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# CHAPTER 6:

# CHARACTERIZING OUTDOOR AIR USING MICROBIAL VOLATILE ORGANIC COMPOUNDS (MVOCS)

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## ABSTRACT

Exposure to bioaerosols containing airborne microorganisms and their by-products from outdoor environments such as industrial, urban or agricultural sites is of great concern as it is linked to adverse health effects in humans including respiratory diseases and infections. The risk exposure from outdoor emissions is difficult to quantify in real-time as the microbial concentration in air is low and varies depending on meteorological factors, anthropogenic activities, and sampling conditions. In addition, the collection of sufficient amount of sample to generate statistically distinguishable and reproducible patterns to characterize and quantify bioaerosols is still a challenge, and this analysis cannot be performed in real time yet. Microbial volatile organic compounds (MVOCs) can be used to chemically characterize ambient bioaerosols and identify pathogens early in air overcoming the inherent limitations of culturing. This book chapter aims to critically review the sampling techniques and analytical approaches that are currently available for the study of MVOCs from industrial, agricultural and rural emissions. Current challenges in MVOCs sample collection, analytical and speciation analysis are addressed, and recommendation for the implementation of a rapid, reproducible and sensitive analytical framework for fingerprinting bioaerosols is provided.


## 6.1 INTRODUCTION

Bioaerosols are defined as the biological particles present in aerosols that originate from plants, animals and microbes [1]. They are ubiquitous in the environment, and their small particle size ranging between 10 nm and 2.5 μm means that they easily propagate in air [2]. Among bioaerosols, some are pathogens, and recent studies have shown that chronic exposure can induce adverse human health effects such as respiratory diseases, infections [3] and asthma [4,5]. Consequently, bioaerosols emissions from industrial, urban and agricultural environments such as wastewater treatment plants (WWTP), composting facilities and other bio-waste processing plants have raised public concerns as the concentrations can be potentially high and cause a negative impact on local air quality [6,7].

Current bioaerosol monitoring methods can be classified into cultural, molecular and chemical techniques [8,9] (Table 6. 1). Although less than 1% of viable microbes are cultivable under standard laboratory conditions, conventional culturing methods have been widely used for bioaerosol studies. These techniques are easy to use and can allow microbial identification at species level, but the results often underestimate the microbial quantity and diversity, are labor intensive and not very reproducible [10]. Molecular techniques are more expensive but are very reproducible allowing the

microbial detection at very low concentrations. These techniques include immunochemistry, flow cytometry, microscopy [11], pyrosequencing, DNA and RNA techniques [12] as well as phospholipid fatty acids (PLFAs) [7,13]. Molecular techniques have the advantage compared to culture techniques of being reproducible and that microbial species can be identification at species level (except with PLFAs analysis that are only specific to a certain microbial group and they give information about the structure of the microbial community). The disadvantages of molecular techniques are, for instance, that the extraction protocols are long and expensive and the data cannot be obtained in real time. Chemical techniques are getting increased interest for the characterization of bioaerosols as they offer reproducible, accurate and reliable analysis, are sensitive allowing the detection of the chemicals in low concentrations and are rapid, inexpensive and can perform real time analysis. Chemical markers used for bioaerosol characterization include biological particles, species-specific proteins, and MVOCs. Chemical techniques focusing on the discrimination between biological and non-biological particles are the fluorescent aerodynamic particle sizer (FLAPS) [7], Spectral Intensity Bioaerosol Sensor (SIBS) [14] and Wideband Integrated Bioaerosol Sensor (WIBS) [15]. These sensors operate using light scattering and fluorescence detection and can work over long periods of time offering real time results but disadvantageously are very heavy and produce a large amount of data which is difficult to process. Carbon nanotube based biosensor is another sensing technique that monitors continuously two different airborne fungal species simultaneously [9]. This device is not designed to be portable yet, and it needs to be configured to detect more microbial species. There are also biosensors based on microfluidic techniques that collect the air sample directly into the liquid of a microfluidic cartridge and concentrate it (from L to μL) prior the detection with an integrated biosensing [16,17]. Mass spectrometers are also applied for the chemical characterization of bioaerosols. The Bioaerosol Mass Spectrometer (BMAS) discriminates between microbial species by the mass spectrum analysis of species-specific proteins and peptides[18]. Conventional GC-MS allow the identification and quantification of VOCs and MVOCs by their mass spectra [19]. Raman spectroscopy techniques are used to obtain information about the average size of the molecules although they do not give information about the sample composition. Fourier Transform infrared spectroscopy (FT-IR) and near-infrared spectroscopy (NIR) have also been used in bioaerosol studies to differentiate the molecules by the absorption of infrared light [20,21]. Although no single method is preferred over another, the selection is generally determined by the objectives of the research, and the difference in sampling methodologies can hamper data comparison.

MVOCs are secondary metabolites resulting from microbial fermentation. They are characterized by low boiling points, high vapor pressures and low molecular weights [22]. As

microbial communities exhibit different MVOCs depending on which environment they are in [23] and fingerprinting analytical techniques have evolved considerably, chemical analysis of MVOCs has the potential to be a reliable and rapid approach for the characterization of ambient bioaerosols [24,25]. Furthermore, a rapid detection of pathogenic fungi and bacteria present in the air could be implemented by the detection of selective MVOCs [26].

MVOCs are produced by all the microorganisms, and not all compounds are uniquely produced by a single microbial species. Species-specific MVOCs have a potential to be used as chemical biomarkers for the selective detection and identification of bacterial and fungal species in ambient air, being untargeted metabolomics, therefore, a potential way forward for the identification of new compounds. Although human risk exposure to MVOCs is linked to the type and concentration of the microbial species, to date is still difficult to perform a real time identification and quantification of the microorganisms present in air [27–29].

**Table 6. 1 Advantages and disadvantages of available sampling techniques to study bioaerosols.**

| Techniques | Target compounds/ molecules | Advantages | Disadvantages | Ref. |
|---|---|---|---|---|
| **Cultural techniques**<br>- Plating<br>- Microscopy<br>- Flow cytometry | - CFU<br>- Number of live/dead cells | - Easy to use<br>- Identification at species level | - Labor intensive<br>- Expensive<br>- Low reproducibility<br>- No real time<br>- Underestimation | 8–10,30 |
| **Molecular techniques**<br>- Immunochemistry<br>- PCR, Q-PCR<br>- Pyrosequencing<br>- DNA/RNA-based techniques<br>- Phospholipids analysis | - Proteins<br>- DNA<br>- PLFAs | -Identification at species level<br>- Reproducible analysis<br>- Detection at low level | - Expensive<br>- Long laboratory protocols<br>- No real time | 12,13,18,7 |
| **Chemical techniques**<br>- FLAPS<br>- Sensing techniques<br>- MVOCs<br>- FT-IR<br>- FT-NIRS<br>- Raman spectroscopy<br>- Mass spectrometry | -Particles<br>-MVOCs<br>- Proteins | - Reproducible analysis<br>- Detection at low level<br>- Accurate and reliable<br>- Rapid, non-destructive, and inexpensive<br>- Possibility of real time | - Biosensors not easy to transport<br>- MVOCs collection techniques are easily portable | 9,14–16,18 |

This book chapter aims to critically review the sampling techniques and analytical approaches that are available at the moment for the study of microbial VOCs from industrial, agricultural and rural emissions. Current challenges on MVOCs sample capture, analytical and speciation analysis are addressed, and directions for the implementation of a rapid, reproducible and sensitive working mechanism for fingerprinting bioaerosols looking at MVOCs are given.

## 6.2 MICROBIAL VOLATILE ORGANIC COMPOUNDS

### 6.2.1. MVOCs in the environment

The analysis of MVOCs has already been applied in different fields such as in forensics and security to detect drugs, explosives or warfare [31], for fungal detection at composting sites [32], as well as in health care to detect *Aspergillus fumigatus* in breath samples [33] or to diagnose Crohn's disease from urine [34]. However, to date, most of the studies have been focused on indoor built environments, and there is limited characterization of MVOCs profiles from outdoor environments apart from animal farms or bio-waste facilities. There is a need therefore of baseline fingerprint of different environments to understand the true contribution of anthropogenic activities and potential health issues as well as seasonal changes of MVOCs and differences in emissions depending on the source or dynamics of the site activity.

MVOCs are present in outdoor air at trace levels, and as a result of their high diversity, they are grouped in 13 different chemical groups (Table 6. 2) [22]: alkanes, alkenes, nitrogen compounds, sulphur compounds, aldehydes, ketones, alcohols, organic acids, ethers, esters, furans, aromatic compounds and terpenes. MVOCs differ in their physicochemical properties as well as in their chemical structure, so efficient sampling methods are required to be able to collect as many compounds as possible.

Table 6. 3 summarizes the most common MVOCs identified from outdoor (compost facilities, municipal solid waste management, and a WWTP) and indoor (houses, buildings and broiler sheds) ambient air [35]. In every study, samples were collected with different techniques i.e. Tenax TA, GR tubes, Carbopack B with sampling times varying between 30 min to 3-5 h and flow rates between 55-100 ml $min^{-1}$ and in one of the studies the sampling technique was not mentioned at all. The wide range of MVOCs found between studies can be attributed to the inconsistency in sample collection devices and sampling settings (flow rate and sampling time). Monitoring and measuring MVOCs in an indoor environment is easier than outdoors as the concentrations are usually higher

and it is a more constant environment. Outdoor MVOCs concentrations instead are much lower, and there are several co-founding factors and environmental parameters that influence on the sample capture such as weather conditions or activity that is being carried out on the site. Due to the low outdoor environment concentrations, it is difficult to differentiate MVOCs profiles among sites or to identify a contaminated site from a non-contaminated site.

**Table 6. 2 Chemical group, physicochemical properties and microbial origin of the predominant MVOCs present in the environment. Table modified from García-Alcega *et al.* [35].**

| Chemical group | Compound | Chemical formula | Molecular weight (g $mol^{-1}$) | log $K_{ow}$ | Boiling point (°C) at 101.3 kPa | Vapor pressure (kPa at 25°C) | Microbial origin |
|---|---|---|---|---|---|---|---|
| Acids | Butanoic acid | $C_4H_8O_2$ | 88.1 | 0.8 | 163.75 | 0.74 | Bacteria and fungi |
| | Propanoic acid | $C_3H_6O_2$ | 74.07 | 0.3 | 141.15 | 0.47 | Bacteria |
| Aldehydes | Acetaldehyde | $C_2H_4O$ | 44.05 | -0.3 | 20.2 | 101 | Bacteria and fungi |
| | Furfural | $C_5H_4O_2$ | 96.08 | 0.41 | 162 | 0.15 | Bacteria and fungi |
| Ethers | 2-methylfuran | C5H60 | 82.1 | 1.85 | 65 | 23.48 | Bacteria and fungi |
| | 3-Methylfuran | $C_5H_6O$ | 82.1 | 1.91 | 65-66 | 21.46 | Bacteria and fungi |
| Sulphur and nitrogen compounds | Dimethyl-sulfide | C2H6S | 62.134 | 0.977 | 188.8 | 53.7 | Bacteria and fungi |
| | Dimethyl disulfide | $C_2H_6S_2$ | 94.19 | 1.77 | 109.8 | 3.83 | Bacteria and fungi |
| | 2-isopropyl-3-methoxypyrazine | $C_8H_{12}N_2O$ | 152.2 | 2.37 | 210.8±30.0 | 0.036 | Bacteria |
| Ketones | 2-heptanone | $C_7H_{14}O$ | 114.19 | 2.03 | 150.6-151.5 | 0.213-0.28 | Bacteria and fungi |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2-hexanone | $C_6H_{12}O$ | 100.16 | 1.38 | 126-128 | 1.47,0.36 | Bacteria and fungi |
| | 3-Octanone | $C_8H_{16}O$ | 128.21 | 2.22 | 157-162 | 0.267 | Bacteria and fungi |
| Terpenes | Geosmin | $C_{12}H_{22}O$ | 182.31 | 3.57 | 252.4±8.0 | 0.00041 | Bacteria |
| | Borneol | C10H18O | 154.25 | 2.3 | 213 | 0.009 | Bacteria |
| | 2-methylisorbenol | $C_{11}H_{20}O$ | 168.28 | 3.31 | 208.7±8.0 | 0.0065 | Bacteria |
| | β-Caryophyllene | $C_{15}H_{24}$ | 204.35 | NA | 254 | NA | Bacteria |
| | α-Pinene | $C_{10}H_{16}$ | 136.23 | 2.8 | 155 | 0.4 | Bacteria and fungi |
| | Camphene | $C_{10}H_{16}$ | 136.23 | 3.3 | 159 | NA | Fungi |
| | Camphor | $C_{10}H_{16}O$ | 152.23 | 2.2 | 209 | 0.53 | Bacteria and fungi |
| Alcohols | 2-methyl-1-propanol | $C_4H_{10}O$ | 74.12 | 0.65-0.83 | 108 | 1.33 | Bacteria and fungi |
| | 2-methyl-1-butanol | $C_5H_{12}O$ | 88.15 | 1.29 | 128 | 0.416 | Bacteria and fungi |
| | 3-methyl-1-butanol | $C_5H_{12}O$ | 88.15 | 1.16 | 130.5 | 0.316 | Bacteria and fungi |
| | 3-methyl-2-butanol | $C_5H_{12}O$ | 88.15 | 1.28 | 111.5 | 1.22 | Bacteria |
| | 3-octanol | $C_8H_{18}O$ | 130.23 | 2.73 | 169 | 0.068 | Fungi |
| | 1-octen-3-ol | $C_8H_{16}O$ | 128.21 | 2.6 | 180 | 0.071 | Bacteria and fungi |
| | 2-octen-1-ol | $C_8H_{16}O$ | 128.21 | 2.59 | 195.8±8.0 | 0.014 | Bacteria and fungi |
| | 2-pentanol | $C_5H_{12}O$ | 88.15 | 1.19 | 119.0-119.3 | 0.815 | Bacteria and fungi |
| | Methanol | $CH_4O$ | 32.04 | -0.5 | 64.7 | 32 | Bacteria and fungi |

*log $K_{OW}$ = Octanol-Water partition coefficient

**Table 6. 3 Most frequent MVOCs in outdoor and indoor environments and range of concentrations (ng m-3).**

| MVOCs | Outdoor environments | | | Indoor environments | | | |
|---|---|---|---|---|---|---|---|
| | **Compost facilities [a]** | **MSW [b]** | **WWTP [c]** | **Normal buildings [d]** | **Living environments [d,e]** | **Problem buildings [f]** | **Broiler sheds [g]** |
| | **Sampling technique** | | | | | | |
| | Tenax TA and GR tubes (sampling conditions n.s.) | Tenax GR tubes filled with adsorption resin (200 mg) @ 100 ml/min; sampling time n.s. | Tenax GR tubes filled with adsorption resin (200 mg) @ 100 ml/min; sampling time n.s. | Tenax TA tubes (30 min @ 100 ml/min) TD tubes (adsorbent and time n.s.) | 300-400 mg Tenax TA-Carbopack B tubes (45-55 ml/min during 3-5h) TD tubes 30 min@ 100 ml/min (adsorbent and time n.s.) | n.s. | Tenax TA tubes (30 min @ 100 ml/min) |
| Ethanol | na | 250 | na | na | na | na | na |
| 2-propanol | na | 120 | na | na | na | na | na |
| 2-methyl-1-butanol | 170-1400 | na | na | na | na | na | na |
| 2-methyl-1-propanol | na | na | na | 340-1380 | 3000-10400 | nd-1740 | na |
| 3-methyl-1-butanol | 300-35000 | na | na | 8700-110000 | 3000 | 175-260000 | nd-25000 |
| 3-methyl-2-butanol | nd-70 | na | na | nd-160 | 3610 | 190-1190 | na |
| 3-octanol | nd-140 | na | na | nd-40 | 5330-8800 | nd-8860 | na |
| 1-octen-3-ol | nd-1900 | na | na | nd-7000 | 5240-11800 | nd-904000 | 300-6000 |
| 2-octen-1-ol | nd-6820 | na | na | nd-14000 | 5240-21500 | 1560-266000 | na |
| 2-pentanol | na | na | na | 1700 | 3610-4800 | nd-1400 | na |
| 2-methylfuran | 75-1500 | na | na | na | 6300 | na | na |
| 3-methylfuran | nd-110 | na | na | nd-160 | 3360 | nd-1800 | na |
| 2-penthylfuran | 85-1240 | na | na | na | 5100 | na | na |
| 2,3-butanedione | na | 90 | na | na | na | na | 3000-324000 |
| 2-butanone | | | 13700 | | | | |
| 3-Hydroxy-2-butanone | na | 140 | na | na | na | na | na |
| 2-heptanone | nd-3000 | na | na | nd-1200 | 4670-16900 | nd-97 | na |
| 2-hexanone | nd-800 | na | na | nd | 4100 | 25-8800 | na |
| 3-octanone | nd-2000 | na | na | nd-3000 | 5240-11600 | nd-3020 | na |
| Acetic acid | na | 60 | na | na | na | na | na |
| Ethyl acetate | na | 110 | na | na | na | na | na |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Nonane | na | 80 | na | na | na | na | na |
| Decane | na | 110 | na | na | na | na | na |
| Undecane | na | 320 | nd | na | na | na | na |
| borneol | 160-7000 | na | na | na | 6900 | na | na |
| geosmin | nd-10 | na | na | nd-50 | 6000-7460 | nd-550 | na |
| 2-methyli-sorbenol | nd-1180 | na | na | nd-560 | 6880 | nd-2800 | na |
| dimethylsulfide | <50-3300 | na | 26400 | na | 1700 | na | nd-1700 |
| dimethyl disulfide | nd-6000 | na | 22500 | nd-710 | 3850-263000 | 16-90 | nd-263000 |
| 2-isopropyl-3-methoxy-pyrazine | nd-340 | na | na | nd-3 | 6220 | nd-9500 | na |

[a]Compost facilities [27,36]; [b] MSW =Municipal solid waste treatment[37]; [c] WWTP= Waste water treatment plant (at sludge dewatering site)[38]; [d]Normal buildings= Without damp problems or non-complaint areas [32,44,45]; [e] Living environments = Houses [32,44,43]; [f]Problem buildings=Buildings with damp problems [39,41]; [g]Broiler sheds [43]; *n.s. = not specified; nd= not detected

Apart from this, researchers do not analyze and report same MVOCs [35], and MVOC contaminant concentration threshold and concentration limits are not consistent. For example, in a study of indoor air from buildings, Lorenz *et al.* [44] identified the MVOCs 1-octen-3-ol, dimethyl disulfide and 3-methylfuran as the main indicators of microbial growth. The authors determined that there is an indoor microbial source of contamination when the detection of one of these MVOCs is present at concentrations above 50 ng $m^{-3}$ or when the sum of eight MVOCs (1-octen-3-ol, 3-methylfurane, dimethyl disulfide, 3-methyl-1-butanol, 2-pentanol, 2-hexanone, 2-heptanone, 3-octanone) together with at least one of the 3 main MVOCs indicators of microbial growth equals or exceeds 500 ng $m^{-3}$. Opposite to this, Korpi *et al.* [44] suggested other limits for 3-methylfurane (≥ 200) and 1-octene-3-ol and 3-methyl-1-butanol ≥ 10000 ng $m^{-3}$.This author also listed another MVOCs different from the ones reported by Lorenz *et al.* (geosmin ≥ 50, 2-isopropyl-3- methoxypyrazine ≥ 400, 2-methyl1-propanol and 2-methylisoborneol ≥ 1500, 2-octen-1-ol ≥ 15000 ng $m^{-3}$).

### 6.2.2 Species-specific MVOCs

The identification of species-specific MVOCs is difficult because first, not all the MVOCs present in the air have solely microbial origin and second because most of the reported MVOCs with microbial origin are not species-specific [28,45–47]. The microbial origin of the most frequent outdoor MVOCs is summarized in Table 6. 3. 2-hexanone and 3-methyl-1-butanol are MVOCs uniquely related to microbial sources. However, they are produced during the metabolism of every fungi and bacteria and therefore are not specific to any microbial species. 3-octanol for instance is only emitted by fungi, and other MVOCs like 3-methyl-2-butanol, geosmin, borneol, 2-methyl-isorbenol, and 2-isopropryl-3-methoxypyrazine are uniquely produced by bacteria. Nevertheless, none of them can be attributed to any specific microorganism. There are inconsistencies about which is the microbe producer of which MVOCs. As an example, 3-octanone was reported to be only emitted by *Aspergillus fumigatus* but lately, it has been discovered that *Aspergillus flavus* also produces it [48]. Additionally, Gao *et al.* [49] suggested that 2-pentyl furan was a metabolite exclusively from *Aspergillus fumigatus,* and not long ago other researchers revealed that there are other fungi that also produce it (*Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Scedosporium apiospermum* and *Fusarium spp* respectively) as well as the bacteria *Streptococcus pneumonia* [33,50].

Vishwanath *et al.* [51] tried to discriminate species specific MVOCs from anthropogenic VOCs present in dust from houses and waste management facilities. They did not succeed in their study due to the ambiguity of MVOCs and the lack of a certified reference material reporting MVOCs concentrations in environmental samples. In another recent study, Choi *et al.*

[52] analyzed in parallel dust and air samples from buildings and houses to investigate the anthropogenic and microbial sources of 28 VOCs that are considered to have a microbial origin. They concluded that the Σ28 VOCs identified in the samples which were commonly accepted as MVOCs, were, in fact, more associated to anthropogenic sources such as the phthalates 2,2,4-trimethyl-1,3-pentanediol mono isobutyrate (widely used as plasticizers), propylene glycol and propylene glycol ethers (both used in paints) than to microbial sources ($P \leq 0.003$). Other MVOCs such as terpenes and sesquiterpenes can also be emitted from both anthropogenic or non-microbial sources (fruits, cleaning products, cosmetics, woods, etc.) and from microbes [46]. The same occurs with methyl-furanes, which apart from being produced by microbes, they are also emitted during the pyrolysis of tobacco components [45]. There are also several other MVOCs that are related to VOCs liberated from building materials as well [28].

Based on a critical review of the literature over the last decade and existing MVOCs metabolic databases such as the *mVOC* from Lemfack *et al.*[25] and the Kyoto Encyclopedia of Genes and Genomes (KEGG) [53], a list of MVOCs potentially specific to a microbial species is summarized in Table 6. 4. The chemical properties of these MVOCs and the bacterial or fungal producer species are detailed in this table. These MVOCs are less often reported in indoor and outdoor studies but could be an approach for the identification of microbial species by their identification.

**Table 6. 4 Physicochemical properties of some potentially species-specific MVOCs. (adapted from García-Alcega *et al.* [35].)**

| Microbial Species | Specific MVOCs | Molecular formula | Molecular weight (g $mol^{-1}$) | Ref. |
|---|---|---|---|---|
| *Aspergillus flavus* | cis2-octen-1-ol | $C_8H_{16}O$ | 128.21 | [54] |
| *Aspergillus fumigatus* | 2,4-Pentadione (Acetylacetone) | $C_5H_8O_2$ | 100.12 | [54] |
| | 3-Methyl-1,3-pentandione | $CH_3COCH(CH_3)COCH_3$ | 114.14 | |
| | p-Mentha-6,8-dien-2-ol acetate | $C_{12}H_{18}O_2$ | 194.27 | |
| *Aspergillus versicolor* | Trimethylnonanoic acid methylester | $C_{14}H_{28}O_2$ | 228.37 | [55] |
| | 1-(3-Methylphenyl)-ethanone | $C_9H_{10}O$ | 134.18 | |
| *Aspergillus candidus* | 3-Cyclohepten-1-one isomer | $C_{18}H_{32}O_2$ | 280.45 | [56] |
| *Emericella nidulans* | beta-Fenchyl alcohol | $C_{10}H_{18}O$ | 154.25 | [56] |

| Microbial Species | Specific MVOCs | Molecular formula | Molecular weight (g $mol^{-1}$) | Ref. |
|---|---|---|---|---|
| | 2-Methyl-butanoic acid methyl ester | $C_7H_{14}O_2$ | 130.18 | |
| | 4,4-Dimethyl-pentenoic acid methyl ester | n/a | n/a | |
| *Penicillium clavigerum* | Bicyclooctan-2-one | $C_8H_{12}O$ | 124.18 | 56 |
| *Penicillium crustosum* | 2-Ethyl-5-methyl-furan | $C_7H_{10}O$ | 110.15 | 56 |
| | 4-Ethylbutan-4-olide ((S)-gamma-hexalactone) | $C_6H_{10}O_2$ | 114.14 | |
| | Isopropylfuran | $C_7H_{10}O$ | 110.15 | |
| *Penicillium cyclopium* | 2-Methyl-2-bornene isomer | n/a | n/a | 56 |
| | delta-2-Dodecanol | n/a | n/a | |
| | 4-Methyl-2-(3-methyl-2-butenyl)-furan | $C_{10}H_{14}O$ | 150.22 | |
| *Penicillium roqueforti* | beta-patchoulene-isomer | $C_{15}H_{24}$ | 204.35 | 54 |
| | beta-elemene-isomer | $C_{15}H_{24}$ | 204.36 | |
| | (1,1-dimethylethyl)-2-methylphenol | n/a | n/a | |
| | Butanoic acid, 2-methyl-2-methylpropyl ester | $C_8H_{16}O_2$ | 144.21 | |
| | alpha-selinene | $C_{15}H_{24}$ | 204.35 | |
| | 1-methyl-4-(1-methylethyl) benzene (p-Cymene) | $C_{10}H_{14}$ | 134.22 | |
| | Propanoic acid 2-methyl-2-methylpropyl ester (or Propanoic acid, 2-methyl-3-methylbutyl ester or Isobutyric acid) | $C_8H_{16}O_2$ | 144.21 | |
| | alpha-chamigrene | $C_{15}H_{24}$ | 204.35 | |
| *Paecilomy cesvariotii* | 3,5,7-Trimethyl-2E,4E,8E-decatetraene | $C_{13}H_{20}$ | 176.3 | 7 |
| | 2-Methyl-2,4-hexadiene | $C_7H_{12}$ | 96.17 | |
| | delta-4-Carene | $C_{10}H_{16}$ | 136.23 | |

| Microbial Species | Specific MVOCs | Molecular formula | Molecular weight (g $mol^{-1}$) | Ref. |
|---|---|---|---|---|
| *Trichodema pseudokoningii* | 2-Methyl-pentane | $C_6H_{14}$ | 86.18 | 7 |
| *Muscodor crispans* | Hexane, 2,3-dimethyl- | $C_8H_{18}$ | 114.23 | 7 |
| | Formamide, N-(1-methylpropyl) | $C_7H_{15}NO_2$ | 145.2 | |
| | Cyclohexane, 1,2-dimethyl-3,5-bis(1-methylethenyl) | $C_{14}H_{24}$ | 192.34 | |
| *Arthrobacter globiformis* | 2-Phenylethylamine | $C_8H_{11}N$ | 121.18 | 7 |
| *Mycobacterium* | 5-Methylhexan-3-ol | $C_7H_{16}O$ | 116.2 | 7 |
| | 7-Methyloctan-3-one | $C_9H_{18}O$ | 142.24 | |
| | 5-Methyl-4-hexen-3-one | $C_7H_{12}O$ | 112.17 | |
| | Cyanoisoquinoline | $C_{10}H6N_2$ | 154.17 | |
| *Bacillus spp.* | (2R,3R)-Butane-2,3-diol | $C_4H_{10}O_2$ | 90.12 | 7 |
| *Geobacillus stearothermophilus* | Dimethyl ditelluride | $C_2H_6Te$ | 157.67 | 7 |
| | Methanetellurol | $CH_4Te$ | 143.64 | |
| | dimethylselenodisulfide | n/a | 173.15 | |
| | dimethyltellurenalsulfide | n/a | 189.73 | |
| *Paenibacillus polymyxa* | 2-(2-Methylpropyl)pyrazine | $C_9H_{14}N_2O$ | 166.22 | 7 |
| | 2,6-Diisobutylpyrazine | $C_{12}H_{20}N_2$ | 192.3 | |
| | 2-Methyl-5-isobutylpyrazine | $C_9H_{14}N_2$ | 150.22 | |
| *Stapahylococcus aureus* | 2,3,4,5-tetrahydropyridazine | $C_4H_8N_2$ | 84.12 | 7 |
| | 4-methylhexanoic acid | $CH_3CH_2CH(CH_3)CH_2CH_2COOH$ | 130.18 | |
| | Butyl butanoate (butyl butyrate) | $C_8H_{16}O_2$ | 144.21 | |
| *Pseudomonas sp.* | 2,4-Diacetylphloroglucinol | $C_{10}H_{10}O_5$ | 210.18 | 57 |
| *Pseudomonas trivialis* | Undecadiene | $C_{11}H_{20}$ | 152.28 | 58 |
| | Benzyloxybenzonitrile | $C_{14}H_{11}NO$ | 209.25 | |
| *Escherichia coli* | Pentylcyclopropane | $C_8H_{16}$ | 112.21 | 58 |

| Microbial Species | Specific MVOCs | Molecular formula | Molecular weight (g $mol^{-1}$) | Ref. |
|---|---|---|---|---|
| *Acinetobacter calcoaceticus* | Sulfoacetaldehyde | $C_2H_4O_4S$ | 124.12 | [58] |
| *Klebsiella sp.* | Pentylbutanoate (or pentyl butyrate) | $C_9H_{18}O_2$ | 158.24 | [58] |
| *Streptomyces citreus* | Dihydroagarofuran (sesquiterpenoid) | $C_{15}H_{26}O$ | 222.37 | [58] |
| | Bicyclogermacrene | $C_{15}H_{24}$ | 204.35 | |
| | betabourbonene | $C_{15}H_{24}$ | 204.35 | |
| | delta-elemene | $C_{15}H_{24}$ | 204.36 | |
| *Alternaria alternata* | 6-Methylheptanol | $C_8H_{18}O$ | 130.23 | [58] |
| *Rhizopus stolonifer* | 1-Octene | $C_8H_{16}$ | 112.24 | [58] |
| | 3-Methyl-3-buten-1-ol | $CH_2=C(CH_3)CH_2CH_2OH$ | 86.13 | |

## 6.3 SAMPLING COLLECTION DEVICES

Electronic noses, activated charcoal pads, thermal desorption (TD) tubes, cyclones and glass impingers are the most used techniques for sampling MVOCs (Table 6.5). TD tubes are preferred among the rest of the techniques as they are directly loaded into the GC-MS without needing sample preparation. MVOCs collected by activated charcoal pads, cyclones and glass impingers, for instance, need additional extraction steps. Electronic noses provide a real time analysis of MVOCs, but the sensitivity is not good enough for the detection of these compounds at environmental levels.

There is information available in the literature about which sampling technique should be used to study airborne microorganisms, odors and MVOCs at composting facilities and or its surroundings [29] but there are still lacking guidelines for sampling in other environments like a farm or a park [35]. Monitoring bioaerosols is difficult because there are several factors that interfere in the sample capture such as the type of activity that is being carried out on the site (turning the compost, incorporation of green waste, no activity at all) as well as the weather conditions (wind speed and wind direction, temperature, relative humidity and atmospheric conditions) or the distance from the emission source. For this reason, a recent guideline from the Environment Agency for sampling bioaerosols from composting sites suggest to collect samples upwind, downwind and at the nearest sensitive receptor in order to be able to compare the different concentrations and also to monitor the weather parameters with a weather station [59]. When interpreting the MVOCs data all the weather variables and site activities need to be taken

into consideration via multivariate analysis to understand the MVOCs patterns concentrations accordingly.

### 6.3.1 Electronic nose

Electronic noses function by electronic chemical sensors combined with an information processing unit and pattern recognition software. Volatile organic compounds are then qualitatively recognized from a reference library [60,61] which can be constructed for each specific category of microbes (fungi and bacteria) [62]. Because advantageously these devices are portable and identify MVOCs in real time, they have often been used to detect fungal contamination in indoor environments as well as in food production processes, agriculture and pathological and clinical diagnoses [28,61,63]. Unfortunately, this technique is not suitable yet for the detection and identification of MVOCs at trace levels because electronic noses are not very sensitive and the sensors cannot discriminate between structurally similar compounds. Moreover, the sensor can easily be activated by random noise and give false identifications or false-positive results. Another inconvenient of this device is the inability of identifying VOCs from complex mixtures where interferences need to be removed by clean-up and pre-concentration steps which involve a potential loss of sample [28].

### 6.3.2 Activated charcoal pads

Charcoal pads (Figure 6.1) are light diffusive samplers which are available at low price and are easy to use as they work without electricity [64]. There are not many studies in the literature using charcoal pads for MVOCs analysis. This technique is advantageous for monitoring an area during long times (from hours to weeks) as the device can be left in the field without the need of controlling it [55,64]. Sample preparation is easy as MVOCs are solvent extracted with carbon disulfide [55], but there is a probability of losing sample and, in fact, the sensitivity of charcoal pads is low. Charcoal pads should be stored in the fridge prior analysis and samples should be analyzed within a month [64].

Figure 6. 1 Charcoal pad passive sampler

### 6.3.3 Cyclones and glass impingers

Cyclones and glass impinger sampling devices collect the air sample by suction into a liquid medium avoiding the loss of microbes due to impaction and dryness [65]. Another of the advantages of impingers and cyclones is that they can collect a high volume of sample during a relatively short sampling time (10-30 min). However, studies have shown that 30 min sampling time can result in 10 and 15% loss of the collection fluid when using cyclones and glass impingers respectively[66]. When considering outdoor sampling, cyclone devices are better suited as they are easier to transport and sterilize than glass impingers. They are also less fragile, and there is no risk of having broken glass. There are a great variety of cyclone samplers used to monitor bioaerosols including the SpinCon air sampler (Specter Industries, Inc.), the BioGuardian air sampler (InnovaTek, Inc.), the BioCapture 650 (MesoSystems Technology, Inc.) and Coriolis®μ (Figure 6.2) [67]. The advantage of the Coriolis®μ sampler among the other cyclones is its lighter weight (3 kg) and the high sample volume (20 ml). The SpinCon sampler is quite heavy (20 kg), and it operates at a high flow rate (400-450 ml $min^{-1}$). The BioGuardian air sampler is lighter (7.7 kg), but it works at a low flow rate (90 ml $min^{-1}$). The Biocapture is also quite light (4-4.5 kg), but the collection volume is small (2-7 ml). One of the most widely used glass impingers is the BioSampler (SKC, Inc.) [11] and there are few studies using the AGI-30 (Ace Glass Inc.). This last one, for instance, is not so efficient collecting samples at shorter time as the BioSampler and also has problems of sample loss at high and low temperatures [68]. Liquid samples collected from cyclones and glass impingers should be kept at -20°C until use and analysis should be carried out within a month.

**Figure 6. 2 Coriolis®µ cyclone**

### 6.3.4 Thermal desorption tubes

Thermal desorption (TD) tubes (Figure 6.3) are the most widely used technique for sampling MVOCs in outdoor environments. The advantage of this technique over the others is that it does not require sample preparation, only the addition of an internal standard, often Toluene-d[8] [34]. Since TD tubes are directly desorbed into the GC-MS, there is no sample loss and allows the detection of outdoor MVOCs at pg level and obtaining good recoveries [27,42,69]. TD tubes coated with Tenax® or Tenax®-Carbotrap 50/50 v/v are the most common sorbents [69] although MVOCs are more efficiently collected with a multi-sorbent bed of carbonaceous adsorbents rather than with solely Tenax® [70] (Figure 6.4). Longer sampling times can be applied without breakthrough of the tubes when using activated charcoal (Anasorb®) coatings, but these are only effective for the collection of very volatile MVOCs, obtaining poor recoveries of aldehydes, unsaturated hydrocarbons, phenols and amines, i.e., the reactive and less volatile MVOCs [39,55]. Carbonyl compounds are better sampled with TD coated with Sep-Pak® but poor recoveries are obtained for the rest of the MVOCs (Korpi et al. 1998; Korpi et al. 2009). TD tubes should be kept at 4°C before analysis for one month, but the caps of the tubes need to be tightened up after being 1 h in the fridge as they can become loose and samples can be lost.


**Figure 6. 3 Thermal desorption tubes**

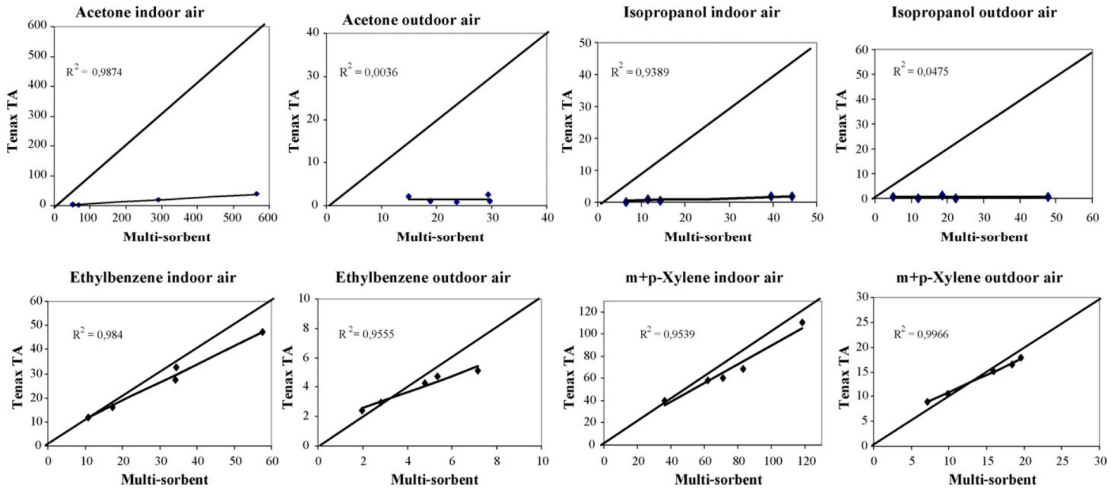


**Figure 6. 4 Comparison of different compound concentrations (µg $m^{-3}$) using multi-sorbent bed tubes (Carbotrap, Carbopack X and Carboxen 569) and Tenax TA tubes (from Gallego et al. [70])**

**Table 6. 5 Advantages and limitations of different sampling techniques (adapted from García-Alcega *et al.*[35])**

| | Advantages | Limitations | Reference |
|---|---|---|---|
| Activated charcoal pads | - Each sample can be analyzed more than once<br>- Good capacities for hydrocarbons, esters, ethers, alcohols, ketones, glycol ethers and halogenated hydrocarbons<br>- Allows long sampling time: MVOCs emissions | - Poor recoveries for less volatile and reactive compounds (amines, phenols, aldehydes, and unsaturated hydrocarbons)<br>- Long sampling times (>1h to days or weeks)<br>- Low sensitivity | 55,64,72–74 |

| | | | |
|---|---|---|---|
| | can be monitored over the time (weeks or days)<br>- Cheap, light and easy to use<br>- Are operated without electricity | - Solvent extraction and heat produce VOC degradation products | |
| Impingers | - High sample volume can be collected<br>- MVOCs are collected in a liquid so the microbes do not get dried<br>- Easily portable | - Possibility of losing sample liquid due to high flow rate<br>- More variability in concentrations between replicates because the sampling time is shorter | 65,67,75–77 |
| Tenax® Desorption tubes | - Quick as sample preparation is no needed<br>- Good recoveries and precision<br>- Easily portable | - Each sample can only be analyzed once<br>- 100 times more sensitive than solvent extraction | 34,42,49,78,79 |
| Activated charcoal Desorption tubes | - Collection of very volatile MVOC<br>- Easily portable | - Each sample can only be analyzed once<br>- Poor recoveries for less volatile and reactive compounds (amines, phenols, aldehydes, and unsaturated hydrocarbons)<br>- 100 times more sensitive than solvent extraction | 39,55 |
| Sep-Pak cartridges | - Good for sampling carbonyl compounds | - Not good recoveries for rest of MVOCs | 80 |
| Electronic nose | - Real time analysis<br>- Portable<br>- User friendly | - Limited detection and identification of MVOCs in the environment<br>- Miss classification and false positive results<br>- Sample pre-concentration and clean up to remove the interferences<br>- Only recognizes compounds present in the library or in the pattern recognition software | 28,54,61,81–83 |

# 6.4 ANALYTICAL TECHNIQUES AND CHEMOMETRICS

## 6.4.1 Chromatographic techniques

The most suitable technique for the analysis of MVOCs is gas chromatography coupled to mass spectrometry (GC-MS) due to the volatile nature of these chemicals [20]. Moreover, trace levels of

MVOCs from complex environmental matrices can be identified and quantified by GC-MS due to its low limit of detection (LOD) (pg $m^{-3}$) and good chromatographic separation. Different sample injection needs to be performed depending on the sampling accessory that is used (e.g., solvent injection, SPME or TD-GC-MS). Few studies have published MVOCs results using liquid chromatographic techniques (LC-MS), but this is only efficient for carbonyl MVOCs such as aldehydes, ketones, carboxylic acids, carboxylic esters and amines [84] and nonvolatile microbial compounds [51].

MVOCs are solvent extracted from charcoal pads, and the extracts are injected into the GC-MS. The main limitation of this technique is that VOCs are degraded during the solvent extraction, and the heat [55] and MVOCs do not have good recoveries due to the sample loss.

TD tubes, for instance, are more appropriate as they are directly loaded into the thermal desorber and connected to the GC-MS without producing any loss of the volatile compounds [85]. When air samples are collected into liquids, headspace solid phase micro-extraction (HS-SPME) is the most common and established MVOC extraction method allowing detection at trace levels (pg $L^{-1}$ ) [86]. The extraction of MVOCs from the liquid sample occurs in a short fused silica fiber without solvents and then they are desorbed at high temperatures and analyzed into the GC-MS. Although this is a robust technique and it offers a high separation capacity, the extraction efficiency is limited to some MVOCs [54,55]. HS techniques need an SPME fiber in the injector to concentrate the MVOCs even though they are not as sensitive as TD-GC-MS. The advantages and limitations of the different analytical techniques are detailed in Table 6.6.

### 6.4.2 Spectrometric techniques

There are few studies using Fourier Transform Infrared Spectroscopy (FT-IR) for analysis of ambient VOCs. This technique measures the frequencies at which the compounds absorb the IR radiation and the intensities of this absorption [87]. There is an EPA database available with the infrared spectral information of the compounds. The advantages of the FT-IR are its low cost, short analysis time and the possibility of real time analysis. The equipment can be coupled with a thermal desorber so it can analyze TD tubes. The main disadvantages are its low reproducibility between samples and the interferences from $H_2O$ and $CO_2$ in the ambient. There are special traps, but they cannot fully solve this problem. For samples with a large amount of compounds, the data analysis can be complicated as a single compound can have more than one peak [88].

Fourier Transform Near Infrared Spectroscopy (FT-NIRS) has been used to detect bacterial contamination in water based pharmaceutical preparations [21]. To the author's knowledge this technique has not been used to study MVOCs yet and could be explored as it provides a very fast analysis, is environmentally friendly and low cost. Disadvantageously, it has the same limitations as FT-IR, the low reproducibility, and repeatability due to the temperature, water and $CO_2$ interferences [21].

**Table 6. 6 Advantages and limitations of different analytical techniques. Table modified from García-Alcega *et al.*[35]**

| | Advantages | Limitations | Reference |
|---|---|---|---|
| GC-MS | - Very good separation High sensitivity | - Relatively non-portable and slow analysis;<br>- Depending on the source used (Electron ionization or Chemical ionization) some compound can be detected (i.e., 2-methyl-1 –butanol is not detectable when EI used; 2-pentanol is not detectable when CI used) | [20,54,55,86,89] |
| GC-HS-SPME-MS | - High sensitivity at trace levels (pg·L−1 to ng·L−1)<br>- Robust and reproducible analytical method<br>- Not sensitive to matrix effects<br>- No solvent is needed<br>- No sample preparation is needed<br>- powerful separation capacity | - Limited extraction efficiency and some MVOCs might not be detected | [54,86,90–93] |
| LC-MS | - Good for analysis of carbonyl compounds | - No suitable for volatile compounds | [84,94,95] |
| FT-IR and FT-NIRS | - Low cost<br>- Fast analysis<br>- Environmental friendly | - Low reproducibility and repeatability<br>- Interferences from $H_2O$, $CO_2$, and temperature | [21,87,88] |

Since different compounds require the use of different ionization sources, for example, the identification of 2-pentanol can only be achieved by electron impact ionization (EI) and 2-methyl-1-butanol by chemical impact (CI) [55,86], it would be recommendable to use a combination of both EI and CI to be able to scan a wider range of MVOCs in the environment.

### 6.4.3 Using chemometrics for analyzing MVOCs profiles

Chemometrics is the mathematical and statistical analysis of the chemical data obtained from the chromatogram of a sample. By chemometrics, the maximum information about the compounds of study is extracted by optimizing signal and data analysis processes and performing multivariate analysis in order to study chemical trends [96]. The use of this approach to identify microorganisms that are in the air is receiving increased attention as chemometrics is

a cost effective and fast analysis in comparison with the more traditional molecular or cell culturing techniques [25].

The schematic procedure for the analysis of the samples is represented in Figure 6. 5. The procedure of the analysis of MVOCs from environmental air starts with the noise removal of the chromatograms (using SNIP baseline detector for example) followed by peak deconvolution which allows accurate mass spectra identification from complex chromatograms (e.g., AMDIS). Then, it proceeds with the identification of all the peaks within the chromatograms of the samples, and this can be done with NIST or free databases such as mzCloud or METLIN. The mzCloud uses a new third generation spectra correlation algorithm to search and identify the compounds. METLIN is a metabolomics database useful for the identification of metabolites, which are linked to the KEGG database[53] to see the metabolic pathways and the microorganisms producers. The MVOCs analysis for ambient air is complex because not all MVOCs have solely microbial origin; these also can be anthropogenic or produced by fruits or vegetables. To discriminate MVOCs from VOCs there is an approach available looking at metabolical databases such as KEGG database[53] or *mVOC* database [25]. After identifying the chromatogram peaks, the *m/z* spectra of the MVOCs are analyzed statistically by multivariate analysis [22], hierarchical Cluster Analysis (HCA) or multidimensional scaling analysis (MDS) and/or principal component analysis (PCA) to study the MVOCs patterns and trends across the samples [22,92]. These analysis can be done using chemometric software such as among others SpectConnect, ACD/MS Manager, OpenChrom, Mass Profiler Professional, or commercial statistical software (MATLAB, ADAPT, etc.) [97]. These statistical analyses help us to identify the key and more representative compounds per site, giving us a preliminary idea of the potential markers for each site. Then, the microbial identity of these potentially species-specific markers should be verified by correlation with DNA sequencing analysis. Specific microbial markers could be used in the future for the identification of microbes in air ideally with a sampling device that gives real time data.

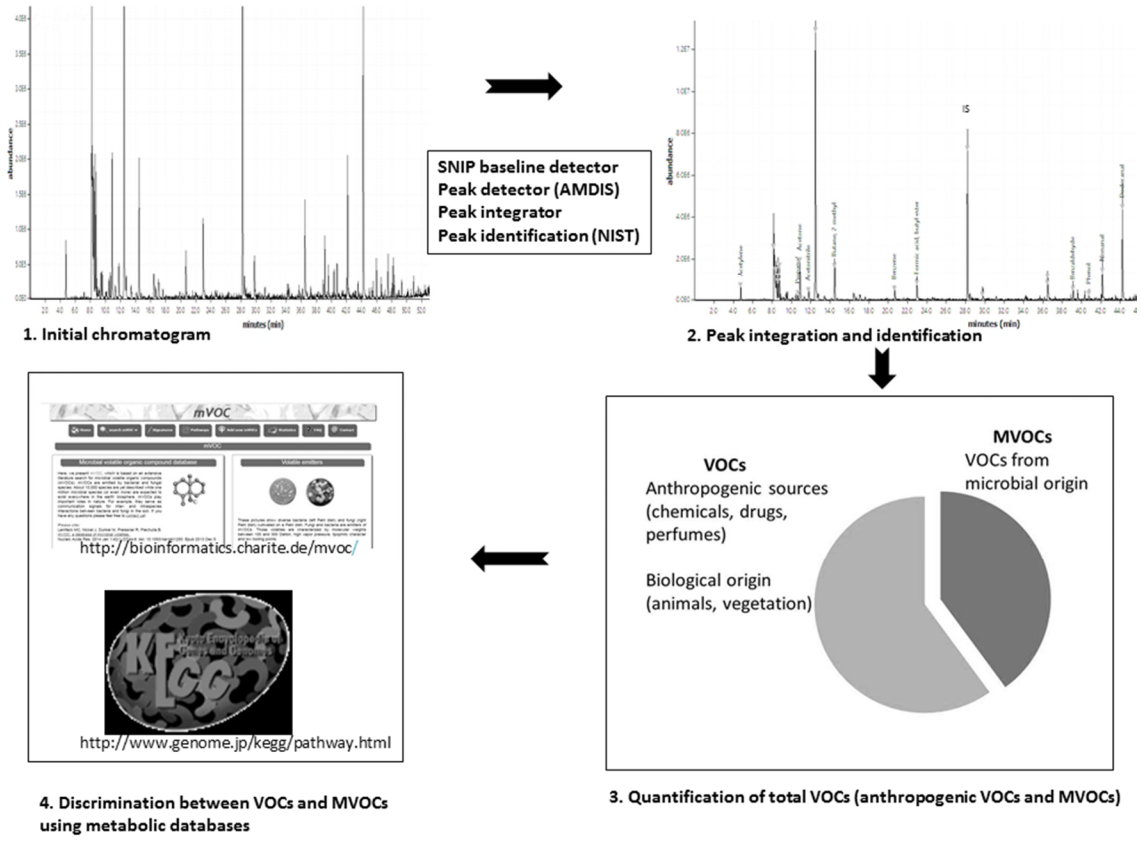


**Figure 6. 5 Treatment of a chromatogram from a WWTP air sample for peak identification and separation of MVOCs from VOCs with online databases.**

Previous researchers have demonstrated the feasibility and potential of identifying fungal species by chemotaxonomy or chemotyping of MVOCs emissions from *in vitro* cultures [74,98–100]. Wihlborg *et al.* (2008) [101] cultured different fungi species and detected 118 different MVOCs. The PCAs obtained from the MVOCs profiles demonstrated that the clustering of the various fungi types depends more on the taxonomy rather than on the culturing medium although this also affected the MVOCs production. Following this work, more emphasis needs to be done towards the development of a species-specific data set containing MVOC and their concentrations from outdoor environments such as industrial (composting facilities, WWTPs, waste facilities), urban (parks, neighborhoods) and rural (farms, countryside) areas.

# CONCLUSIONS AND FUTURE PERSPECTIVES

Although bioaerosols have been studied over the last 30 years, the understanding of their composition is still in its infancy, and further inputs are needed to improve the identification, characterization, and quantification of bioaerosols emitted from urban, rural and industrial environments. Ambient bioaerosol characterization looking at MVOCs biomarkers seems to be

a fast and a reproducible approach as current analytical techniques are more sensitive and allow the detection of chemicals at environmental concentrations. There are numerous variables interfering in the capture of bioaerosols such as weather conditions (wind speed and wind direction, temperature, relative humidity and atmospheric conditions) and activity that is being carried out on the site. When interpreting the MVOCs data these variables need to be taken into consideration via multivariate analysis in order to understand the MVOCs patterns concentrations.

Currently, there is a lack of standardizing methodology for collecting and analyzing MVOCs in a rapid, reliable and reproducible way. Analytical techniques offer a faster and more economical analysis compared to the traditional molecular and microbial ways but the techniques available at the moment are laboratory based, and the data acquisition is not in real time. TD sampling tubes coupled with GC-MS analysis appears to be the most widely used approach as well as it is also a very sensitive and robust technique but the analysis cannot be offered in real time. To obtain accurate real time outdoor MVOCs measurements, an upgraded electronic nose with smaller size, improved and more specific sensors and algorithms for detection and identification of these chemicals at trace levels should be developed.

The next steps for the identification of species specific MVOCs involve both, the chemical characterization of bioaerosols and the speciation analysis simultaneously. Forthcoming directions should point towards the progression of the development of a rapid analysis and assessment of different outdoor air from rural, industrial and urban environments with a combination of a powerful electronic nose and a standardized database with a list of species-specific MVOCs from contrasting outdoor environments. The aim of this database would be to accelerate the process of identifying pathogen microorganisms in the air without molecular or cell culture techniques.

# REFERENCES


[1] Gómez-Domenech, M.; García-Mozo, H.; Alcázar, P.; Brandao, R.; Caeiro, E.; Munhoz, V.; Galán, C. Evaluation of efficiency of the Coriolis air sampler for pollen detection in south Europe. *Aerobiologia (Bologna)* 2010, *26*, 149–155

[2] Prospero, J. M.; Blades, E.; Mathison, G.; Naidu, R. Interhemispheric transport of viable fungi and bacteria from Africa to the Caribbean with soil dust. *Aerobiologia (Bologna)* 2005, 21, 1–19.

[3] Sharma, A.; Clark, E.; McGlothlin, J. D.; Mittal, S. K. Efficiency of Airborne Sample Analysis Platform (ASAP) bioaerosol sampler for pathogen detection. *Front Microbiol* 2015, 6.

[4] O'Connor, D. J.; Daly, S. M.; Sodeau, J. R. On-line monitoring of airborne bioaerosols released from a composting/green waste site. *Waste Manag* 2015, 42

[5] Ghosh, B.; Lal, H.; Srivastava, A. Review of bioaerosols in indoor environment with special reference to sampling, analysis and control mechanisms. *Environ Int* 2015, 85, 254–272.

[6] Macklin, Y.; Kibble, A.; Pollitt, F. *Impact on Health of Emissions from Landfill Sites: Advice from the Health Protection Agency*; 2011.

[7] Pankhurst, L. J.; Whitby, C.; Pawlett, M.; Larcombe, L. D.; Mckew, B.; Deacon, L. J.; Morgan, S. L.; Villa, R.; Drew, G. H.; Tyrrel, S.; et al. Temporal and Spatial Changes in the Microbial Bioaerosol Communities in Green-Waste Composting. *FEMS Microbiol Ecol* 2012, 79, 229–239.

[8] Kim, Y.; Platt, U.; Gu, M. B.; Iwahashi, H. *Atmospheric and biological environmental monitoring*; Springer Science & Business Media, 2009.

]9] Kim, J.; Jin, J.-H.; Kim, H. S.; Song, W.; Shin, S.-K.; Yi, H.; Jang, D.-H.; Shin, S.; Lee, B. Y. Fully Automated Field-Deployable Bioaerosol Monitoring System Using Carbon Nanotube-Based Biosensors. *Environ Sci Technol* 2016, 50, 5163–5171.

[10] Oliver, J. D. The viable but nonculturable state in bacteria. *J Microbiol* 2005, 43, 93–100.

[11] Mandal, J.; Brandl, H. Bioaerosols in Indoor Environment - A Review with Special Reference to Residential and Occupational Locations. *Open Environ Biol Monit J* 2011, 41, 83–96.

[12] Yoo, K.; Lee, T. K.; Choi, E. J.; Yang, J.; Shukla, S. K.; Hwang, S.; Park, J. Molecular approaches for the detection and monitoring of microbial communities in bioaerosols: A review. *J Environ Sci* 2016, 51, 1-15.

[13] Willers, C.; Jansen van Rensburg, P. J.; Claassens, S. Phospholipid fatty acid profiling of microbial communities–a review of interpretations and recent applications. *J Appl*

*Microbiol* 2015, 119, 1207–1218.

[14] Schmidt, M. S.; Bauer, A. J. R. Preliminary correlations of feature strength in spark-induced breakdown spectroscopy of bioaerosols with concentrations measured in laboratory analyses. *Appl Opt* 2010, 49, 101–C10.

[15] Toprak, E.; Schnaiter, M. Fluorescent biological aerosol particles measured with the Waveband Integrated Bioaerosol Sensor WIBS-4: laboratory tests combined with a one year field study. *Atmos Chem Phys* 2013, 13, 225–243.

[16] Pardon, G.; Ladhani, L.; Sandström, N.; Ettori, M.; Lobov, G.; van der Wijngaart, W. Aerosol sampling using an electrostatic precipitator integrated with a microfluidic interface. *Sensors Actuators B Chem* 2015, 212, 344–352.

[17] Sandström, N.; Shafagh, R. Z.; Vastesson, A.; Carlborg, C. F.; van der Wijngaart, W.; Haraldsson, T. Reaction injection molding and direct covalent bonding of OSTE+ polymer microfluidic devices. *J Micromechanics Microengineering* 2015, 25, 75002-75014.

[18] Srivastava, A.; Pitesky, M. E.; Steele, P. T.; Tobias, H. J.; Fergenson, D. P.; Horn, J. M.; Russell, S. C.; Czerwieniec, G. A.; Lebrilla, C. B.; Gard, E. E. Comprehensive Assignment of Mass Spectral Signatures from Individual Bacillus trophaeus Spores in Matrix-Free Laser Desorption/Ionization Bioaerosol Mass Spectrometry. *Anal Chem* 2005, 77, 3315–3323.

[19] Lavine, B. K.; Mirjankar, N.; LeBouf, R.; Rossner, A. Prediction of mold contamination from microbial volatile organic compound profiles using solid phase microextraction and gas chromatography/mass spectrometry. *Microchem J* 2012, 103, 37–41.

[20] Siddiquee, S.; Al Azad, S.; Bakar, F. A.; Naher, L.; Kumar, S. V. Separation and identification of hydrocarbons and other volatile compounds from cultures of Aspergillus niger by GC–MS using two different capillary columns and solvents. *J Saudi Chem Soc* 2015, 19, 243–256.

[21] Quintelas, C.; Mesquita, D. P.; Lopes, J. A.; Ferreira, E. C.; Sousa, C. Near-infrared spectroscopy for the detection and quantification of bacterial contaminations in pharmaceutical products. *Int J Pharm* 2015, 492, 199–206.

[22] Schenkel, D.; Lemfack, M. C.; Piechulla, B.; Splivallo, R. A meta-analysis approach for

assessing the diversity and specificity of belowground root and microbial volatiles. *Front +Plant Sci* 2015, 6, 707-718.

[23] Konuma, R.; Umezawa, K.; Mizukoshi, A.; Kawarada, K.; Yoshida, M. Analysis of microbial volatile organic compounds produced by wood-decay fungi. *Biotechnol Lett* 2015, 37, 1845–1852.

[24] Mason, S.; Cortes, D.; Horner, W. E. Detection of Gaseous Effluents and By-Products of Fungal Growth that Affect Environments (RP-1243). *HVAC&R Res* 2010, 16, 109–121.

[25] Lemfack, M. C.; Nickel, J.; Dunkel, M.; Preissner, R.; Piechulla, B. mVOC: a database of microbial volatiles. *Nucleic Acids Res* 2014, 42, 744-748.

[26] Araki, A.; Kanazawa, A.; Kawai, T.; Eitaki, Y.; Morimoto, K.; Nakayama, K.; Shibata, E.; Tanaka, M.; Takigawa, T.; Yoshimura, T.; et al. The relationship between exposure to microbial volatile organic compound and allergy prevalence in single-family homes. *Sci Total Environ* 2012, 423, 18–26.

[27] Müller, T.; Thißen, R.; Braun, S.; Dott, W.; Fischer, G.; Muller, T.; Thißen, R.; Braun, S.; Dott, W.; Fisher, G. (M) VoC and Composting Facilities Part 1:(M) VOC Emissions from Municipal Biowaste and Plant Refuse. *Environ Sci Pollut Res* 2004, 11, 91–97.

[28] Kuske, M.; Romain, A. C.; Nicolas, J. Microbial volatile organic compounds as indicators of fungi. Can an electronic nose detect fungi in indoor environments? *Build Environ* **2005**, *40* (6), 824–831 DOI: 10.1016/j.buildenv.2004.08.012.

[29] Albrecht, A.; Fischer, G.; Brunnemann-Stubbe, G.; Jäckel, U.; Kämpfer, P. Recommendations for study design and sampling strategies for airborne microorganisms, MVOC and odours in the surrounding of composting facilities. *Int J Hyg Environ Health* 2008, 211, 121–131.

[30] Usachev, E. V; Pankova, A. V; Rafailova, E. A.; Pyankov, O. V; Agranovski, I. E. Portable automatic bioaerosol sampling system for rapid on-site detection of targeted airborne microorganisms. *J Environ Monit* 2012, 14, 2739–2745.

[31] Eiceman, G. A.; Stone, J. A. Ion mobility spectrometry in homeland security. *Anal Chem* 2004, 76, 390–397.

[32] Fischer, G.; Muller, T.; Thißen, R.; Braun, S.; Dott, W. Process-dependent emission of

airborne fungi and MVOC from composting facilities. *Gefahrstoffe Reinhaltung Der Luft* 2004, 64, 160–167.

[33] Chambers, S. T.; Bhandari, S.; Scott-Thomas, A.; Syhre, M. Novel diagnostics: progress toward a breath test for invasive Aspergillus fumigatus. *Med Mycol Off Publ Int Soc Hum Anim Mycol* 2011, 49, 54–61.

[34] Cauchi, M.; Fowler, D. P.; Walton, C.; Turner, C.; Waring, R. H.; Ramsden, D. B.; Hunter, J. O.; Teale, P.; Cole, J. a.; Bessant, C. Comparison of GC-MS, HPLC-MS and SIFT-MS in conjunction with multivariate classification for the diagnosis of Crohn's disease in urine. *Anal. Methods* 2015, 7, 8379-8385.

[35] Garcia-Alcega, S.; Nasir, Z. A.; Ferguson, R.; Withby, C.; Dumbrell, A. J.; Colbeck, I.; Gomes, D.; Tyrrel, S.; Coulon, F. Fingerprinting outdoor air environment using microbial volatile organic compounds (MVOCs) - A review. *TrAC Trends Anal Chem* 2016, 86, 75-83.

[36] Fischer, G.; Albrecht, A.; Jäckel, U.; Kämpfer, P. Analysis of airborne microorganisms, MVOC and odour in the surrounding of composting facilities and implications for future investigations. *Int J Hyg Environ Health* 2008, 211, 132–142.

[37] Lehtinen, J.; Tolvanen, O.; Nivukoski, U.; Veijanen, A.; Hänninen, K. Occupational hygiene in terms of volatile organic compounds (VOCs) and bioaerosols at two solid waste management plants in Finland. *Waste Manag* 2013, 33, 964–973.

[38] Lehtinen, J.; Veijanen, A. Odour Monitoring by Combined TD–GC–MS–Sniff Technique and Dynamic Olfactometry at the Wastewater Treatment Plant of Low H2S Concentration. *Water, Air, Soil Pollut* 2011, 218, 185–196.

[39] Ström, G.; West, J.; Wessén, B.; Palmgren, U. Quantitative analysis of microbial volatiles in damp Swedish houses. *Heal Implic fungi indoor Environ* 1994, 1, 291–305.

[40] Mehrer, A.; Lorenz, W. Potential influences on MVOC measurements. *Proc Indoor Air* 2005, 2, 2444–2449.

[41] Korpi, A.; Järnberg, J.; Pasanen, A.-L. Microbial volatile organic compounds. *Crit Rev Toxicol* 2009, 39, 139–193.

[42] Ryan, T. J.; Beaucham, C. Dominant microbial volatile organic compounds in 23 US

homes. *Chemosphere* 2013, 90, 977–985.

[43] Murphy, K. R.; Parcsi, G.; Stuetz, R. M. Non-methane volatile organic compounds predict odor emitted from five tunnel ventilated broiler sheds. *Chemosphere* 2014, 95, 423–432.

[44] Lorenz, W.; Diederich, T.; Conrad, M. Practical experiences with MVOC as an indicator for microbial growth. In *Proceedings of Indoor Air* 2002, 341–346.

[45] Schleibinger, H.; Laussmann, D.; Bornehag, C. G.; Eis, D.; Rueden, H. Microbial volatile organic compounds in the air of moldy and mold-free indoor environments. *Indoor Air* 2008, 18, 113–124.

[46] Dunkel, M.; Schmidt, U.; Struck, S.; Berger, L.; Gruening, B.; Hossbach, J.; Jaeger, I. S.; Effmert, U.; Piechulla, B.; Eriksson, R. SuperScent—a database of flavors and scents. *Nucleic Acids Res* 2009, 37, 291–294.

[47] Bos, L. D. J.; Sterk, P. J.; Schultz, M. J. Volatile Metabolites of Pathogens: A Systematic Review. *PLoS Pathog* 2013, 9, 1–8.

[48] Stotzky, G.; Schenck, S. Volatile organic compounds and microorganisms. *CRC Crit Rev Microbiol* 1976, 4, 333–382.

[49] Gao, P.; Korley, F.; Martin, J.; Chen, B. T. Determination of unique microbial volatile organic compounds produced by five Aspergillus species commonly found in problem buildings. *Am Ind Hyg Assoc J* 2002, 63, 135–140.

[50] Syhre, M.; Scotter, J. M.; Chambers, S. T. Investigation into the production of 2-Pentylfuran by Aspergillus fumigatus and other respiratory pathogens in vitro and human breath samples. *Med Mycol* 2008, 46, 209–215.

[51] Vishwanath, V.; Sulyok, M.; Weingart, G.; Kluger, B.; Täubel, M.; Mayer, S.; Schuhmacher, R.; Krska, R.; Täubel, M.; Mayer, S.; et al. Evaluation of settled floor dust for the presence of microbial metabolites and volatile anthropogenic chemicals in indoor environments by LC-MS/MS and GC-MS methods. *Talanta* 2011, 85, 2027–2038.

[52] Choi, H.; Schmidbauer, N.; Bornehag, C. G. Non-microbial sources of microbial volatile organic compounds. *Environ Res* 2016, 148, 127–136.

[53] Kanehisa Laboratories. Kegg pathway Database. http://www.kegg.jp/kegg/pathway.html

(accessed Apr 20, 2016).

[54] Morath, S. U.; Hung, R.; Bennett, J. W. Fungal volatile organic compounds: A review with emphasis on their biotechnological potential. *Fungal Biol Rev* 2012, 26, 73–83.

[55] Matysik, S.; Herbarth, O.; Mueller, A. Determination of microbial volatile organic compounds (MVOCs) by passive sampling onto charcoal sorbents. *Chemosphere* 2009, 76, 114–119.

[56] Fischer, G.; Schwalbe, R.; Ostrowski, R.; Dott, W. Airborne fungi and their secondary metabolites in working places in a compost facility. *Mycoses* 1998, 41, 383–388.

[57] Macnaughton, S. J.; Jenkins, T. L.; Wimpee, M. H.; Cormiér, M. R.; White, D. C. Rapid extraction of lipid biomarkers from pure culture and environmental samples using pressurized accelerated hot solvent extraction. *J Microbiol Methods* 1997, 31, 19–27.

[58] Mette, A.; Zervas, A.; Tendal, K.; Lund, J. Microbial diversity in bioaerosol samples causing ODTS compared to reference bioaerosol samples as measured using Illumina sequencing and MALDI-TOF. *Environ Res* 2015, 140, 255–267.

[59] Environment Agency. Environmental monitoring bioaerosols at regulated facilities. 2017.

[60] Wilson, A. .; Baietto, M. Applications and advances in electronic-nose technologies. *Sensors* 2009, 9, 5099–5148.

[61] Wilson, A. D.; Baietto, M. Advances in Electronic-Nose Technologies Developed for Biomedical Applications. *Sensors* 2011, 11, 1105–1176.

[62] Wilson, A. D.; Lester, D. G.; Oberle, C. S. Development of Conductive Polymer Analysis for the Rapid Detection and Identification of Phytopathogenic Microbes. *Phytopathology* 2004, 94, 419–431.

[63] Wilson, A. D. Diverse applications of electronic-nose technologies in agriculture and forestry. *Sensors* 2013, 13, 2295–2348.

[64] Özden Üzmez, Ö.; Gaga, E. O.; Döğeroğlu, T. Development and field validation of a new diffusive sampler for determination of atmospheric volatile organic compounds. *Atmos Environ* 2015, 107, 174–186.

[65] Verreault, D.; Gendron, L.; Rousseau, G. M.; Veillette, M.; Massé, D.; Lindsley, W. G.; Moineau, S.; Duchaine, C. Detection of airborne lactococcal bacteriophages in cheese manufacturing plants. *Appl Environ Microbiol* 2011, 77, 491–497.

[66] Willeke, K.; Lin, X.; Grinshpun, S. A. Improved aerosol collection by combined impaction and centrifugal motion. *Aerosol Sci Technol* 1998, 28, 439–456.

[67] Langer, V.; Hartmann, G.; Niessner, R.; Seidel, M. Rapid quantification of bioaerosols containing L. pneumophila by Coriolis® μ air sampler and chemiluminescence antibody microarrays. *J Aerosol Sci* 2012, 48, 46–55.

[68] Springorum, a. C.; Clauß, M.; Hartung, J. A Temperature-Controlled AGI-30 Impinger for Sampling of Bioaerosols. *Aerosol Sci Technol 2011, 45*, 1231–1239.

[69] Claeson, A. .; Levin, J. . .; Blomquist, G.; Sunesson, A. . . Volatile metabolites from microorganisms grown on humid building materials and synthetic media. *J Env Monit* 2002, 4, 667-672.

[70] Gallego, E.; Roca, F. J.; Perales, J. F.; Guardino, X. Comparative study of the adsorption performance of a multi-sorbent bed (Carbotrap, Carbopack X, Carboxen 569) and a Tenax TA adsorbent tube for the analysis of volatile organic compounds (VOCs). *Talanta* 2010, 81, 916–924.

[71] Korpi, A.; Järnberg, J.; Pasanen, A.-L. Microbial volatile organic compounds. *Crit Rev Toxicol* 2009, 39, 139–193.

[72] Elke, K.; Begerow, J.; Oppermann, H.; Krämer, U.; Jermann, E.; Dunemann, L. Determination of selected microbial volatile organic compounds by diffusive sampling and dual-column capillary GC-FID - A new feasible approach for the detection of an exposure to indoor mould fungi? *J Environ Monit* 1999, 1, 445–452.

[73] Park, J. S.; Ikeda, K. MVOC Emissions from fungi in HVAC system. *Indoor Air* 2002, 335–340.

[74] Hung, R.; Lee, S.; Bennett, J. W. Fungal volatile organic compounds and their role in ecosystems. *Appl Microbiol Biotechnol* 2015, 99, 3395–3405.

[75] Han, T.; Mainelis, G. Design and development of an electrostatic sampler for bioaerosols with high concentration rate. *J Aerosol Sci* 2008, 39, 1066–1078.

[76] He, Q.; Yao, M. Integration of high volume portable aerosol-to-hydrosol sampling and qPCR in monitoring bioaerosols. *J Environ Monit* 2011, 13, 706–712.

[77] Dybwad, M.; Skogan, G.; Blatny, J. M. Comparative testing and evaluation of nine different air samplers: end-to-end sampling efficiencies as specific performance measurements for bioaerosol applications. *Aerosol Sci Technol* 2014, 48, 282–295.

[78] Müller, T.; Thißen, R.; Braun, S.; Dott, W.; Fischer, G.; Muller, T.; Thißen, R.; Braun, S.; Dott, W.; Fisher, G. (M) VoC and Composting Facilities Part 1:(M) VOC Emissions from Municipal Biowaste and Plant Refuse. *Environ Sci Pollut Res* 2004, 11, 91–97.

[79] Van Huffel, K.; Heynderickx, P. M.; Dewulf, J.; Van Langenhove, H. Measurement of Odorants in Livestock Buildings: SIFT-MS and TD-GC-MS. *Nose 2012 3Rd Int Conf Environ Odour Monit Control* 2012, 30, 67–72.

[80] Korpi, A.; Jarnberg, J.; Pasanen, A. L.; Järnberg, J.; Pasanen, A. L.; Jarnberg, J.; Pasanen, A. L.; Järnberg, J.; Pasanen, A. L.; Jarnberg, J.; et al. Microbial volatile organic compounds. *Crit Rev Toxicol* 2006, 39, 139–193 .

[81] Hurst, C.; Longhurst, P.; Pollard, S.; Smith, R.; Jefferson, B.; Gronow, J. Assessment of municipal waste compost as a daily cover material for odour control at landfill sites. *Environ Pollut* 2005, 135, 171–177.

[82] Betancourt, D. A.; Krebs, K.; Moore, S. A.; Martin, S. M. Microbial volatile organic compound emissions from Stachybotrys chartarumgrowing on gypsum wallboard and ceiling tile. *BMC Microbiol* 2013, 13, 1–10.

[83] Suchorab, Z.; Guz, Ł.; Łagód, G.; Sobczuk, H. The Possibility of Building Classification for Mould Threat Using Gas Sensors Array. *Adv Mater Res* 2015, 1126, 161-168.

[84] Korpi, A.; Pasanen, A. L.; Pasanen, P. Volatile Compounds Originating from Mixed Microbial cultures on building materials under various humidity conditions. *Appl Environ Microbiol* 1998, 64, 2914–2919.

[85] Rodríguez-Navas, C.; Forteza, R.; Cerdà, V. Use of thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) on identification of odorant emission focus by volatile organic compounds characterisation. *Chemosphere* 2012, 89, 1426–1436.

[86] Wady, L.; Bunte, A.; Pehrson, C.; Larsson, L. Use of gas chromatography-mass spectrometry/solid phase microextraction for the identification of MVOCs from moldy building materials. *J Microbiol Methods* 2003, 52, 325–332.

[87] Simonescu, C. C. M. *Application of FT-IR spectroscopy in environmental studies. In Advanced Aspects of Spectroscopy;* 2012, p 49.

[88] Lampert, C. Analysis of volatile organic compounds in air samples by infrared spectroscopic analysis of sorbent tube samples. PhD Dissertation, Middle Tennessee State University, 2013.

[89] Došen, I.; Nielsen, K. F.; Clausen, G.; Andersen, B. Potentially harmful secondary metabolites produced by indoor Chaetomium species on artificially and naturally contaminated building materials. *Indoor Air* 2016, 27, 1–13.

[90] Demeestere, K.; Dewulf, J.; De Witte, B.; Van Langenhove, H. Sample preparation for the analysis of volatile organic compounds in air and water matrices. *J Chromatogr A* 2007, 1153, 130–144.

[91] Zhang, Z.; Li, G. A review of advances and new developments in the analysis of biological volatile organic compounds. *Microchem J* 2010, 95, 127–139.

[92] Sun, D.; Wood-Jones, A.; Wang, W.; Vanlangenberg, C.; Jones, D.; Gower, J.; Simmons, P.; Baird, R. E.; Mlsna, T. E. Monitoring MVOC Profiles over Time from Isolates of Aspergillus flavus Using SPME GC-MS. *J Agric Chem Environ* 2014, 3, 48–63.

[93] Zhang, Q.; Zhou, L.; Chen, H.; Wang, C.; Xia, Z. Solid-Phase microextraction technology for in vitro and in vivo metabolite analysis. *Trends Anal Chem* 2016, 80, 57–65.

[94] Cohen, D. D.; Garton, D.; Stelcer, E.; Hawas, O.; Wang, T.; Poon, S.; Kim, J.; Choi, B. C.; Oh, S. N.; Shin, H.-J. Multielernental analysis and characterization of fine aerosols at several key ACE-Asia sites. *J Geophys Res D-Atmospheres* 2004, 109.

[95] Kildgaard, S.; Mansson, M.; Dosen, I.; Klitgaard, A.; Frisvad, J. C.; Larsen, T. O.; Nielsen, K. F. Accurate dereplication of bioactive secondary metabolites from marine-derived fungi by UHPLC-DAD-QTOFMS and a MS/HRMS library. *Mar Drugs* 2014, 12, 3681–3705.

[96] Vivó-Truyols, G.; Torres-Lapasió, J. R.; van Nederkassel, A. M.; Vander Heyden, Y.; Massart, D. L. Automatic program for peak detection and deconvolution of multi-overlapped chromatographic signals part I: peak detection. *J Chromatogr A* 2005, 1096, 133–145.

[97] Murphy, K. R.; Wenig, P.; Parcsi, G.; Skov, T.; Stuetz, R. M. Characterizing odorous emissions using new software for identifying peaks in chemometric models of gas chromatography-mass spectrometry datasets. *Chemom Intell Lab Syst* 2012, 118, 41–50

[98] Polizzi, V.; Delmulle, B.; Adams, A.; Moretti, A.; Susca, A.; Picco, A. M.; Rosseel, Y.; Kindt, R.; Van Bocxlaer, J.; De Kimpe, N.; et al. JEM Spotlight: Fungi, mycotoxins and microbial volatile organic compounds in mouldy interiors from water-damaged buildings. *J Environ Monit* 2009, 11, 1849–1858.

[99] Hussain, A.; Tian, M.-Y.; He, Y.-R.; Lei, Y.-Y. Differential fluctuation in virulence and VOC profiles among different cultures of entomopathogenic fungi. *J Invertebr Pathol* 2010, 104, 166–171.

[100] Wihlborg, R.; Pippitt, D.; Marsili, R. Headspace sorptive extraction and GC-TOFMS for the identification of volatile fungal metabolites. *J Microbiol Methods* 2008, 75, 244–250.

[101] Wihlborg, R.; Pippitt, D.; Marsili, R. Headspace sorptive extraction and GC-TOFMS for the identification of volatile fungal metabolites. *J Microbiol Methods* 2008, 75, 244–250.