RESEARCH REPORT
ENVIRONMENT

Title: Protocol Development for Measurement of Trace Gases Associated with Manure Storage and Odor – **NPB# 00-004**

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General Summary

As concerns surrounding livestock production shift from nuisance concerns to public health concerns, it is increasingly important to have a suitable means of quantifying odors and concentration of compounds emitted from animal facilities. Particularly from a health standpoint, it may be necessary to have the ability to measure concentrations of specific compounds. To that end, development of accurate and easy methods of quantifying these analytes is needed. One approach is the use of solid phase microextraction (SPME) procedures using portable field samplers. These procedures have been widely used in liquid phase samples for a variety of purposes as well as headspace sampling (see appendix A for list of references). This sampling technique is user-friendly, allows for identification and quantification of a wide range of analytes, allows for shipment of samples to an analytical laboratory for analysis, and does not require the disposal of solvents. Our intent was to evaluate SPME against other methods in ambient air sampling and develop a protocol for use of SPME. Sampling criteria were developed into a recommended practice for scientists using SPME portable field samplers.

Abstract

Quantification of odorous compounds associated with livestock odors has been difficult in the past due to sensitivity limitations of available instrumentation. Advances in instrumentation have helped to some extent although for many potential contributors to malodor instrumental detection limits still exceed that of the human nose. Equally important to instrumentation development in quantification efforts is the selection of the method used to capture, transport, and analyze air samples. Solvent extraction and solid phase microextraction (SPME) were compared for their ability to adsorb compounds in air samples collected from livestock facilities (n = 128). Sampling occurred from August – September 2000 and again from May – June 2001. Five different solvent extraction tubes, in duplicate, were used, each comprised of different materials thereby having affinity for various compound classes. Tube contents were extracted using both hexane and methanol to represent the range in solvent materials. The SPME samplers used were coated with carboxen and polydimethylsiloxane.

Results showed that adsorption of air to a single SPME sampler eluted more compounds than that observed from the combination of all five solvent extraction tubes. The SPME fibers, although more expensive than the solvent tubes, are reusable and require less labor in addition to avoiding the accumulation of solvent materials that require proper disposal.

Introduction

Quantification of odorous compounds associated with livestock odors has been difficult in the past due to sensitivity limitations of available instrumentation. Advances in instrumentation have helped to some extent although for many potential contributors to malodor instrumental detection limits still exceed that of the human nose. Equally important to instrumentation development in quantification efforts is the selection of methods used to capture, transport, and analyze air samples.

Uniformity in research procedures, particularly in basic research such as the analytical techniques employed in air analysis, allows for result comparison between laboratory groups. Currently, a standard procedure is not available for quantifying trace gas emissions from livestock operations. This project addressed this issue by comparing various methods of sample collection and processing. Methods used to collect air samples included drawing a fixed volume of air through an adsorbent tube followed by solvent extraction of the compounds trapped by the tube for analysis by GC-MS. A shortcoming of solvent desorption is that some compounds may elude with the solvent peak and not be separated sufficiently for identification nor quantification. Solid phase microextraction (SPME) is a relatively new technique that has demonstrated considerable promise in liquid and headspace analysis and eliminates the tedious desorption process (Miller and Stuart, 1999).

Objectives

- *Evaluate methods for measurement of analytes associated with odor*
  Two methods will be compared; solvent extraction and SPME. Use of solvent extraction will require pre-concentration of air samples by pulling a volume of air through sorbent tubes. Prior to analysis by gas chromatography the compounds trapped within the sorbent tubes are chemically extracted with an organic solvent. The SPME method utilizes exposure of fibers within the SPME apparatus to an air stream for a period of time followed by direct injection into a gas chromatograph where the desorption follows.
- *Evaluate sampling methods for collection of air samples from manure storage facilities*
  Multiple methods of sample collection will be tested, considering the influence on time of exposure, volume of air, and transport mechanisms. Direct exposure of the SPME fibers will be compared to collection of the air stream in a Tedlar bag or glass bulb followed by injection of the SPME fibers into the bag.
  - *Develop a standard procedure for collection and analysis of trace compounds associated with odors from livestock facilities*

  Based on our findings in the air sampling and air analysis stages we incorporate the best-identified methods into a standard procedure for consideration of adoption as an ASAE *Standard*.

Procedures

Solvent extraction vs. SPME

To accomplish the set forth objectives air samples from animal housing facilities were collected during August and September 2000 and again in May and June 2001. Solvent

extraction tubes of each of the following sorbent materials were collected: carboxen, Tenax, carboseive, activated silica gel, and carbotrap (Orbo tube numbers 328, 402, 91, 52, and 101, respectively; Supelco Inc. Bellefonte, PA). Selection of solvent tubes was based on a survey of the literature for identification of components of air collected from in and near livestock facilities. Each tube was collected in duplicate using a portable air sampler pump (Supelco Inc. Bellefonte, PA) for extraction in 5 ml of both methanol and hexane. Following extraction, a 1ml sample of the extractant was placed in an autosampler vial for analysis by gas chromatography coupled to mass spectrometry (GC/MS). In addition, solid phase microextraction samplers were exposed to the air space in each facility for direct injection into the GC/MS. A total of 128 samples were simultaneously collected with each adsorbent tube and with the SPME samplers. This exceeds the proposed number of samples and associated costs by 60%. The SPME samplers were analyzed by the GC/MS within 24 hr of collection. Solvent tubes were extracted and preserved until all samplings were complete within each of the two sampling periods. Following completion of the seasonal sampling, all extraction tubes were analyzed by the GC/MS.

Sampling time and volume and sample collection
Within a separate study, collection of SPME samplers, onsite, was compared to air collection in 1-L Tedlar bags and 200-ml glass sampling bulbs. The purpose of this study was to evaluate the suitability of transporting the air followed by SPME compared to transportation of the air previously adsorbed to the SPME portable field sampler. Twenty samples were collected employing each method. All samples were transported to the GC/MS by car without refrigeration. Recovery of analytes were compared.

Sampling times of SPME field samplers were compared. Times considered for air exposure were 0.5, 2, 8, 16, and 20 min. Because air moves over the SPME field samplers at a constant rate sampling volume is directly related to sampling time. Eighteen sampling events occurred where SPME field samplers were exposed for each of the above sampling times. Following collection, all samplers were transported back to the laboratory for analysis by GC/MS. Standards were exposed to SPME samplers for corresponding time periods to establish prediction equations for analyte concentrations.

Development of a standard procedure
Based on findings from the above objectives, a standard operating procedure will be developed for collection of air samples using SPME samplers if proven to be equivalent to or superior to solvent extraction methods.

Results

Solvent extraction vs. SPME
Table 1 depicts compounds in the standard solution that was used to quantify identified analytes. A total of 32 compounds, if present, could be quantified with additional analytes identified by GC-MS. Table 2 illustrates compounds that were identified by each the five solvent tubes and by the SPME samplers. Only tubes extracted with methanol are depicted. Extraction of the solvent tubes with hexane resulted in elution of fewer compounds than with methanol; indicating that methanol was the preferred solvent for the materials contained within the selected solvent tubes. Note that not all of the compounds present in the stock standard were present in collected samples. In fact, many of the compounds commonly thought to be highly associated with odor (e.g. butyric acid, 3-methylindole) occurred at concentrations below the instrumental level of detection, which is in the low ppb – high ppt range. In addition, only Orbo tubes 52 and

91 (activated silica gel and carboseive, respectively) resulted in consistent identification of analytes. Recovery between the two materials was similar. The remaining materials (carboxen, Tenax, and carbotrap) did not appear effective in trapping volatile fatty acids, phenolics, indoles, alkanes, thiols, or sulfides that were of interest in this study. However, these materials are often cited as the materials of choice for air sampling. Our findings did, however, mimic findings recently reported by others (Kim-Yang et al., 2001; Koziel et al., 2001) suggesting that SPME samplers are, in fact, a suitable method for identifying and quantifying compounds emitted from livestock facilities. Recovery from the SPME samplers, compared to the Orbo tubes, was superior. Other research has suggested use of thermal desorption tubes as superior to SPME samplers (Zahn et al., 1997) however, our work resulted in elution of a greater number of compounds than observed by Keener et al. (unpublished) observed when using thermal desorption tubes.

We did observe some seasonal and site differences. Gamma-butyrolactone was observed only in samples collected in the late summer sampling (August – September 2000). Also during this season, propanoic acid was commonly observed in greater than one site whereas during the early summer sampling (May – June 2001) propanoic acid was only identified at a single site, though the identification occurred during each visit to that site.

We intend to publish the findings of this project in a peer-reviewed journal. As part of that publication average concentrations of identified analytes will be considered. However, daily variation within each sampling site makes this a difficult task. Concentration may need to be reported as a function of temperature and season. The intent of the project to date was to compare methodology for analyzing air sample components. Future efforts will focus on the quantification of the identified analytes.

Sampling time and volume and sample collection

Collection of air samples using Tedlar bags and glass bulbs was compared to on-site collection of the SPME samplers. Based on the 20 samples used for this study, Tedlar and glass appeared to perform similarly, but less effectively than onsite collection of the SPME samplers. While all compounds eluted in each case, greater concentrations were observed with onsite adsorption, perhaps to due adherence of compounds to the glass and Tedlar. Assuming 100% recovery from SPME, recovery of alkanes was 76% and 72% for glass bulbs and Tedlar bags, respectively; 85% and 86% for volatile fatty acids; 83% and 64% for phenolics and indoles; and 75% and 62% for sulfides. Although adsorption to the samplers occurs as well, the entire SPME sampler is desorbed into the GC precluding adsorption from causing problems. In our procedure, all bulbs and bags were re-used following flushing with N_2 gas. Flushing occurred three times before the respective materials were considered ‘clean’. Use of new bulbs and bags each sampling may have improved compound recovery, though this is not likely; failure to adequately clean the materials should have resulted in a build-up of adhered compounds thereby improving apparent recovery. We did not observe this. From a procedural perspective, use of new bulbs and bags would be cost prohibitive therefore we only considered cleaning followed by re-use. Samples adsorbed from the Tedlar bags indicated the presence of a number of compounds that were not in our standard solutions nor observed from glass bulbs or direct adsorption of air samples onto the SPME samplers. These are likely artifacts from the Tedlar material itself and suggest that use of a procedure where samples are sent in Tedlar for later desorption also employ analysis of a blank Tedlar bag to account for any artifacts present.

Use of SPME with liquid samples requires that the SPME material be exposed to the sample for only a brief period of time. Prolonged exposure can result in an adsorption-desorption process whereby some analytes adsorb and are then desorbed by analytes present in greater concentration (Koziel et al., 2001). This can be detected in standard mixtures of analytes by failure to observe a linearity of response (peak area) when analyzed by GC/MS. Short exposure times have also proved possible when sampling small headspaces. However, in the case of air sampling, a greater contact time may be needed to account for reduced concentrations and less surface area of the adsorption material relative to the exposure area. When SPME samplers were exposed at each of 18 sampling sites for 0.5, 2, 8, 16, and 20 min we found that the 0.5 and 2 min exposures resulted in recoveries less than that recovered following 8, 16, and 20 min exposure time ($P<.001$ and $P<.05$, respectively) for all but the volatile fatty acids. Recoveries of the volatile fatty acids were not different between 2, 8, 16, and 20 min exposure times. Exposure for 8 min recovered slightly less but there was not statistical difference from 16 and 20 min recoveries, across all analytes ($P=0.09$). Recoveries of liquid standards and headspace exposure to the standards were similar across all exposure times. The inability to recover as much sample as the reduced exposure time suggests that for ambient air samples, an exposure time of greater than 2 min is advisable. While 20 min exposure time may appear too long, exposure of standards in liquid or a confined headspace did result in a linear exposure of each analyte ($R^2 > 0.90$) indicating that random adsorption-desorption was not occurring. Based on these findings we feel that an exposure time of 10 – 15 min may serve as the best compromise between adequate recoveries and rapid sampling.

Development of a standard procedure

Taking the findings of this project, we have developed a procedure for air sample collection using SPME (see attached). This procedure has been forwarded to researchers at the USEPA for their evaluation and consideration for adoption. Following acceptance of the use of SPME by USEPA, we will pursue adoption of the procedure as an ASAE Standard. Acceptance by USEPA is a necessary first step. However, we have little control over this process beyond our introduction of the procedure to the researchers. Those researchers have indicated an interest in evaluating the procedure and plan to do so. However, we will have no involvement in the evaluation process.

Limitations

While our results and that of others (Kim-Yang et al., 2001; Koziel et al., 2001) show that SPME is a method competitive with other methods drawbacks to the method can be identified and are listed below:

- Cost of samplers, though re-usable
- Samplers are easily broken
- As with other methods, identification of analytes are limited by compatability of adsorbent material
- Currently not an EPA-approved method

At this time, research evaluating the use of this method for ambient air work is quite limited as our lab is one of only a few to consider use of this procedure and no peer-reviewed data exists documenting comparison of SPME to solvent extraction with ambient air samples.

Literature Cited

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Table 1. Compounds included in stock standard solutions for quantification by gas chromatography.

Acetic acid	Pentane	Carbon disulfide
Propanoic acid	Nonane	Dimethyl disulfide
iso-Butyric	Decane	
Butanoic	Undecane	Ethanethiol
3-Methylbutanoic	Dodecane	Propanethiol
Pentanoic	Tridecane	Butanethiol
	Tetradecane	
Phenol		Indole
3-Methylphenol	1-Decene	2-Methylindole
4-Methylphenol		3-Methylindole
2-Ethylphenol	Nonanal	4-Methylindole
3-Ethylphenol		
4-Ethylphenol	Gamma-butyrolactone	
2,6-*bis*(1,1-dimethylethyl)phenol		

Table 2. Compounds identified in collected air samples by each of the Orbo solvent extraction tubes employed and by adsorption of air samples onto solid phase microextraction (SPME) samplers.

	Orbo Tube Number					
Analyte	328	402	91	52	101	SPME
Acetic acid						X
Propanoic acid						X
iso-Butyric						
Butanoic						
3-methylbutanoic						
Pentanoic						
Phenol						X
3-Methylphenol						
4-Methylphenol						X
2-Ethylphenol						
3-Ethylphenol						
4-Ethylphenol						
2,6-*bis*(1,1-dimethylethyl)phenol						
Pentane						
Nonane			X	X		X
Decane			X	X		X
Undecane			X	X		X
Dodecane						X
Tridecane						X
Tetradecane						X
1-Decene						X
Nonanal						X
Gamma-butyrolactone						X
Carbon disulfide						X
Dimethyl disulfide						X
Ethanethiol						
Propanethiol						
Butanethiol						
Indole						
2-Methylindole						
3-Methylindole						X
4-Methylindole						

STANDARD OPERATING PROCEDURE

Solid Phase Microextraction Portable Field Sampler

Adsorption material
75 um PDMS/Carboxen fiber SPME Portable Field Sampler (Supelco Cat. No. 504831, Supelco Inc., Bellefonte, PA)

Sample collection
1. Condition each fiber prior to use by heating the adsorption material in the GC inlet (230 C) for 20 min; retract fiber into the sampler
2. Onsite, expose adsorption fiber to sample area for 12 min
3. Immediately retract adsorption fiber into the sampler
4. Refrigerate until analysis

Analysis
1. Inject SPME sampler into GC inlet; expose adsorption fiber
2. Run GC method (method is lab-specific based on column, flows, preferred peak separation)
3. Calculate individual analyte concentrations using the prediction equation developed from the standard curve.

Development of standard curve
1. Prepare stock standard solutions of analytes of interest. From the stock standard(s) prepare working standards over a range of concentrations (ie. 5%, 10%, 25%, 50%, 75%, 100% of stock standard).
2. Analyze each working standard using the developed GC method (lab specific procedure).
3. Develop a linear equation for each analyte based on known concentration of the analyte for each working standard and the corresponding peak area
4. Calculate the regression coefficient for each analyte. An R^2 of at least 0.95 is expected for each analyte. Coefficents below this value indicate that standards should be made again.

Appendix A

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