Analysis of sulfur and nitrogen odorants using solid-phase microextraction and GC–MS

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Abstract An analytical method involving solid-phase micro-extraction (SPME) and gas chromatography–mass spectrometry (GC–MS) was applied to analyze biosolids odors. A selective ion monitoring (SIM)-based MS method was developed, using SPME injections of odorant standards under the full-scan mode to select the quantification and confirmation ions for each odorant. The odorants analyzed in this study include: dimethylsulfide, dimethyldisulfide, methyl mercaptan, hydrogen sulfide, carbon disulfide, trimethylamine and dimethylamine. We have used this method to quantify parts-per-billion levels of odorant vapors produced during anaerobic incubation of digested wastewater sludge. Important considerations for expedient and accurate calibration under static and dynamic flow conditions are discussed. The SPME–GC–MS method may give a positive intercept in the calibration curve, especially under static sampling conditions, which sets a practical detection limit for odor analysis.

Keywords GC–MS; odor; sludge; SPME

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Introduction

Odors emanating from wastewater sludge are not only unpleasant, but may also have adverse health effects at high concentrations. These odors can limit sludge treatment and disposal options, such as land application of sludge as biosolids. As a result, odor production has become one of the key issues in sludge management. Many odorants derived from sludge are sulfur and nitrogen compounds with very low odor thresholds. To minimize production of these odors, it is essential to understand the mechanisms through which they are generated in biosolids. However, research has been hindered in part by the lack of a robust technique that offers reliable and consistent analysis of low concentrations of sludge odors.

The most common approach in odor measurement has been the use of olfactometry or odor panels, since the human olfactory system is highly sensitive to a wide range of odorous compounds. However, olfactometry has several drawbacks, including its high operational cost and the difficulty of collecting and maintaining representative samples. Odor panels are also less reliable, due to variable extents of human bias and adaptation to odors (Nimmermark, 2001). Other methods for odor measurement include the electronic nose (a method under development that involves use of electronic chemical sensors), H_2S meters, and Draeger tubes. The latter methods can analyze selected odors, but may lack compound specificity and have high detection limits (Lue-Hing *et al.*, 1992).

We developed a method for odor analysis using solid-phase micro-extraction (SPME) and gas chromatography–mass spectrometry (GC–MS) with selective ion monitoring (SIM). SPME is a relatively new and simple technique described in 1989 by Pawliszyn and Belardi (Haberhauer-Troyer *et al.*, 1999). This solvent-free technique is an alternative to traditional sample preparation methods, involving extraction of low concentrations of organic analytes. In SPME, a sorbent-coated fiber contained in a syringe needle is exposed to a sample to allow sorption of analytes to the fiber coating. The sorbed analytes are then thermally desorbed upon insertion of the needle into a GC injection port (Supelco, 1998).

The purpose of this study was to optimize the SPME–GC–MS method for the analysis of selected odors generated from digested sludge. We used this method to quantify parts-per-billion levels of sulfurous odor vapors such as dimethylsulfide (DMS), methyl mercaptan (MM), dimethyldisulfide (DMDS), hydrogen sulfide (H_2S), and carbon disulfide (CS_2). DMS, DMDS, and MM have been studied widely in marine science and food science, due to the role of these compounds in the sulfur cycle and their contribution to the aroma of certain food products such as cheese, wine, and raw milk (Dias and Weimer, 1998; Jansen, 2000). We also used this method to analyze two nitrogen odorants: dimethylamine (DMA) and trimethylamine (TMA).

Methods

SPME fiber

A 75-μm Carboxen-polydimethylsiloxane (CAR–PDMS)-coated fiber was chosen for this study because of its relatively high efficiency in extracting vapors and low-molecular-weight analytes, especially sulfur compounds (Mestres, 1999; Supelco, 1997). SPME fiber holders and CAR–PDMS fibers were purchased from Supelco (Bellefonte, PA). Prior to use, the fiber was conditioned at 300°C for two hours as specified by Supelco.

Calibration of SPME with odor standards

Odorant standards in Teflon permeation devices were obtained from VICI Metronics (Houston, TX). To calibrate for each odorant, the permeation device of that odorant was placed in the thermostatted chamber of a Dynacalibrator® (Model 230, VICI Metronics), and the temperature of the chamber was set at a specified value for which the permeation rate of the odorant had been determined. The carrier flow conveying the odorants from the permeation chamber was maintained at a constant rate of 76 ml/min. Different odorant vapor concentrations were obtained by diluting the odor concentration through the permeation chamber with different flow rates of dilution air. The dilution flow rates were adjustable from 0 to 7,520 ml/min with different float settlings, giving dilution rates ranging over two orders of magnitude. The air carrying the odorant vapor then passed through a Teflon™ sampling chamber (Savillex, Minnetonka, MN) via Teflon™ tubing, with the effluent from the chamber vented in a fume hood. The odorant permeation device was left in the chamber for at least 30 minutes before a sample was taken. Initially, the by-pass valve was closed, and other valves on both sides of the sampling chambers were kept open, in order to direct the odor vapor flow to the sampling chamber and to have the desired odor concentration in the chamber used for SPME sampling. After 32 volumes (approximately 32 min with the lowest total flow rate and 20 seconds with the highest total flow rate), a constant concentration of odorant vapor in the chamber was assumed. Sampling was performed by inserting a SPME needle into the sampling chamber through a Teflon™-lined septum. Figure 1 depicts the set-up used for odor vapor calibration using SPME. Odor compounds can be calibrated with SPME, using static or dynamic sampling, depending on which method better simulates the actual sampling conditions.

SPME sampling time

Sorption time is important for SPME analysis, because equilibrium is not always reached between the sample and the fiber coating. The equilibrium time may range from minutes to hours, depending on sampling conditions and analyte characteristics. For instance, volatile compounds have higher transport rates and require shorter times to reach equilibrium than do semi-volatile compounds (Lord and Pawliszyn, 2000). Kim *et al.* (2002) reported that three hours were required for TMA, CS_2 and DMS to reach equilibrium, whereas 10 hours were insufficient for DMDS. Muller *et al.* (1997) also observed long equilibration times,

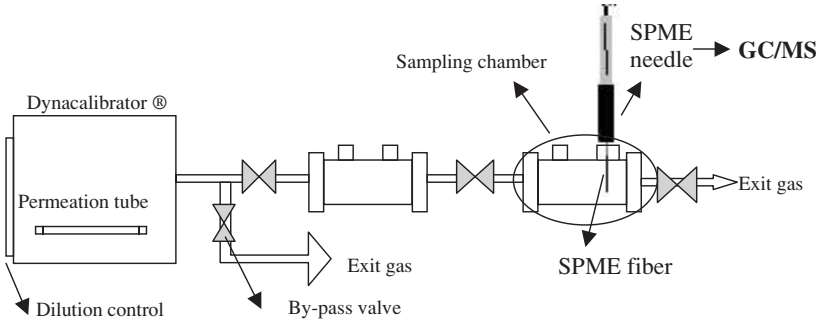

Figure 1 Set-up used for SPME calibration for odor analysis

and suggested that it is not necessary to sample under equilibrium conditions as long as constant extracting conditions are maintained.

Studies have shown that the amount of sorbed analyte increases rapidly in the first 10 minutes of extraction (Muller *et al*., 1997; Popp *et al*., 1999; Kim *et al*., 2002). Pawliszyn (1999) suggested that shorter extraction times can be used when equilibration times are excessively long. To sample under non-equilibrium conditions, however, the extraction time and mass-transfer conditions must be strictly controlled. This is because even small differences in extraction time and conditions may result in significant variations in extracted analyte mass, due to the sharp increase in sorbed concentration in the initial extraction phase (Pawliszyn, 1999). Based on the previous studies, we selected a sample extraction (sorption) time and a GC injection (desorption) time of five minutes for analysis of all calibration standards and odor samples.

Analytical method

Analysis of odorants was performed using a Hewlett-Packard 5890 gas chromatograph coupled to an HP 5970 mass spectrometer. A 30-m DB-5MS column (0.25 mm ID, 0.5 μm film thickness) was used with the following temperature program: isothermal at 32°C for 4.5 min, ramped at 15°C/min to 110°C and then at 30°C/min to 250°C, and held at 250°C for 1 min. Injector and detector temperatures were 240 and 280°C, respectively. A selective-ion monitoring (SIM)-based GC–MS method was developed by performing a series of test injections with permeation tubes of odor standards under the full-scan mode to obtain the optimal GC conditions, retention times, and targets (quantification), and confirmation (qualification) ions for each compound. Before each sampling, blank SPME injections were performed to ensure a clean baseline. The analytes and their retention times, target and confirmation ions, and human threshold limits are shown in Table 1.

Results and discussion

Static and dynamic sampling

The effect of flow conditions on the extraction efficiency of SPME fiber was investigated under dynamic (continuous flow during sampling) and static (no flow) conditions. Dynamic sampling was performed by passing a known flow rate of odor standards continuously through the calibration chamber while a SPME needle was inserted. For static sampling, the inlet and outlet valves were switched to divert the gas standard flow (to a fume hood) and seal the calibration chamber before an SPME needle was inserted. The gas standard was allowed to flow through the calibration chamber for more than 30 minutes before sealing the chamber to ensure that the odor vapor concentration in the chamber was identical to that of the gas standard.

Table 1 Analytes, GC retention times, SIM ions, and odor thresholds for human detection

Analyte	Molecular mass (g/mol)	Confirmation/target ion (m/z)	GC retention time (min)	Odor threshold (ppm)*
H_2S	64	–/34	1.77	0.00047
MM	48	47/48	2.105	0.0011
DMA	45	–/45	2.18	0.023
TMA	59	58/59	2.254	0.00021
DMS	62	47/62	2.80	0.001
CS_2	76	–/76	3.10	0.02
DMDS	94	79/94	8.00	0.0003

*Lue-Hing *et al*. (1992)

Results of dynamic and static calibrations showed that a smaller amount of sorbed analyte was consistently obtained with static sampling. The calibration curves under static and dynamic conditions for TMA, DMS, and DMDS are shown in Figure 2. The slopes of the static and dynamic calibration curves are similar for TMA, and the dynamic conditions gave a significantly higher response, as has been observed by Tuduri *et al*. (2002). The ratios of peak areas obtained from dynamic and static sampling were greater than one for the three compounds (Figure 3). In addition, static tests for TMA gave a calibration curve with an intercept at a positive TMA concentration. In contrast, DMS and DMDS calibration curves have different slopes but similar intercepts.

The difference between TMA and DMS/DMDS calibration curves cannot be explained to date, but it is clear that dynamic tests gave higher concentrations in all cases. Thus, any gas measurements taken from a dynamic system, such as a flux chamber, will have to be quantified based on dynamic standard calibrations, and vice versa.

The difference between static and dynamic results may be explained by either of two mechanisms: (1) depletion of the odor sample in the static procedure, or (2) increased transport rate of analyte to sorption sites in the dynamic procedure. If (2) is the case, then flow rates are inherently linked to measured concentrations, and *any* dynamic measurements are suspect unless the flow rate and mixing conditions in the standard and sample are assured to be identical.

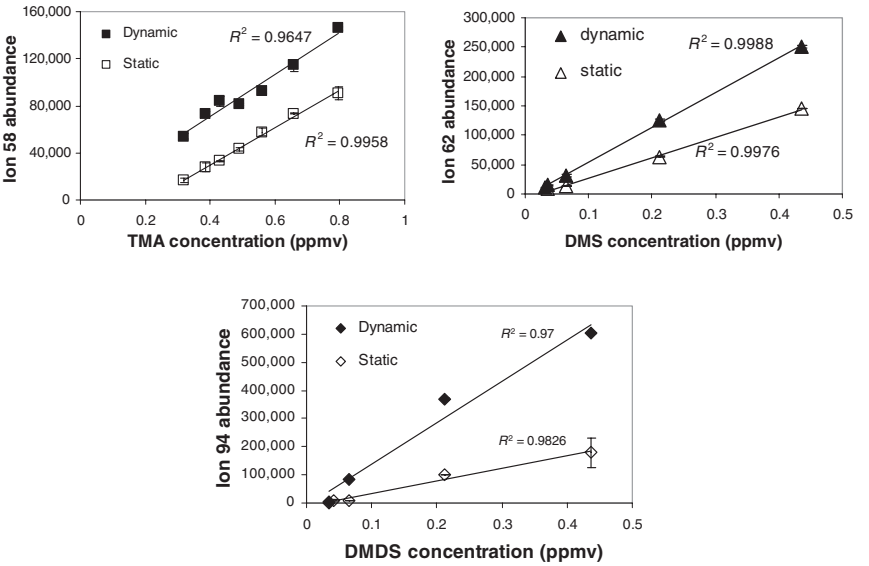

Figure 2 Response under dynamic and static sampling modes for TMA, DMS, and DMDS

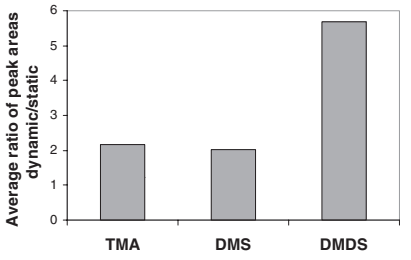

Figure 3 Dynamic/static peak area ratios

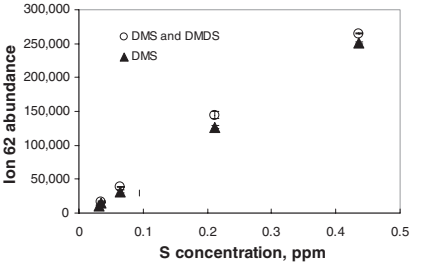

Figure 4 Multi-adsorbate effect on DMS analysis

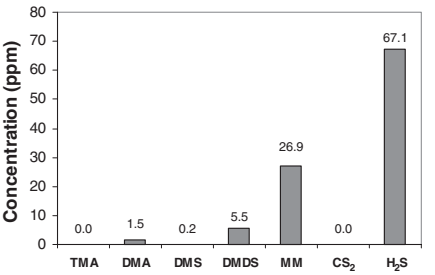

Figure 5 Odor concentrations in a digested biosolids sample measured using the SPME–GC–MS method. Concentrations of TMA and CS_2 were below detection

Multi-adsorbate effect

To test whether sorption of an odor vapor by SPME fibers is affected by the presence of other vapors, we compared the calibration curves of DMS obtained using a DMS permeation device alone and also in the presence of a DMDS permeation device in the calibration chamber. Permeation tubes of DMS and DMDS were placed in the permeation chamber at 30°C, the recommended temperature for DMS calibration. Sampling and desorption times were both 5 minutes. As shown in Figure 4, the amounts of sorbed DMS with and without DMDS were essentially the same, suggesting that the presence of DMDS vapor did not noticeably affect the sorption of DMS to the SPME fiber. Alternatively, the lack of interference might be in part due to the fact that DMDS has a high molecular weight and a relatively low vapor pressure compared to DMS.

The SIM-based SPME–GC–MS method, as described in this paper, is being used in our laboratory to investigate odor production mechanisms from digested biosolids. The biosolids samples are prepared in an anaerobic glove bag, and then incubated in closed 250-ml amber glass bottles. Odor compounds in each sample bottle are extracted by five-minute SPME sampling in the headspace. The SPME fiber is then inserted into the GC injection port at 240°C to desorb the extracted odorants. The same temperature program used for odor calibration was used for odor measurements. Odor concentrations measured in a digested biosolids sample are exemplified in Figure 5, which illustrates the applicability of the SPME–GC–MS method to the analysis of odors in biosolids.

Conclusions

SPME is a simple technique for concentrating volatile odor compounds, and provides low detection limits for the tested sulfur and nitrogen odorants. Use of SPME for odor analysis may facilitate research in this important area in wastewater and sludge management. Sample preparation and extraction are simple with the SPME method, as compared to other methods. However, precise and constant sampling times are essential to minimize the errors associated with sample extraction. Although we observed no competitive sorption

effect between DMDS and DMS, more experiments to examine additional compounds are necessary to ascertain the errors associated with multi-sorbate competition. Finally, additional improvements/modifications of the method are needed to further lower the detection limits to that of the human nose.

Acknowledgement

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