SAMPLING MICROBIAL VOLATILE ORGANIC COMPOUNDS: OPTIMISATION OF FLOW RATE AND SAMPLING TIME

SONIA GARCÍA ALCEGA, SEAN TYRREL & FRÉDÉRIC COULON
School of Water, Energy and Environment, Cranfield University, UK

ABSTRACT

The impact of bioaerosols emissions from urban, agricultural and industrial environments on local air quality is of growing policy concern. However, there is no standardised protocol established yet, despite a large number of bioaerosols sampling methods in use. Additionally, capturing sufficient amounts of material to allow reproducible separation and detection of molecular patterns is still difficult. Chemical fingerprint analysis of microbial volatile organic compounds (MVOC) is a potentially rapid and reproducible approach for the early detection and identification of outdoor contamination as it has been shown to be a successful approach for indoor environments and it can be done on a fine-scale, allowing the identification of species-specific volatiles that may serve as marker compounds for the selective detection of pathogens. In this study we have tested the number and concentration of MVOCs collected using different sampling conditions: 10 min sampling time with variable flow rate (100, 500 and 1000 ml min^{-1}) and 100 ml min^{-1} flow rate during 10, 20 and 30 min using Tenax®-Carbotrap thermal desorption (TD) tubes attached to portable GilAir® air pumps. Our aim was to determine the best sampling conditions in order to get enough material allowing reproducible data of the microbial markers present in outdoor environments. Substantial loses (>50%) of MVOCs occurred when sampling at flow rates higher than 100 ml min^{-1}. 10 min sampling time allowed the collection of most of the MVOCs present in the air (~96%). The optimal sampling settings that allowed the collection of higher concentrations of MVOCs without breakthrough was 10 min sampling at 100 ml min^{-1} flow rate. Ketones were the predominant group of MVOCs identified in the WWTP (34–42%), acetone being the compound present at higher concentration (6476–11731 ng m^{-3}).

Keywords: MVOCs, bioaerosols, thermal desorption, chemometrics, outdoor environments.

1 INTRODUCTION

Bioaerosols are the biological particles within the aerosols formed by plant and animal origin as well as by microbes. The small size of bioaerosols (<2.5 μm) make them easily transportable and ubiquitous in ambient air [1]. Occupational activities involving high levels of biological material such as wastewater treatment plants, biowaste facilities, animal farms and agriculture are of a great concern especially for workers. Recent studies have shown that respiratory diseases such allergies, asthma or chronic obstructive pulmonary disease among others can be linked to bioaerosols exposure [2].

Microorganisms are known to produce a range of volatile organic compounds so called microbial VOC (MVOC). MVOC are secondary metabolites produced by fermentation and their volatility is due to their physicochemical properties such as low molecular weight, low boiling point and high vapour pressure [3]. Since microorganisms produce different MVOC depending on which environment they are thriving in and the concentration of the MVOC is directly correlated to the microbial concentration, bioaerosols emission from outdoor environments can be rapidly assessed and characterised by chemical analysis and chemometrics [4]. Furthermore, the identification and quantification of species-specific MVOCs can be implemented for the selective detection of pathogenic microbes in the environment.

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To date, the best currently available technique to collect environmental air samples is thermal desorption tubes (TD) coated with Tenax/Carbotrap 50/50 (v/v). This is a sensitive technique which allows the detection of MVOC at pg levels in indoor environment allowing a fast analysis without sample preparation [4], [5]. However, a wide range of sampling conditions is being used and there is no standardised protocol established yet (see Table 1). Also the optimisation, sensitivity and reproducibility of this technique has not been yet assessed and validated for outdoor environments and there is no evidence of which conditions are the most appropriate for this purpose [5].

The sampling time used often varies between 30 min and 24 h and the flow rates between 0.7 and 100 ml min^{-1}. Depending on these settings, the air volume sampled ranged between 0.25 and 3.45 L which altogether can significantly affect the sampling analysis and the reproducibility of the samples collected and therefore induced a bias towards interpretation and comparison of studies. Also there are studies that do not specify the sampling conditions that have been used [6] despite it is well known that differences in sampling time and flow rate used lead to different concentration of VOCs and MVOCs [4]. Thus, the objective of this study was to fine-tune the sampling conditions for bioaerosols emission from a waste water treatment plant (WWTP) using thermal desorption technique.

2 MATERIALS AND METHODS

2.1 Sampling sites description and sampling location

Air samples were collected from a WWTP located at the University of Cranfield, UK (Fig. 1). This site was chosen due to the easiness of the access as it is located at walking distance from Cranfield University campus. This WWTP is a traditional treatment plant comprising of balancing tank, 1st lamella clarifier, 1st rough filter, 2nd lamella clarifier, 2nd and 3rd trickling filter, tertiary filter and sedimentation tank. At this WWTP the wastewater from the campus is treated and then transferred to the Brook River, located opposite the WWTP. Samples for this experiment were collected on site, 100 m downwind and 100 m upwind the WWTP. For a method development work, an environment with high concentration of MVOCs such a WWTP was preferred. On site and downwind locations, high levels of MVOCs were expected whereas upwind much lower levels were anticipated. It was important to collect samples in all the three sites with variable concentration of MVOCs for the optimisation of the technique.

Table 1: Range of sampling conditions for MVOCs analysis using TD-tubes in contrasting indoor and outdoor environments.

Sampling location	*Flow rate (ml min^{-1})*	*Time*	*Volume sampled (L)*	*Reference*
Municipal solid waste treatment plants	69	50 min	3.45	[7]
Municipal solid waste treatment plants	Not specified	Not specified	Not specified	[6]
Broiler sheds	100	30 min	3	[8]
Mould homes	3	24 h	4.3	[9]
Emission chamber	100	30 min	3	[10]
Compost facilities	0.7	6 h	0.252	[11]
Ships	100	30 min	3	[12]

Figure 1: Cranfield wastewater treatment.

2.2 Air sample collection

Air sample collection was done using thermal desorption tubes (Markes, Llantrisant, UK). TD tubes were coated with tenax and carbotrap 50/50 v/v. The tubes were conditioned before sampling at 330°C for 45 min at 1.4 bar. TD tubes were attached to a GilAir® plus air sampling pump (Sensydine, LP-Clear water, Florida, US) with Tygon® tubes. Sampling was done at each site in triplicate with different settings as detailed in Table 2.

2.3 MVOCs analysis

Collected air samples were analysed by thermal desorption and gas chromatography–mass spectrometry (TD–GC/MSD) using a Almsco TOF coupled to a TD autosampler (Markes International Limited, Llantrisant, UK) and a 6890 N Network GC System (Agilent Technologies, Palo Alto, USA). TD tubes containing air samples were loaded with 0.5 ng of toluene-d^8 as internal standard with helium at 400 ml min^{-1} flow rate. External multilevel calibrations ranging between 0.001 and 1 ng μl^{-1} was carried out using a pull of individual MVOCs including dimethyl sulphide, 2-methyl-furan, 2-pentanol, 2-methyl-1-butanol, dimethyl disulphide, 2-heptanone, 2-pentyl furan and 2-methyl-1-propanol (Sigma-Aldrich,

Table 2: Sampling time (minutes), flow rate (ml min-1) and sampling volume conditions tested.

Conditions tested	*Sampling time (min)*	*Flow rate (ml min^{-1})*	*Air volume sampled (L)*
Fixed Sampling time and variable flow rate	10	100	1
	10	500	5
	10	1000	10
Variable sampling time and fixed flow rate	10	100	1
	20	100	2
	30	100	3

Dorset, UK). 1 µl of each concentration were loaded in new conditioned TD tubes followed by 0.5 ng of toluene-d^8 as internal standard. The GC-MS was coupled with a 78 m × 250 µm × 0.5 µm Agilent column. The initial oven temperature was set at 35°C and increased to 75°C at 2°C min^{-1}. Then the temperature was increased to 140°C at 2°C min^{-1}. The final ramp was set at 300°C at 10°C min^{-1}. Ions were monitored in full scan mode. MVOCs were identified by NIST mass spectral library. Semi-quantitation of the compounds was carried out with the internal standard toluene-d^8 and when possible, a full quantitation with pure standards was undertaken.

2.4 Accuracy and precision

An empty TD tube and a blank containing toluene-d^8 as internal standard were run as QC every 15 samples.

2.5 Statistical analysis

Differences between averages of MVOCs concentration collected with the sampling conditions tested were analysed using Microsoft Excel 2010 with *t*-tests assuming equal variances. A *p*-value of <0.05 was considered to indicate statistical significance.

3 RESULTS AND DISCUSSION

3.1 Variable sampling time

The difference in MVOC concentration during 10 min sampling compared to 20 min was 3-fold on site and upwind and 2-fold downwind. These differences where only significant when sampling upwind from the WWTP, which was the location that presented lower MVOCs concentration ($p<0.05$) (Table 3). Higher numbers of MVOCs were identified in the chromatograms corresponding to the samples collected during 10 min than during 20 min upwind and on site (10 and 5 MVOCs more respectively). Sampling time between 10 and

Table 3: Total number of MVOCs and total MVOCs concentration (ng m^{-3}) collected at constant sampling flow rate (100 ml min^{-1}) during 30, 20 and 10 minutes at Cranfield waste water treatment plant (on site, upwind and downwind).

Sampling location	*Flow rate (ml min^{-1})*	*Sampling time (min)*	*Sample volume (L)*	*Nb MVOCs*	*ΣMVOCs (ng m^{-3})*	*SD*
Upwind	100	30	3	35	21028	8007
	100	20	2	25	27614	7782
	100	10	1	35	72745	17735
Site	100	30	3	23	88787	7282
	100	20	2	36	67646	33861
	100	10	1	41	183916	19321
Downwind	100	30	3	38	43486	9999
	100	20	2	34	46966	7630
	100	10	1	34	80226	35900

20 min did not affect in the number of MVOCs collected downwind from the source. 30 min sampling on site allowed the collection of 4 more different MVOCs than during 10 and 20 min, which correspond to the heavier and less volatile ones (1-propene, 2-methyl octanol, benzophenone, hexadecane-1-ol respectively) as heavier compounds have less migration capacity and need more time to enter into the tube. The amount of the most volatile MVOCs was considerably reduced from sampling 20 and 30 min compared to 10 min (50%). Only 40% of the MVOCs identified when sampling for 10 min where collected during 30 min sampling time.

Since thermal desorption technique allows the detection of compounds present in air at low concentrations (pg), captured amount should not exceed ng levels. This means that when collecting high sample volumes of MVOCs these can breakthrough or purged off the adsorbent during sample collection. [13]. Despite the lower efficiency in collecting heavier MVOCs, sampling time of 10 min was found to be the optimal time for a reliable chemical characterisation of bioaerosols as the majority of the MVOCs present in outdoor air are volatile and are collected at higher concentration than at 20 and 30 min.

3.2 Variable flow rate

Data of sampling at a variable flow rate (100, 500 and 1000 ml min^{-1}) and constant sampling time of 10 min showed that total and individual MVOCs concentrations (ng m^{-3}) from Cranfield WWTP were 2 folds higher when sampling at 100 ml min^{-1} compared to 500 ml min^{-1} flow rate upwind, downwind and on site (p<0.05) (Table 4). Sampling using 1000 ml min^{-1} flow rate resulted in lower concentration of MVOCs captured than when sampling at 100 ml min^{-1} on site (4 times lower downwind and 10 times lower upwind). It has been recommended in the literature that for both indoor and outdoor air the average of total VOC captured should not exceed 100 µg m^{-3} (i.e. 400 ng maximum mass adsorbable in 4 L sample). Given that the adsorbents from the TD tubes have a reduced capacity but a highly efficient extraction efficiency, a small sample volume is required in order to avoid the breakthrough of the compounds [13].

Table 4: Total number of MVOCs and total MVOCs concentration (ng m^{-3}) collected at constant sampling time (10 min) and variable flow rate (100, 500 and 1000 ml min^{-1}) at Cranfield waste water treatment plant (on site, upwind and downwind).

Sampling location	*Flow rate (ml min^{-1})*	*Sampling time (min)*	*Sample volume (L)*	*MVOCs*	*ΣMVOCs (ng m^{-3})*	*SD*
Site	100	10	1	12	30579	3492
	500	10	5	12	14321	129
	1000	10	10	13	7913	728
Upwind	100	10	1	12	32864	3337
	500	10	5	13	7848	760
	1000	10	10	12	3351	315
Downwind	100	10	1	13	39040	3782
	500	10	5	11	9157	884
	1000	10	10	10	3747	410

3.3 MVOCs analysis and distribution

Among all the chemical groups of MVOCs identified at the Cranfield WWTP, ketones were the predominant MVOC group (34–42%) followed by aldehydes, alcohols and alkanes which shared similar proportions (11–22%) (Fig. 2).

Similarly, the highest concentrations of individual MVOCs were for acetone (6476–11731 ng m^{-3}) followed by nonanal, decanal, phenol, hexadecen-1-ol, 2-methyl-butane, pentane and hexane respectively (~1000 ng m^{-3}, see Table 5).

4 CONCLUSIONS

Our experimental data showed that 10 min sampling and 100 ml min^{-1} flow rate were the best settings for the chemical characterisation of bioaerosols. Due to the limited adsorption capacity of the adsorbents, higher flow rate than 100 ml min^{-1} and sampling times longer than 10 min (air volumes over 1 L) saturated the adsorbent of the TD tubes and VOCs started desorbing (breakthrough). The difference in sampling time indeed affected either the number of MVOCs collected or the amount of each compound captured. However, heavier compounds which have less migration capacity, needed more time to enter into the TD tube and were better sampled during 30 min. Since these heavier compounds represent <4% of the total MVOCs, 10 min sampling at 100 ml min^{-1} flow rate seems the best compromise for characterising and quantifying MVOCs from outdoor environments. Ketones were the chemical group of MVOCs most predominant in Cranfield WWTP (34–42%), being acetone the compound present at higher concentration (647611731 ng m^{-3}). Aldehydes, alcohols and alkanes shared similar proportions (11–22% of the total MVOCs) being nonanal, decanal, phenol, hexadecen-1-ol, 2-mthyl-butane, pentane and hexane the compounds present at higher levels respectively.

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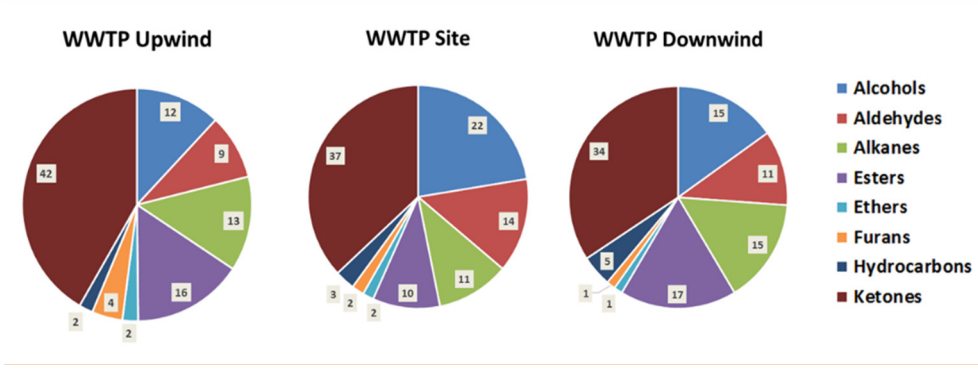

Figure 2: Distribution of chemical groups of MVOCs at the WWTP.

Table 5: MVOCs (ng m^{-3}) identified in Cranfield WWTP upwind, on site and downwind.

Chemical group	*Compound*	*Cranfield WWTP upwind*	*Cranfield WWTP site*	*Cranfield WWTP downwind*
Alcohol	Isopropyl alcohol	373	492	698
	2-Ethyl-1-hexanol	275	248	406
	Phenol	1158	3267	3005
	1-Nonanol	nd	931	235
	1-Dodecanol	153	nd	130
	Hexadecen-1-ol	383	1131	1343
Aldehyde	2-Propenal	309	nd	nd
	Butanal	66	nd	nd
	Hexanal	68	nd	nd
	Octanal	144	236	227
	Heptanal	54	nd	nd
	Nonanal	388	1138	1476
	Decanal	748	2183	2495
	Undecanal	nd	149	nd
Alkane	2-Methyl-butane	767	661	690
	Pentane	749	571	650
	2-Methyl-pentane	nd	nd	352
	Hexane	655	902	720
	Heptane	nd	nd	122
	Octane	nd	nd	361
	Nonane	214	135	779
	Decane	nd	nd	231
	Undecane	191	182	238
	(Z)-6-Methyl-2-	nd	nd	177
	Tridecane	nd	nd	350
	Hexadecane	nd	285	677
	Nonadecane	nd	nd	383
	1-Iodo-nonane	nd	166	125
Ester	Acetic acid methyl	440	nd	nd
	Ethyl acetate	1092	583	1758
	Butanoic acid	nd	nd	246
	Hexanoic acid	nd	nd	1975
	Propanoic acid	1502	1729	2621
	Pentafluoropropio	nd	359	nd
Ether	Dimethyl ether	181	nd	nd
	2-Methoxy-2-	248	439	480
Furan	1-(2-Furanyl)-3-	477	490	505
	3-Methyl-2(5H)-	205	nd	nd
	Dihydro-3-methyl-	160	nd	nd
Hydrocarbon	o-Xylene	384	540	346
	Ethylbenzene	nd	nd	347
	p-Xylene	nd	nd	702
	1-3-Dimethyl	nd	252	358
Ketone	Acetone	6476	9102	11731
	2-Butanone	378	599	554
	6-Methyl-5-	560	nd	214
	2-Butanone	499	nd	636
	Butyrolactone	223	nd	nd
	2,2,5-Trimethyl-3-	nd	298	nd

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