forming meristematic structures. Such physiological flexibility and melanin pigmentation enables them to colonize a wide range of hostile environments, so that many species are considered as polyextremophilic eukaryotic microorganisms [13]. Black yeasts have primarily been classified into two clearly delimited orders: *Dothideales*, and *Chaetothyriales*. Yet, several members in the *Dothidiales* have recently been assigned to the *Capnodiales* on the basis of new molecular evidence [14].

Dothidialean/capnodialean species tend to be isolated from the environment in relation to conditions of extreme pH and temperature, high salinity, desiccation, and radiation. Some species have also been isolated from environments that are rich in aromatic hydrocarbons and

phenols, ranging from the specialized *Neohortaea acidophila* growing on brown coal at pH as low as 0.6 [15], to the cosmopolitan *Cladosporium cladosporioides*, which has been found on creosoted wood but also on a wide variety of plants, and is very common in the indoor air [16]. A very few species in this group are opportunistic human pathogens, but virulence is comparatively low, mostly limited to superficial skin infections and allergic reactions when inhaling spores [17]. Conversely, the vast majority of the black yeasts isolated from clinical cases of severe deep mycoses belong to the *Chaetothyriales* and, in particular, to the family *Herpotrichiellaceae*. One of the most surprising findings of the recent years is the consistent isolation of herpotrichiellaceous fungi from environments that are contaminated with volatile alkylbenzene hydrocarbons [12]. The assimilation of bulk industrial pollutants such as toluene and styrene as the sole carbon and energy sources has been demonstrated with different species from the *Herpotrichiellaceae*, primarily in the genera *Exophiala* and *Cladophialophora* (e.g. *C. psammophila*) and its biotechnological application in purposely inoculated air biofilters packed with synthetic supports has already been tested successfully [18].

Despite the benefits of using black yeasts in biofiltration, to our knowledge there are no reports on the application of these fungi for the treatment of indoor air. The present study is aimed at filling this gap by determining the effect of fungal biomass from different melanized fungi on the reduction of a complex VOCs mixture from an indoor environment. The obtained results might shed new insights into the potential contribution of the black yeasts to the SBS, but also on the viability of developing biofiltration units based on these fungi for the treatment of indoor air in CBLSS.

2. MATERIALS AND METHODS

2.1. Fungal cultures and experimental procedure

The fungal strains used in the present study were obtained from the culture collection of the Westerdijke Fungal Biodiversity Institute (Utrecht, The Netherlands). The selected species corresponded to *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (CBS 101367), *Cladophialophora psammophila* Badali, Prefaneta-Boldú, Guarro & de Hoog (CBS 110553, type strain), and *Neohortaea acidophila* (Hölker, Bend, Pracht, Tetsch, Tob.Müll., M. Höfer & de Hoog) Quaedvlieg & Crous, (CBS 113389, type strain). Fungi were cultivated on potato dextrose agar plates (PDA, Pronadisa, Madrid, Spain) from 11 to 28 days, depending on the growth rate of the strain, by inoculating three colonies on the agar per petri dish. Three agar-plate cultures of every fungus were introduced in Nalophan™ gas sampling bags (Foodpack BV, Harderwijk, NL) and filled with 2.0 L of the laboratory ambient air. This procedure was repeated with *C. cladosporioides*, but only one agar plate was introduced into the sampling bag because of the significantly larger colonies formed by this fungus. Two additional bags containing three uninoculated agar plates were prepared, the first was filled with the same laboratory indoor air as an abiotic control, while the second was filled with nitrogen gas (purity > 9.999%, Abelló Linde, Barcelona, Spain) in order to monitor the contribution of the used materials to the VOCs profiles. Both the abiotic and materials controls were processed exactly in the same manner as the bags with fungal cultures were. After 48 hours of incubation at room temperature, 1.5L of the air content from every bag was transferred to stainless-steel tubes (length: 3 in × 0.5 in; o.d.: 0.25 in) filled with a multisorbent bed of 350 mg of Tenax/Carbograph 5TD (Markes International Limited, Llantrisant, UK) through a manual pump (Easy-VOC, Markes International Limited, Llantrisant, UK). Before each use, tubes were conditioned by thermal cleaning (335ºC for 40 minutes) under a nitrogen flow rate of 50 mL min^{-1} (purity 99.999%) by a tube conditioner device (TC20, Markes International Limited, Llantrisant, UK).

2.2. Analytical methods

Full quantitative scans on VOCs from the previously collected samples were carried out in a TD-GC-ToFMS system. This instrument was composed of a thermal desorption unit (Unity, Markes International Limited, Llantrisant, UK), a gas chromatographer (7890, Agilent, USA), and a time-of-flight mass spectrometer (BenchTOF-dx model, Almsco, Germany). A mid polar DB-624 column was used for chromatographic separation (60 m, 250 µm, 1.4 µm; Agilent, USA). The specific parameters and experimental conditions of the analysis have been summarized in Table 1. Desorption tubes were heated to 300ºC with a helium flow rate of 50 mL min^{-1} for 10 min (first desorption stage). Desorbed analytes were then directed to a hydrophobic general purpose cold trap (10ºC, thermoelectric cooling), filled with Tenax TA and graphitised carbon. After flash-heating of the cold trap to 320ºC during 5 min (second desorption stage), analytes were injected into the chromatographic column for further separation, which took about 48 minutes. Molecules reaching the ToFMS detector were fragmented by electron impact ionization (EI) at 70 eV at a mass range of 28-330 amu.

Deuterated toluene-d8 (Sigma Aldrich) was used as an external standard for quantification. This compound was injected (10 ng) into an independent thermodesorption tube and was analyzed by following strictly the same methodology as with the samples. Given the sensitivity of the method, two unused thermodesorption tubes were analyzed as blanks, in order to exclude any potential contamination arising from these materials during the analysis.

2.3. Data processing

The deconvolution process for the chemical identification of the VOCs that were present in each analyzed sample was carried out with the software TargetView v3 (ALMSCO International, Germany). This algorithm identified the compounds of the chromatogram automatically based on an updated version of the NIST11 library [19]. Chemical identifications were confirmed with at least 80% certainly. The identified molecules were

manually check for their presence in the Microbial Volatile Organic Compound Database (mVOC), so that potential microbial metabolites were identified [20]. The air to water partition coefficients (Henry constant) was estimated from the Estimation Programs Interface (EPI) Suite™ v4.1 (US Environmental Protection Agency, USA). General information on the usage and occurrence of the identified chemicals was obtained from the PubChem database [21].

3. RESULTS AND DISCUSSION

3.1. Profiling of the indoor air VOCs

Seventy-one VOCs belonging to 13 chemical families were identified and quantified in the headspace of a Nalophan™ sampling bag filled with a model laboratory indoor air, after 24 hours of incubation in the presence of non-inoculated agar plates (*Indoor* abiotic control; Table 2). The contribution of the agar plates and the sampling bag material to the observed VOCs profile was evaluated in a second bag filled only with nitrogen gas and incubated under the same conditions (*Materials* control). Thirty-three VOCs were measured in this latter control (125.4 $\mu g \cdot m^{-3}$), 20 of which were also found in the *Indoor* control. The most abundant compounds (>10 $\mu g \cdot m^{-3}$) 3-methylbutanal, 2-methylpropanal and acetone have previously been identified as important volatiles released in baked potato flesh [22, 23], and might therefore arise from the PDA cultivation medium used in this study. Other characteristic VOCs from baked potatoes were also detected in minor amounts the *Materials* control (<10 $\mu g \cdot m^{-3}$): pentanal, heptanal, nonanal, ethyl acetate, and methanethiol. Most of the remaining 13 VOCs that were exclusive from the *Materials* control were present at low amounts (<1 $\mu g \cdot m^{-3}$) and might just be below the detection limit in the *Indoor* control. Trace VOCs contained in the *Materials* control might arise from environmental contaminants trapped in the agar.

The measured total VOCs concentration in the *Indoor* control of 1.4 $mg \cdot m^{-3}$ was slightly above most values reported for non-industrial indoor environments, which are usually below 1 $mg \cdot m^{-3}$ [1]. The European Collaborative Action on Indoor Air Quality (ECA-IAQ) recommends a minimum list of 63 VOCs to be included in non-industrial indoor air quality investigations [1]. Yet, only 15 of those reference VOCs were found in the present study (Table 2). This apparent quantitative and qualitative specificity of the studied VOCs profile is because of its representativeness of a laboratory environment, rather than the domestic, office, and public environments discussed in the ECA-IAQ report. The IRTA premises comprise an array of research laboratories on analytical chemistry, molecular biology, plant physiology and animal sciences. It is thus not surprising to find common solvents among the most abundant VOCs (50 – 250 $\mu g \cdot m^{-3}$): pentane, tetrachloroethylene, toluene, dichloromethane, ethanol, trichloromethane (chloroform) and acetone [21]. Low amounts of the laboratory-related chemicals methyl ethyl ketone, hexane, heptane, 1,2,3,4-tetrahydronaphthalene (tetralin) and of the broad-spectrum antibiotic cycloserin, used as an additive in microbial selective cultivation media, were also detected (Table 2).

Ubiquitously present xenobiotics were found as well, like the halomethanes dichlorodifluoromethane (Freon 12), used as a refrigerant and spray propellant prior to the 1994 Montreal Protocol ban because of its ozone layer-depletion potential, and bromodichloromethane primarily formed as a by-product from drinking water chlorination [21]. Several fuel-related chemicals were detected, like BTEX (benzene, toluene, ethylbenzene and xylene isomers) and complex monoaromatic hydrocarbons substituted with multiple alkyl groups present in kerosene and gasoline blends, gasoline oxygenates 2-methoxy-2-methylpropane (MTBE) and 2-ethoxy-2-methylpropane (ETBE), and C4-C5 aliphatic hydrocarbons that probably originate from gas cylinders and spray propellants. Other VOCs might be emitted from manufactured materials from the indoor environment, such as

plastics, resins and rubber (styrene, cyclopentane, dibutyl phthalate, methacrolein, and benzocyclobutane) [21].

A few compounds might have a natural origin like carbonyl sulfide, the most abundant sulfur compound naturally present in the atmosphere (the concentration reported here fits in the range of the natural background), and even biological like the plant metabolite and aroma compound 3-hexenyl benzoate. In fact, 41 of the identified VOCs from the indoor air (58%) have also been reported as microbial volatile metabolites [20], and so they could have a biological origin. It is unlikely that such microbial intermediates arise from the biodeterioration of building materials, but some of them could be released from biological samples that are processed in the aforementioned laboratories (digestate from anaerobic reactors, compost, plant and animal materials, etc.).

3.2. Fungal metabolism of VOCs

Important quantitative and qualitative differences were observed between the VOCs profiles of the *Indoor* sample, used here as an abiotic control, and those from fungal incubations (Tables 2 and 3). The presence of fungal biomass reduced the mass of the VOCs present in the indoor air by 99.6%, 98.9%, and 98.4% when incubated with cultures of *C. cladosporioides*, *C. psammophila*, and *N. acidophila* (Table 4). A few VOCs were reduced by less than 90%, which ranged from the biodegradable hydrophilic methanol and hydrophobic butane, to the more recalcitrant benzene, dibutyl phthalate, and cycloserine. Some of these VOCs are known to be fungal volatile metabolites as well and so a low reduction, and even increase in some cases, might be the result from biosynthesis. In fact, 42 VOCs from the fungal incubations were not detected in the abiotic control and, thus, must be considered as de novo biosynthesized fungal metabolites (Table 3). The fact that only 18 of these fungal metabolites are reported as such in the Microbial Volatile Organic Compound Database

(mVOC) highlights our limited knowledge on the chemical diversity of volatile fungal metabolites. Information compiled in the mVOC database is limited to relatively few fungal species that are relevant in the production of aromas and as spoilage bioindicators in food, biodeterioration of materials, as semiochemicals in biotic interactions, and in medical diagnostics [20].

Cladosporium cladosporioides displayed both the highest elimination of indoor VOCs and emission of volatile metabolites (49.5 µg·m^{-3}, 17 compounds; Tables 3 and 4). Such observation could be because of the larger biomass/air contact area of this fungus, which also grows at a much faster rate than the other tested strains. *Cladosporium cladosporioides* has frequently been reported in SBS cases and was included in this study as a reference. A previous comparative qualitative assessment concluded that *C. cladosporioides* produced far less volatile metabolites than other common SBS species, such as *Aspergillus versicolor*, *Paecilomyces variotii*, and *Penicillium commune* [24]. The VOCs profile of *C. cladosporioides* in that study comprised 1-octene, 3-methylfuran, 3-pentanone and minor amounts of an unidentified sesquiterpene. The sesquiterpene caryophyllene was later proposed as a bioindicator of apple contamination with this fungus [25]. Here, we were able to quantify 17 VOCs for *C. cladosporioides*, which included relatively high concentrations of 1-octene (15.5 µg·m^{-3}), while 3-methylfuran and caryophyllene were also found in minor amounts (Table 3). Trace contents of other terpenes (ß-pinene, D-limonene, and *o*-cymene) were also detected as specific metabolites of *C. cladosporioides*. Interestingly, iodomethane was the most abundant fungal metabolite from the whole study (24.6 µg m^{-3}) and was also produced exclusively by this fungus. Biogenic emissions of halomethanes were previously reported for ectomycorrhizal fungi and have been considered as an important natural source of ozone-depleting gases [26]. *Cladosporium cladosporioides* has also been associated with an accumulation of carbonyl sulfide, which was already present in the indoor air. This compound

was detected in filters from HVAC units colonized with *Cladosporium* and *Penicillium* species as compared with non-colonized units [27].

The concentration of newly formed VOCs reached its lowest value with *C. psammophila*, but the number of identified compounds was instead the most numerous (8.6 µg m^{-3}, 20 compounds; Table 3). Interestingly, in the comparative assessment mentioned earlier [24], no volatile metabolites were detected for closely related *Phialophora fastigiata*, also in the *Chaetothyriales*. Here, the most abundant metabolite, which appears to be exclusive of *C. psammophila*, was 3-methyl-1-butanol (2.4 µg m^{-3}). An increased concentration (1.8 µg m^{-3}) of 2-methyl-1-propanol, already present in the indoor air, was also detected along with trace amounts (< 1 µg m^{-3}) of the isomer 2-methyl-1-butanol. All these three organic alcohols have been reported among the most commonly produced volatile metabolites by SBS fungi [24]. Methylamine was detected as the second most abundant metabolite (1.1 µg m^{-3}) and was also emitted by the other studied fungi, though in lower amounts. Its presence might be explained as product of the arginine demethylation pathway in eukaryotic organisms [28]. The remaining metabolites were below 1 µg·m^{-3} and were characterized by the occurrence of several aliphatic and alkylbenzene hydrocarbons, largely absent in the other fungi. Whether or not this specific profile is related to the metabolic capacity of *C. psammophila* to grow on certain alkylbenzenes as the sole source of carbon and energy [29], remains as an interesting research question.

The slowest growing cultures of *N. acidophila* displayed a somewhat intermediate profile in quantitative terms in the emission of new VOCs (15.8 µg m^{-3}, 16 compounds; Table 3). The main metabolite 1-(dimethylamino)-2-propanone (10.1 µg m^{-3}) was present well above the rest of the emitted volatile compounds (<1 µg m^{-3}). Specific metabolites for this fungus included decanal, 2-methyl-1-pentene, octane, nonane, acetophenone, dimethyl sulfide, methanethiol, diisobutyl phthalate, 2-methyl-2-oxazoline, and (dimethylamino)acetone. Other

minor metabolites were shared with *C. psammophila*: 1-pentene, 1-heptene, nitroethane, and cyclofenchene. Several of these compounds are already known from previous research on the SBS [3, 5, 30, 31] and on fungal metabolites [20], but some others might have been detected for the first time thanks to the very high sensitivity of the implemented GC-ToFMS analytical approach.

3.3. Potential application of melanized fungi for removing indoor VOCs

To this day, a satisfactory technology for the removal of indoor VOCs has yet to be developed [10, 11]. Physicochemical processes have the disadvantage of requiring relatively high amounts of energy and chemicals (UV light, ozone), expensive maintenance and materials (membranes), or generate wastes that require further treatment (activated carbon). Biological processes might therefore be preferred, particularly in completely confined environments where resources are scarce. However, the VOCs elimination efficiency in conventional biofilters is affected in the long term by clogging due to the excess of biomass. Using specialized slow-growing fungi such as *Cladophialophora psammophila* and *Neohortaea acidophila*, rather than the generalist and faster-growing *Cladosporium cladosporioides*, might therefore be an advantage (Table 4). The mass-transfer of hydrophobic contaminants from air to the liquid/biofilm phase can also limit the biofiltration process, particularly in the treatment of indoor air [32]. The air/water partition coefficient (Henry's Law constant; K_{AW}) has been proposed as an indicator for the VOCs susceptibility of being removed by biological systems, so that the biofiltration treatability limit would be for VOCs with a $K_{AW} < 10$ [33]. Yet, we have previously shown that in scarcely irrigated biofilters packed with hydrophobic supports, VOCs with a very high K_{AW} can still be removed from complex mixtures [34]. Sustained operations under such conditions requires the enrichment of xerophilic microorganisms that are able to thrive and biodegrade the adsorpted COVs.

The results from the present study with melanized fungi are very promising in that the balance between elimination of indoor VOCs and volatilization of metabolites was clearly in favor of the first (Table 4). Considering both elimination and generation, the fungal cultures reduced the total VOCs content by 96.1% (*C. cladosporioides*), 98.3% (*C. psammophila*), and 97.3% (*N. acidophila*). The very hydrophobic aliphatic hydrocarbons ($K_{AW} > 10$) were effectively removed below the detection limit (0.1 $\mu g \cdot m^{-3}$; Table 2). The most likely explanation for these very high removal efficiencies is because of the adsorption of VOCs to the hydrophobic fungal wall. A comparative study between a number of melanized and hyaline fungal strains [35], showed that the highest hydrophobicity occurred in strains of *C. cladosporioides* and *Cladosporium minourae*. The second species has recently been reclassified as *Cladophialophora minourae*, within the order *Chaeothyriales* [36], and is a close relative of *C. psammophila*. Our results would also agree with previous observations in HVAC units of a multi-story office, in that colonization with *Cladosporium* spp. (along with *Penicillium* spp.) was related to less VOCs emissions, compared to other units with no fungal presence [27].

Cell hydrophobicity might be an evolutionary trait of the black yeasts for scavenging traces of volatile substrates and survive on very little available organic carbon, thus explaining oligotrophy in several species in the group. Recent laboratory evidence based on fungal species in both *Chaetothyriales* and *Capnodiales* suggest that, besides a protective role, melanin might also act as an antenna pigment that captures high energy photons (from ultraviolet to gamma rays) and yields metabolic energy for growth [37]. The concurrence of such extraordinary physiological traits: xerotolerance, oligotrophy, radiotrophy, low emissions of volatiles, cell-wall hydrophobicity, and biodegradation capacity, makes black yeasts ideal candidates for the biofiltration of indoor air in CBLSS.

However, biosafety assurance is a key issue in the implementation of biological solutions for the treatment of indoor air. All strains tested in this study have been classified as BSL1, category that comprises well-characterized microbes which do not cause disease in healthy humans. Yet, there are some specific issues that must be considered when selecting a candidate species for application. The main concern with *C. cladosporioides* (*Capnodiales*) is its fast growth and profuse production of spores, which are prone to aerosolization. A vacuolar serine protease has been identified as a major allergen in this fungus, which might cause rhinitis and even severe asthma when spores are inhaled [38]. This fungus has sporadically been reported in cutaneous and subcutaneous infections, generally in immunocompromised individuals [17].

The biosafety of antropized environments regarding the presence of black yeasts from the *Herpotrichiellaceae* family (*Chaetothyriales*) is currently under discussion. Extremophilicity and utilization of toxic alkylbenzenes, but also the capacity to cause severe infections in otherwise healthy individuals appear to be common ecological traits in several species from this group [12]. Recent studies have demonstrated that herpotrichiellaceous black yeasts are more common in the domestic environment than previously thought [39]. Conversely to the countless number of *C. cladosporioides* strains that are available in public culture collections, to this day only one isolate of *C. psammophila* is known [29]. This fungus was isolated from a gasoline contaminated soil and has de capacity to grow on alkylbenzenes as sole carbon and energy sources, feature that appears to be shared by other related species in the genus *Exophiala* that are classified under BSL2 [40]. No direct evidence of alkylbenzene assimilation has been reported for the related and very dangerous BSL3 pathogenic *Cladophialophora bantiana* and *Rhinocladiella mackenziei*, known exclusively from clinical cases, but recent genomic evidence points to the existence of specific genes for the

assimilation of alkylbenzenes in the later species [41]. However, despite its phylogenetic proximity to *C. bantiana, C. psammophila* has proven to be non-pathogenic [29].

While phylogeny and ecophysiology delimitations in the *Herpotrichiellaceae* are still in progress, the use of specialized and slow-growing fungi from the *Capnodiales* offers an alternative with minimum biohazard. *Neohortaea acidophila* is also known from a single isolate obtained from extremely acidic lignite and so it might display a highly specialized ecology [15]. It excretes and acidotoloerant laccase for the biodegradation of phenolic compounds and forms slimy non-sporulating yeast-like colonies under common culture conditions. Niche specialization, low growth rates under common culture conditions, and non-sporulation might also prevent dispersion. Growth under acidic conditions might prevent microbial contamination with other species and favor the absorption of ammonia, a common inorganic indoor air contaminant. However, the related halophilic species *Hortaea werneckii* can grow in saturated NaCl solutions but it also causes tinea nigra, a rare superficial and non-invasive skin infection [42].

4. CONCLUSIONS

A total of 71 volatile chemicals (total concentration 1.4 $mg \cdot m^{-3}$) were identified and quantified above the detection limit (0.1 $\mu g \cdot m^{-3}$) in a model laboratory indoor air. Such complex VOCs profile contained several xenobiotic and biogenic compounds, and thus might act as a proxy indoor air for highly technified locked environments, such as those in prolonged space missions. The total VOCs content from this model indoor air was reduced by more than 96% when incubated in the headspace of confined pre-grown agar cultures of black yeasts, after 48 hours of exposure and in relation to the abiotic control. The hydrophobicity of the fungal cell wall is proposed as a major factor in the removal of VOCs from the gas phase. The three assayed species belonging to the orders *Capnodiales* and *Chaetotyriales* also

produced 42 putative volatile metabolites, which were rather species-specific. Most of these fungal metabolites were present at the low concentration range (<1 $\mu g \cdot m^{-3}$) and had a little quantitative impact on the final total VOCs concentration. The VOCs emission profile of *C. cladosporioides* (*Capnodiales*), a ubiquitous and well-known species often associated to the SBS, was consistent with previous literature reports. The other two selected species, *C. psammophila* (*Chaetothyriales*) and *N. acidophila* (*Capnodiales*) known from single isolations, display a rather specialized ecophysiology associated to the biodegradation of recalcitrant compounds with no history of clinical cases, and are characterized by slow-growing yeast-like colonies in agar cultures. Therefore, these species might represent suitable biocatalysts for the treatment of indoor air polluted with VOCs. Further research is still needed on the phylogeny and ecophysiology of the black yeasts in order to guarantee the biosafety and scalability of biofiltration applications based on these fungi.

Acknowledgements

This research was supported by the CERCA Programme / Generalitat de Catalunya and by the IRTA Seed Research Fund Program for the application of fungi in space biotechnology.

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TABLES

Table 1. Analytical parameters of the TD-GC-ToFMS method implemented in the present study.

Unit	Parameter	Value	Unit
Thermal Desorption	He flow	50	$mL \cdot min^{-1}$
	Desorption time	10	min
	Desorption temperature	300	ºC
	Desorption flow	50	$mL \cdot min^{-1}$
	Cold trap temperature	10	ºC
	Final cold trap temperature	320	ºC
	Trap hold	5	min
Gas-Chromatographer	Initial temperature	40	ºC
	Time	5	Min
	Rate	2	$ºC \cdot min^{-1}$
	Final temperature	45	ºC
	Time	48.5	Min
	Rate	5	$ºC \cdot min^{-1}$
	Final temperature	230	ºC
	Time	4	Min
Mass Spectrometer	He flow	1.6	$mL \cdot min^{-1}$
	Column type	DB-624 (60 m; 250 µm; 1.4 µm)	
	Filament	1.6	V
	m/z range	28-330	uma
	Transfer line temperature	250	ºC
	Ion Source temperature	230	ºC

Table 2. Quantification of VOCs detected after 48 hours of confined incubation of non-inoculated agar plates with nitrogen gas (Materials) and the tested indoor air (Indoor), as well as agar plate cultures of *C. cladosporioides* (*Ccl*), *C. psammophila* (*Cps*), and *Neohortaea acidophila* (*Nac*) with the indoor air. The air/water partition coefficient and environmental occurrence have also been indicated.

Chemical family and compound	CAS nr	K_{AW} [a]	Occurrence [b]	Concentration ($\mu g \cdot m^{-3}$) [c]				
				Materials	Indoor	*Ccl*	*Cps*	*Nac*
Alcohols								
Methanol	67-56-1	1.74E-04	*Fm, Bm*	4.8	4.1	1.4	0.5	7.4
Ethanol	64-17-5	2.32E-04	*Fm, Bm*	19.4	151.3	–	–	–
1-Propanol	71-23-8	3.08E-04	*Fm, Bm*	0.1	–	–	–	–
1-Butanol	71-36-3	4.08E-04	*SBS, Fm, Bm*	–	10.0	–	–	–
2-Butanol	78-92-2	4.08E-04	*Fm, Bm*	–	0.1	–	–	–
2-Methyl-1-propanol	78-83-1	4.08E-04	*Fm, Bm*	0.1	0.8	–	1.8	0.2
Propargyl alcohol	107-19-7	2.40E-05	–	–	0.3	–	–	–
Aldehydes								
Acetaldehyde	75-07-0	2.77E-03	*Fm, Bm*	–	9.3	2.6	4.5	6.9
2-Methylpropanal	78-84-2	4.89E-03	*Fm, Bm*	17.3	17.4	–	–	–
Methacrolein	78-85-3	2.29E-03	–	–	5.3	–	–	–
Propanal	123-38-6	3.68E-03	*Fm, Bm*	0.4	–	–	–	–
2-Propenal	107-02-8	1.46E-03	–	0.1	–	–	–	–
Butanal	123-72-8	4.89E-03	*SBS, Fm*	4.2	10.2	–	–	–
3-Methylbutanal	590-86-3	6.49E-03	*Fm, Bm*	35.5	109.8	–	–	–
2-Methylbutanal	96-17-3	6.49E-03	*Fm, Bm*	–	38.9	–	–	–
Pentanal	110-62-3	6.49E-03	*SBS, Fm, Bm*	0.3	0.1	–	–	–
Heptanal	111-71-7	1.14E-02	*Fm, Bm*	0.1	–	–	–	–
Nonanal	124-19-6	2.02E-02	*SBS, Fm, Bm*	0.7	0.9	–	–	–
Benzeneacetaldehyde	122-78-1	2.24E-04	*Fm, Bm*	–	0.8	–	–	–
Carboxylic acids								
Acetic acid	79-20-9	2.24E-05	*Fm*	0.1	–	–	–	–
Formic acid	107-31-3	3.07E-05	–	0.1	–	–	–	–
Isovaleric acid	503-74-2	5.24E-05	*Bm*	0.1	–	–	–	–
cis-3-Hexenyl benzoic acid	25152-85-6	5.15E-03	–	–	0.7	–	–	0.4
Aliphatic hydrocarbons								
Isobutane	75-28-5	3.96E+01	–	5.6	2.3	–	0.2	–
Butane	106-97-8	3.96E+01	*Bm*	1.2	2.6	0.3	0.7	–
Propane	74-98-6	2.98E+01	*Fm, Bm*	1.4	–	–	–	–
(E)-2-Butene	624-64-6	9.82E+00	–	–	0.1	–	–	–

2-Methyl-1-propene	115-11-7	9.82E+00	–	–	1.7	–	–	–
2-Methyl-butane	78-78-4	5.26E+01	–	–	0.7	–	–	–
3-Methyl-1-butyne	598-23-2	1.16E+00	–	–	0.1	–	–	–
Pentane	109-66-0	5.26E+01	*Fm*	–	107.7	–	–	–
n-Hexane	110-54-3	6.98E+01	*SBS, Fm*	–	12.5	–	2.1	0.5
Heptane	142-82-5	9.27E+01	*SBS, Fm, Bm*	–	8.9	–	–	–
Aromatic hydrocarbons								
Benzene	71-43-2	2.20E-01	*SBS, Fm*	–	2.1	–	4.8	5.1
Toluene	108-88-3	2.43E-01	*SBS, Fm, Bm*	–	203.0	–	–	–
Ethylbenzene	100-41-4	3.23E-01	*SBS, Fm, Bm*	–	3.0	–	–	–
m-, *p*-Xylene	108-38-3/ 106-42-3	2.68E-01	*SBS, Fm, Bm*	–	3.4	–	–	–
Styrene	100-42-5	1.13E-01	*SBS, Fm, Bm*	–	84.2	–	–	–
Propylbenzene	103-65-1	4.28E-01	*SBS*	–	0.7	–	–	–
1-Ethyl-3-methylbenzene	620-14-4	3.56E-01	*Fm, Bm*	–	1.2	–	–	–
1,2,3-Trimethylbenzene	526-73-8	2.96E-01	*Bm*	–	0.2	–	0.4	–
1-Ethyl-4-methylbenzene	622-96-8	3.56E-01	–	–	0.2	–	–	–
(1-Methylpropyl)-benzene	135-98-8	5.69E-01	–	–	0.1	–	–	–
1,2,4-Trimethylbenzene	95-63-6	2.96E-01	*SBS, Fm, Bm*	–	0.3	–	–	–
1,3-Diethylbenzene	141-93-5	4.73E-01	–	–	0.6	–	–	–
1-Ethenyl-2-methylbenzene	611-15-4	1.25E-01	–	–	0.6	–	–	–
Butylbenzene	104-51-8	5.69E-01	–	–	0.3	–	–	–
1,2-Diethylbenzene	135-01-3	4.73E-01	–	–	0.2	–	–	–
1-Ethyl-2,3-dimethylbenzene	933-98-2	3.93E-01	–	–	0.8	–	–	–
1,2,4,5-Tetramethylbenzene	95-93-2	3.27E-01	–	–	0.3	–	–	–
2-Ethenyl-1,3-dimethylbenzene	2039-90-9	1.38E-01	–	–	0.6	–	–	–
Cyclic hydrocarbons								
Cyclopropane	75-19-4	4.46E+00	–	–	0.8	0.1	0.1	0.5
Cyclopentane	287-92-3	7.86E+00	–	–	0.7	–	–	–
1,2,3,4-Tetrahydronaphthalene	119-64-2	2.08E-01	*Bm*	–	0.5	–	–	–
1-Methyl-indan	767-58-8	2.08E-01	–	–	0.9	–	–	–
Esters								
Methyl acetate	79-20-9	1.17E-03	*Fm*	0.1	0.1	–	–	–
Ethyl acetate	141-78-6	9.52E-03	*SBS, Fm, Bm*	1.6	2.7	–	–	–
Dibutyl phthalate	84-74-2	5.00E-05	*Bm*	–	0.3	–	0.3	–
Ethers								
Dimethyl ether	115-10-6	3.52E-02	*Fm, Bm*	1.1	0.1	–	–	–

2-Methoxy-2-methylpropane	1634-04-4	8.25E-02	–	–	0.1	–	–	–
2-Ethoxy-2-methylpropane	637-92-3	1.09E-01	–	–	2.7	–	–	–
Ketones								
Acetone	67-64-1	2.03E-03	*Fm, Bm*	22.6	64.8	–	–	–
Methyl ethyl ketone	78-93-3	2.69E-03	*Fm, Bm*	4.0	6.1	–	–	–
Methyl isobutyl ketone	108-10-1	4.74E-03	*Fm, Bm*	–	0.1	–	–	–
Furans								
Furan	110-00-9	2.20E-01	*Fm, Bm*	1.1	2.3	–	–	–
2-Methylfuran	534-22-5	2.43E-01	*Fm, Bm*	–	0.2	–	–	–
2-Ethyl-furan	3208-16-0	3.22E-01	*Fm, Bm*	–	0.2	–	–	–
Organohalogen compounds								
Dichloromethane	75-09-2	3.74E-01	–	0.5	185.1	–	–	–
Trichloromethane	67-66-3	1.32E-01	*Fm, Bm*	0.2	73.8	–	–	–
Dichlorodifluoromethane	75-71-8	1.17E+01	–	–	0.2	–	–	–
Bromodichloro-methane	75-27-4	4.31E-02	–	–	0.2	–	–	–
Tetrachloroethylene	127-18-4	6.76E-01	*SBS, Bm*	–	232.9	–	–	–
Benzoyl bromide	618-32-6	3.16E-03	–	–	8.7	–	–	–
Nitrogen organic compounds								
Acetonitrile	75-05-8	1.25E-03	*Fm, Bm*	–	15.4	–	–	–
Butyronitrile	109-74-0	2.20E-03	–	0.1	–	–	–	–
Isobutyronitrile	78-82-0	2.20E-03	–	–	0.1	–	–	–
2-Methylbutanenitrile	18936-17-9	2.92E-03	–	0.1	–	–	–	–
Cycloserine	68-41-7	2.65E-10	–	–	0.5	–	–	0.3
Organosulfur compounds								
Methanethiol	74-93-1	1.07E-01	*Fm, Bm*	0.1	–	–	–	–
Carbonyl sulfide	463-58-1	2.01E+00	–	–	0.3	0.8	–	–
Carbon disulfide	75-15-0	1.24E+00	*Fm, Bm*	0.9	14.5	–	–	–
Dimethyl disulfide	624-92-0	4.96E-02	*Fm, Bm*	0.1	0.8	–	–	1.1
Organosilicon compounds								
Hexamethylcyclotrisiloxane	541-05-9	2.60E+00	*Bm*	1.0	–	–	–	–
Octamethylcyclotetrasiloxane	556-67-2	3.57E+00	–	0.4	–	–	–	–
			Total concentration:	125.4	1413.5	5.2	15.4	22.4
			Number of compounds:	33	71	5	10	9

[a] Obtained from the EPISuit™ interface (bond estimate).

[b] *SBS*: priority pollutant in relation to the SBS, as listed in the ECA-IAC report [1]. *Fm*: fungal metabolite and *Bm*: bacterial metabolites according to the mVOC database [20].

[c] Quantification in relation to deuterated toluene, added as an external standard.

– : not reported or not detected.

Table 3. Quantification of fungal volatile metabolites detected after 48 hours of confined incubation of cultures of *C. cladosporioides* (*Ccl*), *C. psammophila* (*Cps*), and *Neohortaea acidophila* (*Nac*). The air/water partition coefficient and environmental occurrence have also been indicated.

Chemical family and compound	CAS nr	K_{AW} [a]	Occurrence [b]	Concentration (µg·m^{-3}) [c]		
				Ccl	*Cps*	*Nac*
Alcohols						
2-Methyl-2-propanol	75-65-0	4.08E-04	–	0.2	0.2	0.2
3-Methyl-1-butanol	123-51-3	5.42E-04	*Fm, Bm*	–	2.4	–
(S)-2-Methyl-1-butanol	1565-80-6	5.42E-04	–	–	0.7	–
Aldehydes						
Benzaldehyde	100-52-7	5.49E-04	*SBS, Fm, Bm*	0.5	–	0.5
Decanal	112-31-2	2.68E-02	*Fm, Bm*	–	–	0.5
Aliphatic hydrocarbons						
1-Pentene	109-67-1	1.10E+01	*Fm*	–	0.1	0.1
2-Methyl-pentane	107-83-5	6.98E+01	*SBS, Fm*	0.2	–	–
2-Methyl-1-pentene	763-29-1	1.73E+01	–	–	–	0.2
1-Heptene	592-76-7	1.95E+01	*Fm*	–	0.4	0.8
1-Octene	111-66-0	2.58E+01	*Fm*	15.5	–	–
Nonane	111-84-2	1.63E+02	*SBS, Fm, Bm*	–	–	0.3
2,2-Dimethyl-octane	15869-87-1	2.17E+02	–	–	0.1	–
Aromatic hydrocarbons						
1,3,5-Trimethylbenzene	108-67-8	2.96E-01	*Fm, Bm*	–	0.1	–
2-Ethyl-1,4-dimethylbenzene	1758-88-9	3.93E-01	–	–	0.2	–
1-Ethyl-2,4-dimethylbenzene	874-41-9	3.93E-01	*Fm*	–	0.8	–
1,2,3,4-tetramethylbenzene	488-23-3	3.27E-01	–	–	0.3	–
Cyclic hydrocarbons						
Cyclopentene	142-29-0	2.04E+00	*Bm*	0.1	–	–
Cyclooctene	931-88-4	4.77E+00	*Fm*	0.8	–	–
Cyclofenchene	488-97-1	6.30E+00	–	–	0.3	0.3
Esters						
Benzyl acetate	140-11-4	5.79E-04	*Bm*	0.4	–	–
Methyl benzoylformate	15206-55-0	5.65E-06	–	–	0.3	–
Diisobutyl phthalate	84-69-5	5.00E-05	–	–	–	0.3
Ethers						
1,3-Dioxolane	646-06-0	9.12E-04	–	0.1	–	–

2-Methyl-1,3-dioxolane	497-26-7	1.21E-03	–	4.0	0.5	–
Ketones						
Cyclohexanone	108-94-1	2.09E-03	*SBS, Bm*	–	0.2	–
Acetophenone	98-86-2	4.01E-04	*SBS, Fm, Bm*	–	–	0.4
1-Cyclopropyl-ethanone	765-43-5	1.57E-03	–	–	0.1	–
Furans						
3-Methylfuran	930-27-8	2.43E-01	*Fm, Bm*	0.2	–	–
Tetrahydrofuran	109-99-9	3.45E-03	*SBS*	–	0.2	–
Organohalogen compounds						
Iodomethane	74-88-4	2.25E-01	*Bm*	24.6	0.1	–
1,3,5-Trifluorobenzene	372-38-3	3.50E-01	–	0.1	0.1	–
Nitrogen organic compounds						
Nitroethane	79-24-3	2.29E-03	–	–	0.4	0.3
2-Methyl-2-oxazoline	1120-64-5	1.36E-03	–	–	–	0.1
(Dimethylamino)acetone	15364-56-4	2.00E-05	–	–	–	10.1
Methylamine	74-89-5	3.10E-04	*Bm*	0.6	1.1	0.7
Organosulfur compounds						
Dimethyl sulfide	75-18-3	3.21E-02	*Fm, Bm*	–	–	0.9
Terpenes						
ß-Pinene	127-91-3	6.59E+00	*SBS, Fm, Bm*	0.4	–	–
D-Limonene	5989-27-5	1.55E+01	*SBS*	0.1	–	–
o-Cymene	527-84-4	4.73E-01	*Fm, Bm*	0.4	–	–
ß-Caryophyllene	87-44-5	2.82E+01	*Fm*	1.1	–	–
			Total concentration:	49.5	8.6	15.7
			Number of compounds:	17	20	16

[a] Obtained from the EPISuit™ interface (bond estimate).

[b] *SBS*: priority pollutant in relation to the SBS, as listed in the ECA-IAC report [1]. *Fm*: fungal metabolite and *Bm*: bacterial metabolites according to the mVOC database [20].

[c] Quantification in relation to deuterated toluene, added as an external standard.

– : not reported or not detected.

Table 4. Fungal growth kinetics and VOCs mass balance in PDA plate cultures of *C. cladosporioides* (*Ccl*), *C. psammophila* (*Cps*), and *Neohortaea acidophila* (*Nac*) after confined incubations with 2 L of a model laboratory indoor air during 48 hours.

Fungal growth kinetics / VOCs mass balance	*Ccl*	*Cps*	*Nac*
Pre-growth incubation time (d)	11	21	28
Radial growth rate ($mm \cdot d^{-1}$)	2.10	0.45	0.14
Apparent fungal/air contact area during VOCs exposure (cm^2)	50.2	25.0	4.6
Indoor air VOCs mass (ng)	5.2	15.4	22.4
Fungal volatile metabolites mass (ng)	49.5	8.6	15.7
Total VOCs mass (ng)	54.7	24.0	38.1
Indoor air VOCs removal efficiency (%) [a]	99.6	98.9	98.4
Overall VOCs removal efficiency (%) [a, b]	96.1	98.3	97.3

[a] In relation to the total VOCs mass of 2827 ng present in the indoor air of the abiotic control.

[b] Including the *de novo* generated fungal metabolites.