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Odorous VOCs Identification from Ageing Dewatered Anaerobically Stabilised Biosolids from Polish WWTP by Two-dimensional Gas Chromatography Couple with Time-of-Flight Mass Spectrometry

Radosław J. Barczak\*, Hubert Byliński\*\*, Tomasz Dymerski\*\*\*, Jacek Gębicki\*\*\*, Jacek Namieśnik\*\*\*

\* UNSW Water Research Centre, School of Civil and Environmental Engineering, The University of New South Wales, NSW 2052, Sydney, Australia; Warsaw University of Technology, Faculty of Building Services, Hydro and Environmental Engineering, Nowowiejska 20 Street, 00-653, Warsaw, Poland

\*\* Gdansk University of Technology, Faculty of Civil and Environmental Engineering, Narutowicza 11/12 Street, 80-233, Gdańsk, Poland

\*\*\* Gdansk University of Technology, Faculty of Chemistry, Narutowicza 11/12 Street, 80-233, Gdańsk, Poland

r.barczak@unsw.edu.au

Odour from biosolids emission is a complicated matrix. Still a little is known about chemical composition of odours from biosolids and which compounds are responsible for overall odour. Apart from well-known odorants such as sulfur compounds and ammonia, participation of volatile organic compounds (VOCs) in overall odour may be of importance. Especially, if those chemicals have a low or unknown odour threshold, exist in low concentrations and their identification possesses an analytical challenge. In this work two anaerobically stabilised dewatered biosolids samples from wastewater treatment plant in Poland were stored over 21 days under ambient conditions. Emissions from biosolids samples were collected in triplicate onto Tenax TA sorbent tubes using a U.S. EPA flux hood method on days 1, 7, 10, 14 and 21. The 55 VOCs coming from different chemical compounds categories such as aliphatic and aromatic hydrocarbons, alcohols, aldehydes, ketones, sulphides and esters were identified by two-dimensional gas chromatography couple with time-of-flight mass spectrometry. The results for both biosolids samples vary. For example, during the first day of the biosolids storage 2-methylthiophene and 2-methylheptane were detected in both biosolids cakes whilst methanethiol and methyl ethyl sulphide in the biosolids cake no. 1 only. Only a few compounds were presented during the entire storage period: toluene, styrene, dimethyl sulphide (apart from the first day from biosolids cake no 1) and hexadecanal (apart from the seventh day from biosolids cake no 2). Most of the compounds were detected on an irregular basis across the whole measurement period.

* 1. Introduction

Odours are a common occurrence at and around biosolids processing facilities and biosolids application locations (Visan & Parker, 2004). In order to develop strategies for minimising odour impact on local communities (Hayes et al., 2017), it is necessary to identify odorous compounds and their fate during the biosolids processing, storage and land application. Odour from biosolids emission is a complicated matrix which produces many odorous chemical compounds, mainly volatile sulphur compounds, volatile nitrogenous compounds and volatile organic compounds (VOCs) or others (Fisher et al., 2018a; Byliński et al., 2019; Fisher et al., 2017). Still a little is known about chemical composition of odours from biosolids and which compounds are responsible for overall odour (Barczak et al., 2018). Due to the variability in individual chemical properties of odorants from the biosolids emission, no analytical technique can be applied universally for odorants assessment. None of the methodologies can be self-sufficient to obtain the most complete and exhaustive comprehension of the odorants emission taking into account the necessary high-time resolution, sensitivity, reliability, and reproducibility (Giungato et al., 2018). Apart from the well-known odorants associated organic matter degradation, such as sulfur compounds and ammonia, the participation of other VOCs in the overall odour may be of importance (Barczak et al., 2019). Especially, if those chemicals have a low or unknown odour threshold, exist in low concentrations and their identification possesses an analytical challenge. Such compounds can contribute to the overall odour properties of emissions, as even low-impact odorants that are considered unimportant in odour perception can have great significance in total odour perception (Ryan et al., 2008). It is therefore essential to identify and quantify the trace VOCs, as these compounds can contribute to the overall odour (Zhou et al., 2016). For example, Barczak et al. (2019) reported importance of 2,4,6-Trichloroanisole (TCA) as a potentially important odorant in the emissions from anaerobically stabilized dewatered biosolids. In that study TCA was hardly detected by GC-MS, whilst commonly detected by sensory examination.

To obtain a full picture of the odour and its odorants components require using different analytical methods with highest resolution and accuracy. In the case of separation of complex mixtures of chemical compounds, one-dimensional chromatographic analysis seemed to be insufficient for the identification of a wide spectrum of the substances presented in the samples. Better resolution allows two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC–TOF–MS). The GCxGC technique provides the information about a composition of the investigated samples characterized by a complex matrix composition. The aim of this paper was to qualitatively identified VOCs from an emission from ageing biosolids samples.

The knowledge about full chemical speciation will allow to elaborate effective deodorization methods. Odour production in biosolids is a complex process influenced by several factors including, but not limited to, process variables within anaerobic digestion and dewatering processes, as well as the relationships between odour perception and the concentration of odorants (Adams, 2004).

* 1. Methodology

Biosolids samples were collected from a WWTP with average daily flow Qav equal to 92 200 m3/d in Gdańsk, Poland. The mechanical part of WWTP consists of four mechanical screens (two hook-belt screens with 6-mm clearance and two scraper screens with 10-mm clearance), two sand traps (30 m × 10.4 m × 4.5 m thickness) and three primary settling tanks (diameter ca. 50 m each). The six bioreactors (summary 158,100 m3) and secondary settling tanks are part of the biological section. Primary sludge and waste activated sludge stabilization process is conducted in an anaerobic mesophilic fermentation in the temperature 37 °C in the closed digester chambers with the retention time of 21–28 days. The two dewatered biosolids cakes, namely B1 and B2 with the approximate capacity of 10L were collected from the conveying line connecting thickener and combustion chamber. The dry mass of dewatered biosolids was in the range of 19 - 22%. Sampling was conducted in two consecutive days in the springtime. Biosolids samples were transported in polypropylene bucket to the laboratory. Each sample was stored in a bucket, creating about 15 cm thin layer of biosolids. The samples were processed for the first emission analysis within 6 hours of sampling. Emission from the stored biosolids samples were captured using the US EPA dynamic flux hoods. The hood volume was 0.03 m3 and enclosed 0.13 m2 of surface area. Emission samples were collected at a constant flow rate (100 mL/min for 10 mins) onto Tenax TA sorbent tubes in triplicate. Nitrogen gas, with the constant flow at 5 L/min, was used to purge the hood for 30 mins before the emission samples, which allowed the flux hood headspace to change five times. Aged emission samples were captured on day 1, 7, 10, 14, and 21. Between emission sampling, the trays were loosely sealed with a lid allowing moisture loss. The individual samples were stored in aerobic conditions without stirring.

Analysis of samples containing odorous VOCs released from biosolids was performed using two-dimensional gas chromatography (Agilent Technologies, Palo Alto, CA, USA) coupled with a mass spectrometer and fragment ion passage time analyser (LECO Corp., St. Joseph, MI, USA). The set of chromatographic columns consisted of the first column (30 m x 0.25 mm x 0.25 µm; stationary phase type Equity 1 (Sigma Aldrich, USA)) and the second column (2.0 m x 0.10 mm x 0.10 µm; stationary phase sol gel type Wax (SGE Analytical Science, Australia)). The temperature program used during the chromatographic analyses is summarized in Table 1.

The total time of a single chromatographic analysis was 65 minutes. Hydrogen supplied to the system with a constant flow rate of 1 cm3 / min was used as the carrier gas. The modulation time was 5 seconds. The detector voltage was 1600V and the mass range recorded during the analyses was m/z = 40-500 amu. Data obtained as a result of the chromatographic analyses were processed using the LECO ChromaTOF ™ 4.44 software (LECO Corporation St. Joseph, MI, USA). With the minimum probability of 85% chromatographic peaks were tentatively identified by comparing the spectra obtained with those in the NIST (National Institute of Standards and Technology) spectrum library and information on the retention times of individual compounds.

Table 1: Two-dimensional gas chromatography analyses parameters

|  |  |  |
| --- | --- | --- |
| Parameter | first column | second column |
| Initial temperature | 40°C | 45°C |
| Initial temperature holding time | 1min | 1min |
| First temperature programe | raised at a rate of 10°C/min to 90°C | raised at a rate of 10°C/min 95°C |
| Second temperature programe | raised at a rate of 3°C/min to 240°C | raised at a rate of 3°C/min to 245°C |
| Temperature holding time | 5 min | 5min |
| Transfer line temperature | 250°C |
| Ion source temperature | 250°C |

* 1. Results and discussion

Figure 1 presents an example of a chromatogram illustrating the degree of separation obtained that would not have been possible to achieve using classical one-dimensional gas chromatography.Most of the present studies related to analysis of volatile odorous compounds base on one dimensional GC (Fisher et al., 2018a; Wu et al., 2018; Brattoli et al., 2013). The technique of two-dimensional gas chromatography coupled with a TOF-MS is an analytical tool that allows achieving a high degree of separation of complex mixtures of chemical compounds.

The proposed two-dimensional GC allow to identify compounds which likely would be co-eluted using only one chromatographic column. It allowed to separate pairs of compounds with the same retention times (RT) at the first chromatographic column, such as 1-propanol and 2-butene, 2-methylthiophene and toluene, heptanal and o-xylene, tetradecane and 3-octanone.



*Figure 1: Chromatogram obtained as a result of GCxGC analysis (the peak is derived from octadecanal).*

Obtained results of biosolids emission analyses using GCxGC–TOF–MS are presented in the Table 2. The 55 volatile odorous organic compounds belonging to different chemical groups were identified. The variety of chemical odorous compounds are representatives of aliphatic and aromatic hydrocarbons, alcohols, aldehydes, ketones, esters, sulphides or others. This shows the complexity of the volatile fraction from stabilized dewatered biosolids. Similar groups of chemical compounds detected in anaerobic digested dewatered biosolids were reported in works of Fisher et al. (Fisher et al., 2018a; Fisher et al., 2017). However, some potential odorants of concern detected by other authors such as volatile fatty acid, indole, skatole and p-cresol were not detected in this study. Moreover, using the method proposed in this study was not possible to detect inorganic odorous compounds such as hydrogen sulphide and ammonia.

Results for both biosolids samples vary. For example, during the first day of the biosolids storage 2-methylthiophene, 2-methylheptane, indane, tetrahydrolinalool and 3-methylheptane were detected in both biosolids cakes whilst methanethiol and methyl ethyl sulphide in the biosolids cake B1 only. Only a few compounds were presented during the entire storage period: toluene, styrene, dimethyl sulphide and hexadecanal. Only the exemptions of presence of dimethyl sulfide and hexanal were the first day from biosolids cake B1 and the seventh day from biosolids cake B2, respectively. Additionally, chemical compounds have not been detected in every of the triplicate emission samples. Most of the compounds were detected on an irregular basis across the whole measurement period. In general, the majority of the odorants were detectable from the beginning of the storage time and its presence decreased following the storage period. However, it is not the rule for all identified chemicals. For example, 1-heptane was detected from day 7 whilst acetophenone was present in both biosolids cakes from day 14.

In the work of Fisher (Fisher et al., 2017) commonalities between the two cakes were seen in the gradual reduction in presence of most odorants. Concentrations in the top layer decrease as the compounds are stripped with ageing, thereby limiting emission of compounds from the surface.

Table 2: Frequently detection of volatile odorous compounds in biosolids emission samples. Three “+” mean that a given compound was present in each of the 3 replicates, two mean that it was present in 2 out of 3 replicates, while 1 means that only one of 3 replicates. The sign "-" means that the compound was present in the sample at concentrations below the limit of detection (<LOD).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Day of storage** | 1 | 7 | 10 | 14 | 21 |
| **Biosolids sample no** | B1 | B2 | B1 | B2 | B1 | B2 | B1 | B2 | B1 | B2 |
| **No** | **1st RT/s**  | **2nd RT/s**  | **Compound name**  |  |  |  |  |  |  |  |  |  |  |
| 1 | 278  | 1.23  | Methanethiol  | ++ | - | - | - | - | - | - | - | - | - |
| 2 | 282  | 1.79  | Ethanol  | + | ++ | ++ | +++ | ++ | ++ | +++ | +++ | +++ | + |
| 3 | 298  | 1.26  | Dimethyl sulfide  | - | + | +++ | +++ | +++ | ++ | ++ | ++ | + | ++ |
| 4 | 306 | 1.25 | Carbon disulfide | ++ | +++ | - | + | - | ++ | ++ | +++ | - | + |
| 5 | 310  | 2.31  | 1-Propanol  | +++ | + | - | - | - | - | + | + | - | +++ |
| 6 | 310 | 1.10 | 2-Butene | ++ | ++ | - | - | + | - | ++ | - | ++ | + |
| 7 | 338 | 1.40 | Methyl ethyl sulfide | +++ | - | - | - | - | - | - | - | - | - |
| 8 | 394 | 1,89 | Pentanal | ++ | ++ | + | + | - | - | ++ | - | ++ | - |
| 9 | 398 | 1,23 | 1-heptene  | - | - | - | ++ | ++ | - | ++ | - | +++ | + |
| 10 | 410 | 1,19 | Heptane | +++ | +++ | ++ | ++ | ++ | - | ++ | - | - | + |
| 11 | 442  | 2.26  | Dimethyl disulfide  | +++ | - | - | - | + | - | - | + | - | - |
| 12 | 478 | 2,26 | 2-methylthiophene | +++ | + | - | - | - | - | - | - | - | - |
| 13 | 478  | 1.89  | Toluene  | +++ | ++ | + | + | +++ | ++ | +++ | +++ | +++ | - |
| 14 | 486 | 1,22 | 2-methylheptane | +++ | ++ | - | - | - | - | - | - | - | - |
| 15 | 494 | 3,00 | Pyridine | ++ | + | - | - | - | - | + | + | - | - |
| 16 | 498 | 1,22 | 3-methylheptane | +++ | ++ | - | - | - | - | - | - | - | - |
| 17 | 510 | 2,08 | Hexanal | +++ | +++ | - | - | - | - | +++ | + | ++ | - |
| 18 | 626 | 2,12 | m-xylene | ++ | +++ | + | - | ++ | + | + | + | ++ | + |
| 19 | 658 | 2,87 | Styrene | +++ | +++ | ++ | + | ++ | ++ | ++ | ++ | ++ | + |
| 20 | 666 | 2.24 | Heptanal | +++ | ++ | - | - | - | - | +++ | + | ++ | + |
| 21 | 666 | 2,28 | o-xylene | ++ | ++ | ++ | +++ | - | ++ | ++ | ++ | + | ++ |
| 22 | 742 | 1,47 | α-Phellandrene | +++ | +++ | - | ++ | - | + | - | +++ | - | +++ |
| 23 | 746 | 1,39 | Propylcyclohexane | ++ | +++ | - | - | - | - | - | - | - | - |
| 24 | 758 | 1,27 | 2,6-dimethyloctane | +++ | ++ | - | - | - | - | - | - | - | - |
| 25 | 778 | 1,55 | Camphene | +++ | +++ | - | + | - | - | - | ++ | + | - |
| 26 | 826 | 1.61 | α-Pinene  | +++ | ++ | + | ++ | + | - | - | + | +++ | ++ |
| 27 | 846 | 1,48 | 2,6-dimethyl-2,-octadiene | +++ | ++ | - | - | - | - | - | - | - | - |
| 28 | 870 | 2,06 | Hexyl acetate | ++ | ++ | - | - | - | - | - | + | - | - |
| 29 | 894 | 2,49 | 1,3,5-Trimethylbenzene | +++ | ++ | - | - | - | ++ | + | + | + | - |

*Continuation of Table 2.*

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 30 | 914 | 2,68 | Indane | ++ | ++ | - | - | - | - | - | - | - | - |
| 31 | 926 | 2,13 | p-cymene | + | +++ | - | ++ | - | - | - | + | - | - |
| 32 | 938 | 1,30 | Undecane  | ++ | - | - | - | + | - | + | - | - | - |
| 33 | 954 | 1,96 | Acetophenone | - | + | - | - | - | + | +++ | + | + | + |
| 34 | 986 | 2,84 | Dihydromyrcenol | +++ | + | - | - | - | - | - | - | - | - |
| 35 | 998 | 2,24 | o-cymene | ++ | - | - | - | + | - | - | - | - | - |
| 36 | 1022 | 1,29 | Dodecane | + | ++ | - | - | + | - | ++ | - | - | - |
| 37 | 1038 | 2,34 | Tetrahydrolinalool | +++ | +++ | - | - | - | - | - | - | - | - |
| 38 | 1042 | 1,39 | 1-undecene | + | - | - | - | ++ | - | - | - | + | - |
| 39 | 1106 | 1,31 | 3,7-Dimethyldecane | +++ | + | - | - | - | - | - | - | - | - |
| 40 | 1142 | 2,30 | 2-pentylthiophene | +++ | +++ | - | - | - | - | - | - | - | - |
| 41 | 1230 | 1,33 | Tetradecane | + | +++ | + | ++ | - | ++ | - | + | + | - |
| 42 | 1230 | 1,90 | 3-octanone | ++ | +++ | - | - | - | - | - | - | - | - |
| 43 | 1258 | 1,31 | 2,6-dimethylundecane | ++ | ++ | - | - | - | - | - | - | - | - |
| 44 | 1358 | 1,30 | 2,3,7-trimethyloctane | +++ | +++ | - | - | - | - | - | - | - | - |
| 45 | 1366 | 0,50 | 1-Methylnaphthalene | +++ | +++ | - | - | - | - | - | - | - | - |
| 46 | 1374 | 1,43 | 3-tetradecen | ++ | + | - | - | - | - | - | - | - | - |
| 47 | 1430 | 1,32 | Tridecane | + | ++ | - | - | + | - | - | - | - | - |
| 48 | 1494 | 1,34 | 4-Methyl-tridecane | +++ | +++ | - | - | - | - | - | - | - | - |
| 49 | 1546 | 2,17 | Dodecanal | ++ | ++ | + | + | +++ | - | ++ | - | +++ | - |
| 50 | 1550 | 1,33 | Heptadecane | + | - | - | ++ | - | - | + | - | + | - |
| 51 | 1642 | 1,95 | α-humulene | +++ | +++ | - | - | - | - | - | - | - | - |
| 52 | 1842 | 2,14 | Octadecanal | ++ | ++ | ++ | + | ++ | ++ | - | ++ | + | - |
| 53 | 2110 | 2,11 | Hexadecanal | ++ | ++ | ++ | - | ++ | ++ | +++ | ++ | ++ | ++ |
| 54 | 2246 | 1,97 | Methyl palmitate | + | ++ | - | - | - | - | ++ | - | +++ | - |
| 55 | 2442 | 3,64 | Farnesol | - | + | ++ | - | - | ++ | - | + | - | + |

At the first day of storage all detected terpenes (α-pinene, camphene, p-cymene, o-cymene, α-phellandrene and α-humulene) were observed and as the biosolids were stored their presence mostly decreased. Only two terpenes were observed until the end of the measurement campaign: the first one α-pinene, however without day 14 and α-phellandrene, however in the biosolids cake B2 only. Contradictory to works of other authors, limonene was not detected in this study (Fisher et al., 2017, Maulini-Duran et al., 2015, Yager et al., 2014).

Some odorants such as farnesol, methyl palmitate, hexyl acetate, 2-pentylothiophene, 3-octanone were seldom reported in other studies. Moreover, not any reliable data for their odour detection threshold exists in literature.

The differences with identification of VOCs from biosolids cakes B1 and B2 emission might be related with the batch coming from different digesters. A variability in individual chemical compounds emission was observed in other studies (Fisher et al., 2018b). It was likely affected by the complex interactions and changes in the chemical compound concentrations due to the varying microbial pathways and activities. The variability may also be affected by the inconsistency of the processes because of different inputs at different times. Differences between emission sample replicates from the same cakes are likely related to presence of chemical compounds with concentrations closed to method detection limits. The triplicates sample was not taken simultaneously, but the next one following the previous one. In the trace analysis those time discrepancies might affect the concentrations of chemical substances.

* 1. Conclusions

In this study, 55 odorous VOCs were detected. Apart from the well-known odorants such as methanethiol, ethanol, dimethyl sulphide, dimethyl disulphide, styrene, pyridine, α-pinene was identified some odorants without known odour detection threshold in air such as acetophenon. The range of odorous VOCs identified as the anaerobically stabilised biosolids cakes are aged suggests they are present because of a range of factors, such as biotic and/or abiotic production as well as household or industrial inputs to the sewer catchment. The different identification of odorous VOCs between the biosolids cakes suggests temporal variation in biosolids properties should be an area of further research. The proposed GCxGC-TOF-MS methodology allowed better identification of odorants compare to a GC-MS instrumentation.

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