Stable isotopes to investigate decay processes in farm wastes.

Utilisation des isotopes stables pour l'étude des processus de dégradation des déjections animales.

Philip J Hobbs, Richard Johnson and David Chadwick.
Institute of Grassland and Environmental Research,
North Wyke, Okehampton, Devon, EX20 2SB. UK
E-mail : phil.hobbs@bbsrc.ac.uk

Abstract

Decay rates of organic matter within farm wastes have been determined using deuterated phenol (d_5) and acetic acid (d_4) over a 10 day period. Production and decay rates of compounds responsible for odour and pollution were also quantified, these were the volatile fatty acids, phenols and indoles as well as methane and carbon dioxide. The relative rates of emission, bio-regeneration and bio-decay were calculated. 70% of acetic acid was lost through emission and 30% decayed within the waste to products such as methane. Regeneration was 10% of the total lost. The emission of methane was 4.0 g t^{-1} d^{-1}. The ratio of methane generated to the decay of the acetic acid concentration was 1:66 respectively. Phenol concentration demonstrated a maximum of 85 mg l^{1} after 175 hr. Ammonia was emitted at a rate of 4.7 g m^{-2} d^{-1}.

Keywords : *biochemical decay processes, stable isotopes, odour production, gaseous emissions.*

Résumé

Utilisant le phénol deuterié et l'acide acétique on a déterminé les taux de décomposition de la matière organique dans les déjections animales (lisiers) pendant dix jours. Aussi, on a mesuré la production et les taux de décomposition de composés responsables des odeurs et de la pollution atmosphérique. Ce sont les acides gras volatils, les phénols, les indoles et aussi le méthane et l'ammoniac. On a calculé les taux d'émission relatifs ainsi que de bio-regénération. Dans ces lisiers, 70% d'acide acétique se sont dégradés par l'émission et 30% sont transformés en méthane (taux d'émission mesuré de 4.0 g t^{-1} d^{-1}). La proportion de méthane produit par la décomposition de la concentration d'acide acétique était 1:66 respectivement. La concentration en phénol démontrait un maximum de 85 mg l^{-1} après 175 heures. L'ammoniac s'était émis à un taux de 4.7 g m^{-2} d^{-1}.

Mots-clés : procédés de dégradation biochimique, isotopes stables, production odeurs, émissions gazeuses.

1. Introduction

There decay processes in wastes that are not well described and if we are to progress in reducing emissions, then dynamic interactions involving emission, bio-decay and production of odorants should be quantified. For example, although we can measure odour and compounds emitted from the surface, we do not know the rate at which the slurry concentration may be reduced by bio-decay or increased by biological production from the slurry.

Previous investigators have noted the declining concentration of volatile fatty acids (VFAs) in pig wastes (Caunt and Hester 1989) and found it to be zero order with respect to (or independent of) concentration. Ishaque *et al* (1985) identified that degradation of phenolic compounds does not occur in anaerobically stored wastes and oxygen was required to facilitate breakdown. Phenols, indoles and branched chain fatty acids are by-products of the decay of protein (Spoelstra 1980) and it is these decay processes, that, if minimised, would reduce air pollution. These processes are therefore indicative of the magnitude of anaerobic decay or mineralisation of organic forms to inorganic forms of nitrogen and phosphorus which exists as phospholipids in viable bacteria.

Behaviour of the populations and their interactions are obviously difficult to ascertain, however the results of their actions are recognised as changes in concentration of substrate (or energy source) and the by-products. Decline of odorants due to emissions has proved difficult to measure, even when using a purpose built emissions chamber (Hobbs *et al* 1997).

Investigation of the kinetic rates as relationships between emission, bio-decay and production are of significance to waste management as we can optimise the time of spreading. We investigated these relationships using deuterated compounds and analysis by gas chromatography-mass spectrometry (GC-MS). To clarify our approach the decline of the initial concentration of odorant (C_o) to a new concentration C, is equal to the subtraction of the emission rate (ER) and the bio-decay rate (DR), plus the production rate (PR).

$$C = C_o - ER - DR + PR \qquad \text{Eq - 1}$$

The changing concentration of an odorant in the slurry will be determined by the production rate of odorant and subtracting the emission rate and the biological decay rate. The latter two are determined by the declining concentration in the synthetic slurry, and the bio-decay rate determined from the decay of the deuterated odorant concentration in the slurry, respectively.

2. Materials and methods

2.1. Experimental design

Nine 1 litre Kilner jars (Fischer Scientific UK, Loughborough, Leicestershire, UK) contained accurately about 350 ml of finishing pigs slurry from collected beneath the slatted floors after three weeks accumulation. The volume to surface ratio was 350 cm^3 to 72 cm^2, being about the same for the average slurry store. Equal quantities of both deuterated acetic acid-(d_4) (Sigma-Aldrich Co Ltd, Poole Dorset UK) and phenol-(2,3,4,5,6)-d_5 (Sigma-Aldrich) were added to three Kilner jars (slurry d4d5) and the same quantities of acetic acid and phenol were added to a further three Kilner jars (slurry A&P). Three remaining slurry samples were left unchanged (slurry). Three addition Kilner jars each contained accurately about 330 mls of an artificial slurry as described in Table 1 making 12 jars in all. Air was drawn over the slurry surface at 0.6 litres min^{-1} by an electric air pump and then though a gas meter to confirm the volume. The experiment ran for 10 days at 15 ^oC.

Odorant	mg l^{-1}
Acetic acid d_4	2005
Acetic acid	2070
Propanoic acid	510
2-methyl propanoic acid	505
Butanoic acid	301
3-methyl butanoic acid	104
2-methyl butanoic acid	116
Pentanoic acid	108
Phenol	66.6
Phenol d_5	73.0
4-methyl phenol	68.6
4-ethyl phenol	39.2
Indole	7.3
3-methyl indole	5.9

* Buffered to pH 8.5 with 0.880 ammonia

Table 1
*Composition of artificial slurry**

The decline of the concentration of the odorants in the synthetic slurry will give the emission rate for the individual odorants as there are no biological means of decay. The difference between the concentration decline due to emission from the synthetic slurry and the deuterated acetic acid and phenol in the slurry should give their respective bio-decay rates. Differences between the initial concentration in the real slurry (unless otherwise stated descriptions refer to the unadulterated slurry sample) and the emission and bio-decay rates should give an estimation of the production rate for this particulate set of conditions. Emission rates of other VFAs and phenols may be calculated by their changing concentration in the artificial slurry and also their summated regeneration and bio-decay by subtraction of the ER from the real slurry samples.

2.2. Sampling

3.0 ml aliquots were taken from each sample every day (except weekends) for analysis by GC-MS for odorant concentration. The weight of the samples and headspace samples for quantification of ammonia, methane and carbon dioxide were taken at the same time. The pH measurement was taken every other day. Three replicate samples were taken at the beginning and the end to determine the total N, ammoniacal N (for a mass balance), dry weight (%), oil (acid hydrolysed), water soluble carbohydrate (WSC), total ash and crude fibre. Results are presented as an average of three replicates.

2.3. Odorant and gas analysis

Compounds that contributed to the odour were identified by concentrating a known volume of headspace above the pig slurry (Hobbs *et al* 1996) before identification using GC-MS. Odorants subsequently identified were quantified in the slurry using GC-MS. Prior to the analysis 3 ml aliquots were centrifuged at 5000 G and 1.5 mls of supernatant was pipetted into a auto-sampler vial and 0.1 ml of 2-methyl phenol was added as internal standard.

A Hewlett Packard (hp) 5890 II Series gas chromatograph and a 5972A mass selective detector (MSD II) were used to determine odorant concentrations in the slurry samples.

2.4. Slurry analysis

A range of analyses were performed, they included total nitrogen determination by the Kjeldahl method, and ammonium-N (Searle 1984). Water soluble carbohydrates, crude fibre(acid detergent), acid hydrolysed oils, pH and dry matter were also determined using methods common to food analysis.

3. Results and discussion

3.1. Volatile fatty acids

Exponential curves were the best expression of the declining concentrations for all VFAs from the range of samples (Fig 1), with half lives of 85 to 144 hr^{-1} as shown in Table 2. The declining concentration rates of other VFAs was not reduced when extra acetic acid was added for both the deuterated and additional acetic and phenol replicates, indicating that declining concentration rates of acetic acid was independent from that of other VFAs.

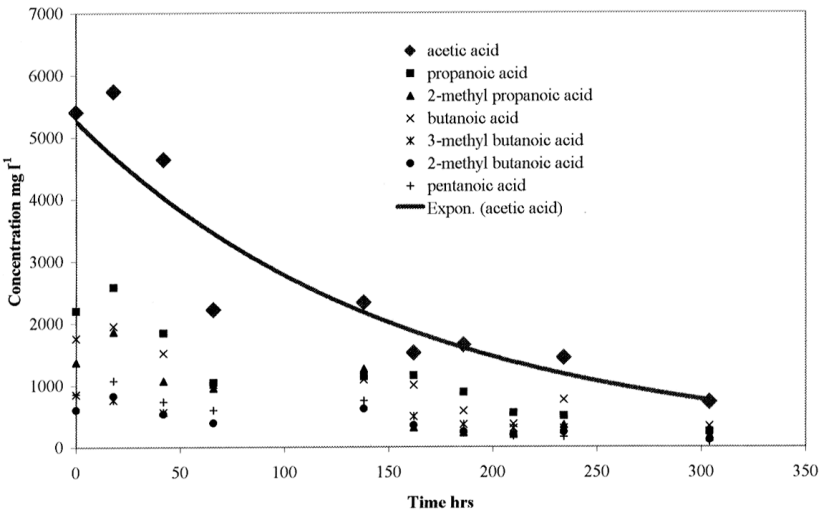

Figure 1
Exponential decline of the VFA concentrations in slurry.

The VFA concentration time profiles were exponential indicating that the decline was concentration dependant, (Table 2) and therefore first order. This contrasts with the concentration independent, or zero order reaction mechanism identified by Caunt and Hester (1989) and Jolicoeur and Morin (1987) for acetic and propanoic acids for aerobic digestion processes. However we included the initial concentration changes which declined quickly and were significant in defining an exponential curve as the best fit (Fig 1) and no mechanical assistance was used in our experiment to improve diffusion of oxygen into the slurry. If we were to ignore the first few time periods, as did the latter authors, a zero order curve was also appropriate ($P<0.001$) for our data. However there are conditions when a pseudo-first order occurs when the substrate concentration is much less than the reaction rate (Skoog *et al* 1992). This may be the case here as our zero order reaction rates for acetic acid are 0.0012 g l^{-1} hr^{-1} and are less than those in the publications above of 0.176 and 0.033 g l^{-1} hr^{-1} respectively.

Odorant	Initial slurry concentration mg l^{-1}	Reaction order	Half life hrs
Acetic acid	5844	1.04	98
Propanoic acid	2484	1.08	102
2-methyl propanoic acid	1294	1.03	95
Butanoic acid	1876	0.97	126
3-methyl butanoic acid	836	0.88	144
2-methyl butanoic acid	701	0.92	124
Pentanoic acid	1047	0.97	85

Table 2
Summary of the declining concentration rates for the volatile fatty acids

3.2. Acetic acid dynamics

The initial acetic acid concentration in the pig slurry was 5,800 mg l^{-1} and this declined to 700 mg.l^{-1}. As the initial concentrations of acetic acid varied for samples, the half-life of the exponential curves were determined and used to assess the relative concentration decline in these particular conditions. These were then used to generate a comparative graph (Fig 2) showing decline due to emission and biological function. These rates varied with time but emission was the major reason for decline (half-life of 158 hours) and bio-decay reduced the half life to 95 hours. Little, if any, production of acetic acid occurred increasing the overall half-life to 98 hours.

The reaction order was also determined for the bio-decay and emission in the slurry samples. As the declining acetic acid concentration rate was much greater than the production rate, it is appropriate to apply a differential method (Mahler and Cordes 1969) where the slope gives the reaction order and the intercept the reaction rate. There was no significant difference for the reaction order between the emission rate and the bio-decay rate with a mean of 1.02 $\pm$ 0.03. Certainly if Henry's Law was true then the emission should be concentration dependant. In addition, if the bio-decay of acetic acid was performed by enzymes either from microbial sources, or in solution, then the order should be bimolecular or pseudo-first order (Mahler and Cordes 1969). However it was surprising there was no difference between the reaction order for the two processes.

The relationship of the bio-decayed mass of acetic acid to the mass of methane was in good agreement ;

$m = 0.015.A - 1.190$ Eq-2

($P<0.001$) where A and m are the masses in mg of acetic acid and methane respectively, indicating that about 1 mg of methane was generated for every 66 mg that bio-decayed within the waste. A greater mass of carbon dioxide was produced than the amount of acetic acid that bio-decayed (1.88 mg mg^{-1} of acetic acid) ($P<0.001$);

$CO_2 = 1.88.A + 475$ Eq-3

3.3. Phenolic dynamics

The concentration of phenol peaked during the experiment with a maximum at 85mg l^{-1} after 175 hours. The initial and final concentration of phenol was 30 mg l^{-1} and 50 mg l^{-1} respectively. Not only did the concentration have a maximum, but the initial emission and bio-decay rates were of opposite proportions to acetic acid at 10 % (0.017mg l^{-1} hr^{-1}) and 90 % (0.144 mg l^{-1} hr^{-1}) emission and bio-decay respectively, with regeneration at 1.20 mg l^{-1} hr^{-1} being greater than the sum of the two described means of loss at zero time.

The profile of the decline of 4-methyl phenol within the pig slurry can be described by a half life of 495 hours ($P<0.100$) identifying different mechanism(s) of production and/or bio-decay than those of phenol. However Ishaque et al (1985)

shows comparable bio-decay rates for the two phenols and states that the availability of oxygen is the limiting factor in phenolic bio-decay.

No significant changes in concentration were observed for 4-ethyl phenol and the indoles. Analysis of the slurry showed that the WSC had increased 6 fold for all slurry containing samples ($P<.001$) suggesting that limited hydrolysis of polysaccharides had occurred. No significant changes or differences were observed between samples or during the experiment for the acid hydrolysed oil, crude fibre, total ash or dry matter content (Table 3).

Sample No	Total nitrogen g l^{-1}	Carbohydrates W/S g l^{-1}	Oil Acid Hydrolysed g l^{-1}	Crude fibre g l^{-1}	Total Ash g l^{-1}	Ammonia nitrogen g l^{-1}	Dry matter g l^{-1}
Prior to experiment slurry	6.20	0.10	6.37	8.67	17.50	3.16	66.70
After experiment :							
. slurry	5.07	0.67	8.87	9.33	18.10	2.39	60.71
. slurry+acetic4 & phenol-d5	4.53	0.60	7.70	7.67	16.53	2.27	65.23
. artificial slurry	np	np	np	np	np	1.25	np
. slurry+acetic & phenol	5.10	0.67	6.70	6.00	15.10	2.76	66.01

np not performed

Table 3
Analysis of slurry contents

3.4. Ammonia dynamics

Ammonia concentrations in all replicates reduced throughout the experiment. Greater rates of reduction were observed for the artificial slurry of 1.91 g l^{-1} over the 10 day period, indicating that factors other than the VFAs are responsible for retaining ammonia in pig slurry. The initial concentration of total N and ammoniacal N in the slurry was at 6.20 and 3.16 g l^{-1} respectively (Table 3). Over the experiment about 1.13 g l^{-1} of N was lost and the concentration of ammonia in the pig slurry depleted by 0.85 g l^{-1}, indicating that an additional unaccounted for organic fraction of nitrogen was lost from the slurry, most probably as ammonia.

When expressed as a surface emission, the loss of ammonia-N was 4.7 g m^{-2} d^{-1}, a value very similar to that of Sommer *et al* (1993). These authors measured emission rates of ammonia-N from stored pig slurry with similar total N, total ammoniacal-N and dry matter content to those used in our study during Autumn/Winter and Spring/Summer periods resulting in 3.9 and 4.6 g m^{-2} d^{-1}, respectively. These measurements were made from stores with a surface area and volume of 2.6 m^2 and 4.3 m^3 respectively over a 3 month period.

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5. References

Caunt P, Hester K W 1989 A kinetic model for volatile fatty acid biodegradation during aerobic treatment of piggery wastes. *Biotech and Bioeng* **34** 126-130.

Hobbs P J, Misselbrook T H and Pain B F 1997 Characterisation of odorous compounds and emissions from slurries produced from weaner pigs fed dry feed and liquid diets. *J Sci Food Agric* **73** 437-445.

Hobbs P J, Pain B F, Kay R M, Lee P A 1996 Reduction of odorous compounds n fresh pig slurry by dietary control of crude protein *J Sci Food Agric* **71** 508-514.

Ishaque M, Bisaillon J-G, Beaudet R, Sylvestre M 1985 Degradation of phenolic compounds by microorganisms indigenous to swine waste. *Ag Wastes* **13** 229-235.

Jolicoeur P, Morin A 1987 Isolation of Acinetobacter calcoaceticus strains degrading the volatile fatty acids of swine wastes *Biol Wastes* **19** 133-140.

Mahler H R, Cordes E H 1969 Chemical Kinetics In:*Biological Chemistry* Harper & Row, NY pp219-228.

Searle P L 1984 The Bertholot or indophenol reaction and its use in the analytical chemistry of nitrogen. *Analyst* **109** 549-568.

Skoog D A, West D M, Holler F J 1992 Chemical methods of analysis In: *Fundamentals of Analytical Chemistry* Eds Skoog D A, West D M Holler, Saunders Publishing Orlando Florida. pp 640 -664.

Sommer S G, Christensen B T, Nielsen N E, Schjorring J K 1993 Ammonia volatilization during storage of cattle and pig slurry: effect of surface cover. *J Agric Sci* **121** 63-71.

Spoelstra S F 1980 Origin of objectionable odorous components in piggery wastes and the possibility of applying indicator components for the study of odour development. *Agric Environ* **5** 241-260.