

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 μ L) 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 - Plating - Microscopy - Flow cytometry	- CFU - Number of live/dead cells	- Easy to use - Identification at species level	- Labor intensive - Expensive - Low reproducibility - No real time - Underestimation	8–10,30
Molecular techniques - Immunochemistry - PCR, Q-PCR - Pyrosequencing - DNA/RNA-based techniques - Phospholipids analysis	- Proteins - DNA - PLFAs	- Identification at species level - Reproducible analysis - Detection at low level	- Expensive - Long laboratory protocols - No real time	12,13,18,7
Chemical techniques - FLAPS - Sensing techniques - MVOCs - FT-IR - FT-NIRS - Raman spectroscopy - Mass spectrometry	- Particles - MVOCs - Proteins	- Reproducible analysis - Detection at low level - Accurate and reliable - Rapid, non-destructive, and inexpensive - Possibility of real time	- Biosensors not easy to transport - 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.*

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 ₄ H ₈ O ₂	88.1	0.8	163.75	0.74	Bacteria and fungi
	Propanoic acid	C ₃ H ₆ O ₂	74.07	0.3	141.15	0.47	Bacteria
Aldehydes	Acetaldehyde	C ₂ H ₄ O	44.05	-0.3	20.2	101	Bacteria and fungi
	Furfural	C ₅ H ₄ O ₂	96.08	0.41	162	0.15	Bacteria and fungi
Ethers	2-methylfuran	C ₅ H ₆ O	82.1	1.85	65	23.48	Bacteria and fungi
	3-Methylfuran	C ₅ H ₆ O	82.1	1.91	65-66	21.46	Bacteria and fungi
Sulphur and nitrogen compounds	Dimethyl-sulfide	C ₂ H ₆ S	62.134	0.977	188.8	53.7	Bacteria and fungi
	Dimethyl disulfide	C ₂ H ₆ S ₂	94.19	1.77	109.8	3.83	Bacteria and fungi
	2-isopropyl-3-methoxypyrazine	C ₈ H ₁₂ N ₂ O	152.2	2.37	210.8±30.0	0.036	Bacteria
Ketones	2-heptanone	C ₇ H ₁₄ O	114.19	2.03	150.6-151.5	0.213-0.28	Bacteria and fungi

	2-hexanone	C ₆ H ₁₂ O	100.16	1.38	126-128	1.47,0.36	Bacteria and fungi
	3-Octanone	C ₈ H 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	C ₁₀ H ₁₈ O	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 ₄ H ₁₀ O	74.12	0.65-0.83	108	1.33	Bacteria and fungi
	2-methyl-1-butanol	C ₅ H ₁₂ O	88.15	1.29	128	0.416	Bacteria and fungi
	3-methyl-1-butanol	C ₅ H ₁₂ O	88.15	1.16	130.5	0.316	Bacteria and fungi
	3-methyl-2-butanol	C ₅ H ₁₂ O	88.15	1.28	111.5	1.22	Bacteria
	3-octanol	C ₈ H ₁₈ O	130.23	2.73	169	0.068	Fungi
	1-octen-3-ol	C ₈ H ₁₆ O	128.21	2.6	180	0.071	Bacteria and fungi
	2-octen-1-ol	C ₈ H ₁₆ O	128.21	2.59	195.8±8.0	0.014	Bacteria and fungi
	2-pentanol	C ₅ H ₁₂ O	88.15	1.19	119.0-119.3	0.815	Bacteria and fungi
	Methanol	CH ₄ O	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-pentylfuran	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-methyl-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

^aCompost facilities ^{27,36}; ^bMSW =Municipal solid waste treatment ³⁷; ^cWWTP= Waste water treatment plant (at sludge dewatering site) ³⁸; ^dNormal buildings= Without damp problems or non-complaint areas ^{32,44,45}; ^eLiving environments = Houses ^{32,44,43}; ^fProblem buildings=Buildings with damp problems ^{39,41}; ^gBroiler sheds ⁴³; *n.s. = not specified; nd= not detected

Apart from this, researchers do not analyze and report same MVOCs³⁵, 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-methylfuran, 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-methylfuran (≥ 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-methyl-1-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-isopropyl-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 it⁴⁸. 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 Σ 28 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 .
<i>Aspergillus flavus</i>	cis2-octen-1-ol	C ₈ H ₁₆ O	128.21	54
<i>Aspergillus fumigatus</i>	2,4-Pentadione (Acetylacetone)	C ₅ H ₈ O ₂	100.12	54
	3-Methyl-1,3-pentandione	CH ₃ COCH(CH ₃)COCH ₃	114.14	
	p-Mentha-6,8-dien-2-ol acetate	C ₁₂ H ₁₈ O ₂	194.27	
<i>Aspergillus versicolor</i>	Trimethylnonanoic acid methylester	C ₁₄ H ₂₈ O ₂	228.37	55
	1-(3-Methylphenyl)-ethanone	C ₉ H ₁₀ O	134.18	
<i>Aspergillus candidus</i>	3-Cyclohepten-1-one isomer	C ₁₈ H ₃₂ O ₂	280.45	56
<i>Emericella nidulans</i>	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 ₇ H ₁₄ O ₂	130.18	
	4,4-Dimethyl-pentenoic acid methyl ester	n/a	n/a	
<i>Penicillium clavigerum</i>	Bicyclooctan-2-one	C ₈ H ₁₂ O	124.18	56
	2-Ethyl-5-methyl-furan	C ₇ H ₁₀ O	110.15	
<i>Penicillium crustosum</i>	4-Ethylbutan-4-olide ((S)-gamma-hexalactone)	C ₆ H ₁₀ O ₂	114.14	56
	Isopropylfuran	C ₇ H ₁₀ O	110.15	
	2-Methyl-2-bornene isomer	n/a	n/a	
<i>Penicillium cyclopium</i>	delta-2-Dodecanol	n/a	n/a	56
	4-Methyl-2-(3-methyl-2-butenyl)-furan	C ₁₀ H ₁₄ O	150.22	
	beta-patchoulene-isomer	C ₁₅ H ₂₄	204.35	
	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 ₈ H ₁₆ O ₂	144.21	
<i>Penicillium roqueforti</i>	alpha-selinene	C ₁₅ H ₂₄	204.35	54
	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 ₈ H ₁₆ O ₂	144.21	
	alpha-chamigrene	C ₁₅ H ₂₄	204.35	
<i>Paecilomyces variotii</i>	3,5,7-Trimethyl-2E,4E,8E-decatetraene	C ₁₃ H ₂₀	176.3	7
	2-Methyl-2,4-hexadiene	C ₇ H ₁₂	96.17	
	delta-4-Carene	C ₁₀ H ₁₆	136.23	

Microbial Species	Specific MVOCs	Molecular formula	Molecular weight (g mol ⁻¹)	Ref .
<i>Trichodema pseudokoningii</i>	2-Methyl-pentane	C ₆ H ₁₄	86.18	7
<i>Muscodor crispans</i>	Hexane, 2,3-dimethyl-	C ₈ H ₁₈	114.23	7
	Formamide, N-(1-methylpropyl)	C ₇ H ₁₅ NO ₂	145.2	
	Cyclohexane, 1,2-dimethyl-3,5-bis(1-methylethenyl)	C ₁₄ H ₂₄	192.34	
<i>Arthrobacter globiformis</i>	2-Phenylethylamine	C ₈ H ₁₁ N	121.18	7
<i>Mycobacterium</i>	5-Methylhexan-3-ol	C ₇ H ₁₆ O	116.2	7
	7-Methyloctan-3-one	C ₉ H ₁₈ O	142.24	
	5-Methyl-4-hexen-3-one	C ₇ H ₁₂ O	112.17	
	Cyanoisoquinoline	C ₁₀ H ₆ N ₂	154.17	
<i>Bacillus spp.</i>	(2R,3R)-Butane-2,3-diol	C ₄ H ₁₀ O ₂	90.12	7
<i>Geobacillus stearothermophilus</i>	Dimethyl ditelluride	C ₂ H ₆ Te	157.67	7
	Methanetellurol	CH ₄ Te	143.64	
	dimethylselenodisulfide	n/a	173.15	
	dimethyltellurenalsulfide	n/a	189.73	
<i>Paenibacillus polymyxa</i>	2-(2-Methylpropyl)pyrazine	C ₉ H ₁₄ N ₂ O	166.22	7
	2,6-Diisobutylpyrazine	C ₁₂ H ₂₀ N ₂	192.3	
	2-Methyl-5-isobutylpyrazine	C ₉ H ₁₄ N ₂	150.22	
	2,3,4,5-tetrahydropyridazine	C ₄ H ₈ N ₂	84.12	
<i>Staphylococcus aureus</i>	4-methylhexanoic acid	CH ₃ CH ₂ CH(CH ₃)CH ₂ CH ₂ COOH	130.18	7
	Butyl butanoate (butyl butyrate)	C ₈ H ₁₆ O ₂	144.21	
	2,4-Diacetylphloroglucinol	C ₁₀ H ₁₀ O ₅	210.18	
<i>Pseudomonas trivialis</i>	Undecadiene	C ₁₁ H ₂₀	152.28	58
	Benzyloxybenzonitrile	C ₁₄ H ₁₁ NO	209.25	
<i>Escherichia coli</i>	Pentylcyclopropane	C ₈ H ₁₆	112.21	58

Microbial Species	Specific MVOCs	Molecular formula	Molecular weight (g mol ⁻¹)	Ref .
<i>Acinetobacter calcoaceticus</i>	Sulfoacetaldehyde	C ₂ H ₄ O ₄ S	124.12	58
<i>Klebsiella sp.</i>	Pentylbutanoate (or pentyl butyrate)	C ₉ H ₁₈ O ₂	158.24	58
<i>Streptomyces citreus</i>	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	
<i>Alternaria alternata</i>	6-Methylheptanol	C ₈ H ₁₈ O	130.23	58
<i>Rhizopus stolonifer</i>	1-Octene	C ₈ H ₁₆	112.24	58
	3-Methyl-3-buten-1-ol	CH ₂ =C(CH ₃)CH ₂ CH ₂ OH	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 surroundings²⁹ but there are still lacking guidelines for sampling in other environments like a farm or a park³⁵. 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 station⁵⁹. 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 sample²⁸.

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 electricity⁶⁴. 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 disulfide⁵⁵, 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 month⁶⁴.



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 respectively⁶⁶. 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-d⁸ 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

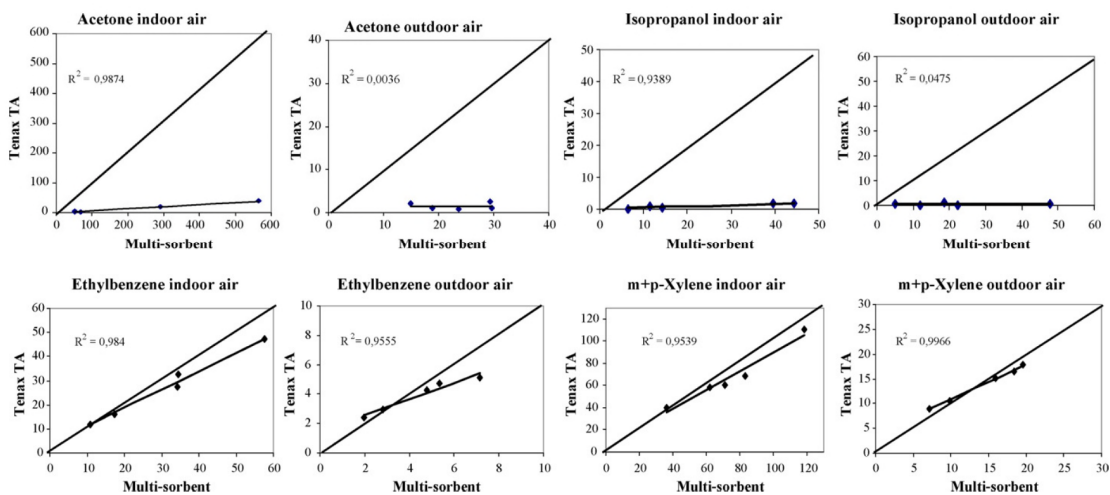


Figure 6. 4 Comparison of different compound concentrations ($\mu\text{g m}^{-3}$) using multi-sorbent bed tubes (Carbotrap, Carboxen 569) and Tenax TA tubes (from Gallego *et al.* ⁷⁰)

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

	Advantages	Limitations	Reference
Activated charcoal pads	<ul style="list-style-type: none"> - Each sample can be analyzed more than once - Good capacities for hydrocarbons, esters, ethers, alcohols, ketones, glycol ethers and halogenated hydrocarbons - Allows long sampling time: MVOCs emissions 	<ul style="list-style-type: none"> - Poor recoveries for less volatile and reactive compounds (amines, phenols, aldehydes, and unsaturated hydrocarbons) - Long sampling times (>1h to days or weeks) - Low sensitivity 	55,64,72–74

	can be monitored over the time (weeks or days) <ul style="list-style-type: none"> - Cheap, light and easy to use - Are operated without electricity 	<ul style="list-style-type: none"> - Solvent extraction and heat produce VOC degradation products 	
Impingers	<ul style="list-style-type: none"> - High sample volume can be collected - MVOCs are collected in a liquid so the microbes do not get dried - Easily portable 	<ul style="list-style-type: none"> - Possibility of losing sample liquid due to high flow rate - More variability in concentrations between replicates because the sampling time is shorter 	65,67,75–77
Tenax® Desorption tubes	<ul style="list-style-type: none"> - Quick as sample preparation is no needed - Good recoveries and precision - Easily portable 	<ul style="list-style-type: none"> - Each sample can only be analyzed once - 100 times more sensitive than solvent extraction 	34,42,49,78,79
Activated charcoal Desorption tubes	<ul style="list-style-type: none"> - Collection of very volatile MVOC - Easily portable 	<ul style="list-style-type: none"> - Each sample can only be analyzed once - Poor recoveries for less volatile and reactive compounds (amines, phenols, aldehydes, and unsaturated hydrocarbons) - 100 times more sensitive than solvent extraction 	39,55
Sep-Pak cartridges	<ul style="list-style-type: none"> - Good for sampling carbonyl compounds 	<ul style="list-style-type: none"> - Not good recoveries for rest of MVOCs 	80
Electronic nose	<ul style="list-style-type: none"> - Real time analysis - Portable - User friendly 	<ul style="list-style-type: none"> - Limited detection and identification of MVOCs in the environment - Miss classification and false positive results - Sample pre-concentration and clean up to remove the interferences - 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 amines⁸⁴ and nonvolatile microbial compounds⁵¹.

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 heat⁵⁵ 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 compounds⁸⁵. 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 absorption⁸⁷. 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 peak⁸⁸.

Fourier Transform Near Infrared Spectroscopy (FT-NIRS) has been used to detect bacterial contamination in water based pharmaceutical preparations²¹. 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 CO₂ interferences²¹.

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

	Advantages	Limitations	Reference
GC-MS	<ul style="list-style-type: none"> - Very good separation - High sensitivity 	<ul style="list-style-type: none"> - Relatively non-portable and slow analysis; - 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	<ul style="list-style-type: none"> - High sensitivity at trace levels (pg·L⁻¹ to ng·L⁻¹) - Robust and reproducible analytical method - Not sensitive to matrix effects - No solvent is needed - No sample preparation is needed - powerful separation capacity 	<ul style="list-style-type: none"> - Limited extraction efficiency and some MVOCs might not be detected 	54,86,90–93
LC-MS	<ul style="list-style-type: none"> - Good for analysis of carbonyl compounds 	<ul style="list-style-type: none"> - No suitable for volatile compounds 	84,94,95
FT-IR and FT-NIRS	<ul style="list-style-type: none"> - Low cost - Fast analysis - Environmental friendly 	<ul style="list-style-type: none"> - Low reproducibility and repeatability - Interferences from H₂O, CO₂, 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 trends⁹⁶. 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 database⁵³ 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 database⁵³ 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.

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.

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