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PRACTICAL SECTION FOR GROWERS

This report covers progress during the third year of the project and is divided into three parts, with the overall objective of developing a quantifiable method of producing odour-free mushroom composts.

The three sections involve developing:

- alternatives to broiler poultry manure as a nitrogen source in mushroom compost
- methods for quantifying mushroom composting odours
- microbial inocula which metabolise the odorants produced during composting

Part 1: Alternative Nitrogen Sources
Poultry and horse manures are the main sources of sulphur in the generation of odorous sulphides from composting. Experiments in Years 1 and 2 using aerated tunnels showed that spent hop powder could be used in place of poultry manure, with significantly less odour but comparable mushroom yields and compost density. Cocoa meal was also a promising material, but the release of nitrogen was more delayed. Degradation using inorganic nitrogen sources (urea or ammonium sulphate) was slower than with poultry manure. However, the nitrogen from urea was readily available. The experiments also showed that rape straw, which has a high nitrogen content, could be used in place of wheat straw with a lower inclusion of poultry manure . The substitution of wheat straw with other straw types would increase the availability of straw for organic production.

Experiments in Year 3 had the following objectives:

- to determine if mixtures of organic and inorganic N sources could be used
- to determine if wheat straw could be replaced by other straw types (bean and linseed)
- to examine the replacement of poultry manure with alternative N sources in a conventional windrow composting system
- to examine the performance of the composts and composting methods on commercial sites
- to examine new alternative N sources which have become available, using bench-scale composting equipment.

Replacing wheat straw with rape straw resulted in a significant reduction in odour in both windrow and aerated tunnel composts without affecting mushroom yield or compost density. Rape straw has a higher nitrogen content than wheat straw and required a lower inclusion of poultry manure. Mushroom yield from bean straw and linseed straw composts were lower than from wheat straw or rape straw composts. Rape and bean straw could be used in organic mushroom compost, particularly if there was a shortage of organic wheat straw.

Substituting 50% of poultry manure N with cocoa meal or urea reduced mushroom yield, although cocoa meal was better than urea. Hop powder with ammonium sulphate produced a good mushroom yield (260 kg/tonne) when the compost N was less than 3% of dry matter. Using inorganic N sources (urea or ammonium sulphate) resulted in lower compost bulk density. Substituting poultry manure by 50% with organic (spent hop powder, cocoa meal) or inorganic (ammonium sulphate or urea) nitrogen sources resulted in significant reductions in odour and sulphide concentrations.

Odour concentrations from windrow composts were higher than from aerated tunnel composts using similar composting materials.
Nitrogen sources which produced similar mushroom yields to poultry manure in flask composting equipment were cocoa meal + calcium hydroxide and molasses waste (including ammonium chloride).

Part 2: Odour Quantification Techniques
Odour samples obtained from eleven composting sites in the first two years of the project showed that sulphur containing compounds were found to be the major contributors to compost odours. There was a close correlation between the compost odour concentration (OC) of the pre-wet and Phase I compost air samples and the combined hydrogen sulphide and dimethyl sulphide concentrations using gas detector tubes. In order to measure sulphide concentrations of less than 0.1 ppm (100 ppb) eight electronic sulphide detectors were assessed. Only two instruments responded to low sulphide concentrations in compost odour samples:

(i) Laboratory-based pulsed fluorescence sulphide analyser, manufactured by Thermo Environmental Instruments Ltd
(ii) Semi-portable colorimetric hydrogen sulphide analyser, manufactured by Zellweger.

Experiments in Year 3 had the following objectives:

- to further investigate the use of the above electronic sensors for measuring low sulphide concentrations
- to determine the relationship between low odour and sulphide concentrations, around and downwind of composting sites
- to validate the odour/sulphide relationship with further compost samples from different composting sites and synthetic compost odours.

Air samples were obtained from five composting sites, at increasing distances downwind from the Phase I composting stacks, to the site boundary and beyond.

A pulsed fluorescence analyser was found to be sensitive to sulphides in composting odours at 10 ppb. There was a good correlation between the instrument readings and odour concentration. The sensitivity of the analyser enabled it to detect odour plumes at the boundary sites, about 50 m from the Phase I composting stacks.
For measurements of odour and sulphide (hydrogen sulphide and dimethyl sulphide) concentrations of compost yards in Year 3, there was good agreement with the odour/ sulphide relationship which was previously found.

Odour concentrations on two windrow composting sites were lower than in samples in previous years. The reductions were probably due to more frequent turning of the stacks and lower compost moisture content, reducing the amount of anaerobic compost.

Synthetic pre-wet and Phase I odours were prepared from sulphides, ammonia and other odour compounds which closely simulated real composting odours when presented to an odour panel. This shows that the most important odour compounds in mushroom composting have been identified.

Part 3: Microbial Degradation of Composting Odours

Ten bacterial isolates were obtained from mushroom compost which were able to remove odorous sulphur containing compounds from compost air. The bacterial isolates belong to the following species: *Pseudomonas putida*, *Pseudomonas fluorescens*, *Bacillus cereus/thuringiensis* and *Hyphommicrobium* spp. Several

compost systems were compared for testing the use of the bacteria in removing hydrogen sulphide and dimethyl sulphide from compost air. All the systems produced highly variable levels of sulphides and were therefore unsuitable for testing the efficacy of the bacterial isolates in removing odour from large-scale systems. A more controllable experimental composting system for producing uniform sulphide and odour levels is being developed.

Action Points for Growers

1. Replacing wheat straw with rape straw reduces the poultry manure requirement by about 10%, since the rape straw has a higher nitrogen content. This reduces odour emissions without affecting mushroom yield or compost bulk density.
2. Replacing poultry manure with other organic nitrogen sources (spent hop pellets or cocoa meal) also reduces compost odours without affecting mushroom yield or compost density.
3. Replacing poultry manure with inorganic nitrogen sources reduces mushroom yield and compost bulk density although urea is better than ammonium sulphate.
4. Aeration of pre-wet and Phase I areas, more frequent turning of windrows and reduction in compost moisture all reduced anaerobic compost and emissions of odours.
5. Sulphides are the main cause of mushroom composting odours, and there is a good correlation between odour and sulphide concentration in the air of composting sites. A pulsed fluorescence analyser can be used to measure sulphide concentrations as low as 10 ppb on site boundaries.

Project Milestones

Task	Target Date	Milestones
1/1	12 months	Methodology to monitor and sample odours developed: Relationship between laboratory-based odour quantification techniques and subjective methods established (HRI, IGER, Aromascan plc)
1/2 1/3	12 months	Organic waste N sources analysed and processed into odour-free forms (HRI, Bulrush, Holdsworthy Bioplant)
1/4	12 months	Different N sources compared in laboratory-based composting processes; N balance in substrate and its biomass and mushrooms determined (HRI)
	12 months	Consortium to decide whether odour quantification techniques are sufficiently reliable to use in intermediate and large scale composting experiments or whether further laboratory development is needed. Decision on which N sources should be taken into intermediate scale facilities.
2/1	18 months	If Task 1/1 completed on time, odour quantified in intermediate-scale facilities and bulk chamber composts using GC-MS, olfactometry and electronic nose (HRI-W, Aromascan, IGER)
2/2	24 months	Composting temperature and aeration regimes developed for bulk chambers (HRI)
2/3	24 months	If Tasks 1/1 and 1/2 were completed on time, quantitative methods for measuring smell at commercial sites will have been developed (Industrial partners a – f, HRI, IGER, Aromascan)
2/4	24 months	If Task 2/3 was completed on time, odour of conventional composting will have been quantified (Industrial partners a – f, IGER, Aromascan)
2/5	24 months	Evaluation of N sources in bulk chamber experiments completed (HRI)
	24 months	Consortium to decide on which temperature/aeration regime(s) and composting system(s) to be used for larger scale experiments, or whether further intermediate scale experiments are needed to develop the regimes. Decision on which N sources are suitable for larger scale experiments.

	24 months	Decision on whether odour quantification of conventional composting sites is reliable or whether further assessments or development of quantification techniques are needed.
3/1	30 months	Influence of microbial inoculation on odour emissions and degradation and additives to enhance water retention determined in laboratory conditions. (HRI)
3/2	30 months	Compost odours synthesised in the laboratory to test the parameters of the electronic nose (IGER).
	30 months	Consortium to assess the performance of microbial inocula in reducing odour levels. Decision on whether larger scale experiments with microbial inocula can commence.
3/3	36 months	If Task 2/2 was completed on time, temperature and aeration regimes will have been evaluated at commercial sites (Industrial Partners a – f, HRI).
3/4	36 months	Evaluation of N sources in commercial scale experiments completed (HRI, Bulrush, Holdsworthy Bioplant, Industrial Partners a – f).
	36 months	Consortium to compare conventional and experimental composting odour levels and to decide what levels of improvement are necessary in the experimental and commercial systems. Consortium to decide which further developments in N sources are required.
4/1	42 months	If Tasks 3/1 was completed on time, the effect of microbial additives and methods for enhancing compost water retention in bulk chamber experiments will have been determined (HRI).
4/2	42 months	Modified N sources further examined in laboratory experiments; methods for reducing N losses from compost determined (HRI).
	46 months	Consortium to decide on the format of the manual and preparation of remaining publications.
4/3	47 months	Combined effect of new composting regimes, N sources, microbial additives and methods of enhancing water retention determined at commercial sites (Ind. Partners a-f; HRI)
4/4	48 months	Production of a manual on odour assessment, compost preparation and N sources in compost (HRI, IGER and Industrial Partners)

All the milestones to the end of Year 3 have been achieved. The remaining milestones should be achieved within the timescales specified.

SCIENCE SECTION

Part 1: Alternative Nitrogen Sources

Poultry and horse manures are the main sources of sulphur in the generation of odorous sulphides from composting. Experiments in Years 1 and 2 using aerated tunnels showed that spent hop powder could be used in place of poultry manure, with significantly less odour but comparable mushroom yields and compost density. Cocoa meal was also a promising material, but the release of nitrogen was more delayed. Degradation using inorganic nitrogen sources (urea or ammonium sulphate) was slower than with poultry manure. However, the nitrogen from urea was readily available. The experiments also showed that rape straw, which has a high nitrogen content, could be used in place of wheat straw with a lower inclusion of poultry manure . The substitution of wheat straw with other straw types would increase the availability of straw for organic production.

Experiments in Year 3 had the following objectives:

- to determine if mixtures of organic and inorganic N sources could be used
- to determine if wheat straw could be replaced by other straw types (bean and linseed)
- to examine the replacement of poultry manure with alternative N sources in a conventional windrow composting system
- to examine the performance of the composts and composting methods on commercial sites
- to examine new alternative N sources which have become available, using bench-scale composting equipment.

Materials and Methods

Bench-scale composting equipment

Substrate ingredients were composted in 'Quickfit' multiadapter flasks immersed in thermostatically controlled water baths, each holding two 10-litre flasks (Noble *et al*, 1997). The prepared ingredients (3 kg samples) were placed on a perforated stainless steel platform within each flask and the flasks immersed in the waterbaths such that the water level was above the level of the enclosed substrate. Each flask was connected to ancillary equipment providing independent aeration of the compost. The oxygen concentration in the substrate was controlled regularly by adjusting the airflow through the compost in each flask within the range 0 - 16 litres kg^{-1} substrate h^{-1} by means of flow meters. The temperature of the substrate in the flasks was monitored with Squirrel multipoint temperature loggers (Grant Instruments Ltd, Cambridge, UK).

For the first 48 h of the composting process, the thermostat of the waterbath was set at 47°C to allow a natural succession and gradual build-up of microorganisms. The substrate temperature was then increased to 72°C for 5 days, after which the substrate was re-mixed and the temperature reduced to 47°C for the remainder of the composting period, which was seven days, or prolonged until the air in the flask was clear of ammonia. An oxygen concentration of 11(± 1.5)% v/v was maintained in the substrate.

Aerated bulk composting tunnels

Bales of straw were wetted and formed into stacks using a compost turning machine. Further water was added to the straw in a separate turn to achieve a moisture content of 70%. After 4 d, 50% of the required N source was mixed into the stack; the remaining N source and gypsum at 30 kg $tonne^{-1}$ fresh compost ingredients were mixed into the stack after a further 2 d. Water was added in a further three turns after day 4, to achieve a moisture content of 78%. Stack temperatures were monitored with platinum resistance sensors and data logger. The preparation time for the blended compost ingredients, before filling into the tunnels was seven days.

Six aerated bulk composting tunnels at HRI Wellesbourne were used for the experiments. Compost was filled on to a slatted base in the tunnels, mounted above an air plenum through which a controlled flow of fresh and/or recirculated air could be blown. Two of the tunnels consisted of modified insulated cargo containers. Both of these tunnels had a vertical partition, which did not extend into the air plenum below, to enable two different composts to be filled into each tunnel (Type A). The other four tunnels consisted of insulated polythene tunnels, inside which were two parallel walls, joined by a wall at one end (Type B). The compost was enclosed by a removable end wall, which fitted across the sidewalls. Details of the tunnels, temperature, oxygen and air flow measurement and control, methods for filling and emptying the tunnel and methods for measuring ammonia concentrations are given in Noble & Gaze (1994 & 1998).

The tunnel composting regime consisted of three stages, designated Phases 0, I and II. The tunnels were filled with 4 t batches of blended compost ingredients to a height of 1.5 m. The Type A tunnels were each filled with two 4 t batches of blended compost ingredients separated by a central partition. In the Type B tunnels the airflow was set at 9 m^3h^{-1}, unless the oxygen concentration in the compost fell below 6%, in which case the airflow was increased to 13 m^3h^{-1} until the oxygen concentration was above 6%. The respective airflows in the Type A tunnels were 12 and 26 m^3h^{-1}. After five days (Phase 0), the compost was emptied from the tunnels, mixed and if necessary re-wetted to achieve a moisture content of 77%, and then re-filled. The subsequent 6-day Phase I was similar to the Phase 0 regime.

For the Phase II pasteurisation regime, the tunnels were filled with 2.5 t of material from the Phase I stage to a height of 0.9 – 1.1 depending on the ingredients. Following a 20 h equalisation of compost temperature at 45 - 48°C, the composts were pasteurised at 58 - 60°C for 6 h. Compost temperatures were then reduced to 46 - 49°C (conditioning). A minimum oxygen concentration of 13% was maintained during Phase II. Composting was completed when the compost temperature had fallen to within 1°C of the air temperature and ammonia could no longer be detected in the compost. Details of temperature and airflow control during Phase II are given in Noble & Gaze (1998).

Windrow composting

Straw bales were formed into windrows and wetted on day 0 without a pre-wetting procedure. Nitrogen sources were added on day 2 and water applications and windrow turns were made on alternate days during a 16 day period. Water applications after day 10 were varied according to compost moisture content. Compost moisture content at filling of bulk pasteurisation tunnels was 76%.

Both aerated and windrow composts were pasteurised at 58 C for 6 hours and conditioned at 45-48 C.

Compost analysis

Analyses were conducted on freeze-dried, finely milled samples of the compost ingredients and of the substrates before and after processing in the flask composting equipment. Dry matter (DM), N, ammonium (NH^+_4) and ash contents and pH were determined as described previously (Noble and Gaze, 1994).

Odour analysis

Odour samples were collected in 20 L Teflon bags as follows:
(i) from flasks by evacuating the air in the flask
(ii) from aerated tunnel composts, 0.2 m downwind of the compost during the emptying of the Phase 0 tunnel stage.
(iii) from windrow composts, 0.2 m downwind of the stack during turning.

The odour samples were then transported to IGER North Wyke and analysed within 24 h. Odour concentration (OC) was determined by an odour panel using dilution olfactometry and volatile organic compounds detected by gas chromatography – mass spectrometry (GC – MS), as described in the Year 2 Annual Report. A Dräger Accuro bellows pump with appropriate detector tubes (Drägerwerk, Lübeck, Germany) was used for on-site measurement of ammonia and specific sulphides, in the same way as sampling odours for collection in Teflon bags. Two replicate measurements were made for each sampling.

Mushroom cropping procedure

(i) *Bench-scale flask composts*
At the end of the composting period, the material in each flask was weighed. After samples were taken for analysis, 2 kg of the residual material was inoculated with mushroom spawn (spawned) at two percent of the fresh weight of compost with *Agaricus bisporus* spawn (Hauser A15) and filled into plastic pots, 230 mm diameter x 220 mm depth. The pots were placed in polythene bags in an incubator at 25°C and when the substrate was fully colonised with mushroom mycelium, about 15 days after spawning, the containers were covered (cased) with a moist mixture of peat and sugar beet lime (900 g). When mushroom mycelium was visible on the surface of the casing, the containers were transferred to a controlled environment chamber with an air temperature of 18°C, relative humidity of 90% and a CO_2 concentration of 0.1% to induce fruiting. Mushrooms were harvested daily over a 30 day period (cap diameter 25-30 mm).

(ii) *Large-scale, aerated tunnel and windrow composts*
The cropping procedure is outlined in Noble & Gaze (1998). The composts were inoculated with mushroom spawn ("spawned") using the Hauser A15 (Sylvan Spawn Ltd, Peterborough, UK) and 2100 (Amycel-UK Ltd, Burton-on-Trent, UK) strains. Half the compost spawned with each strain was supplemented with the soya meal-based "Betamyl 1000" (Sylvan Spawn Ltd) at a rate of 1% of compost fresh weight. Spawned trays were stacked four high in cropping sheds, with four replicate trays of each spawn and supplement sub-treatment from each of eight compost treatments

(128 trays per shed with 16 trays from each of the compost treatments). A split-plot design was used with compost treatments allocated to main plots, which were arranged in a Latin square design, and spawn/supplement treatments allocated to sub-plots within each main plot. Mushrooms were picked as large buttons (diameter 30-40mm) over a 24-days period (three flushes of mushrooms). The yields of each run were analysed separately and the means of each treatment were incorporated into the analysis structure of the tunnel composting stage of the experiment.

Percentage dry matter content of each batch (treatment and run) of mushrooms from the first and second flushes was calculated from the fresh weight of 20 mushrooms, and the dry weight after oven drying (Burton & Noble, 1993). The N and ammonium (NH_4^+) contents of mushrooms from the first and second flush were determined on freeze-dried samples of 20 mushrooms from each batch (Noble & Gaze, 1994).

(iv) Commercial farm tests

Samples of compost were transported in bulk bags to tray and shelf farms. The compost was spawned with the same strain used on the farm for standard compost, and received the same cultural treatment. The commercial trays contained x kg of compost, and a similar amount of compost was filled into the ends of shelves on the shelf farm.

Bags were also filled with samples of compost, spawned with same strains used on the commercial farms. Batches of 20 bags, each containing 20 kg compost, were sent to the commercial farms.

Experiments

Bench-scale flask composts

Experiment 1: Effect of substrate N from difference N sources on mushroom yield

Compost ingredients were prepared from wheat straw and the following N sources:

i. Broiler poultry manure (control)
ii. Brewers' grains
iii. Molasses waste liquid including ammonium chloride (a sugar production waste)
iv. Mycelium by-product (a waste from citric acid production)
v. Amide waste (from oilseed rape processing)
vi. Cocoa shells
vii. Cocoa meal (with and without calcium hydroxide)
viii. Sporavite (molassed fibrous meal with added urea).
ix. Analyses of the above N sources are shown in Table 1.

Composts were prepared with two levels of N (1.6 and 2.3% of dry matter) and a moisture content of 77%, and were filled into flasks for composting as previously described. Analyses of the flask composts at filling and spawning are shown in Tables 2 and 3.

Aerated tunnel composting

Experiment 2: Use of different compost nitrogen and sources and straw types

The substrates were prepared from wheat (*Triticum aestivum*), oilseed rape (*Brassica napus*) and field bean (*Vicea faba*) straws as the main carbon (C) source. Rape and bean straws were only used with broiler poultry manure. Wheat straw was used with poultry manure (control treatment), cocoa meal + urea and spent hop powder + urea.

In the latter two formulations, the organic and inorganic (urea) N sources each provided 50% of the supplemented N. The analysis of the raw ingredients and the quantities used in the formulations are shown in Table 4. The proportions of C and N sources in the formulations were calculated on the basis of their N and dry matter contents using the formula in Noble & Gaze (1994) to achieve blended ingredients with an N content of 1.8 to 2.1% of dry matter. The moisture content of the composts at filling of the Phase 0 tunnels was 77 - 78%. Analyses of the composts at filling of the Phase II tunnels and at spawning are shown in Tables 5 and 6.

Windrow composting

Experiment 3: Use of different compost nitrogen and sources and straw types

Substrates were prepared from wheat, oilseed rape, bean and linseed/ flax (*Linum usitatissimum*) straws. All the straws were used with poultry manure. Wheat straw was also used with two other organic N sources, cocoa meal and spent hop powder, in combination with two inorganic N sources, urea and ammonium sulphate. Linseed straw was used only once. The combinations of straw types, organic and inorganic N sources are shown in Table 5. Analyses at filling of Phase II tunnels and at spawning are shown in Tables 7 and 8. Two replicate runs of the experiment were produced, with the positions of the compost stacks in the yard and allocation of treatments to composting tunnels changed between replicate runs.

Results

Bench-scale flask composts

Experiment 1: Effect of substrate N from difference N sources on mushroom yield

Analysis of materials

Analyses of the N sources used are shown in Table 1. The molasses waste (+ammonium chloride), Croda amide waste and mycelium by-product had N contents above 3% of dry matter, although the molasses waste and mycelium by-product had higher moisture contents. The cocoa shells waste and brewers grains had lower N contents than poultry manure or the cocoa meal waste used in previous experiments (Table 4). The N content of hop haulm waste on a fresh weight basis was significantly lower than that of the other alternative N sources. Hemp straw had a similar N content to wheat straw. These latter materials were therefore not tested in the composting experiment.

Ammonia losses during flask composting of poultry manure, brewers' grains, molasses waste, amide waste and Sporavite were similar (Table 2, Figs. 1 - 4). Emission of ammonia from cocoa meal (with or without calcium hydroxide), and mycelium by-product was significantly less, and little ammonia was released from cocoa shells compost (Table 2, Figs. 1 & 4).

At spawning, the molasses waste, mycelium by-product and Sporavite composts all had high ammonium N contents, particularly at the high inclusion rate (Table 3). The pH of the cocoa shells, cocoa meal, amide and Sporavite composts was higher than that of the poultry manure compost. The brewers' grains compost had a significantly lower pH.

Mushroom yield

The N sources which produced mushroom yields similar to poultry manure composts were cocoa meal + calcium hydroxide and molasses waste (+ammonium chloride) at

the lower inclusion rate. At the higher inclusion rate, there was high ammonium N content at spawning and reduced mushroom yield.

Aerated tunnel composts

Experiment 2: Use of different compost nitrogen and sources and straw types

Analysis of composts

Analyses of the materials used in the experiment are shown in Table 4. The cocoa meal and spent hop powder had similar N contents on a fresh weight basis to the poultry manure. Rape straw had a significantly higher N content than wheat, bean or linseed straw.

During composting the poultry manure/wheat straw and poultry/manure rape straw composts reached the highest temperatures and released the most ammonia (Table 9). Sulphides were only detected with gas detector tubes from the poultry manure/wheat straw compost, which also had a significantly stronger odour than the other composts.

Analyses of the composts at filling of Phase II tunnels and at spawning are shown in Table 5. The composts had similar N contents at spawning, although the bean straw and spent hop powder + urea composts had higher ammonium N contents. The bulk density of the rape straw compost was higher than that of the wheat straw compost, whereas the bean straw and spent hop powder + urea composts had significantly lower bulk densities (Table 5).

Mushroom yield and dry matter

Mushroom yields from the poultry manure/wheat straw and rape straw composts were similar (Table 10). Yields from the bean straw, cocoa meal + urea and hop powder + urea composts were lower. Overall, supplementation of compost with Betamyl did not affect mushroom yield. Mushroom dry matter content from the poultry manure/wheat straw composts was lower than from the other composts (Table 11).

Windrow composting

Experiment 3: Use of different compost nitrogen and sources and straw types

Composting process and compost analysis

Maximum temperature reached during windrow composting of the different materials were similar, except linseed straw and rape straw composts (also bean straw in Run 2) which reached lower temperatures. Linseed straw was very difficult to compost and breakdown; it was not repeated in Run 2.

Ammonia evolution during composting was significantly higher from rape and bean straw composts than from wheat straw composts (with poultry manure) (Fig.5 and Table 12). Ammonia losses from composts with ammonium sulphate were lower than from composts containing urea or other N sources (Fig. 6 and Table 12). Wheat straw/poultry manure composts produced the highest sulphide (H_2S and dimethyl sulphide DMS) and odour concentrations. Sulphide and odour concentrations from composts in which 50% of the poultry manure was replaced by urea or cocoa meal were lower (Table 12). The odour concentrations were lower from composts prepared from rape or bean straw (and poultry manure) or with wheat straw and the alternative N sources. None of these composts produced sulphide levels which could be detected with gas detector tubes. Odour concentrations from the windrow composts (Table 12)

were higher than from aerated tunnel composts using similar composting materials (see Table 5, Report Year 2).

Analyses of the windrow composts at filling of Phase II tunnels and at spawning are shown in Tables 7 and 8. The cocoa meal composts had higher N contents at spawning than the other materials due to the lower ammonia losses.

The bulk density of composts containing ammonium sulphate was lower than that of the other composts due to reduced degradation. Bulk densities of other composts were similar. There was no difference in bulk density between windrow and aerated tunnel composts.

Mushroom yield and dry matter

Yields from the wheat straw and rape straw (with poultry manure) composts were similar but yields from the bean straw and linseed straw composts were significantly lower (Table 13). Substitution of 50% of the poultry manure N with cocoa meal resulted in a slightly lower yield, and substitution with urea resulted in a slightly lower yield in Run 2 and much lower yield in Run 1. Hop powder with ammonium sulphate produced a good yield in Run 2 but a poor yield in Run 1. This was probably due to the high N and ammonium N in the compost in Run 1. Cocoa meal with urea or ammonium sulphate produced yields of 200 kg/tonne or less.

Mushroom dry matter content was variable between the two replicate runs of the experiment, although mushroom grown on rape or bean straw composts generally had a higher dry matter content than those grown on wheat straw composts (Table 15).

Conclusions - Part 1

1. Replacing wheat straw with rape straw resulted in a significant reduction in odour in both windrow and aerated tunnel composts without affecting mushroom yield or compost density. Rape straw has a higher nitrogen content than wheat straw and required a lower inclusion of poultry manure.
2. Substituting 50% of poultry manure N with cocoa meal or urea reduced mushroom yield, although cocoa meal was better than urea.
3. Mushroom yield from bean straw and linseed straw composts were lower than from wheat straw or rape straw composts.
4. Hop powder with ammonium sulphate produced a good mushroom yield (260 kg/tonne) when the compost N was less than 3% of dry matter.
5. Using inorganic N sources (urea or ammonium sulphate) resulted in lower compost bulk density.
6. Substituting poultry manure by 50% with organic (spent hop powder, cocoa meal) or inorganic (ammonium sulphate or urea) nitrogen sources resulted in significant reductions in odour and sulphide concentrations.
7. Odour concentrations from windrow composts were higher than from aerated tunnel composts using similar composting materials.
8. Nitrogen sources which produced similar mushroom yields to poultry manure in flask composting equipment were cocoa meal + calcium hydroxide and molasses waste (including ammonium chloride).

Table 1. Analysis of new alternative N sources used in bench-scale flask composting equipment.

N source	DM, %	% of DM		
		Total N	NH_4^+	Ash
Hop haulm waste	25.9	2.07	0.09	13
Cocoa shell waste	98.0	2.60	0.12	9
Brewers' Grains	24.2	2.68	0.04	5
Molasses waste (+ ammonium chloride)	60.3	6.32	3.37	9
Croda amide waste	99.8	3.55	0.08	-
Mycelium by-product	23.1	3.03	0.05	3
Hemp straw	89.2	0.52	0.01	5
Sporavite	74.7	7.40	2.30	16

Table 2. Analysis of flask composts at filling

Treatment*	DM %	% of DM N	% of DM NH_4^+	% of DM Ash	pH	Ammonia losses, g/tonne compost
Poultry manure (control)	22.5	1.63	0.39	10	8.0	14696
	21.2	2.46	0.59	10	8.4	31143
Cocoa shells waste	21.4	1.59	0.10	10	7.9	700
	25.5	2.18	0.17	12	8.0	0
Cocoa meal	22.5	1.26	0.08	10	8.1	1528
	27.8	2.31	0.31	10	8.3	1495
Cocoa meal + calcium hydroxide	27.4	1.61	0.17	11	8.0	706
	23.6	2.03	0.24	11	7.8	1410
Brewers' grains	20.5	1.65	0.14	9	7.6	21739
	19.3	2.09	0.37	12	7.2	19402
Molasses waste (+ ammonium chloride)	24.6	1.58	0.84	13	8.0	12435
	31.5	2.88	1.53	24	8.0	46032
Mycelium by-product	23.6	1.49	0.04	9	6.2	43
	20.1	2.63	0.04	9	5.9	868
Croda amide waste	33.1	1.60	0.05	9	6.9	13466
	-	-	-	-	-	-
Sporavite	26.3	1.76	0.60	12	8.2	7112
	24.1	2.54	0.91	12	8.3	130992

* wheat straw was used for all the composts

Table 3. Analysis of flask composts at spawning

Treatment*	DM %	N (% of DM)	NH_4^+ (% of DM)	Ash (% of DM)	pH	Mushroom yield g/kg compost
Poultry manure	24.3	2.20	0.05	15	7.5	236
(control)	25.3	2.61	0.05	15	7.5	147
Cocoa shells	29.3	1.60	0.07	10	8.5	59
waste	22.1	2.30	0.05	12	8.8	124
Cocoa meal	28.5	1.86	0.03	12	8.5	179
	28.7	2.88	0.20	9	8.3	29
Cocoa meal +	30.8	2.02	0.09	13	8.7	199
calcium hydroxide	32.1	2.98	0.07	13	8.3	242
Brewers' grains	31.5	1.70	0.10	12	7.1	85
	28.9	1.87	0.04	13	7.0	130
Molasses waste	29.4	2.62	0.21	14	7.5	205
(+ ammonium chloride)	35.5	3.42	1.50	15	7.6	96
Mycelium by-	29.5	1.74	0.18	11	7.6	87
product	28.1	1.77	0.39	11	7.5	68
Croda amide	30.4	1.81	0.04	11	8.4	50
waste	-	-	-	-	-	-
Sporavite	31.1	1.76	0.42	14	8.3	131

* wheat straw was used for all the composts

Table 4. Analysis of straw and N sources (NS) and quantities used in bulk tunnel experiments. Analyses are means of three replicate samples.

Straw and N source			**% of DM**		
Ingredients	**% W/W**	**DM%**	**Total N**	**NH_4^+**	**Ash**
Wheat straw	100-NS	86	0.50	0.02	6
Rape straw	100-NS	87	1.00	0.02	9
Linseed straw	100-NS	87	0.65	0.01	4
Bean straw	100-NS	88	0.58	0.01	4
Poultry manure	36.2	74	5.31	0.90	17
Spent hop powder	22.2*	94	4.00	0.05	8
Shell extracted cocoa meal	21.2*	95	4.50	0.07	4
Ammonium sulphate	1.5*	100	21.2	27.24	-
Urea	0.9*	100	46.7	-	-

* 50% of supplemented N source (NS)

Table 5. Analysis of aerated tunnel composts at filling of Phase II tunnels

Treatment*	% DM	% of DM			pH
		Total N	NH_4^+	Ash	
Poultry manure (control)	23.8	1.78	0.23	15	7.8
Rape straw + poultry manure	21.2	2.15	0.38	13	7.7
Bean straw + poultry manure	22.4	2.30	0.30	12	8.1
Cocoa meal + urea	22.2	2.14	0.30	11	8.2
Spent hop powder + urea	21.7	1.90	0.31	9	7.9

* Wheat straw was used except where stated

Table 6. Analysis of aerated tunnel composts at spawning.

Treatment*	% DM	% of DM			pH	Bulk density kg m^{-3}
		Total N	NH_4^+	Ash		
Poultry manure (control)	28.5	2.89	0.08	26	7.9	475
Rape straw + poultry manure	25.7	2.52	0.04	20	7.8	525
Bean straw + poultry manure	26.5	2.71	0.27	20	8.1	425
Cocoa meal + urea	25.9	2.59	0.03	24	7.7	500
Spent hop powder + urea	25.2	2.27	0.20	17	8.0	425

* Wheat straw was used except where stated

Table 7. Analysis of turned windrow composts at filling of Phase II tunnels

Treatment*	% DM	% of DM			pH
		Total N	NH_4^+	Ash	
Run 1					
Poultry manure (control)	23.5	2.27	0.21	21	7.9
Rape straw + poultry manure	24.1	2.10	0.18	23	7.5
Bean straw + poultry manure	25.3	2.56	0.07	17	7.6
Linseed straw + poultry manure	25.4	2.15	0.22	14	8.4
Cocoa meal + urea	22.5	2.75	0.54	14	8.0
Cocoa meal + ammonium sulphate	21.1	3.01	0.26	17	7.2
Hop powder + ammonium sulphate	22.6	2.55	0.24	17	7.5
Poultry manure + urea	23.7	2.05	0.05	21	7.5
Cocoa meal + poultry manure	24.7	2.65	0.31	19	8.2
Run 2					
Poultry manure (control)	24.2	1.95	0.24	21	8.0
Rape straw + poultry manure	22.7	2.37	0.57	19	8.0
Bean straw + poultry manure	22.7	1.83	0.31	16	7.8
Cocoa meal + urea	20.8	2.15	0.22	16	7.7
Hop powder + ammonium sulphate	20.5	2.51	0.32	15	7.9
Poultry manure + urea	23.3	2.49	0.17	17	7.7
Cocoa meal + poultry manure	21.5	2.80	0.31	16	7.7

* Wheat straw was used unless stated

Table 8. Analysis of turned windrow composts at spawning.

Treatment*	% DM	% of DM			pH	Bulk density kg m^{-3}
		Total N	NH_4^+	Ash		
Run 1						
Poultry manure (control)	27.6	2.49	0.11	27	7.8	450
Rape straw + poultry manure	31.4	2.52	0.03	28	7.8	475
Bean straw + poultry manure	27.2	3.17	0.05	24	7.8	475
Linseed straw + poultry manure	26.5	1.76	0.12	16	7.7	400
Cocoa meal + urea	25.1	3.66	0.07	22	7.6	475
Cocoa meal + ammonium sulphate	25.2	3.41	0.17	22	6.2	450
Hop powder + ammonium sulphate	24.7	3.02	0.24	33	7.3	450
Poultry manure + urea	25.4	2.70	0.25	27	7.9	450
Cocoa meal + poultry manure	24.3	4.11	0.14	25	7.8	475
Run 2						
Poultry manure (control)	29.4	2.58	0.03	26	7.8	475
Rape straw + poultry manure	26.4	2.98	0.04	23	7.7	475
Bean straw + poultry manure	27.3	2.31	0.08	16	8.2	450
Cocoa meal + urea	25.0	3.27	0.04	19	8.1	475
Hop powder + ammonium sulphate	26.7	2.76	0.06	19	8.2	450
Poultry manure + urea	27.7	3.03	0.07	22	8.0	525
Cocoa meal + poultry manure	25.4	3.29	0.02	22	8.0	500

* Wheat straw was used unless stated

Table 9. Maximum temperatures during yard blending, Phase O and Phase I and ammonia losses during Phase O and Phase I of aerated tunnel composting.

Treatment*	Maximum temperature, °C			Ammonia losses, mg/tonne compost
	Yard	Phase O	Phase I	
Poultry manure (control)	67	81	75	31738
Rape straw + poultry manure	51	85	83	40413
Bean straw + poultry manure	59	74	57	17583
Cocoa meal + urea	46	78	64	3321
Spent hop powder + urea	72	73	55	3817

* Wheat straw was used unless stated

Table 10. Mushroom yields (kg t^{-1} spawned compost) from aerated tunnel composts with different straw types and N sources; two spawn strains with and without Betamyl supplementation.

Treatment*	Spawn strain / Supplementation			
	A15 Betamyl		2100 Betamyl	
	-	+	-	+
Poultry manure (control)	281	254	261	226
Rape straw + poultry manure	235	255	254	239
Bean straw + poultry manure	204	165	195	150
Cocoa meal + urea	135	122	128	111
Spent hop powder + urea	159	143	141	177

* Wheat straw was used except where stated

Table 11. Mushroom dry matter contents from aerated tunnel composts prepared from different straw and N sources, strain A15 unsupplemented compost. Mean of flushes 1 and 2

Treatment*	Mushroom dry matter, %
Poultry manure (control)	7.82
Rape straw + poultry manure	9.13
Bean straw + poultry manure	8.24
Cocoa meal + urea	9.08
Spent hop powder + urea	9.39

- Wheat straw was used unless stated

Table 12. Maximum temperatures during windrow composting and time taken, and ammonia losses during composting.

Treatment*	Max temp, °C	Time to reach max temp., h.	ppm		Odour conc. OUm^{-3}	Ammonia losses, mg/tonne compost
			H_2S	DMS		
Run 1						
Poultry manure (control)	72	225	1.7	3.2	9947	9110
Rape straw + poultry manure	63	270	0	0	4101	35620
Bean straw + poultry manure	74	153	0	0	3906	45701
Linseed straw + poultry manure	55	360	-	-	-	-
Cocoa meal + urea	72	336	0	0	1764	7659
Cocoa meal + ammonium sulphate	75	258	-	-	-	3480
Hop powder + ammonium sulphate	72	160	0	0	550	4085
Poultry manure + urea	70	168	0.1	0	3925	9021
Cocoa meal + poultry manure	78	252	0.3	0.1	5217	4586
Run 2						
Poultry manure (control)	76	216	2.0	3.8	14406	2618
Rape straw + poultry manure	67	165	0	0	3011	9429
Bean straw + poultry manure	60	146	0	0	2418	8677
Cocoa meal + urea	75	167	0	0	1691	49
Hop powder + ammonium sulphate	72	252	0	0	2420	0
Poultry manure + urea	73	170	0.5	0.1	3482	8718
Cocoa meal + poultry manure	75	168	0.4	0	6779	4798

* Wheat straw was used unless stated

Table 13. Mushroom yields (kg t^{-1} spawned compost) from windrow composts with different straw types and N sources.

Treatment*	A15		2100	
	-	+	-	+
Run 1				
Poultry manure (control)	270	276	253	264
Rape straw + poultry manure	285	263	248	255
Bean straw + poultry manure	177	182	144	138
Linseed straw + poultry manure	83	103	71	105
Cocoa meal + urea	210	196	-	-
Cocoa meal + ammonium sulphate	79	70	43	76
Hop powder + ammonium sulphate	164	141	147	120
Poultry manure + urea	181	183	128	131
Cocoa meal + poultry manure	248	256	258	264
Run 2				
Poultry manure (control)	290	285	281	286
Rape straw + poultry manure	285	250	271	251
Bean straw + poultry manure	194	169	161	171
Cocoa meal + urea	172	152	185	157
Cocoa meal + poultry manure	251	262	239	254
Hop powder + ammonium sulphate	296	258	262	275
Poultry manure + urea	286	274	258	247

* Wheat straw was used unless stated

Table 14. Mushroom yields from experimental composts on commercial farms

Compost treatment	Test site	Mushroom strain	Mushroom yield Experimental	Mushroom yield Commercial standard
Poultry manure/ wheat straw	h	512	15.4 kg/m^2	17.9 kg/m^2
Cocoa meal + urea	h	512	13.6 kg/m^2	17.9 kg/m^2
Cocoa meal + poultry	f	2100	170 kg/tonne	216 kg/tonne
Hop waste + ammonium sulphate	b	130	9.7 kg/m^2	-
Cocoa meal + ammonium sulphate	b	130	8.9 kg/m^2	

Table 15. Mushroom dry matter contents from windrow composts, mushroom strain A15 unsupplemented compost, mean of flushes 1 and 2

Treatment*	Mushroom DM, %	
	Run 1	Run 2
Poultry manure (control)*	7.75	8.10
Rape straw + poultry manure	8.84	8.31
Bean straw + poultry manure	8.37	8.43
Linseed straw + poultry manure	7.88	-
Cocoa meal + urea	7.00	8.81
Cocoa meal + ammonium sulphate	8.62	-
Hop powder + ammonium sulphate	8.29	7.87
Poultry manure + urea	8.39	7.79
Cocoa meal + poultry manure	7.47	7.84

*wheat straw was used unless stated

Fig. 1 Ammonia emissions during flask composting.
N = 1.6% of DM at filling.

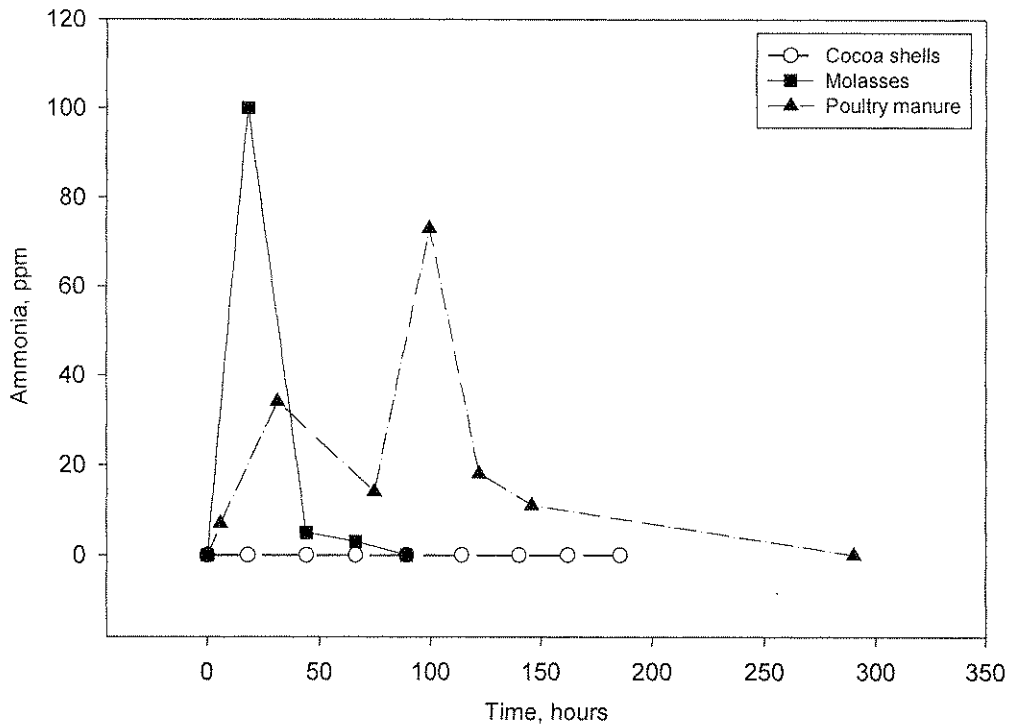


Fig. 2 Ammonia emissions during flask composting.
N = 1.6% of DM at filling

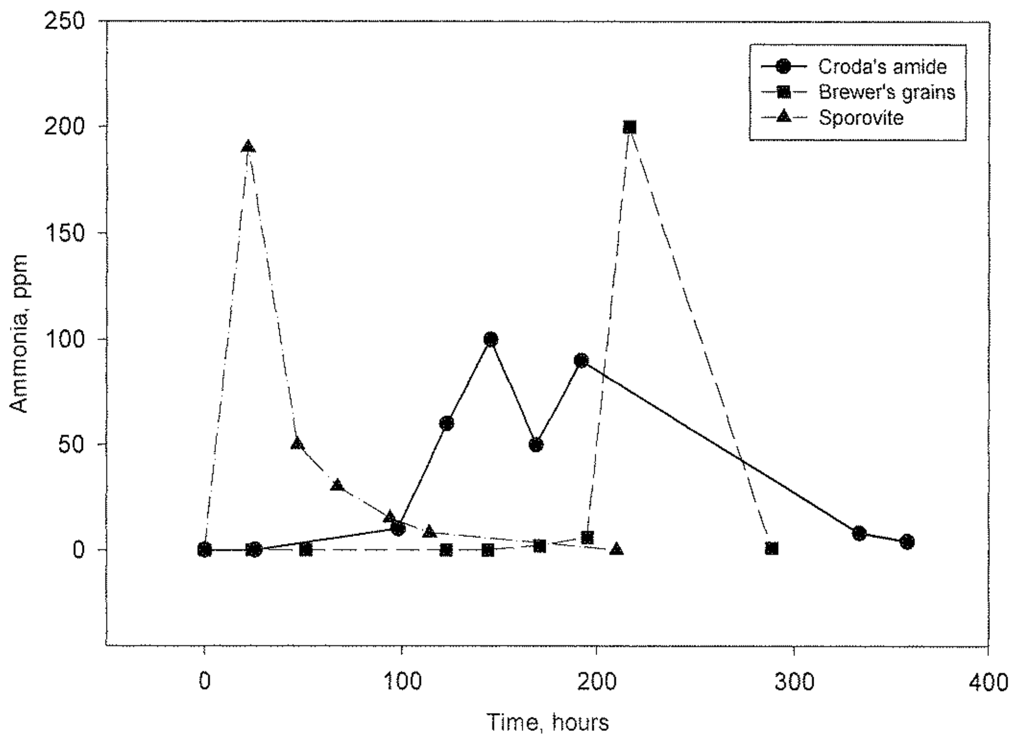


Fig. 3 Ammonia emissions during flask composting.
N = 2.3% of DM at filling.

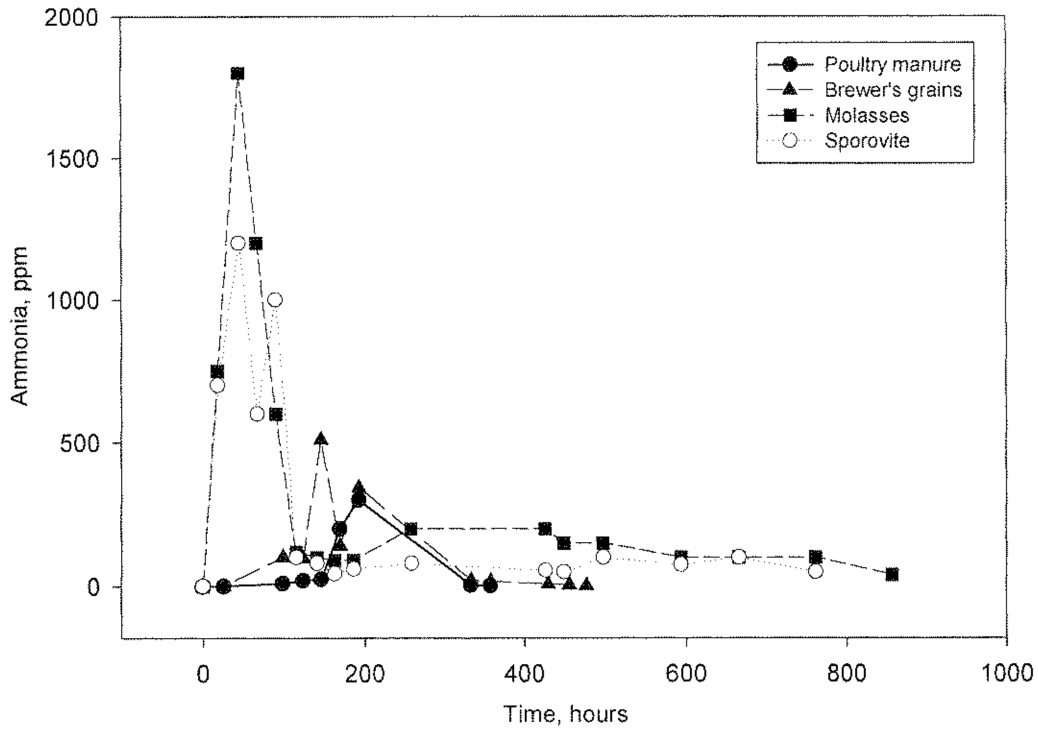


Fig. 4 Ammonia emissions during flask composting.
N = 2.3% of DM at filling.

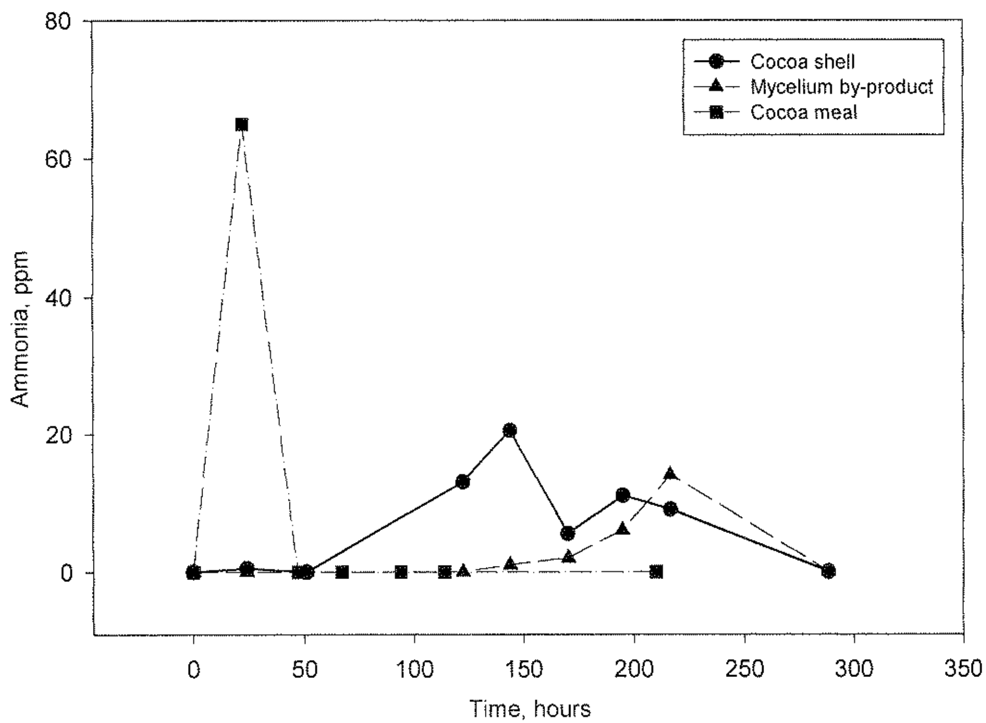


Fig. 5 Ammonia emissions during windrow composting and Phase II.
N = 2.2% of DM at filling of Phase II.

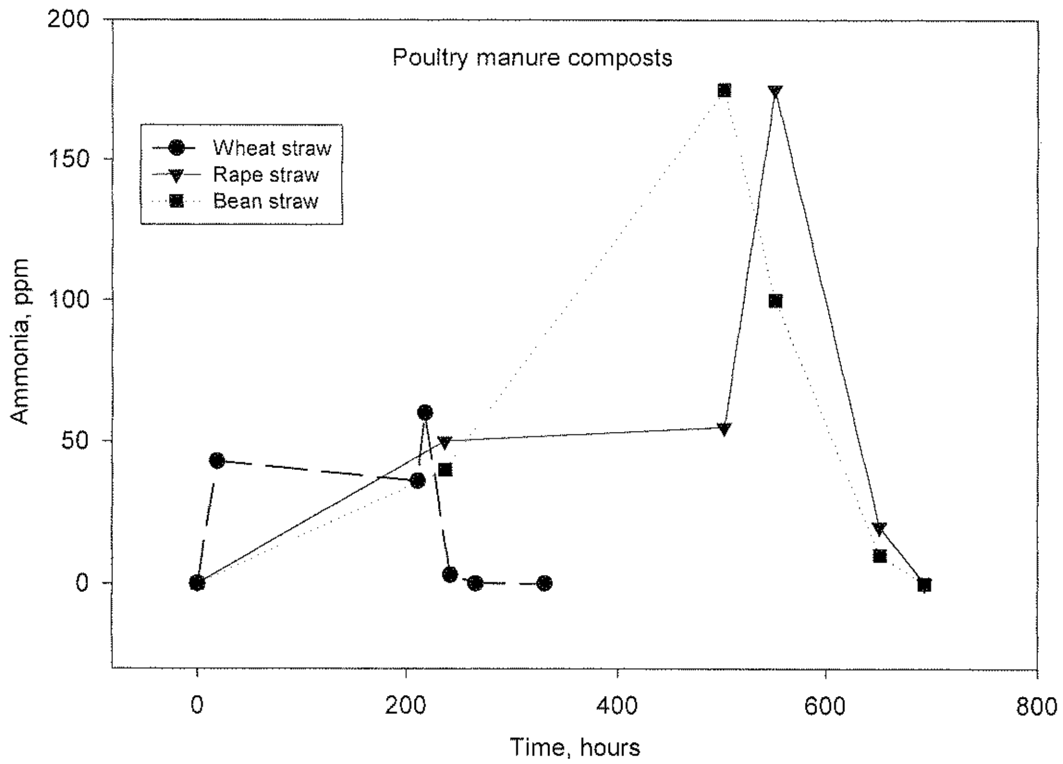
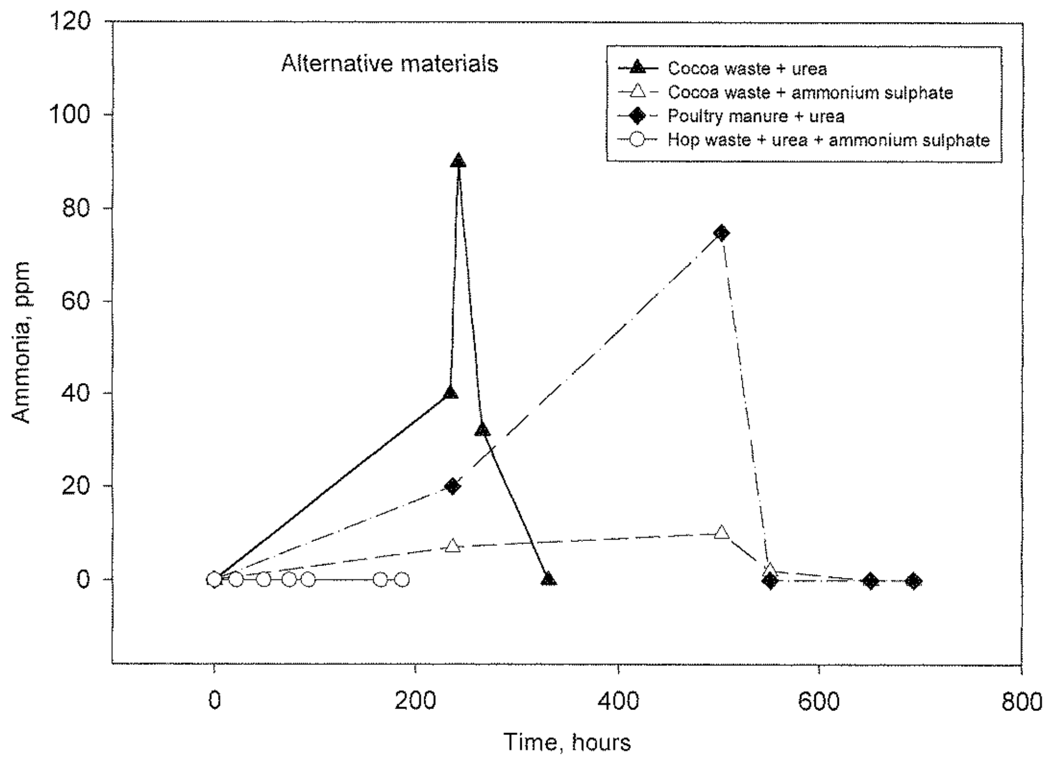


Fig. 6 Ammonia emissions during windrow composting and Phase II.
N = 2.2% of DM at filling of Phase II.



Part 2: Odour Quantification Techniques

Odour samples obtained from eleven composting sites in the first two years of the project showed that sulphur containing compounds were found to be the major contributors to compost odours. There was a close correlation between the compost odour concentration (OC) of the pre-wet and Phase I compost air samples and the combined hydrogen sulphide and dimethyl sulphide concentrations using gas detector tubes. In order to measure sulphide concentrations of less than 0.1 ppm (100 ppb) eight electronic sulphide detectors were assessed. Only two instruments responded to low sulphide concentrations in compost odour samples:

(iii) Laboratory-based pulsed fluorescence sulphide analyser, manufactured by Thermo Environmental Instruments Ltd

(iv) Semi-portable colorimetric hydrogen sulphide analyser, manufactured by Zellweger.

Experiments in Year 3 had the following objectives:

- to further investigate the use of the above electronic sensors for measuring low sulphide concentrations
- to determine the relationship between low odour and sulphide concentrations, around and downwind of composting sites
- to validate the odour/sulphide relationship with further compost samples from different composting sites and synthetic compost odours.

Materials and Methods

Odour sample collection

Air samples were obtained from five composting sites, (Tunnel Tech South, Shepherds Grove, Gateforth Park, HRI Wellesbourne and Monaghan Middlebrook Avon) at increasing distances downwind from the Phase I composting stacks, to the site boundary and beyond. Odour samples were collected in 20 L nalophane bags. Samples were obtained downwind of the compost stacks during turning at distances of about 0.2, 5, 15, 30, 40 and 50 m.

Replicate samples were collected simultaneously. Background samples were collected 200 m upwind of the composting sites. Samples were transported to IGER, North Wyke for gas chromatography - mass spectrometry (GC-MS) and olfactometry analysis. Two replicate samples were collected for each of the analyses, which were conducted 24 h after sampling. A Dräger Accuro bellows pump (Drägerwerek, Lübeck, Germany) was used in conjunction with appropriate detector tubes: acetic acid (6722101), amines (8101061), NH_3 (CH2051 and CH31901), carbon disulphide, CS_2 (8101891), DMS (6728451), H_2S (8101991 and 8101831), mercaptan (thiols) (6728981) and phenol (8101641). Detector tubes were used on-site in the same way as sampling odours for collection in Teflon bags, 24h after on-site sampling. Windspeed at the point of odour sampling was measured with a vane anemometer (Type 949079, Airflow Developments Ltd, High Wycombe, UK).

Details of olfactometry (odour panel assessment) and chemical analysis using GC-MS are given in the Year 2 report. All the samples were tested with a type DTM Project Research, Amsterdam Olfactometer; samples were also tested with a newer Olfactomat "C" Project Research, Amsterdam instrument. The odour panellists also made an assessment of odour intensity of the samples according to Burton et al (1997). A range of dilutions differing by a factor of two each time were presented to

each panellist a number of times through one known port of the olfactometer. The highest dilution was equivalent to the 75% odour threshold value. At each dilution, the panellists were required to indicate the subjective strength of the odour, according to the following scale:
0 No odour
1 Very faint odour
2 Faint odour
3 Distinct odour
4 Strong odour
5 Very strong odour
6 Extremely strong odour.
The relationship between intensity and odour concentration was calculated by linear regression.

Electronic sulphide detectors

Two electronic sulphide detectors were used, which operated on different principles:
(i) Pulsed fluorescence sulphur dioxide (SO_2) analyser (Type 43C, Thermo Environmental Instruments Ltd, Franklin, MA, USA) coupled to a sulphide to SO_2 converter (Type 45C, Thermo Environmental Instruments Ltd). The analyser was supplied by Unicam Chromatography, Cambridge, UK.
(ii) 'Chemcassette' hydrogen sulphide analyser. Electronic optical measurement of gas sensitive colorimetric paper tape (Type 7100 Zellweger Analytics Ltd, Bishops Stortford, Herts.,UK).
Odour samples were drawn into the two analysers using an electric pump. Each analysis took about 2 minutes to complete. The analysers were also tested with gas samples containing known concentrations of three sulphides at concentrations from 10 to 1400 ppb: hydrogen sulphide, dimethyl sulphide and methanethiol.

Validation of the compost odour / sulphide relationship

Relationship between odour and sulphide concentrations
The Year 3 odour concentrations from each of the sites were correlated with sulphide (hydrogen sulphide and dimethyl sulphide) measurements, to test the validity of the relationship which was found in the previous two years.

Comparison of real and synthetic mushroom compost odours
In order to test the reliability of the chemical analysis of composting odours, synthetic odours of typical pre-wet and Phase I compost odours were prepared. These were prepared from the six compounds which exceeded their detection thresholds by the greatest order of magnitude in real pre-wet and Phase I odour samples. These compounds were dimethyl sulphide, H_2S, butanoic acid, methanethiol, butanoic acid (in both the pre-wet and Phase I odours), indole (pre-wet only) and trimethylamine (Phase I only). Since ammonia occurred in all the compost odours, this was also added to both synthetic mixtures. The concentration of each chemical was the average concentration found in real compost odours. Synthetic and real odours were presented to an odour panel in a series of dilutions. Each mixture was presented twice.

Synthetic odour mixtures were also prepared without the ammonia, fatty acids and amine removed in order to test the influence of sulphides with and without other odorants in the air sample.

Results

Performance of electronic sensors

The Zellweger instrument was sensitive to hydrogen sulphide and methanethiol at 50 ppb but was insensitive to dimethyl sulphide at over 1000 ppb. There were no relationships between the Zellweger readings and the sample odour concentration (Fig. 7) or the sulphide readings from the pulsed fluorescence analyser (Fig. 8)

The pulsed fluorescence analyser was sensitive to all three sulphides tested (hydrogen sulphide, dimethyl sulphide and methanethiol) at a concentration of 10 ppb. There was a good correlation between the instrument readings (up to 500 ppb) and odour concentration (Fig.9).

Odour and sulphide concentrations on and around composting sites

The odour and sulphide concentrations decreased logarithmically with increasing distance from the main odour source (turned Phase I stacks) (Fig. 10). The pulsed fluorescence instrument was able to detect sulphides at 10 ppb in odour samples from site boundaries, about 50 m downwind of the Phase I stacks. Both the odour and sulphide concentrations on the downwind site boundaries were significantly above the background (upwind) concentrations (Fig.11).

Validation of the compost odour / sulphide relationship

Relationship between odour and sulphide concentrations
Further odour samples from composting sites in Year 3 of the project are shown as open circles in Fig.11. All the samples show good agreement with the odour/sulphide relationship which was previously found. Odour and sulphide concentrations determined on the seven composting sites in Year 3 are shown in Table 16. Comparison with Year 1 and 2 results for the same compost yards show an overall reduction. This is probably due to more frequent turning and lower initial moisture content leading to reduced anaerobic compost.

Comparison of real and synthetic compost odours
The synthetic pre-wet and Phase I odours smelt similar to poultry manure and the real and synthetic odours had similar detection thresholds. When presented as a series of dilutions, odour intensity score v odour concentration was similar for all the real and synthetic samples (Fig.12). This indicates that the six-compound mixtures were good simulations of pre-wet and Phase I odours, and that the measurement of a large number of compounds is unnecessary.
Removing the fatty acids and trimethylamine from the synthetic compost odour mixtures had little effect on the odour concentration (Table 17). Most of the odour concentration of the synthetic pre-wet mixture was due to the sulphides (H_2S, DMS and methanethiol) since removal of the ammonia also had little effect on the odour concentration. However, removal of the ammonia from the synthetic Phase I mixtures had a significant effect on reducing odour concentration (Table 17).

During the year, a new olfactometer was installed at IGER (Olfactomat "C". Compost odour samples were presented to the panellists through both the original and new olfactometers. Both machines were calibrated with a known concentration of butanol gas. Although there was still a good correlation between sample odour and

sulphide concentrations (Fig.13), the new machine gave a significantly higher odour concentration for samples of an equivalent sulphide concentration. This was probably due to the reduced metal pipe work in the new olfactometer, resulting in reduced absorption of sulphides, and hence higher odour concentrations to the panellists. The emphasises the need to specify the type of olfactometer being used to assess odour concentration of air samples containing sulphides.

Conclusions - Part 2

1. A pulsed fluorescence analyser was found to be sensitive to sulphides in composting odours at 10 ppb. There was a good correlation between the instrument readings and odour concentration.
2. The sensitivity of the analyser enabled it to detect odour plumes at the boundary sites, about 50 m from the Phase I composting stacks.
3. For measurements of odour and sulphide (hydrogen sulphide and dimethyl sulphide) concentrations of compost yards in Year 3, there was good agreement with the odour/ sulphide relationship which was previously found.
4. Odour concentrations on two windrow composting sites (A and I) were lower than in samples in previous years. The reductions were probably due to more frequent turning of the stacks and lower compost moisture content, reducing the amount of anaerobic compost.
5. Synthetic pre-wet and Phase I odours were prepared from sulphides, ammonia and other odour compounds which closely simulated real composting odours when presented to an odour panel. This shows that the most important odour compounds have been identified.
6. Most of the odour concentration of synthetic pre-wet odours could be attributed to the sulphide (hydrogen sulphide, dimethyl sulphide and methanethiol) content, although ammonia contributed strongly to the odour concentration of synthetic Phase I mixtures.
7. The type of olfactometer used in measuring odour concentration should be specified, since different types of machines can give different values.

Table 16. Odour concentrations by olfactometry and total sulphide (hydrogen sulphide and dimethyl sulphide) concentrations for composting yards, Year 3 results

Compost yard	Odour conc., Odour units / m^3		Total sulphides, ppm	
	Pre-wet	Phase I	Pre-wet	Phase I
A	-	4465	-	1.4
B	-	127800	-	172.5
H	-	60856*	-	32.2
I	2248	9518	0.6	7.5
K	4345	-	0.8	-
L	-	7939*	-	4.0
M	4839*	1953*	2.2	0.6

*estimated from the total sulphide concentrations

Table 17. Synthetic odour mixtures used to simulate typical pre-wet and Phase I compost odours

Compound	Concentration in mixture, µg m^{-3}					
	All compounds		Sulphides + ammonia		Sulphides	
	Pre-wet	Phase I	Pre-wet	Phase I	Pre-wet	Phase I
Dimethyl sulphide	1667	3528	1667	3528	1667	3528
Hydrogen sulphide	730	4362	730	4362	730	4362
Methanethiol	211	126	211	126	211	126
Butanoic acid	33	2129	-	-	-	-
3-methyl butanoic-acid	-	869	-	-	-	-
Trimethylamine	-	582	-	-	-	-
Indole	7	-	-	-	-	-
Ammonia	27976	75740	27976	75740	-	-
Odour conc. OU m^{-3}	1359 3442	3955 11378	1829 2623	9837 14191	1851 252	1881 5778

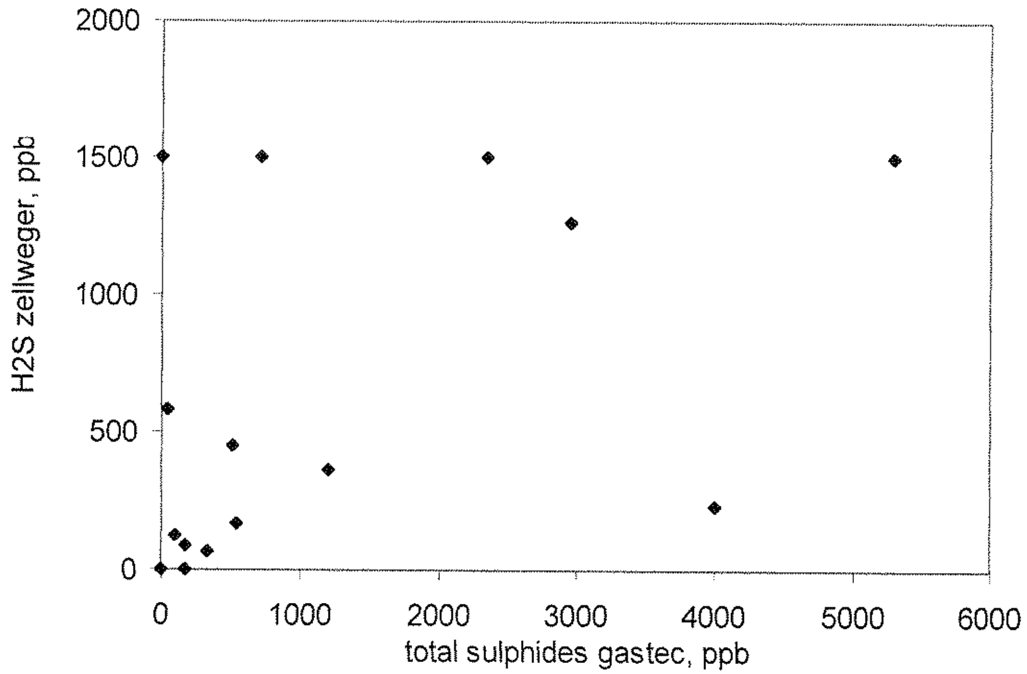


Fig. 7 Measurement of sulphides with Zellweger instrument
H2S zellweger, ppb
2000
1500
1000
500
0
0
1000
2000
3000
4000
5000
6000
total sulphides gastec, ppb

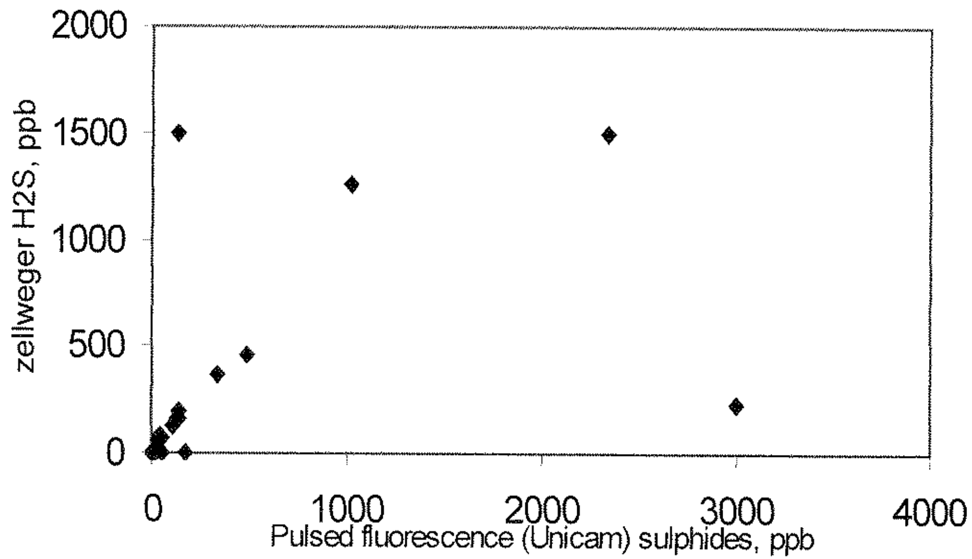


Fig. 8 Measurement of sulphides with Zellweger and Unicam instruments
zellweger H2S, ppb
2000
1500
1000
500
0
0
1000
2000
3000
4000
Pulsed fluorescence (Unicam) sulphides, ppb

Fig. 9 Relationship between pulsed fluorescence sulphides (Unicam analyser) and odour concentration

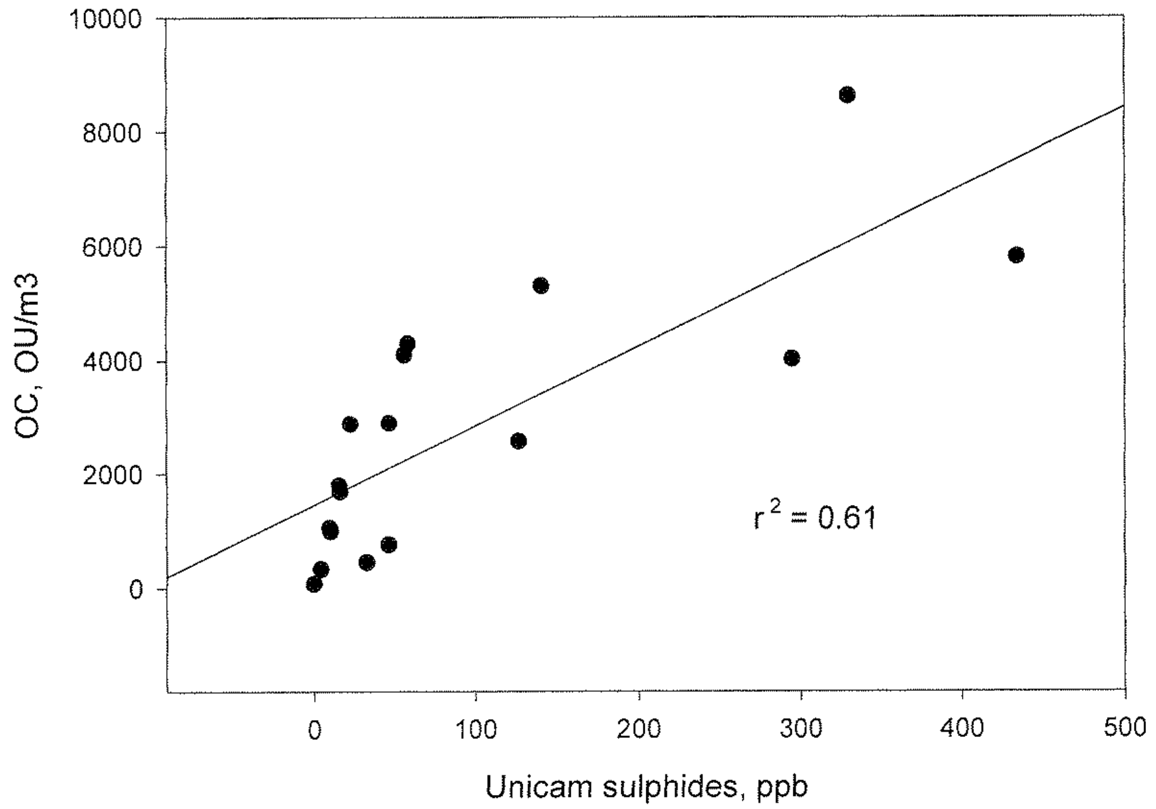


Fig. 10 Odour concentration of plume from Phase I composting stacks

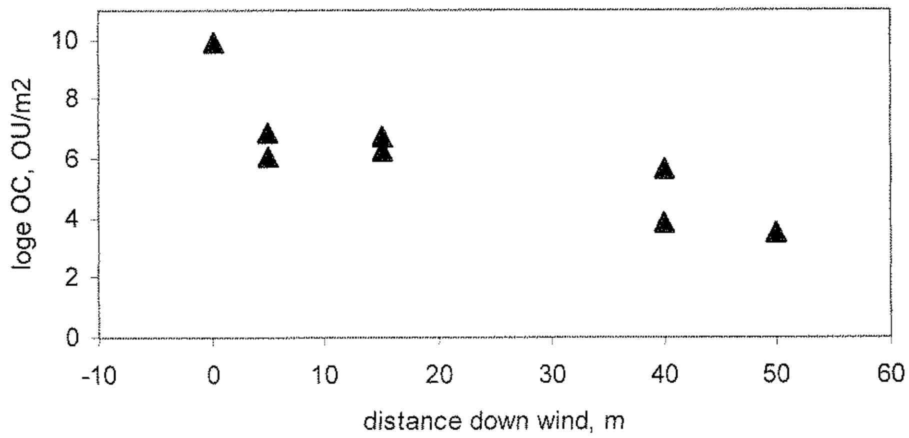
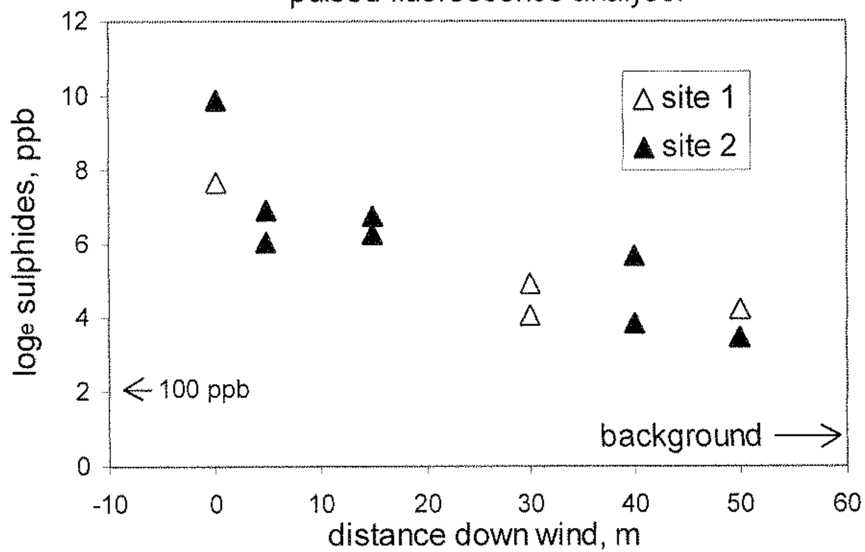


Fig. 11 Total sulphides in odour plume measured with Unicam pulsed fluorescence analyser



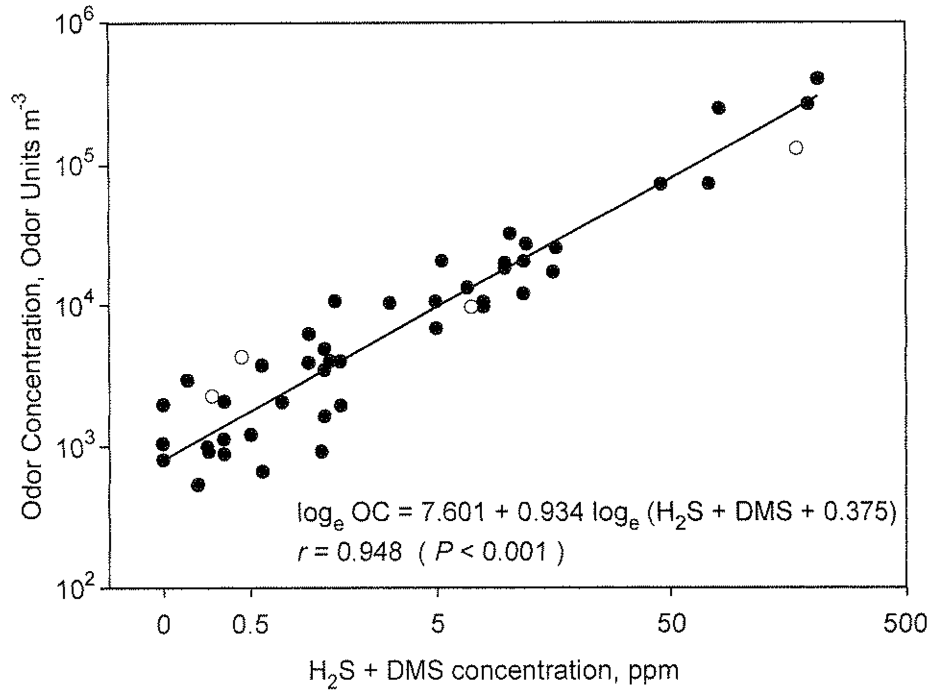


Fig. 12 Relationship between the combined on-site hydrogen sulphide and dimethyl sulphide concentrations and the odor concentration of bag odor samples from mushroom composting yards. Each point is the mean of two sample determinations. Open circles are for 1999/2000 measurements

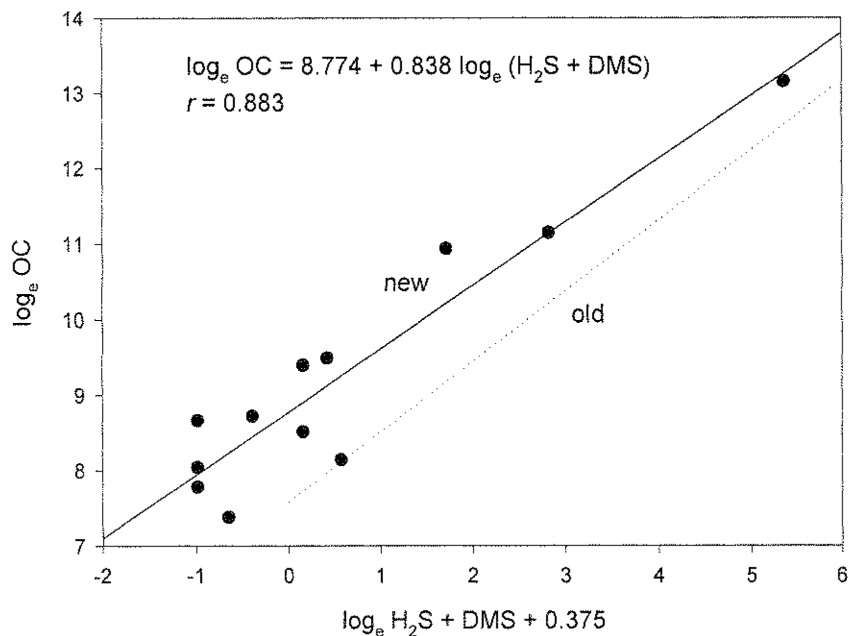
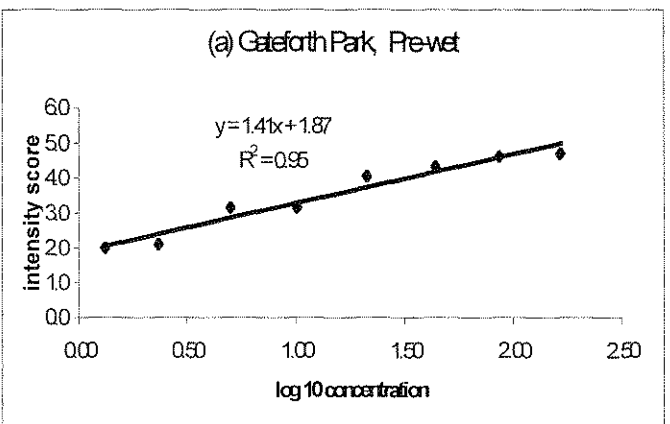
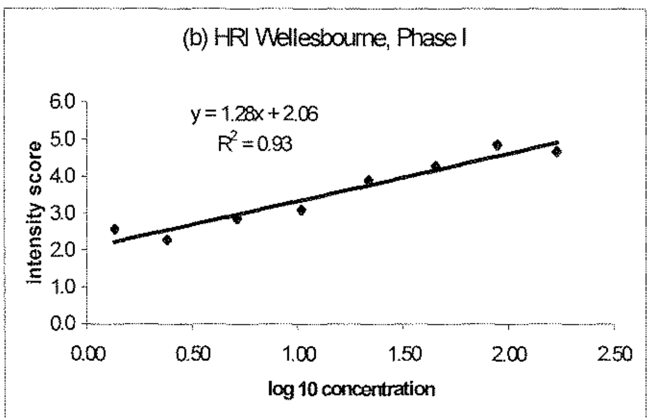
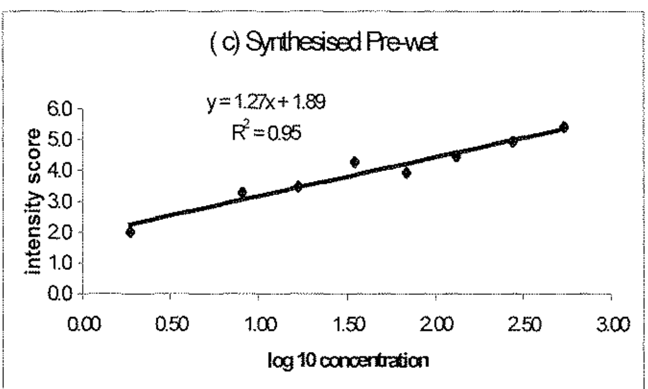


Fig.13 Relationship between the combined hydrogen sulphide and DMS concentrations and odour concentration, new olfactometer

Fig. 14 Relationship between air sample concentration and intensity score (0 to 6 scale) for real compost yard odour samples (a and b) and synthetic compost odour samples produced by mixing the most important odorant compounds in their typical concentrations (c and d). Each value is the mean of six determinations.







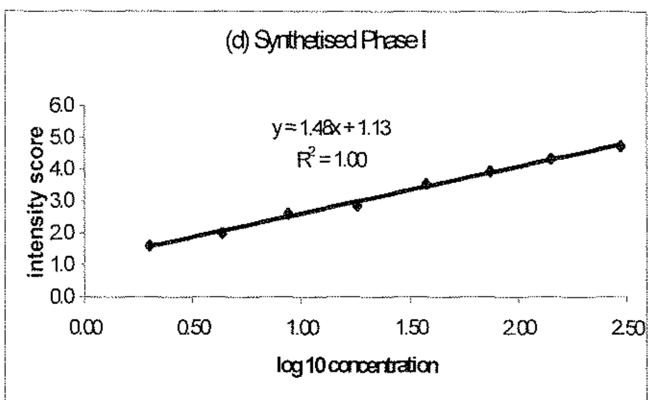


Figure 15. Structure of the 16S rRNA ribosomal gene indicating the area sequenced.

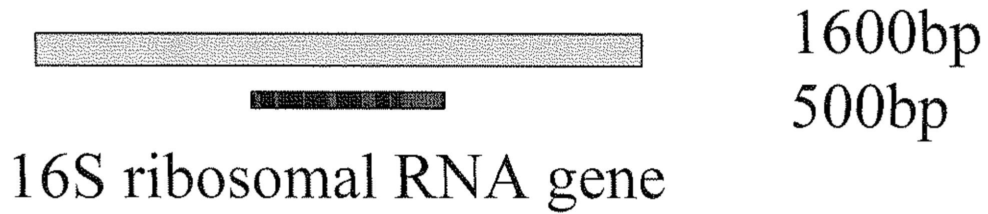
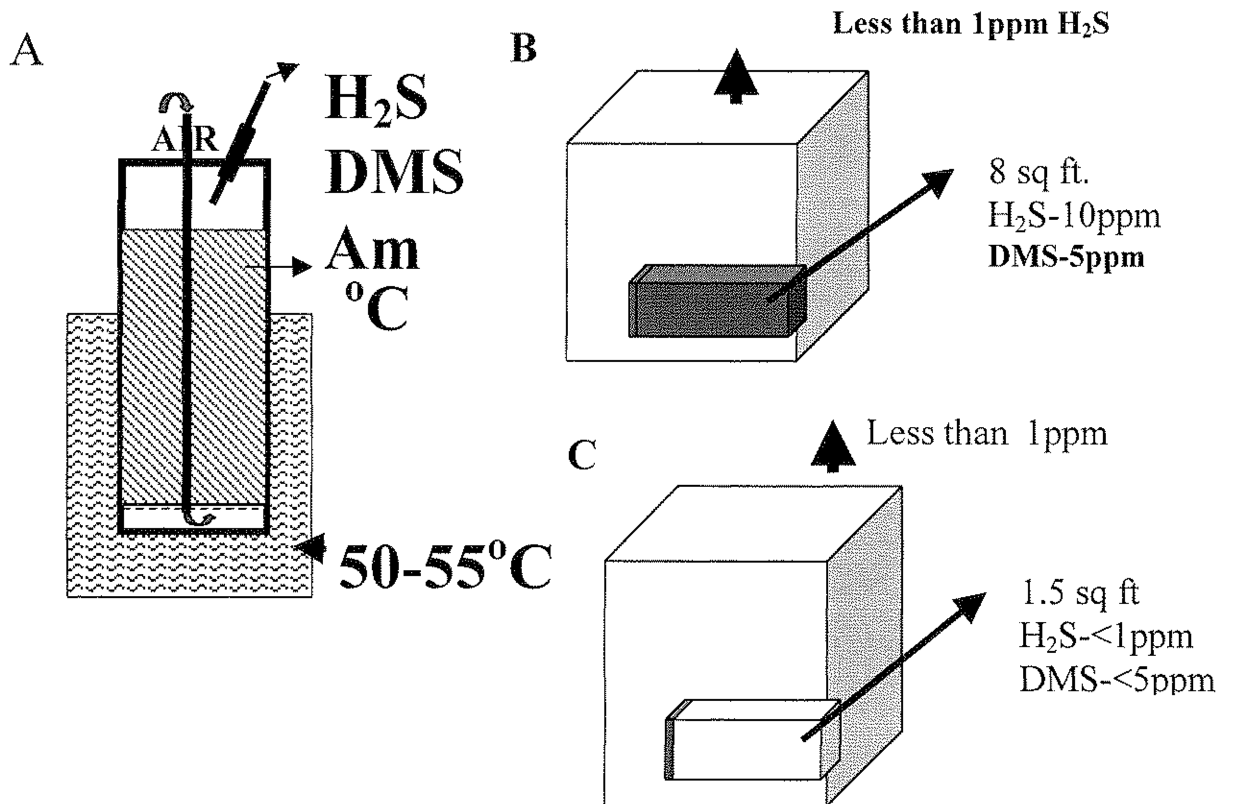


Figure16. Structure of the model systems. A, laboratory microcosm; B, compost stack experiment



Part 3: Microbial Degradation of Odour

Introduction

The target odour compounds identified for removal from compost are H_2S and DMS. Bacterial strains known to remove H_2S have been isolated from environmental samples and when re-applied to a biofiltration system can remove this compound from emissions. The isolates used in these types of study are normally present in a separate unit where ammonia has been removed, the temperature and other conditions within the system have been controlled. Such biofiltration systems are expensive and normally require specialised ducting, equipment and out-buildings. This part of the project aims to isolate bacteria capable of removing H_2S and DMS from the environment, and re-inoculating them on to the compost to test if they can remove these compounds directly from the stack. The isolates would have to function on the outside of the stack or on a matrix applied over its surface. As well as isolating strains, the development of reproducible compost capable of generating stable levels of the target compounds are needed. If successful, these strains would be used on a larger scale to try and remove H_2S and DMS from larger composting systems.

Strains

As a positive control for testing strains isolated from compost, one strain of a *Hyphomicrobium* species (a gift Dr H. Op den Camp, Netherlands) known to remove H_2S in pure culture was used in these studies. The strain was tested for its ability to grow on simple nutrient media at a range of temperatures and large scale laboratory growth conditions in Luria Broth/Luria agar developed. Ten isolates obtained from the outer layer (5cm) of the compost stack were tested for their ability to remove H_2S and DMS. Seven of the ten isolates performed as well as the *Hyphomicrobium* strain, the remaining three isolates significantly reduced the levels of H_2S and DMS from samples, however they were approximately 3 times less active than the other isolates. All 11 strains had the following general characters; they grew between 25 and 40°C, within a pH range of 6.5 and 8.5, and were aerobic. The 16S ribosomal RNA gene of each isolate was amplified using the universal primers 8f and 1654r (Figure 15). A region within the gene of approximately 500bp from each isolate was sequenced. Analysis of the sequence revealed that the isolates fell into the following lineages:

Pseudomonas putida-lineage
Pseudomonas fluorescens-lineage
Bacillus cereus/thuringiensis/etc - lineage
Hyphommicrobium- lineage

Testing isolates on compost odours

The *Hyphomicrobium* isolate was introduced on an agar plate or as a broth suspension into an air sample collected from above an 8-day-old compost sample. At t=0 the compost air sample was found to contain 30ppm H_2S and 5ppm DMS. After 8 hours at 30oC the sample with the Hyphomicrobium strain had no detectable H_2S or DMS, while in the untreated sample 20ppm H2S and no DMS was detected. This indicated that the strain effectively removed the H2S from the sample. Similar results were found for the 10 isolates used on 2 separate treatments, although slightly different

starting conditions for both experiments were obtained (H_2S 15ppm and 25ppm, and DMS 8ppm and 0ppm at the start). The decline in H_2S and DMS detected in the control samples indicated that these molecules were unstable during the incubation period. The results also indicate that the microorganisms removed H_2S from samples of compost air sample. This takes the isolates one step closer to showing that they can function in the compost system they need to work in.

Model compost systems

The isolates now need to be tested to see if they can remove H_2S and DMS once applied directly to the compost surface or to a matrix that is then placed over the surface of the compost. Application directly into the centre of the compost itself is not considered feasible since the temperature conditions (>50°C) and oxygen levels are not suitable for the growth of the bacteria. Model compost systems described in Fig 16 A were set up and found to produce either highly variable results where H_2S measurements ranged from below 1ppm to above 200ppm. Simpler contained systems were established to try and reduce the variability and provide a standard level of H_2S of between 10-20ppm, and DMS of 2-5ppm. These levels are appropriate to those detected above the compost stacks. Self-contained compost systems were established to produce the starting material for this work. In the first run the stack after 2 weeks had developed an anaerobic core with H_2S and DMS levels of 15ppm and 5ppm, respectively (Figure 16 B). Above the stack no H_2S or DMS was detected. The central region was removed and placed in 30L and 500ml sealed containers held at 55°C. In the 30L containers the levels of H_2S and DMS in the headspace decreased to undetectable levels within 5 days. Once the contents of these containers was disturbed higher levels of H2S (5ppm) and no DMS was detected. In the smaller containers the levels of H_2S and DMS were maintained in 5 out of the 10 containers sampled, while in the remaining 5 containers the level of H2S increased to a maximum level of 30ppm H_2S. To test the effect of changes in pH, additions of antibiotics chemicals, and bacterial isolates a repeat stack was set up. In this stack a much smaller area where H_2S and DMS was detected was found (Figure 16 C). On transfer to the smaller containers and incubation for 24 hours no H_2S or DMS was detected in any of the 25 samples and so no treatments were conducted. Two further stacks were set up, this time using recycled 'goodie' water to wet the system, on both occasions a lighter coloured area of compost at the centre of the stack was observed indicating the anaerobic zone. This area was removed and found to be very pungent but H_2S and DMS levels were around 1 ppm. Incubation of one set of these samples for up to 3 days did not result in the detection of any H_2S or DMS in the headspace.

Future Direction - Microbial Inocula

Two areas are being pursued. The first is to develop a model compost system that will deliver reproducible levels of H_2S or DMS to test the effect of bacterial additions. The compost production system at HRI, although produces material that is not odourless does produce material with very low levels of H_2S and DMS. Samples of compost from a local producer, which will be transported to HRI for experiments, will be undertaken. The difficulty with these model systems is that the microorganisms are designed to be applied to the surface of the compost where the temperature and oxygen conditions are more favourable for their activity. The smaller model systems that are available are held in conditions more suited to the centre of the stack where

the bacteria can not function. The development of more complex microcosms to test the ability of the microorganisms under realistic conditions is envisaged. The second area under investigation is to try and isolate bacteria capable of removing H_2S and DMS under conditions similar to those present in the centre of the stack. No isolates like this have been previously described in the literature and they may not exist. Isolation methods using anaerobic growth chambers will be undertaken.

Overall Conclusions

1 Replacing wheat straw with rape straw resulted in a significant reduction in odour in both windrow and aerated tunnel composts without affecting mushroom yield or compost density. Rape straw has a higher nitrogen content than wheat straw and required a lower inclusion of poultry manure.

2. Substituting 50% of poultry manure N with cocoa meal or urea reduced mushroom yield, although cocoa meal was better than urea.

3. Mushroom yield from bean straw and linseed straw composts were lower than from wheat straw or rape straw composts.

4. Hop powder with ammonium sulphate produced a good mushroom yield (260 kg/tonne) when the compost N was less than 3% of dry matter.

5. Using inorganic N sources (urea or ammonium sulphate) resulted in lower compost bulk density.

6. Substituting poultry manure by 50% with organic (spent hop powder, cocoa meal) or inorganic (ammonium sulphate or urea) nitrogen sources resulted in significant reductions in odour and sulphide concentrations.

7. Odour concentrations from windrow composts were higher than from aerated tunnel composts using similar composting materials.

8. Nitrogen sources which produced similar mushroom yields to poultry manure in flask composting equipment were cocoa meal + calcium hydroxide and molasses waste (including ammonium chloride).

8. A pulsed fluorescence analyser was found to be sensitive to sulphides in composting odours at 10 ppb. There was a good correlation between the instrument readings and odour concentration.

9. The sensitivity of the analyser enabled it to detect odour plumes at the boundary sites, about 50 m from the Phase I composting stacks.

10. For measurements of odour and sulphide (hydrogen sulphide and dimethyl sulphide) concentrations of compost yards in Year 3, there was good agreement with the odour/ sulphide relationship which was previously found.

11. Odour concentrations on two windrow composting sites (A and I) were lower than in samples in previous years. The reductions were probably due to more frequent turning of the stacks and lower compost moisture content, reducing the amount of anaerobic compost.

12. Synthetic pre-wet and Phase I odours were prepared from sulphides, ammonia and other odour compounds which closely simulated real composting odours when presented to an odour panel. This shows that the most important odour compounds have been identified.

13. Most of the odour concentration of synthetic pre-wet odours could be attributed to the sulphide (hydrogen sulphide, dimethyl sulphide and methanethiol) content, although ammonia contributed strongly to the odour concentration of synthetic Phase I mixtures.

14. The type of olfactometer used in measuring odour concentration should be specified, since different types of machines can give different values.

15. Ten bacterial isolates were obtained from mushroom compost which were able to remove odorous sulphur containing compounds from compost air. The bacterial isolates belong to the following species: *Pseudomonas putida*, *Pseudomonas fluorescens*, *Bacillus cereus/thuringiensis* and *Hyphommicrobium* spp.

References

Burton CH, Sneath RW, Misselbrook TH & Pain BF 1998 The effect of farm scale aerobic treatment of piggery slurry on odour concentration, intensity and offensiveness. *J. agric. Engng. Res.* **71**, 203 - 211.

Burton KS, Noble R (1993) The influence of flush number, bruising and storage temperature on mushroom quality. *Post harvest Biology and Technology* **3**, 39 – 47.

Noble R, Gaze R H 1994 Controlled environment composting for mushroom cultivation: substrates based on wheat and barley straw and deep litter poultry manure. *J agric Sci, Camb* **123** 71-79.

Noble R, Gaze RH (1998) Composting in aerated tunnels for mushroom cultivation: influences of process temperature and substrate formulation on compost bulk density and productivity. *Acta Horticulturae* **469**, 417 – 426.

Noble R, Fermor T R, Evered C E, Atkey P T 1997 Bench-scale preparation of mushroom substrates in controlled environments. *Compost Sci Utiliz* **5**(3) 32-43.