Variations of odorous VOCs detected by different assessors via gas chromatography coupled with mass spectrometry and olfactory detection port (ODP) system


ABSTRACT

Odorous volatile organic compounds (VOCs) were analysed via a thermal desorption gas chromatograph coupled with a mass spectrometer and olfactory detection port (TD-GC-MS/ODP) to characterize odour emissions from a wastewater treatment plant (WWTP). Three trained ODP assessors, screened according to the European dynamic olfactometry standard (EN: 13725), were used for the olfactory analysis. Their sensitivity to n-butanol varied within the acceptable limits. VOC samples from the WWTP were collected onto Tenax TA sorbent tubes in triplicate and each assessor analysed one tube in the same sample desorbing and analysing conditions. Intensities of odours detected from ODP were scaled from 1 to 4. The ODP assessors used their own odour descriptors based on their own experience as well as referenced descriptors on published compost and wastewater odour wheels. The ODP assessors detected a total of 32 different odorous VOCs; however, the intensities assigned by each assessor to particular VOCs varied. Moreover, some odorous VOCs were not detected by all assessors. For example, geosmin was detected by only two assessors. The use of a TD-GC-MS/ODP system for the analysis of odorous VOCs is valuable when analysed by different assessors, allowing a range of responses to specific odorants in a populations to be investigated.

Key words | odour, olfactory detection port (ODP), sniffer (assessor), VOCs, wastewater

INTRODUCTION

Gas chromatography (GC) equipped with chemical detectors (CD) and an olfactory detection port (ODP) offers the possibility to assess odour-active components in complex mixtures, as well as to obtain specific correlations of odour-active components with chromatographic peaks (d’Acampora Zellner et al. 2008). These combined techniques allow for deeper comprehension of the odour mixture composition. Compounds can be identified and quantified according to both their chemical and olfactory qualities (sensory characters). For each odorant separated by the GC, with his/her nose a human assessor has the potential to detect compounds, to measure the duration of the odour activity, describe the quality of the odour perceived, and to quantify the intensity of the odour (Delahunty et al. 2006).

The application of a GC analytical/sensory technique for the assessment of environmental odours has mainly focused on the identification of priority odorants and the characterization of changes in composition of odorous emissions from various agricultural operations such as swine barns, poultry houses and dairy facilities, municipal waste, and wastewater management operations (Kai & Schäfer 2004; Wright et al. 2005; Wang et al. 2010).

Some compounds with very low odour thresholds might be present in such low concentrations that CD (require compound concentration at level of 10^-13 g) cannot detect them but the assessors could (distinguish some volatile compounds at 10^-17 g) (Acree & Bernard 1984). This means that the human nose has the potential to detect odours even when there is no corresponding chromatogram peak (Khiari et al. 1992; Kai & Schäfer 2004; Knudsen et al. 2007). For example, a 2 ng/L concentration was considered as the limit of detection for geosmin by the mass spectrometer detector (MSD), while the odour could still be
perceived at a level of 0.5 ng/L by the human nose (Hocher-eau & Bruchet 2004).

Cross-tabulation of results between chemical and sensory analysis can develop new insights into singular odorant contributions to overall smell. However, only when the ODP assessors are selected properly with sufficient sensitivity can they distinguish between a variety of odour characters.

The sensory assessment of odour emissions can be based on odour units measured by dilution olfactometry (using the European dynamic olfactometry standard EN: 13725 (CEN 2003) or equivalent national standards). Dilution olfactometry is based on the use of a dilution instrument, called an olfactometer, which presents the odour sample, diluted with odour-free air at precise ratios, to a panel of human assessors. The assessors are selected in compliance with a procedure performed using reference gas n-butanol; only those with an acceptable odour threshold and who meet predetermined repeatability and accuracy criteria are selected as panellists. However, sensitivity for n-butanol may not correspond with sensitivity for other odorants.

This paper shows the variation between ODP assessors in identification and intensity assessment of odorous volatile organic compounds (VOCs) from a wastewater treatment plant (WWTP).

MATERIAL AND METHODS

The biosolids cakes were analysed during a 35-day storage period, so their odour composition in the emission changed. However, this paper only shows variation between ODP assessors and not analytical variation in the emission. Emissions from 218 WWTP dewatered biosolids cake samples were captured using a US Environmental Protection Agency dynamic flux hood operated according to the standard AS/NZS 4323.4 (2009). High purity nitrogen gas was used to purge the chamber to a stable sampling condition before the emission samples were collected onto Tenax TA sorbent tubes in triplicate.

Sample analysis was performed using a gas chromatograph equipped with an MSD (Agilent 7890A GC, 5975C MSD, Agilent Technologies, USA) and an ODP (ODP2, Gerstel GmbH & Co., Germany). A DB-VRX 30 m × 0.25 mm × 1.4 μm column, (Agilent Technologies, USA) was utilized in the gas chromatograph for compound separation, with helium as the carrier gas at a flow rate of 1.6 mL/min. Samples were loaded on an Ultra automatic sampler (Markes International, UK) and then thermally desorbed using a Unity thermal desorber (Markes International, UK). A general-purpose cold trap (U-T11PGC, Markes International, UK) was utilized to concentrate the sample prior to injection. The sorbent tubes were heated at 275 °C for 8 min while high purity helium was passed through the sorbent tubes at the flow rate of 50 mL/min to desorb VOCs, which were then concentrated onto the cold trap at minus 10 °C by a Peltier cooler. Following the sample transfer, the cold trap was heated to 300 °C at 50 °C/min heating rate for 6 min to desorb the VOCs from the cold trap and then injected into the chromatographic column. A split flow of 20.7 mL/min was applied during tube and traps desorption to prevent column overloading. The GC column temperature was initially held at 50 °C for 2 min, then raised at a rate of 15 °C/min to 200 °C, and then held for 5 min.

The eluent from the gas chromatograph was split between a mass spectrometer operating in scan mode and an ODP. For assessors to effectively sniff at the ODP, the split ratio between the MSD and port was set at 2:3 (MSD:ODP) based on the calculation of the Gerstel Column Calculator (Gerstel GmbH & Co., Germany).

Three ODP assessors complying with panellist requirements according to EN: 13725, were used for the olfactory analysis. The assessors included two males – RB and XW – and one female – RF. The n-butanol sensitivity of the ODP assessors was 21, 25 and 41 ppb for RB, RF and XW, respectively. Assessors RB and RF were trained whilst assessor XW had previous experience with ODP analysis. A simplified direct intensity method was employed to record odour descriptors and intensities (Van Ruth 2001; Delahunty et al. 2006). Each ODP assessor analysed one of each triplicate sorbent tube. Intensities of odours detected from ODP were recorded by hand or using a controller device with a scale from 1 to 4 (with 4 being the strongest and 1 the weakest odour intensity) according to the Gerstel ODP Recorder system (Gerstel GmbH & Co., Germany). Moreover, the assessor RB extended the scale for additional half points: 0.5, 1.5, 2.5 and 3.5. ODP measurements were performed in an odour-free room. During the sample analysis, odour descriptors were recorded with voice-recognition software during olfactometry runs. ODP assessors used their own odour descriptors based on their own experience and referenced descriptors on published compost and wastewater odour wheels (Suffet & Rosenfeld 2007). To avoid fatigue and contamination, each analyst was responsible for analysing a maximum of three samples in a session with duration of 17 min for each sample and six samples maximum per day, separated by at least 2 hours between runs.

An ODP result recorded as odorous VOCs was assumed to be valid if an assessor detected a response at a similar GC
retention time and similar odour character across all 656 samples; however, not all odorous VOCs were identified in every sample. Otherwise the results were treated as noise (insignificant). The ODP response could take a few seconds, from the time when the assessor started smelling to the end of the signal. Retention time (RT) refers to the amount of time a solute spends in a chromatographic column. Median RTs are reported to a hundredth of a minute. In Table 1, odorous VOCs were grouped by distinguishing one from another based on the similar RT and similar odour characters. Because of the nature of the ODP analysis the ranges of RTs among odorous VOCs

<table>
<thead>
<tr>
<th>Odorous VOC</th>
<th>Median RT [min. 1/100 of min]</th>
<th>Descriptors</th>
<th>Mass spectra library comparison results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.92</td>
<td>Fishy, garbage, rubber, burned rubber</td>
<td>Trimethylamine</td>
</tr>
<tr>
<td>2</td>
<td>3.76</td>
<td>Rubbish, seaweed, garlic, sulfur</td>
<td>Dimethyl sulfide</td>
</tr>
<tr>
<td>3</td>
<td>6.24</td>
<td>Bad breath, garlic, sulfur</td>
<td>Unknown</td>
</tr>
<tr>
<td>4</td>
<td>6.46</td>
<td>Sulfur, fishy, truffles</td>
<td>Unknown</td>
</tr>
<tr>
<td>5</td>
<td>7.0</td>
<td>Garlic, sulfur, seaweed</td>
<td>Dimethyl disulfide</td>
</tr>
<tr>
<td>6</td>
<td>7.4</td>
<td>Solvent, gasoline, burning plastic</td>
<td>Toluene</td>
</tr>
<tr>
<td>7</td>
<td>7.71</td>
<td>Garlic, sulfur, seaweed</td>
<td>Unknown</td>
</tr>
<tr>
<td>8</td>
<td>7.9</td>
<td>Rubbish, sulfur, seaweed, fishy, astringent, acrid, onion</td>
<td>Unknown</td>
</tr>
<tr>
<td>9</td>
<td>8.09</td>
<td>Sulfur, seaweed, sweet, acrid, astringent, oily popcorn, garlic,</td>
<td>Unknown</td>
</tr>
<tr>
<td>10</td>
<td>8.85</td>
<td>Sweet, soy sauce, chemical, floral, cleaning product, roses, minty, solvent</td>
<td>Xylene</td>
</tr>
<tr>
<td>11</td>
<td>9.69</td>
<td>Yeast, soy sauce, sweet, pinene, floral, roses, onion, lime</td>
<td>a-Pinene</td>
</tr>
<tr>
<td>12</td>
<td>10.10</td>
<td>Mushrooms, urine, chemical, musty</td>
<td>Unknown</td>
</tr>
<tr>
<td>13</td>
<td>10.21</td>
<td>Rotten cabbage, sulfur, porcini mushrooms</td>
<td>Dimethyl trisulfide</td>
</tr>
<tr>
<td>14</td>
<td>10.34</td>
<td>Chemical oily, plastic, chemical paint, chemical glue, solvent, sulfur</td>
<td>Unknown</td>
</tr>
<tr>
<td>15</td>
<td>10.59</td>
<td>Geranium, herbaceous, pinene, grassy</td>
<td>Unknown</td>
</tr>
<tr>
<td>16</td>
<td>11.00</td>
<td>Musty, wet cloth, oily, herbaceous</td>
<td>Unknown</td>
</tr>
<tr>
<td>17</td>
<td>11.15</td>
<td>Urine, fizzy chemical, milk powder, herbaceous, minty</td>
<td>Unknown</td>
</tr>
<tr>
<td>18</td>
<td>11.42</td>
<td>Chemical, herbaceous, soil, musty, sweet, floral,</td>
<td>Unknown</td>
</tr>
<tr>
<td>19</td>
<td>11.57</td>
<td>Sweet, chemical, gasoline, solvent, betadine, hospital, lychee, burning</td>
<td>Unknown</td>
</tr>
<tr>
<td>20</td>
<td>11.78</td>
<td>Musty, wet cloth, oily, herbaceous, sweet, hospital</td>
<td>Unknown</td>
</tr>
<tr>
<td>21</td>
<td>11.99</td>
<td>Sweet, floral, chemical, naphthalene, perfume, mothballs, herbaceous, roses</td>
<td>Unknown</td>
</tr>
<tr>
<td>22</td>
<td>12.33</td>
<td>Pepper, roses, dusty, hay, chicken stock, chilli, wheat</td>
<td>Unknown</td>
</tr>
<tr>
<td>23</td>
<td>12.55</td>
<td>Milk powder, chicken stock, yeast, chocolate, plastic</td>
<td>Unknown</td>
</tr>
<tr>
<td>24</td>
<td>12.71</td>
<td>Wood, pepper, roses</td>
<td>Unknown</td>
</tr>
<tr>
<td>25</td>
<td>12.96</td>
<td>Bread, yeast, oily popcorn</td>
<td>Unknown</td>
</tr>
<tr>
<td>26</td>
<td>13.25</td>
<td>Gasoline, petrol, plastic, machine, burn, solvent, sweet</td>
<td>Unknown</td>
</tr>
<tr>
<td>27</td>
<td>13.52</td>
<td>Machine, chemical, herbaceous</td>
<td>Unknown</td>
</tr>
<tr>
<td>28</td>
<td>14.14</td>
<td>Sweet, floral, faecal</td>
<td>Unknown</td>
</tr>
<tr>
<td>29</td>
<td>14.92</td>
<td>Musty, gasoline, hospital, machine</td>
<td>2,4,6-Trichloroanisole</td>
</tr>
<tr>
<td>30</td>
<td>15.49</td>
<td>Apples, sweet, solvent</td>
<td>Unknown</td>
</tr>
<tr>
<td>31</td>
<td>15.81</td>
<td>Faecal, naphthalene, indole, chemical, chlorine, musty, betadine</td>
<td>Unknown</td>
</tr>
<tr>
<td>32</td>
<td>16.51</td>
<td>Pesticide, geosmin, musty</td>
<td>Geosmin</td>
</tr>
</tbody>
</table>
vary. Odorous VOCs were identified based on commercial mass spectral library NIST 11 (Agilent Chemstation software, Santa Clara, CA, USA) with probability of matches between the fragment sizes greater than 75% and if the odour character described in the literature matched with odour characters reported by the assessor.

**ODP assessor training procedure for intensity method**

ODP assessor training consisted of four steps: 1 – introduction of the ODP method; 2 – vocabulary descriptors training using standard compounds; 3 – training with the reference mixture and learning the use of the scale with ODP; 4 – training with the reference mixture with different concentrations.

During the second step seven pure chemicals with different odour characters/descriptors, which occurred in biosolids emission, were used for panelist training: (R)-(+) limonene as citrus; eucalyptol as pine, turpentine; indole as mothball, burnt, faecal; dimethyl trisulfide as sulfur, fish, cabbage, marshy, swampy; pyridine as putrid; p-xylene as plastic; and dimethyl sulfide as canned corn, cabbage, sulfur, gasoline (Suffet & Rosenfeld 2007; Flavomet 2004). Water-diluted samples of these compounds were presented to the assessors in labelled vials; assessors sniffed every vial and described the character of each compound and then tried to remember the character of each compounds. The procedure was repeated until every assessor had learned all descriptors corresponding to each compound. On the second day assessor awareness of each descriptor was checked by presenting them with the same chemical group, like most sulfur compounds. Or they might be two different compounds with similar odour character belonging to the same chemical substance. Or they might be different isomers of one chemical. The reason is that they might be different in molecular structure, and chemically they were not the same.

After passing vocabulary descriptors training the assessors started ODP intensity training with the reference mixture and learned how to use the scale. Four sorbent tubes with odorants diluted in methanol with decreasing concentrations were presented to every individual assessor through the TD-GC-MS/ODP system. Odorant concentrations which were prepared based on the experiment corresponded with the scale, where 1 means low and 4 extremely high. The final step consisted of performing the odour-descriptive analysis of six model solutions simulating the matrix of the research. Taking into account the results obtained in the ODP intensity training step, assessors were asked to evaluate the intensity, according to their own scale, of the sensory attributes as well as recording the descriptors. Odorant concentrations were unknown to the assessors.

**RESULTS AND DISCUSSION**

In total, 32 potentially different odorous VOCs were found. A summary of the odorous VOCs is presented in Table 1. Only seven of 32 odorous VOCs were relatively easy to identify based on matches with NIST 11 library. These were trimethylamine, dimethyl sulphide, dimethyl disulphide, xylene, α-pinene, 2,4,6-trichloroanisole, and geosmin.

The variations in reported odour descriptors may have been due to subjective associations. Humans can detect and discriminate countless odorants, but can identify few by name, especially if they do not have labels to choose from (Yeshurun & Sobel 2010). Differences in descriptors may also be influenced by the concentration of odorants, and the threshold range of individual analysts. For example, odorous VOC No. 28 was only detected by assessor RF, and was described as sweet, floral and faecal. These disparate descriptors may potentially be associated with the co-elution of two or even more odorants with different odour characters around 14.14 min. Moreover in some cases ODP assessors used different descriptors for the same odorous VOC. For example odour 29 was described as musty, gasoline, hospital by RB and RF and as machine smell by XW.

It is noteworthy that some of the odorous VOCs have similar descriptors, such as No. 16 and No. 20. The potential explanation is that they might be different isomers of one chemical substance. Or they might be two different compounds with similar odour character belonging to the same chemical group, like most sulfur compounds.

To compare responses of the ODP assessors, kernel density estimation (Trosset 2009; Hallgren 2012) was used. This is a useful non-parametric technique for visualizing the underlying distribution of a continuous variable. The kernel density estimation was conducted individually for all assessors using 0.05 smoothing bandwidth as shown in Figure 1. Figure 1 shows how frequent individual assessors detected odorous VOCs, however without distinction of the odour intensity. Figure 1 shows that the probability density function looks different for each assessor.

It is noteworthy that some odorous VOCs are detected only by two or even by one assessor. For example odorous VOCs no. 6, 7, 11, 22, 25 and 26 with median RTs 7.4, 7.71, 9.69, 12.33, 12.96 and 13.25 min, were only detected by RB and RF. While odorous VOCs no. 17, 24, 27 and 32 (geosmin), with median RTs 11.15, 12.71, 13.52 and 16.51 min, were only detected by RF and XW. The reason for this may be because of sensitivity differences of the three assessors where RB and RF are more sensitive than XW, as their n-butanol thresholds indicated.
Additionally, some odorous VOCs were detected very often by one or two operators whilst only a few times by the other participants. For instance, odorous VOC no. 8 with median RT 7.9 min was detected 127 times by RF, 44 times by RB and only seven times by XW; odorous VOC no. 9 with median RT 8.09 min was detected 91 times by RF, 38 times by RB and only two times by XW; and odorous VOC no. 12 with median RT 10.10 min was detected 87 times by RF, 83 times by RB and only five times by XW. Similar situations were seen for odorous VOCs no. 1, 21, 23, 30 and 31. This may be decided by concentration level of samples collected at different time (age of biosolids) and sensitivity of assessors.

In most situations RF detected odorous VOCs most often. Only in a few cases (13, 16, 20) did RF detect less frequently than other assessors. Odorous VOC no. 20 with median RT 11.78 min was detected 140 times by RB, 118 times by XW and only 63 times by RF. Moreover, odorous VOCs no. 15 and 28 with median RTs of 10.59 and 14.14 min, respectively, were detected only by RF. This may imply that sensitivity of assessors to different odours may vary, which means an assessor who is more sensitive to n-butanol may not necessarily be sensitive to another odour.

In Figure 2, the results of odorous VOCs intensities for every odorous VOC and for every ODP assessor are
shown. In most cases, intensities of odorous VOCs reported by XW were lower than intensities reported by the rest of the assessors, which may be consistent with the lower n-butanol sensitivity.

From the group of 32 odorous VOCs identified, some were frequently reported by all ODP assessors and may be considered key odorous contributors to the overall odour emission, especially for VOCs which exhibited intensities extending over the whole range of the scale. Such odorous VOCs are: No. 15 which was detected 184, 189 and 195 times; No. 16 detected 192, 178 and 186 times; No. 18 detected 117, 163 and 161 times; No. 20 detected 140, 63 and 118 times; and No. 29 detected 182, 184 and 104 times, by RB, RF and XW, respectively. Additionally, geosmin was detected relatively often but only by two assessors, 170 and 118 times by RF and XW, respectively.

In previous studies, much GC-MS/ODP environmental research has been conducted using only one assessor. Examples are the characterization of VOCs and odorants associated with swine barn particulate matter (Cai et al. 2006), analysis of compounds causing tastes and odour in drinking water (Young & Suffet 1999), research on occurrence of the main odorous compounds in water sources including rivers, lakes and reservoirs (Yu et al. 2009) and in the evaluation of odorous chemicals in drinking water (Khiairi et al. 1992). It is very likely that in those studies some compounds or a group of compounds could have been missed as only one assessor was used.

There is no standardized methodology for GC-MS/ODP and only a limited number of guidelines for assessors and number of assessors have been proposed. Even with careful selection and training to improve assessor performance and therefore accuracy and precision of the data collected (Vene et al. 2017), it cannot be excluded that one or more assessors may have a specific anosmia (Doty 1979). This consideration is of great importance, especially when identifying compounds responsible for odours.

On the other hand, some environmental studies using more than one ODP assessor have been published. For example, Agus et al. (2012) identified characteristic odorants in municipal wastewater effluent and monitoring compounds that could affect the aesthetics of drinking water. Agus et al. (2011) characterized the occurrence and the fate of odorants at different stages of advanced WWTPs and in potable water reuse systems, while Hochereau & Bruchet (2004) analysed taste and odour episodes in drinking water. However, none of these studies compared the performance of individual assessors for individual odorants. As shown in this study, the influence of assessor variations when identifying key odorants needs to be considered and reported. Future GC-MS/ODP analysis should be based on an agreed-upon minimum number of assessors, e.g. six such as is used for dynamic olfactometry; however, increasing assessor numbers also increases costs. The frequency of detection of specific odorous VOCs by different ODP assessors could be included as a factor relating to the potential for the odorant to be a key odour component. Frequency based methods such as nasal impact frequency or surface of nasal impact frequency values recommend the use of 6–12 people to determine the percentage of people detecting each odour at a given retention time (Brattoli et al. 2013, 2014). Such methods have been used in the food and beverage industry; however, their use is not widely reported in environmental analyses.

CONCLUSIONS

The ODP assessors detected 32 different potentially odorous VOCs from biosolids emissions. However, the frequencies of positive responses and the intensities assigned by each assessor to particular VOCs varied. Moreover, some odorous VOCs were not detected by all assessors, probably because of different sensitivity level of assessors to specific compounds. For example, geosmin was detected by only two assessors. The use of TD-GC-MS/ODP systems for the analysis of odorous VOCs could be valuable when analysed by different assessors, which would allow a range of responses to specific odorants in a population to be investigated. Even with careful selection and training of analysts to detect and describe GC effluent odours, variability was still present in the qualitative odour descriptions and intensity ratings due to the subjective nature of sensory analysis. As individuals’ odour sensitivity to different odorous VOCs may vary, the results from only one assessor within the analysis is not sufficient, especially when the purpose of the research is to identify odorous components. The quality and quantity identification of other compounds and their correlations with the responses from ODP will be the subject of future papers.

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