BIOREDUCTION OF FALLEN STOCK

An evaluation of in-vessel bioreduction for containment of sheep prior to disposal

Final report

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ABSTRACT

The Animal By-Products Regulation (EC No. 1774/2002) that forbids the burial of fallen stock has caused widespread concern across the livestock industry on both economic and environmental grounds. Consequently, more biosecure and economically viable alternatives for dealing with fallen stock need to be developed and validated for use by the livestock sector. The European Commission (EC) may allow novel alternative methods to be permitted as a means of treating fallen stock after consultation of the European Food Safety Authority (EFSA) and provision of robust scientific data. Bioreduction has been proposed as one possible mechanism of storing (and disposing of) fallen stock. However, to date there has been insufficient scientific evaluation and reporting of the disposal system to enable its formal evaluation.

Through joint funding from Hybu Cig Cymru and the Welsh Assembly Government, bioreduction was evaluated under controlled, replicated conditions; and under conditions which simulated those typical of 'on-farm'. The trial was run over twelve months which included two lambing periods and was based at Bangor University's research farm. The trial found negligible numbers of human pathogens in the waste generated from bioreduction. In addition, no pathogens were detected in gaseous emissions from the system, and generation of non-CO_2 greenhouse gases and odorous gases was intermittent. It was found that the bioreduction system could satisfactorily cope with the volume of carcasses normally associated with a sheep flock numbering 1600, so that none had to be disposed of via any other option. The system was relatively costly to install, however, running costs thereafter were relatively low. On a weight basis, cost of waste disposal was considerably less than costs of disposing fallen stock via the conventional method. Our findings indicate that in-vessel bioreduction could potentially offer livestock farmers a sustainable, practical, cost-effective, and biosecure method of containing fallen stock prior to disposal by an approved collector. Further work is probably needed to improve our understanding on some aspects of the system prior to making any formal application to the EC for its legislative approval.

Contents

1 INTRODUCTION

1.1 Current options for disposing of fallen stock

Traditionally, most fallen stock were disposed of by burial in soil. Recent scientific evidence, however, has suggested that this practice does not eliminate the risk of BSE/TSE exposure to both animals and humans. Even after burial, scrapie-infected material can persist in the soil for years and can present a source of infection (Quiquampoix et al., 2008). Improper burial can also cause pollution of ground- and surface-waters and act as a vector for the transmission of disease to man, animals, birds, and insects. Consequently, the Animal By-Products Regulation (EC No. 1774/2002) (ABPR) was implemented on the 1st of July, 2003 (amended September, 2005). In Wales, implementation occurred on the 31st of October, 2003 (amended May, 2006).This regulation does not provide for the burial of fallen stock and requires disposal via three options:

1. Collection and heat-treatment (incineration or rendering) via an approved company;
2. A knackers' yard or hunt kennel; or
3. Small-scale (usually on-farm) incineration in an approved incinerator.

In outbreaks of major epizootic diseases, burial can be implemented but it is under strict Competent Authority control.

The new regulations have caused widespread concern within the livestock industry. In particular, the requirement to dispose of fallen stock off-farm imposes a financial burden, especially on sheep farmers for whom it may account for almost two thirds of farm net margin (Bansback, 2006). Currently, the cost of disposal per sheep carcass via option 1 (above) varies considerably (*ca.* £12-36) throughout the UK, depending on location (Bansback, 2006). However, disposal costs are likely to increase universally in the wake of increasing fuel prices and when the UK government financial contribution (currently at 10%) finishes in November, 2008. Other concerns have been raised on environmental grounds with regards to the collecting scheme. In particular, the movement of people and vehicles between livestock enterprises may have implications for biosecurity through epizootic dispersion (Sánchez et al., 2008). Concerns have also been raised with regards to how farmers should store fallen stock prior to disposal, especially when carcasses cannot be collected immediately (e.g. during peak lambing time when collectors may be stretched). Carcasses need to be stored away from livestock and be secure from vermin (e.g. in 'dolav' boxes); however, not all farmers have such areas or facilities. Nevertheless, there is an overriding requirement to dispose of fallen stock "without undue delay" (Anon 2002).

Only a limited number of farmers have the option of disposal to a local knackers' yard or hunt kennel, and the long term viability of such establishments is somewhat unclear (e.g. due to the Hunting Act, 2004). Whilst some farmers have collaboratively bought small-scale incinerators for disposal of fallen stock, the adoption of this idea appears to be hampered by the initial capital outlay, the

requirement of suitable sites (e.g. away from livestock holdings and housing), the cost entailed with the requirement to dispose of the buy-product (ash) at an approved landfill site, and the energy required to run an incinerator.

Consequently, more environmentally acceptable, biosecure and economically viable alternatives for dealing with fallen stock need to be developed and validated for use by the livestock sector. The EC may allow for novel alternative methods to be permitted as a means of disposing (or as part of the disposal process of) fallen stock after consultation of the European Food Safety Authority (EFSA) and provision of robust scientific data. To permit the introduction of an alternative disposal method requires that satisfactory information is provided to the EC on the following five criteria:

1. The identification and characterisation of the risk materials to be disposed of;
2. The TSE risk reduction by the particular process;
3. The degree of risk containment;
4. The identification of interdependent processes; and
5. The intended end-use of the product.

1.2 Bioreduction

For the purpose of this report, bioreduction is defined as:

"*the aerobic degradation of animal by-products* (sheep, unless otherwise stated) *in a partially sealed vessel, where the contents are heated and aerated*".

Bioreduction has been proposed as one possible mechanism of storing (and disposing of) fallen stock prior to disposal. In this context, disposal means removal from the livestock holding (i.e. farm) to an approved premises. However, to date there has been insufficient scientific evaluation and reporting of the system to enable its formal evaluation (European Commission, 2003; Stanford and Sexton, 2006). Indeed, relatively little scientific literature is available directly on the aerobic liquid-based degradation of animal carcasses; however, some studies have reported success in aerobic treatment of different wastes, including abattoir waste (Johns, 1995; Fransen et al., 1998; Mittal, 2006).

Bioreduction should not be mistaken for biodigestion or composting. The former tends to be an anaerobic process and typically associated with a 'soak away' from the biodigester unit to the soil environment, such as a septic tank system. Composting involves the regular mixing of carcasses with other feedstuffs (e.g. straw, woodchip) in a relatively dry aerobic environment. The physical integrity is maintained within a bioreduction system, with an air-vent being the only opening to the atmosphere. The contents of a vessel used for bioreduction still have to be eventually disposed of following the normal procedure for Category 1 material in accordance to the ABPR (i.e. via incineration or rendering). However, if

bioreduction is successful, the volume of waste and hence its associated disposal cost should be considerably reduced.

1.3 Project history

This project was jointly funded by Hybu Cig Cymru (HCC) and the Welsh Assembly Government (WAG). Prior to the onset of the project, a visit was made to Portugal to see operating bioreduction systems used for non-ruminant species. Specifically, the first site visit was to a university that utilised bioreduction for rabbits, rats, and mice that had been used for experimental purposes. The second site visit was to a pig farm, which evidently utilised bioreduction for pigs and piglets. Another site visit was planned to a local council that used bioreduction for containment of road-kill, but wasn't possible due to time constraints.

Following the visit to Portugal, two bioreduction vessels were imported from the Barcelona-based manufacturer, Resmat, to Henfaes Research Station[1]. Each was of 6500 litre capacity, measuring approximately 2.5 m in diameter, and 3.0 m length. They were constructed of two types of high-density fibreglass: a Class C with 70 g m^{-2} density on the inner side of the vessel, and a M4 binder of density 600 g m^{-2} on the outside. Vat construction is a hand layup process, in which the different layers composing the lining are applied over the mould, hand impregnating the fibreglass with resin.

In previous trials, where a different form of bioreduction was utilised for pigs and rabbits, the loading capacity of such vessels was found to be approximately 2600 kg per year (Lobera et al., 2007ab).

Following arrival at Henfaes, the vessels were placed in a thick visqueen protective layer and placed on a bed of sand in the ground in a suitable location. Finally, the area was fenced off in order to restrict unauthorised access to people and animals (Appendix 1).

1.4 Project aims

The aim of the project was:

"*to evaluate the efficacy and environmental compliance of in-vessel bioreduction as an on-farm containment system for fallen stock from sheep farms prior to disposal*".

[1] Henfaes Research Station is owned by Bangor University and is where the University farm is based. It comprises 46 ha of lowland, 203 ha of upland grazing and 1750 ha of common grazing rights.

The main criteria for assessing the success of the system were:

a) that it provides a secure method of on-farm containment for fallen stock prior to disposal via approved contractors i.e. it is an effective containment (rather than a dissipative) system; and
b) that it affords a degree of treatment to animal by-products without increasing any biological or chemical risk i.e. it reduces pathogen loads, does not expel large volumes of harmful or odorous gases, and that the final product is intrinsically non-hazardous, thus suitable for biosecure removal and treatment/disposal.

This report collates the information gathered over the trial period.

2 METHODS

2.1 Trial management

The bioreduction vessels were managed as according to the manufacturer's instructions (Manvigna, 2006). At the onset of the trial, 2800 litres of water was placed into each of the vessels, so that they were just less than half filled. The water was heated to 40 ± 2 °C by an oil-filled heating element running the length of the vessels. The internal temperature was maintained by a thermostat that regulated the degree of heating required. Air was automatically pumped to the base of the vessels and sparged at a pressure of approximately 0.5 bars, for 45 min $hour^{-1}$. An electricity meter recorded the amount of power (kWh) required per day to run the vessels.

The water was left to reach 40 °C (which took 5 days) before carcass addition. The numbers and weights of carcasses were recorded throughout the trial period. A small incision was made to the abdomen of each adult sheep just prior to placing it in the vessel. For every kg of carcass, 1 g of the commercial 'Ingestor Product' was added to the corresponding vessel. This is a product derived from seaweed (*Ascophyllum nodosum*) and is meant to facilitate bioreduction by serving as a substrate and a nutrient source for microbes as well as accelerating autolysis of cells in the fluid phase (Gutiérrez et al., 2003). The water level was maintained so that ⅔ of each carcass was submerged throughout the trial period.

Both vessels were managed under contrasting regimes. Specifically:

- The first vessel (**V1**) was managed under controlled conditions. All fallen stock placed in V1 were generated by humanely culling barren ewes from a neighbouring farm, on-site. To this vessel, 300 ± 5 kg of material was inputted on a single occasion. It was then left for a period of three months (the length of one experimental 'run'), and the vessel emptied. The vessel was left empty for approximately six weeks, and then the process was repeated twice over. This provided a triplicate dataset for the V1 trial.

- The second vessel (**V2**) was managed as it would on-farm, i.e. fallen stock were inputted as and when they occurred from the farm's flock of 1600 sheep. This amounted to 2816 kg of carcasses over the twelve month trial period (89 adult sheep and 11 bags (25 kg -sized feed bags) of lambs). During peak usage (lambing time at the onset of the trial), 1053 kg of carcasses were placed into V2 in one month, with relatively sporadic additions thereafter. The aim was to assess whether the number of fallen stock generated at the farm would exceed the system's loading capacity, and how often the vessel would require emptying.

2.2 Scientific appraisal of bioreduction

See Appendix 3 for a detailed explanation of materials and methods used for scientific analyses.

2.2.1 Trial validation

Prior to starting, trial management and scientific procedures were validated via external peer-reviewing by a leading scientist, Prof. Ken Killham of the University of Aberdeen and every aspect was deemed satisfactory (Appendix 7.2).

2.2.2 Liquor sampling

Triplicate liquor samples were collected from the top (< 10 cm depth) and bottom (base) of both vessels approximately every three weeks for the duration of the trial. The origin of each liquor sample is indicated by letters: A = top layer of V1; B = bottom layer of V1; C = top layer of V2; D = bottom layer of V2.

2.2.3 Gas sampling

Gaseous emissions were analysed from both vessels on corresponding sampling dates as for liquors. Samples were taken at the opening of the vessels (i.e. approximately 30 cm away from the carcasses), within the chimneys, and 5 m downwind of the vessels.

2.2.4 Sample analysis

2.2.4.1 Liquor

2.2.4.1.1 Physicochemical analysis

Samples were subject to a range of analyses for physicochemical characterisation. Analyses performed included:

- percentage solids;
- pH;
- electrical conductivity;

- nutrient analysis,
    - ammonium,
    - nitrate,
    - phosphate;
- cations,
    - sodium,
    - potassium,
    - calcium; and
- O_2 concentrations were measured monthly.

2.2.4.1.2 Microbiological analysis

Samples were tested for a range of bacteria, including:
- *Salmonellae* spp.;
- *Campylobacter* spp.;
- *E. coli* O157;
- *E. coli*;
- Coliforms;
- Enterobacteriacae; and
- total viable bacterial counts (TVC).

2.2.4.2 Gaseous emissions

2.2.4.2.1 Concentration of gases

Gases were analysed (M40 Multi-gas monitor; Industrial Scientific UK, Turweston, England) for the following:
- oxygen (O_2);
- carbon dioxide (CO_2);
- carbon monoxide (CO);
- ammonia (NH_3);
- methane (CH_4); and
- hydrogen sulphide (H_2S).

The degree of odour was also continuously logged by staff based at Henfaes Research Station and by those staff visiting the site.

2.2.4.2.2 Bioaerosols

Microbiological analyses on gaseous emissions from the vessels were performed as for liquors, with the exception that Enterobacteriacae and TVC. These analyses could not be completed due to the specific culturing methods required (Appendix 3.1.3). Another agar (R2A agar; Oxoid, Ltd., Basingstoke, UK) was trialled as a way of estimating overall bacteria numbers, but proved to be unsuccessful as are many culturing techniques with bioaerosols (Amann et al., 1995; Wang et al., 2001).

2.2.5 Statistical analysis

Data were analysed by a multi-factorial analysis of variance (ANOVA) and *t*-tests functions accordingly within Genstat 8.1 (Rothamsted Experimental Station, Hertfordshire, UK). Significant differences between treatments were identified using Fisher's Least Significant Difference (LSD) test within the same software.

2.3 Practical appraisal of bioreduction

2.3.1 Rate of carcass breakdown

The weight and number of all carcasses placed inside the vessels was recorded by the operators in a spreadsheet, together with the date of addition. The volume of waste sent for disposal was also recorded. Together, this allowed the rate of carcass breakdown to be assessed over time. Internal photographs of V1 were regularly taken over the course of the trial as a form of visual logging of the rate of carcass breakdown.

2.3.2 Issues encountered

A log was kept of any issues that arose during the trial period in terms of the vessels or their operation.

2.4 Economic appraisal of bioreduction

The costs of buying, importing, and installing the bioreduction vessels were recorded (labour and all materials). The volume of water inputted was recorded individually for both vessels throughout the trial period. A meter (AEM 31 Elcomponent) was fitted to record the energy requirement of the system (kWh). The number and type (sheep or lamb) of each carcass was recorded prior to placement within the vessel to calculate comparative costs of disposal via the conventional system. Each carcass was weighed prior to entry into the vessels, so the exact amount of the Ingestor Product to be added (and associated costs) was known. Finally, the volume of waste to be incinerated was recorded to calculate disposal costs.

3 RESULTS

3.1 Scientific appraisal of bioreduction

3.1.1 Liquor

3.1.1.1 Physicochemical characteristics

A summary of the chemical and microbiological characteristics of the wastes from both vessels is presented in Table 1. The characteristics of the liquor collected differed considerably. In particular, samples from V2 were notably thicker than those from V1, especially towards latter stages of the trial by which time a considerable volume of carcasses had been inputted. Samples taken from V1 turned increasingly darker with time; whilst in V2, samples remained a green colour throughout due to frequent input of carcasses. Within each vessel, samples from the upper layer of the vessels were typically of thicker consistency and greater viscosity due to the presence of rumen content and partially-dissolved wool. This was particularly clear in V2, where percentage solids in the liquor were statistically greater in the upper layer relative to the bottom layer (*t*-test, $P < 0.001$, $n = 3$ for each sampling date). The increasing viscosity slightly impeded sample collection towards latter stages of the trial, although this was overcome using sampling bottles of greater diameter.

Liquor temperature remained relatively consistent throughout the trial period. There were no significant differences in temperatures taken from different locations within the vessels, or between the readings taken immediately after collection and that value on the instrument panel ($P > 0.05$). Liquor pH was slightly–moderately basic throughout the trial period (7.94–9.69; mean ± standard error of mean (SEM): 8.68 ± 0.03), and tended to be highest approximately two weeks into an experimental run in V1. There were no significant differences between pH values in V1 and V2. Conversely, electrical conductivity values differed significantly between both vessels (means mS cm^{-1} ± SEM: V1, 9.8 ± 0.1; V2, 24.0 ± 3.1; $P < 0.001$). Specifically, whereas values in V1 remained relatively stable throughout (9.2–11.1 mS cm^{-1}), a considerable range of values were observed in V2 (6.8–46.9 mS cm^{-1}), with very large increases observed after approximately 200 days.

Generally, high levels of nutrients were recovered from the wastes, although there were some differences depending on sample origin (i.e. V1 or V2; top or bottom layers). Although the C-to-N ratios were similar throughout, significantly greater levels of carbon compounds were present in V2 ($P < 0.001$). Most of the nitrogenous compounds were in the form of ammonium in all samples. Overall levels of both ammonium ($P < 0.001$) and nitrate ($P < 0.05$) were significantly greater in samples from V2.

Phosphate levels were greatest in liquor from V2, significantly so in the bottom layers ($P < 0.05$). Notably high levels of cations were present in the waste,

especially potassium. Levels were significantly higher in samples taken from the bottom layers of V2 for each cation ($P < 0.001$) and tended to increase with time. This was also reflected in electrical conductivity values.

Finally, dissolved oxygen levels were low on all sampling dates; and as expected were greatest at the top layers for both vessels. Overall values were significantly greater in V1 ($P < 0.05$).

Table 1. Physicochemical properties of liquor waste samples from both bioreduction vessels (V1 and V2) over the twelve month trial period. Values represent means ± SEM (n = 3 for each sampling point). Letters indicate origin of liquor sample: A = top layer of V1; B = bottom layer of V1; C = top layer of V2; D = bottom layer of V2. Temperature values are those taken from individual samples immediately after collection. Values for chemical analyses are in mg l^{-1}, unless otherwise stated. NPOC = non-purgeable organic carbon.

	Sample				
Parameter	**A**	**B**	**C**	**D**	**Mean**
Temperature (°C)	40.9 ± 1.0	38.7 ± 0.6	41.2 ± 1.1	38.4 ± 0.7	39.8 ± 0.7
pH	8.68 ± 0.12	8.72 ± 0.10	8.59 ± 0.16	8.71 ± 0.17	8.68 ± 0.03
EC (mS cm^{-1})	9.7 ± 0.2	9.8 ± 0.2	17.9 ± 4.3	30.2 ± 3.8	16.9 ± 4.8
Percentage solids (%)	0.7 ± 0.1	0.5 ± 0.1	8.8 ± 2.3	2.3 ± 0.3	3.1 ± 1.9
Total C	2613 ± 77	2670 ± 84	6188 ± 619	8753 ± 598	5056 ± 1489
Total N	903 ± 13	926 ± 35	1729 ± 259	2746 ± 184	1576 ± 434
NPOC	1996 ± 25	2053 ± 79	5169 ± 850	8130 ± 538	4337 ± 1465
C-to-N ratio	2.9	2.9	3.6	3.2	3.2
NO_3^-	0.14 ± 0.01	0.13 ± 0.00	0.48 ± 0.14	0.18 ± 0.01	0.23 ± 0.08
NH_4^+	2.64 ± 0.14	2.55 ± 0.12	9.46 ± 1.07	9.22 ± 0.98	5.96 ± 1.94
PO_4^{3-}	99.6 ± 8.8	87.4 ± 5.4	137.8 ± 25.3	250.5 ± 28.0	143.8 ± 37.2
Ca	149 ± 27	150 ± 21	277 ± 49	466 ± 43	261 ± 75
K	252 ± 13	261 ± 16	432 ± 63	738 ± 39	421 ± 114
Na	162 ± 11	166 ± 9	265 ± 45	471 ± 28	266 ± 72
Dissolved oxygen	0.73 ± 0.1	0.67 ± 0.27	0.43 ± 0.00	0.33 ± 0.13	0.54 ± 0.09

3.1.1.2 Microbiological characteristics

At each sampling point, analogous liquor samples were sent to a UKAS-accredited, independent laboratory for screening for two or more bacterial types as further validation of our methods and results. All results obtained were statistically equal to ours. The results obtained from our analyses are used throughout this report.

No *Salmonellae* spp. or *Campylobacter* spp. from any sample throughout the trial period. *E. coli* O157 was only recovered once during the whole trial, from samples taken at both depths in V1 on day 48. Even so, these numbers were very low (10 colony forming units (CFU) ml^{-1}) and were only detectable following enrichment. Only low numbers of generic *E. coli* were recovered, and none were detected in samples after day 14 in V1 and after day 28 in V2 (Fig. 1). Similarly, coliform bacteria were only recovered at the first sampling point in V2, and not at all in V1 (Fig. 2).

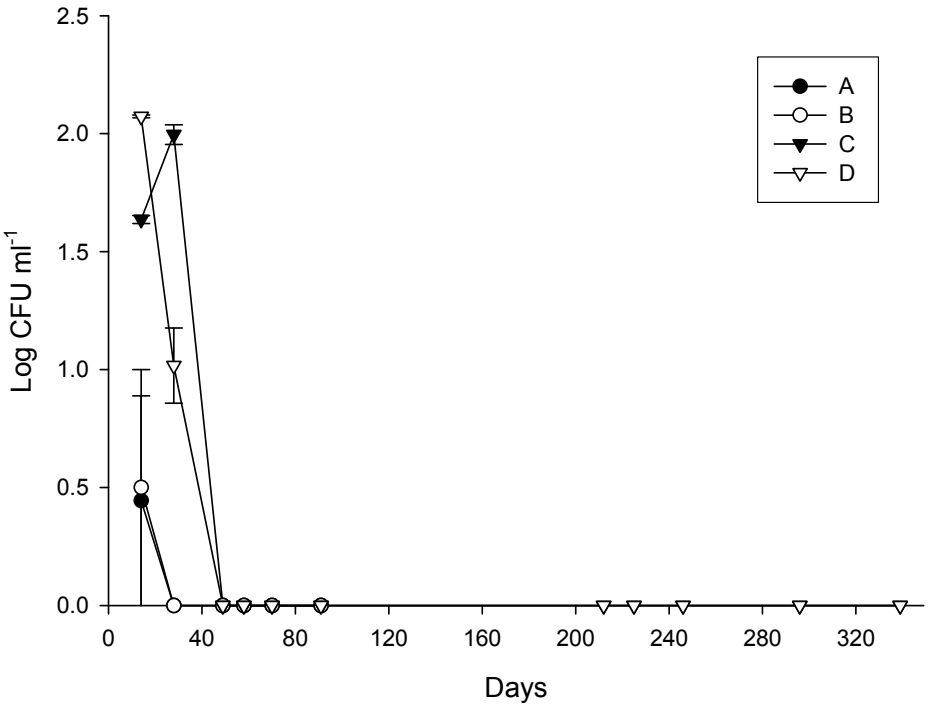

Fig. 1. Numbers of generic *E. coli* recovered from liquor samples from both bioreduction vessels over the trial period.

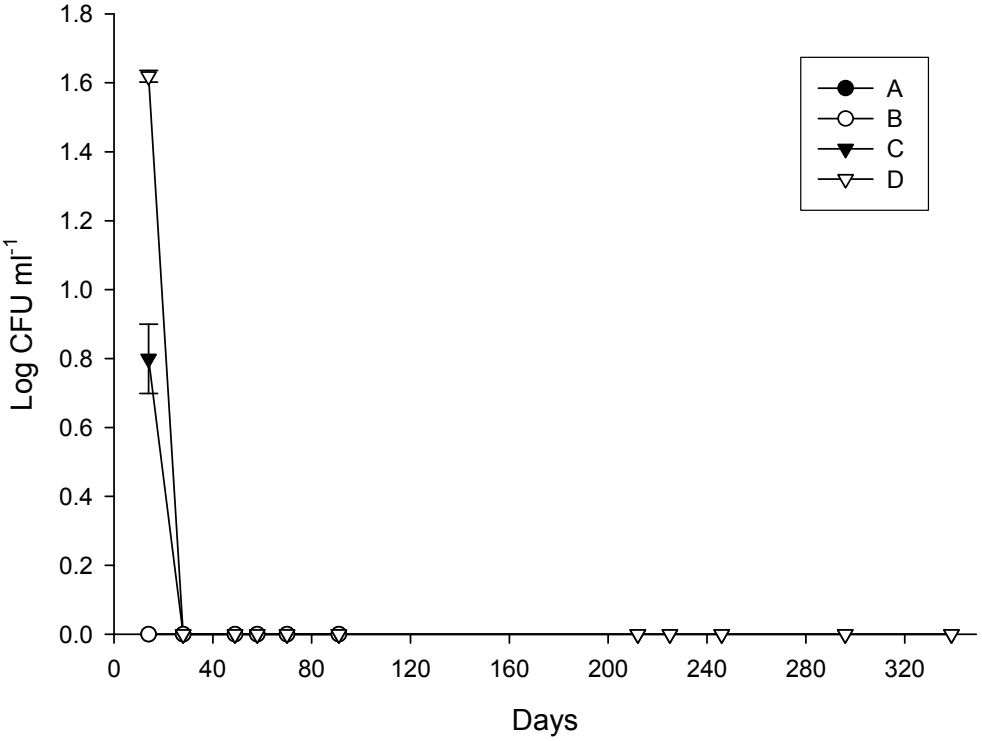

Fig. 2. Numbers of coliform bacteria recovered from liquor samples from both bioreduction vessels over the trial period.

Enterobacteriacae were recovered from both vessels, although there was a general decrease with time in V2 so that none were recovered after 58 days. However, in V1, numbers recovered towards the end of the three month experimental run period after a similar decrease at 58 days (Fig. 3).

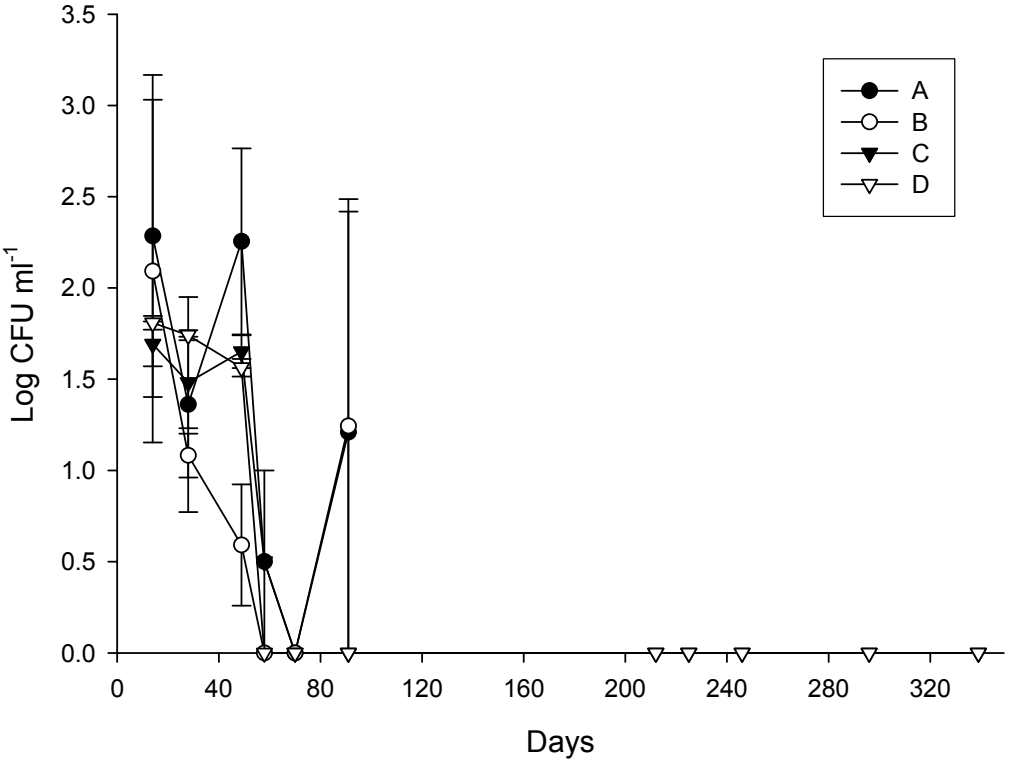

Fig. 3. Numbers of Enterobacteriacae recovered from liquor samples from both bioreduction vessels over the trial period.

After initially high numbers, TVC values decreased in all samples, and stabilised thereafter (Fig. 4). This pattern was particularly noticeable in V2, where numbers decreased by approximately 6 log CFU ml^{-1} values between the first two sampling date and those thereafter. There was a similar decrease in V1, although over a course of approximately seven weeks. Towards the end of each experimental run in V1, numbers started to increase again. Conversely, counts remained relatively stable in both top and lower levels in V2.

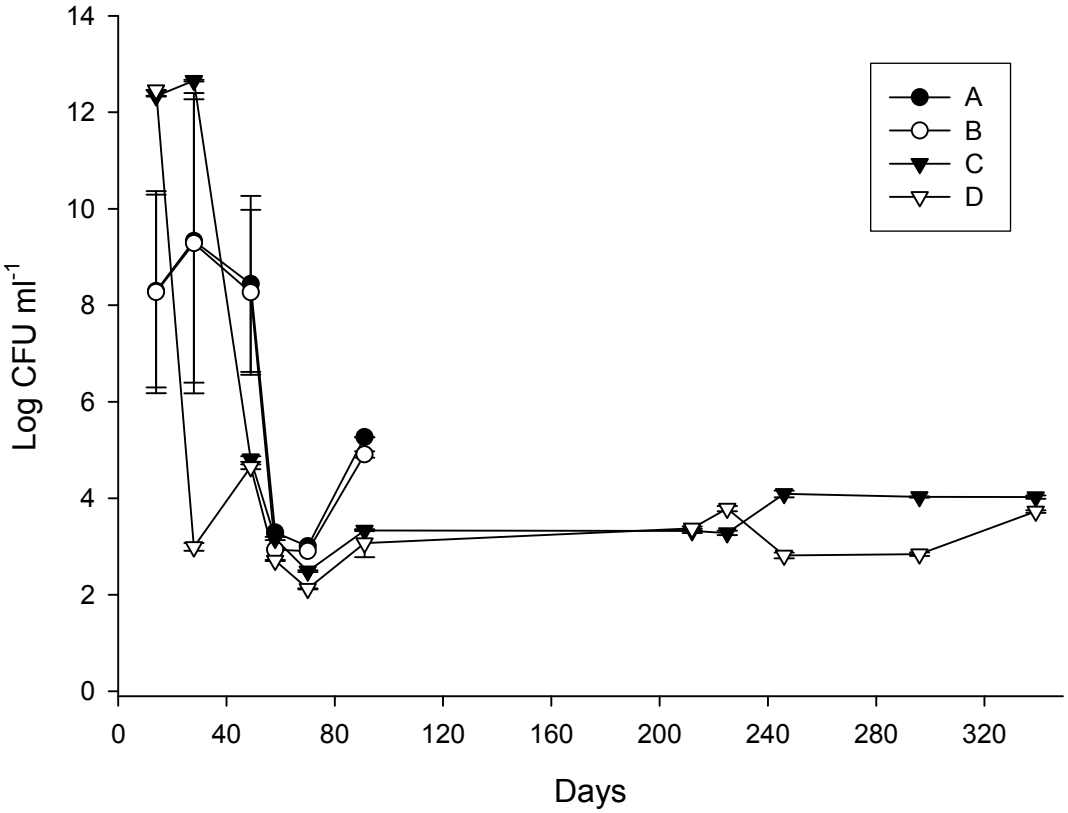

Fig. 4. Total viable counts recovered from liquor samples from both bioreduction vessels over the trial period.

3.1.2 Gaseous emissions

3.1.2.1 Concentration of gases

The omission of odours from the vessels is discussed in Section 3.2.3.3. There were no differences in gaseous composition in comparison to ambient air samples on any occasion at distances of 5 m away from the vessels. No CO was detected at any sampling point throughout the trial period. The greatest increases in CO_2 levels were observed in V2 approximately two months into the trial, when levels increased to 0.10% (from 0.04%) at the vessel opening. On corresponding dates, O_2 levels decreased somewhat (lowest value recorded was 18.8%; mean % ± SEM over trial period, 20.7 ± 0.1). No differences relative to ambient air were detected in CO_2 concentrations within the chimneys on any sampling date. Occasional, sporadic increases were seen in levels of NH_3 and H_2S throughout

the trial period; although again, changes were only detected when the vessel was opened. Elevated concentrations of H_2S were detected at the vessel opening of both V1 and V2 during the first 15 days of the trials (V2 being greatest, although not statistically so), but no differences were detected thereafter. Ammonia levels reached a peak of 23 parts per million (ppm) in V2 on day 91; but no differences in relation to ambient air could be detected on all but three sampling days throughout the trial period. Lastly, increased levels of CH_4 could be detected only twice during the trial period, at the vessel opening during the first week in one experimental run in V1 (0.6%), and on day 8 at the vessel opening in V2. These levels were still well within the percentage lower explosive limit (4.4% for methane).

3.1.2.2 Bioaerosols

No *Salmonellae* spp., *Campylobacter* spp., *E. coli* O157, *E. coli*, or coliforms were recovered from any samples of gaseous emissions throughout the trial period.

3.2 Practical appraisal of bioreduction

3.2.1 Waste disposal

In between experimental runs, V1 was emptied and the waste disposed of as for Category 1 material in accordance to the ABPR. All waste generated during the trial was disposed of via a large waste-processing company. Emptying was done via suction under vacuum to an articulated lorry. Pipes were fitted with a filter to prevent entry of solid material. The whole process took approximately thirty minutes, with the actual removal of contents completed in 5–10 minutes. Waste was then transported for incineration.

V2 was half-emptied near to the second lambing period. Although the top layer was relatively solid and thus not suitable for removal under vacuum, the pipe was placed beneath the viscous layer which allowed suction of the waste of thinner consistency.

3.2.2 Rate of carcass breakdown

For sequential illustrations of carcass breakdown with time in V1, see Appendix 4. In V1, carcasses started to degrade within a matter of days, so that after three weeks, no whole carcasses were evident. At the end of the first three month run when V1 was emptied, only some solid material was present in the form of bones (mainly skulls) at the base of the vessel. However, during the subsequent two experimental runs, some woolly material also remained, suggesting that the rate of breakdown had decreased somewhat.

The rate of carcass breakdown was sufficient in V2 such that no fallen stock had to be disposed of via the conventional system. Over the twelve month trial period, 89 adult sheep and 11 bags (25 kg -sized feed bags) of lambs were placed into V2, equating to 2816 kg of carcasses. After the period of peak usage (where 1053 kg of carcasses were placed into V2 in one month), additions were relatively sporadic thereafter; with the longest period without any input being 69 days. Mean weight of material added each time was 68.7 (± 5.9) kg. As expected, the frequency of carcass addition affected the capacity of the system to accept fallen stock (i.e. rate of carcass addition *vs*. rate of carcass breakdown). Taking this into account, towards the end of the trial period and as the second lambing period approached, half of the waste liquor in V2 was removed as the vessel was approaching its maximum capacity. Up to that point, 2233 kg of carcasses had been inputted over 338 days (equivalent to 6.6 kg day^{-1}). Thereafter, 583 kg of carcasses were added over a period of 27 days (the second lambing period).

3.2.3 Issues encountered

3.2.3.1 The lids

The lids were made of plastic which threaded onto a plastic collar. The plastic was of seemingly low quality and lids tended to warp/bend due to heat from sunlight, occasionally resulting in difficulty in opening or closing. The collar was held in place by four pop-rivets, which left a significant gap between the collar and the vessel. This resulted in gas and foam leakage, and offered a passage to flies. It wasn't possible to lock the lids, although a chain was locked across one lid as an improvised safety measure (Appendix 2).

3.2.3.2 Carbon filters

The small size of the grains within the filter meant they became compacted and therefore restricted air-flow. This effect was exacerbated when grains became saturated with condensed water vapour. As a result, air was forced through the space between the lids or between the pipe coupling-joints (Section 3.2.3.5), resulting in odour loss. The filters were subsequently removed due to their ineffectiveness.

3.2.3.3 Odour

For the majority of the time, little or no noxious odour was detectable until within close proximity (i.e. 5-10 m) of the vessels, and this was frequently remarked upon by visitors. However, it occasionally became evident downwind at distances up to approximately 200 m from the vessels. The smell generated was different to that from conventionally rotting carcasses and invariably tended to 'cling' onto the clothes, hair, and skin of individuals. The incidence of smell seemed to vary according to prevailing weather conditions, e.g. smell tended to circulate around the area on days with little or no wind. It was also apparent that smell was

exacerbated when the air pumps were switched on, especially if they had been off for a longer period than usual (e.g. following breakdown). Increasing the chimneys' heights helped alleviate the issue; however, the dysfunctional filtering system and the amount of gases that escaped at ground-level due to an ineffective seal contributed to the problem.

3.2.3.4 Positioning of piping and electrics

The different piping and electrical connections to the vessel were spaced out far from each other (Appendix 2), which caused some problems during installation. There was also a need to be wary when carrying carcasses to (and loading into) the vessels in case the piping got caught in the carcasses' legs or tripped up the operator.

3.2.3.5 Minor issues

(i) There was no 3 Amp switch for the air pump within the main electrical box, which caused it to heat and led to 'tripping' of the electrical system.
(ii) No regulator was supplied for the air pump and it had to be ordered afterwards after issues with foaming.
(iii) The diameter of the chimney pipe was 125 mm, whereas 110 mm piping is used in the UK. Rubber 'couplings' thus had to be bought to connect the piping, adding costs and meant an ineffective seal was formed; resulting in gas dissipation and smell.
(iv) During initial stages of the trial (when the large volume of water was heated to 40 °C), the piping encasing the heater element became very hot (> 135 °C). Such heating led to oil spillage, generation of smoke, the paint peeling away, and could present a hazard. This stopped after approximately ten days and seemingly caused no mechanical problems.
(v) The length of piping used as a chimney protruded approximately 50 cm on the inside of the vessels. This reduced their capacity by some degree as fewer carcasses could be inputted so as not to block the chimney, even though there was sufficient space within the vessel.
(vi) The oil level within the piping encasing the heater element needed to be maintained at a certain point to ensure effective heating without damage to the element or tripping of the electrical supply. However, the required level was not marked on the interior or exterior of the casing therefore it was difficult to judge whether the level was correct.

3.3 Economic appraisal of bioreduction

By managing V2 as it would be 'on-farm', this project has enabled us to formulate a comparison of bioreduction costs relative to the conventional system for disposal of fallen stock. Costs were calculated for V2 over the twelve month trial period and compared to the costs incurred had the fallen stock generated (89

sheep and 11 bags of lambs (2816 kg)) been disposed via the conventional National Fallen Stock Scheme (Table 2). Omitting the set-up costs, the saving made on animal disposal costs (i.e. the difference between bioreduction and the conventional system) was £1361.75 over the twelve month trial period.

Table 2. Cost of fallen stock (89 sheep, 11 bags of lamb) disposal via bioreduction relative to those incurred via the conventional system over the twelve month trial period, excluding set-up cost. Asterisk denotes cost incurred for both vessels (see below); NFSS = National Fallen Stock Scheme. See Appendix 5 for a fuller cost breakdown.

	Bioreduction		**Conventional system**	
Cost type	**Item**	**Cost (£)**	**Item**	**Cost (£)**
Set-up	1 vessel	3293.83	–	
	Importing from Spain*	1292.50	–	
	Installation, electrical connection, fencing (materials & labour)*	3561.09	–	
Running	Water	4.16	–	
	Electricity	100.15	–	
	Ingestor Product supplement	46.46	–	
Waste disposal	Liquor disposal	225.00	Carcass disposal	1586.75
Other	–		Annual NFSS membership fee	18.00
	Total	**8523.19**		**1604.75**
	Disposal cost per kg of fallen stock (excluding set-up costs)	**0.08**		**0.57**

Whilst some costs have been calculated 'per vessel' (materials, water, electricity, and supplement), others have had to be quoted as for two vessels. For instance, it is not possible to ascertain import and fencing costs for one vessel, although they would evidently be less than for two vessels. The actual costs incurred per vessel will therefore be somewhat less than those stated in Table 2.

Given the annual difference in disposal costs between the conventional and bioreduction systems, a simple economic analysis would indicate that bioreduction would be more cost-effective than the conventional disposal scheme if the vessels had a lifetime of seven years or more (Appendix 5).

4 DISCUSSION

4.1 Scientific appraisal

4.1.1 Liquor analysis

Physicochemical analysis showed that the liquor waste was a moderately basic, nutrient-rich matrix, similar in composition to sheep manure (Williams et al., 2006; 2008ab). Nevertheless, there were some notable differences in liquor characteristics, depending on their origin i.e. V1 or V2. In particular, samples from V2 contained significantly greater levels of nutrients such as ammonium and phosphate, and all cations. These results were expected due to the regular input of carcasses and the release of nutrients and cations during subsequent breakdown (e.g. ammonium from faecal material; calcium from bones; sodium from blood). The C-to-N ratios of samples were much lower than for manure (Williams et al., 2006; 2008ab), which reflect the relatively nitrogen-rich status of the wastes. Much of the nitrogenous compounds were in the form of ammonium, and it is expected that a great deal was generated through ammonification. This is the microbial conversion of organic nitrogen (e.g. from the breakdown of protein material/ muscle) into ammonium (Switzenbaum et al., 1994).

There are many different systems designed for aerobic treatment of waste, all designed to lessen the impact of waste via reducing volumes and/or altering its properties. Numerous studies report how aerobic digestion affects the physicochemical and biological properties of different waste types and the benefits compared to anaerobic digestion. For instance, mesophilic aerobic digestion has been proved to hasten the degradation and attenuation of a range of chemical compounds potentially harmful to man or the environment; including polycyclic aromatic hydrocarbons (Zheng et al., 2007), phytotoxic compounds (McNamara et al., 2008), a range of dioxins (Disse et al., 1995; Field and Sierra-Alvarez, 2008) and endocrine disruptors (Hernandez-Raquet et al., 2007). In a protein-rich environment such as a bioreduction vessel, the known ability of aerobic processes to degrade protein, fats and other carbohydrates (Arvanitoyannis and Ladas, 2008) is particularly noteworthy. Aerobic treatment of

waste is also proven to reduce concentrations of pathogenic bacteria (Skjelhaugen and Donantoni, 1998; Borowski and Szopa, 2007; Arvanitoyannis and Ladas, 2008; Beline et al., 2008) and viruses (Scheuerman et al., 1991). Aerobic digestion of other waste types such as sewage sludge and abattoir wastes has been shown to reduce Chemical and Biological Oxygen Demand and enhance degradation of organic pollutants (Arvanitoyannis and Ladas, 2008). It is also proven to reduce the concentration of sulphurous compounds and associated smell in sewage sludge (Dewil et al., 2008). Lastly, aerobic treatment of piggery waste was found to reduce concentration of nitrogen and phosphorus compounds (Beline et al., 2008; Zupancic and Ros, 2008).

The amount of Ingestor Product was added according to the manufacturer's instructions (Manvigna, 2006). Resmat have manufactured more than one form of Ingestor Product, with some in liquid form and others in powder form such as that used during this trial. Furthermore, some of these products have contained microbes, others not. The version that we trialled did not contain microbes, and one of the main constituents of this product is sodium alginate, which is composed of soluble fibres, proteins, and both poly and oligosaccharides (Gutiérrez et al., 2003). The product is meant to facilitate bioreduction by serving as a substrate and a nutrient source which activates and aids proliferation of microbial flora in aerobic or anaerobic conditions. It is also meant to help accelerate autolysis in the fluid phase (Gutiérrez et al., 2003). However, it is unclear to what extent the rate of bioreduction is dependent upon the addition of the Ingestor Product, especially given that such small quantities are added to the vessels (1 g per kg of carcass) and that the intrinsic bacterial population is so high. Rather, it is expected that the primary drivers of bioreduction will be microbial processes and specific enzymatic catalysts, in addition to cell autolysis and hydrolysis. Specifically, it may be proposed that the rate of bioreduction is largely a function of complex interaction of competition and inhibition between microbial populations, as well as grazing by other micro-organisms such as protozoa. In addition, it is claimed that the alginic acid within the Ingestor Product decreases smell by reducing the concentrations of emitted ammonia and sulphide gases (Gutiérrez et al., 2003). Whilst only very low amounts of these gases were detected during the trial period, this was probably more due to regular air input which decreased prevalence of anaerobic bacteria than the addition of the Ingestor Product. Many products containing both bacteria and enzymes are commercially available that claim to accelerate processes that are microbial-driven, such as composting and waste degradation. It appears that their effectiveness is highly dependent on both the properties of the raw material and the micro-organisms and enzymes applied (Vargas-Garcia et al., 2006; Gruda et al., 2008). Further trials are planned to elucidate the effects of the Ingestor Product supplement on bioreduction (Section 5.1) and to develop an improved product to facilitate bacterial and enzymatic breakdown (Section 5.3).

Clear changes were witnessed in physicochemical properties of the waste over time. This will have led to changes and fluctuations in microbial populations and

possible variation in microbial community composition. However, such changes are not expected to be uniform between all bioreduction vessels as microbial populations may also be affected by a range of other factors. For instance, the rate of carcass input may affect microbial community composition, or the gut microflora of carcasses will alter if animals have recently been administered antihelmintic or antibiotic drugs.

Maintaining a highly-nutritious waste medium such as the liquor within the vessels at a mesophilic temperature is likely to support high bacterial loads. Rumen content from carcasses will act as a significant source of bacteria (Williams et al., 2007a) which are expected to be fundamental drivers in the initial stages of bioreduction (Gutiérrez et al., 2003; Sánchez et al., 2008). Overall bacteria numbers (TVC) over the course of the trial were similar to those found in previous work with abattoir wastes (Avery et al., 2005; Williams et al., 2007a; 2008ab). However, many of the groups of micro-organisms expected in such waste were not recovered at all (*Salmonellae* spp. and *Campylobacter* spp.,), or were only prevalent during initial stages of the trial (*E. coli* O157, *E. coli*, coliforms, and Enterobacteriacae). These findings are in accordance with Gutiérrez et al. (2003) who found no coliforms within waste generated in their bioreduction system. The lack or relatively sudden reduction of such microbes may be explained by a number of factors, as mentioned previously. Firstly, pathogens may be out-competed by other bacteria within the bioreduction system. They are also likely to be subject to intense predation by other micro-organisms such as protozoa, which have been shown to severely impact pathogen numbers in a range of matrices (Chabaud et al., 2006; Bjornlund and Ronn, 2008; Thelaus et al., 2008). A significant proportion of pathogenic micro-organisms are also likely to be eliminated by the predominance of saprophytic flora responsible for microbial decomposition of organic tissues (Sánchez et al., 2008). The slightly alkaline conditions, coupled with high conductivity values in some samples (e.g. bottom of V2) may also affect pathogen prevalence by disrupting cell membranes. Lastly, the bacteria listed derive from the gut of ruminants, which is a highly anoxic environment, and many are classified as facultative anaerobes or microaerophilic organisms (Davis et al., 1973; Singleton and Sainsbury, 1985). Even though dissolved oxygen levels were low within the waste samples, the regular input of air into the bioreduction system may have been sufficient to reduce or eliminate such organisms from the wastes.

Culturing techniques were used throughout the trial period in order to process a sufficient number of samples within the allocated time and resources. It is known that stressed or starved bacterial cells may enter into a viable but non-cultureable (VBNC) state and hence will not be detected using plating methods. However, it is unlikely that cells become starved in such a warm, nutrient-rich environment as a bioreduction vessel. Furthermore, the plating techniques, broths and agars utilised were specific for each bacterial type.

4.1.2 Gaseous analysis

Traditionally, most studies on bioaerosols have utilised culture methods (Huang et al., 2002). Bioaerosols appear to be particularly prone to non-cultivability because of stresses related to collection methods (Wang et al., 2001). However, media that were selective for each micro-organism were used in the study. For future work, incorporating the use of molecular tools (Angenent et al., 2005; Maron et al., 2005) as supplementary methods to culturing may be of benefit.

It appears that different micro-organisms have varying propensities for aerosolisation. Moletta et al. (2007, 2008) found that aerosolisation is not only a random phenomenon and some micro-organisms seem to develop an "active" strategy linked to their metabolism to avoid or to take advantage of the gas vector. Although some work has studied the aerosolisation behaviour of different micro-organisms, the ability of pathogenic bacteria such as those analysed during this trial to enter an aerosolised state is not clear. However, human and animal infections have occurred through bioaerosol formation of pathogenic organisms, including *E. coli* O157 (Varma et al., 2003; Cornick and VuKhac, 2008), *Salmonellae* spp. (Proux et al., 2001; Oliveira et al. 2006) and *Campylobacter* spp. (Posch et al., 2006). It was anticipated that a bioreduction system could generate potentially harmful bioaerosols due to regular air input into waste that may harbour pathogens. Furthermore, survival of air-borne bacteria is often enhanced in humid environments such as that contained within a bioreduction vessel. However, no pathogens were isolated at any instance in gaseous emissions from within the vessel, or downwind. Clearly, it was unlikely that they would be prevalent within gaseous emissions as only a limited number of such micro-organisms were recovered in the waste samples. However, further work is needed on the emissions of bioaerosols, particularly with regards to *Legionella* spp. (e.g. *Legionella pneumophila*), which tend to prevail in warm, dirty water (Eckmanns et al., 2006; Hyland et al., 2008).

Only relatively small and sporadic changes were seen in the concentration of gases compared to ambient air; and all changes were detected at the vessel opening within approximately 30 cm of carcasses. In effect, bioreduction caused no large expulsion of combustible or harmful gases. Air was actively pumped into both vessels throughout the trial period. This may explain why only small differences were recorded between ambient air compared to studies that had previously investigated gaseous emissions from decomposing carcasses under anaerobic conditions (e.g. Gutiérrez et al., 2003; Sánchez et al., 2008). For instance, production of methane occurs through the degradation of lipids, carbohydrates, organic acids and proteins in anaerobic conditions (Husted 1994), whilst hydrogen sulphide is produced from the bacterial breakdown of sulphates in organic matter in the absence of oxygen (Smet et al., 1999; Muyzer and Stams, 2008). It has also been shown that the prevalence of ammonium-oxidizing bacteria is impeded in aerobic waste systems (Parravicini et al., 2008)

and production of greenhouse gases is also reduced in aerobic waste-treatment systems (Beline et al., 2008). Nevertheless, the low levels of dissolved oxygen within the waste indicate periods of anaerobic or partially anaerobic environments. The availability of oxygen may vary in different locations within the vessel, and also on whether the rate of uptake by microbes exceeds that of supply (from the pump and that dissolving from air). Biological oxygen demand will vary according to the rate of carcass addition as addition of new material increases bacteria numbers and respective oxygen demand. This explains why H_2S levels were greatest in the opening of V2 rather than V1. Furthermore, periods of anoxia or limited oxygen and corresponding production of H_2S (rather than SO_2) may also explain the occasional smell generated from the system (Smet et al., 1999). It has been shown that small changes to waste composition or characteristics may lead to considerable alterations in microbial populations and respective gaseous emissions under anaerobic digestion (Chen et al., 2008; Ward et al., 2008). It is inevitable that changes in waste composition would induce such an effect in a bioreduction system. Furthermore, small changes in temperature may affect the amount and composition of gaseous emissions in a waste-degrading environment due to alteration of the microbial population (Chae et al., 2008). Small changes in temperature inevitably occur in a bioreduction system, e.g. at times of carcass or water addition. These factors help to explain the discrepancy in gaseous emissions at different sampling points in the current trial.

4.2 Practical appraisal

4.2.1 Site requirements

Installation of the bioreduction system was unproblematic. However, we established that the system should not be sited too close to households due to the possibility of undesirable odours. Other aspects to consider prior to installation include: (i) accessibility for the operator to use the system and for the contractor during waste disposal; (ii) whether the area is liable to flooding or if groundwater level is higher than the depth needed to install the vessel; (iii) whether the site is within close proximity to a water source and an electricity supply; and (iii) whether the ground is particularly stony hence may damage the vessel during installation. While not all farm holdings would have suitable locations for installing a bioreduction system, a considerable number would have a site(s) which met the required criteria.

4.2.2 Rate of carcass breakdown

As in the current study, aerobic degradation has been shown to considerably reduce waste volumes (Mittal, 2006; Arvanitoyannis and Ladas, 2008; Ichinari et al., 2008). The rate of carcass breakdown was notable when vessels were managed under both controlled (V1) and on-farm (V2) conditions.

The rate of breakdown was easier to visually evaluate in V1 in comparison to V2 due to there being only one input event per experimental run. During the first experimental run, the rate of carcass breakdown in V1 was notable, with only limited solid material remaining at the end (Appendix 4). However, greater amounts remained at the end of the two subsequent experimental runs. As the final weight of solid material couldn't be ascertained, it is not possible to clearly ascertain whether this was due to a reduced rate of breakdown or due to the build-up of solids from the previous experimental run (Section 4.4). However, it was evident that the remaining solid material restricted air flow from the pump into the vessel through blocking the air dispensing holes. This may have affected the environmental conditions (oxygen status) within and hence the rate of breakdown; particularly if the rate of breakdown is primarily driven by aerobic bacteria. Although the system is meant to be aerobic, dissolved oxygen levels measured within the waste were low on each occasion (Table 1) and were comparable to other waste types, such as landfill leachate (Smith et al. 2008). However, oxygen levels are expected to be lower in warm liquid and where biological activity is high, such as the liquor waste within a bioreduction vessel. It is unknown whether the limiting factor with regards to carcass breakdown is the availability of oxygen and whether this explains the seemingly reduced rate witnessed in V1 during latter stages of the trial. Although the effect of waste treatment in aerobic systems has been shown to be dependent on aeration intensity and duration (Bohdziewicz and Sroka, 2005), high rates of carcass breakdown were seen in bioreduction systems with no air input by Gutiérrez et al. (2003). Nevertheless, recent work with meat industry wastes showed that waste degradation rate was greater under aerobic, rather than anaerobic, treatment (Buendia et al., 2008).

All the fallen stock generated from the farm's flock of 1600 sheep during the trial period was placed in V2. The rate of carcass breakdown was sufficient so that no fallen stock had to be disposed of via any other method during this time, although the vessel was half-emptied towards the latter stages. Overall during the twelve months, the number of deaths was 5.5% of total ewe numbers, which is slightly higher than typical sheep mortality rates (HCC, 2004). Typical losses at lambing time may be approximately 10% of total lambs born (dependent on the weather, disease, etc.) and approximately 3% of ewes (HCC, 2004). Sheep mortality is therefore condensed around lambing time and is relatively low thereafter. The trial included a period of low input into the system in between two lambing periods of high input, as it would on-farm.

Due to the irregular nature of carcass addition into V2, it is only possible to estimate the system's maximum capacity in terms of number/weight of animals over a unit time. Up to the point that V2 was half-emptied, 2233 kg of carcasses had been inputted over 338 days. This equates to a loading capacity 6.6 kg day^{-1} or 2409 kg $year^{-1}$ before the vessel had to be emptied. This value is similar to that quoted by Lobera et al. (2007ab) for bioreduction of pigs and rabbits and is

of note as it was expected that the rate of breakdown of sheep would be considerably less due to the presence of wool and relatively large bones. The sheep inputted into V2 were Welsh Mountain sheep weighing a mean of 30.1 kg. With sheep of this size, it can therefore be estimated that the maximum capacity of a bioreduction vessel is 80 sheep per year. Based on an annual mortality of 5% (HCC, 2004), this means that one bioreduction vessel could meet the needs of a farm with a sheep flock of 1600 before it needs emptying:
Total weight of carcasses: 2400 kg
Mean weight of one carcass: 30 kg

The system can therefore accommodate 80 sheep per year; equal to 5% of 1600. Nevertheless, it is unclear whether this rate of carcass breakdown would be sustained into the next twelve months and it appeared to have reduced towards the latter stages of the trial due to the build-up of solid material. To maintain the rate of carcass breakdown, it is therefore likely the vessel would need to be emptied on an annual basis.

Identifying the microbial populations that govern bioreduction is part of a forthcoming project (Section 5.3) which will indicate whether the key microbial drivers are indeed oxygen-limited. If so, alterations to vessel design could be made to enhance oxygen availability and therefore improve the rate of bioreduction. This may have particular relevance to conditions within on-farm vessels (V2 in this trial) where carcasses are regularly inputted. Firstly, the carcasses within such vessels may act as physical barriers at the liquor-air interface hence impede on the degree of oxygen dissolving, and secondly due to elevated biological oxygen demand owing to high bacteria numbers. The importance of air input to the effectiveness of bioreduction will also be explored in future trials (Section 5.1).

The temperature readings from the thermostat displayed within the instrument panel and those taken from samples were statistically similar throughout the trial period (Table 1). It is expected that maintaining the temperature within the vessels at a similar or slightly elevated point to that within a living sheep will aid proliferation of intestinal microbial populations and thus the associated breakdown of carcasses. Chen and Huang (2006) found that lowering the temperature from 55 °C to 35 °C enhanced the stability and performance of anaerobic digestion of poultry mortalities. Further trials are planned to investigate the effectiveness and environmental compliance of bioreduction under periods of no heat input (Section 5.1).

4.2.3 Managing a bioreduction system

As we had no previous experience of bioreduction, the system was evaluated purely on experiences gathered over the twelve month trial period. Whilst this will not identify any long-term problems, it will at least indicate any immediate problems that need resolving.

Some issues arose during the trial period which required technical involvement (e.g. the breakdown of the heater element which causes the electrical supply to trip). However, the system generally required minimal maintenance and was easily managed (Sections 3.2.4). Although specific expertise is required to install such a system (namely the electrical work), utilisation thereafter is inherently simple and requires no specific skills and only limited time input from the operator. This is one of the strengths of bioreduction relative to other waste management options. As discussed previously, disposal of the generated waste was also unproblematic and did not require specialised apparatus from the contractor.

4.2.4 Improving the bioreduction system

The (patented) vessels are well-designed and customised for their purpose. For example, the digestion unit is a single piece construction; all apertures are in the top of the vessel and each aperture is designed to provide access for specific purposes; the heater, thermostat and aerator are all sealed at their point of entry to the vessel; the heating unit is of a type not commonly found; and there is 'after-moulding' fabrication within the vessel to protect the heating element and to prevent the aerator from being clogged. They are easy to handle due to their low weight, yet are durable and seemingly long-lasting. That they are constructed from fibreglass also permits easy repair should damage have occurred prior to installation. Other design features that are not so immediately evident include the design of the aeration unit to give even distribution of air and the external control unit for heating and aeration (Wood, 2007). Furthermore, it is a proven system with full technical/installation backup via David Muir. Above all else, the system is relatively simple and requires only low-level maintenance, which is an important element if they are ever to be commercially available.

However, a number of features could (and should) be altered in future designs to rectify some problems encountered with the vessels and to further improve their efficacy.

Aerobic treatments can be very effective in reducing the concentration of odorous compounds (Skjelhaugen and Donantoni, 1998; Mittal, 2006; Arvanitoyannis and Ladas, 2008). However, given the nature of bioreduction, some exposure to smell is inevitable; especially to those in close contact with the contents of the vessels (e.g. during carcass addition). Smell was relatively unproblematic in the main, being significantly less pungent than several conventional 'farm smells' such as slurry spreading. Nevertheless, effort must be made to limit generation of smells as it may reduce the acceptability of such systems. There is an array of methods to consider that may reduce generation of undesirable odours from bioreduction. First and foremost, a bioreduction system should be sited away from housing or where a congregation of people work, yet be accessible to those that use it, and with vehicle access to allow the waste contractor to remove contents. Choosing a

suitable location which also allows a high-level (> 4 m) exhaust to be placed may be adequate to negate the need for further measures. As air is actively pumped into the system, this should reduce the occurrence of methanogens and the associated odorous gases that they produce. However, the low levels of dissolved oxygen in the liquor waste indicate that the availability of oxygen may actually be limited. This may formulate anaerobic conditions and associated smell. Increasing the rate and/or length of time of air input may therefore alleviate the problem. It may also facilitate the rate of carcass breakdown, as described previously.

Further measures or technologies could be adopted. UV treatment may be effective in treating the malodour, as well as having the benefit of sterilising the gaseous emissions. UV only requires a small residence time and works under warm, humid conditions (i.e. conditions within a bioreducer) and also has low maintenance and running costs (although bulbs can be expensive). Ozone treatment could also be effective, although it is likely to be financially unviable.

One potential method would be to use granular activated carbon (GAC). GAC is an effective and common technology applied in odour (and pollution) control. It works by physisorption, although the GAC could also be dosed with appropriate chemicals to enable simultaneous chemisoption. The GAC could be tailored to suit the malodour to be treated e.g. by altering the pore size of the carbon granules to “fit” the malodorous molecules. High humidity and warm gas streams can reduce effectiveness, although water vapour could be removed through condensing via some form of cooling jacket. In many circumstances, the GAC can be tailored to cope in high humidity conditions. GAC and its associated technologies trap the malodourants and the carbon would then be recycled back to the manufacturer to be thermally treated to reactivate it and remove (and destroy) the odour molecules. Residence treatment times are low to moderate, and the system requires only low-level (but regular) maintenance, depending on the rate of organic loading.

Liquid scrubbing techniques are often used as gaseous treatments. These can be as simple as water scrubbing which can effectively remove ammonia-type odours and some sulphurous-type odours; as well as many other organic odours that are reasonably soluble in water (short carbon chain acids, alcohols, aldehydes etc.). The addition of small amounts of caustic soda can aid the scrubbing of acidic odours such as fatty acids. The addition of oxidising agents such as sodium hypochlorite (as hydrogen peroxide) can also vastly improve odour removal, particularly those caused by sulphurous-type odours. However, addition of such chemicals may affect the chemistry of the liquor within the vessel and hence the biological activity. This may impede the rate of bioreduction.

Other approaches that may warrant further investigation include organic treatment of odours via passage through peat beds (bioreactors), masking of odours (e.g. with flower or pine odours), and neutralising sprays (which differ

from masking in that they react with the pollutant odour to form a new compound with less or no odour).

The lids on the current vessels are one of the greatest issues of concern and contribute to the problem of smell. Furthermore, they are inadequate in terms of safety if the vessels are to be considered for the commercial market. Such problems may be alleviated with lids that resemble that of a traditional 'pickling jar'. This would permit secure and effective locking with a padlock and would form an effective air-tight (rubber) seal. Using piping of 110 mm diameter would also improve gas transfer.

The filter used in the vessels should be radically improved. Firstly, the grains should be larger in size so as not to restrict air movement. The system could improve by changing the actual type/composition of the filters (e.g. to GAC or Activated Carbon Fibres/Filters). Incorporating a pump into the chimney system to actively draw or drive air towards (and through) the filter system may also be considered. Also, as mentioned above, incorporating some form of cooling jacket into the chimney to condense water vapour would avoid saturation of the filter, and the condensed water could be fed directly back into the vessel.

To satisfy Health and Safety requirements, the section of piping that encases the heater element should be sealed or covered within a protective layer and/or warn the operator that it may be hot. It should also be explained in the operators' manual that expulsion of oil and smoke is expected during the initial heating stages. All external piping and electrical connections (apart for the thermostat) should be placed closer together to one side of the vessel so as to reduce the likelihood of damage and to facilitate installation. Other aspects highlighted in the Results section (e.g. provision of a 3 Amp switch and air regulators; reduction of inward pipe length) could be easily rectified. A gauge (dipstick) that indicated the maximum and minimum level of oil required within the piping encasing the heater element could be easily incorporated into the design. Another worthwhile yet simple inclusion would be a clear 'maximum' line on the inside of the vessels to show the operator when to stop inputting carcasses and consider emptying the vessel.

Finally, it is recommended that the bioreduction vessels are placed in a visqueen layer, as in the current trial. This acts as a secondary skin in case of damage and should further enhance the vessels' lifetime as it forms a barrier between the soil and vessel interface.

The manufacturers have assured us that many of the proposed suggestions have been incorporated into the new design of vessel.

4.3 Economic appraisal

In order for bioreduction to appeal to the livestock industry, it must prove to be an economically viable alternative to the conventional fallen stock disposal scheme. Our calculations were based on the number of sheep at Henfaes and the rate that would be paid to the local fallen stock collector, in addition to the rate paid for electricity and water. As explained previously (Section 3.3), it was not possible to work out the exact costs per vessel due to the fact that two vessels were imported and installed for this trial. It is therefore important to note that set-up and installation costs would thus be less than the values quoted here.

As highlighted previously, there is considerable variation in the cost of carcass disposal, depending on location. There will also be a difference depending on whether the farmer is a member of the NFSS. For instance, the cost of disposing fallen stock generated during this trial period via the conventional system would equate to £2006.50 (rather than £1586.75) to non-members. The liquid fraction of the waste generated during this trial was incinerated at a cost of £90 tonne^{-1}. This price may have been reduced as the waste disposal company were *en route* to collect from another nearby site, hence saved on transport costs. Waste or carcass disposal charges in other areas may therefore differ from those paid as part of this study.

Some costs (e.g. water, electricity, supplement, waste disposal) will vary depending on the amount of carcasses added, hence will differ according to farm/flock size. There could also be considerable variation in costs incurred for materials, labour, electricity, and water. Figures for maintenance or depreciation of the vessels have not been included due to the trial having lasted only twelve months. However, it is expected that these will be low due to the relatively low-tech nature of the vessels and the durability of materials used. Finally, the lifetime of the vessels is not known, although again it is envisaged to be at least twenty years given the durability of materials used.

It is clear that the greatest cost of the bioreduction system lies is the initial capital outlay, with relatively low running costs thereafter (Table 2 and Appendix 5). Wood (2007) concluded that there are a significant number of companies in the UK that are manufacturing similar vessels for use in disposal of other wastes (e.g. sewage). However at present, no companies had existing systems to cope with carcasses or provide controlled heat sources. Nevertheless, such companies were interested in manufacturing bioreduction vessels should there be a positive change in the regulatory framework. At present, such companies couldn't establish the final cost as this would depend on sales volumes and the economies of scale. However, demand for larger quantities may make rotomoulding a more economic, efficient and effective production method than the 'hand-laying' process used to build the vessels at present (Gutiérrez et al., 2003; Wood, 2007). Were the system to be legalised, David Muir has registered a UK company to distribute the bioreducers (Biosecurity Systems Ltd.) and plans

to investigate the economics of production under licence in the UK. Evidently, this would significantly reduce transport costs and would also facilitate obtaining supplies of components for repairs should breakdown occur.

It is expected that the cost of carcass disposal via the conventional method will increase due to escalating transport costs and the energy-intensive nature of rendering or incineration. Likewise, it is inevitable that the costs of electricity and hence running the bioreduction system will increase. Nevertheless, on a weight basis and without taking into account the set-up costs and depreciation of the vessel, it is likely that carcass disposal costs will remain considerably cheaper via bioreduction (8 p kg^{-1} waste) compared to the conventional system (57 p kg^{-1} waste; Table 2). Given the foreseeable cost increase via the conventional system as previously discussed, the difference in waste disposal costs in terms of weight may further augment with time.

Based on figures for all costs derived from the current study and the number of fallen stock generated during the twelve month trial period, it can be predicted that the bioreduction system would have a pay-back period of approximately 7 years, should there be no breakdown or other unforeseen costs (Appendix 5).

From our economic appraisal and discussions with farmers' groups, it appears that bioreduction may offer real financial benefits in terms of reducing costs of animal disposal. However, it is evident that it would not be within the financial scope of smaller sheep farms. Nevertheless, a bioreduction system may be accessible if it were to be bought and managed cooperatively by a farmers group. Although this may have regulatory implications in terms of the transport of fallen stock, many on-farm incinerators have been cooperatively purchased. With effective management, it is envisaged that a similar format could be workable for bioreduction.

Whilst the figures obtained during this trial cannot be directly extrapolated to all sheep farms, they do allow us to gain a significant insight into the financial implications of bioreduction. These findings indicate that bioreduction may offer the livestock industry a financially viable and appealing alternative to the current method for disposing of fallen stock.

4.4. Limitations of the study

None of the carcasses placed inside the vessels were firstly screened for the presence of bacterial pathogens; therefore we cannot clearly state that bioreduction eliminated pathogens. Nevertheless, it is known that sheep sporadically harbour a range of bacterial pathogens. For instance, *E. coli* O157 may be prevalent within 40% of flocks (Ogden et al., 2005), and *Salmonellae* spp. and *Campylobacter* spp. have been isolated in 12% and 44% of sheep faeces, respectively (Oloya et al., 2007; Milnes et al., 2008). Concentrations of

such bacteria may reach up to 10^6 CFU g^{-1} faeces (Stanley and Jones, 2003; Ogden et al., 2005). Given the number of carcasses that were placed inside the vessels, it is therefore likely that some did harbour such pathogens, even though our results were negative. The fate of pathogens during bioreduction is to be explored in detail in forthcoming work (Section 5.5).

In previous studies looking at bioreduction of pigs, six months was required for complete breakdown of carcasses (Lössel et al., 2007). In this study, carcasses were left to degrade for 3 months per experimental run in V1, and the vessel was subsequently emptied prior to the next run. However, only the liquid fraction of waste could be removed via suction therefore any solid material remained within the vessel as it would have required manual removal. As a result, there was some accumulation of solids with time, especially at the end of the final run. This may have affected nutrient levels and bacteria numbers recovered within the waste during subsequent sampling. However, a clear window of approximately six weeks was left in between each experimental run so as to limit bacterial carry-over. Furthermore, no or negligible numbers of pathogens were found in samples throughout the trial period returned (Section 3.1.1.2), which confirm that bacterial carry-over didn't occur between runs.

The percentage solids figures represent the liquefied fragment of waste as the presence of large solid material (e.g. bones, wool, and carcasses) prohibited quantifying the 'total' waste within the vessel. Coupled with carry-over of solid waste from one experimental run to the other, this means it is not possible to say exactly what percentage of initial carcass weight remained at the end of each 3 month (i.e. the efficiency of carcass breakdown).

Lastly, the electricity meter was not fitted from the onset of the trial, but after approximately five months. Economic conclusions drawn in terms of energy requirement have therefore been extrapolated over the whole trial period from daily figures obtained following installation of the meter. Nevertheless, power requirement remained relatively consistent throughout the trial period and therefore the figures presented should provide an accurate estimation of running costs.

5 FUTURE DEVELOPMENTS

Preliminary trials indicate that this method of bioreduction offers a practical, cost-effective, and secure method for storing and reducing volumes of carcasses. However, to ensure compliance, further work is necessary to fully assess environmental risk and industry acceptability. As a result, further work is already planned or in progress.

5.1 Trial II (funded by WAG (£100K and HCC (£20K))

Through further funding from WAG and HCC, the next stage of the trial has been developed and is due to commence in October '08. The work will purposefully seek to address questions raised during the first trial. This work falls into two main areas:

A. **Scientific trials of bioreduction**: The validity of bioreduction will be assessed under a wider range of environmental conditions, specifically: (i) without the addition of the Ingestor Product, and (ii) under periods of simulated system breakdown where no air and/or heat input may occur. This will help to achieve greater understanding of bioreduction and evaluate its effectiveness under such conditions.
B. **Stakeholder evaluation of bioreduction**: As part of an on-going dialogue, a formal evaluation will be made of key stakeholders' perceptions of bioreduction. This will involve semi-structured questionnaires with farmers, environmental and government regulators, industry representatives etc. It will help pre-empt any practical, environmental or legislative issues before submission of the scientific case to the EC and also help improve the technology at an early stage.

5.2 Assessing the risks associated with transmissible spongiform encephalopathies (TSEs) in fallen stock containment systems (details not finalised)

The work proposed is the first step towards gaining an in-depth knowledge of TSE behaviour in containment systems. To achieve this, the first step will be a critical desk-based review of the existing literature on TSE and containment technology. This review will be undertaken by a scientific team that includes relevant expertise on TSE behaviour in the environment. The output from the project will include an assessment of the likely risk of TSE proliferation and infection pathways in two containment systems, namely bioreducers and freezers. Secondly, a knowledge gap analysis will identify further areas for experimentally-based research for key pieces of underpinning information which are missing. This information will be encompassed in a final report and project stakeholder meeting with WAG and associated parties (e.g. HCC, DEFRA, and Animal Health).

5.3 PhD studentship (funded by BPEX)

Although the findings of Trial I have demonstrated the considerable rate of carcass degradation within a bioreduction system, there remain many specific questions regarding the microbial and enzymatic processes which drive the process. Further in-depth information on these aspects of bioreduction is required and could aid both in the formal application to the EC for its legislative approval and in optimising the rate of carcass breakdown. The work entailed within this PhD studentship falls into two main areas:

A. **Characterisation of key microbial divers**: To assess microbial community development in the bioreducers and the potential for managing/optimising these through inoculation, etc.
B. **Enzyme profiling**: Molecular and physiological characterisation of the enzymatic processes in the bioreducers and the potential for managing/optimisation.

5.4 Life-Cycle Assessment of bioreduction (funded by NFSCo and others)

A life-cycle assessment (LCA) is a systems analysis tool that provides information on the environmental effects of a product from its cradle (acquisition of raw materials) to its grave (waste management). It gathers information on all the inputs and outputs to and from a product system, and assesses the potential environmental impacts associated with these inputs and outputs (e.g. global warming potential, eutrophication potential, acidification potential). All direct, on-site emissions as well as indirect emissions incurred off-site (e.g. during the manufacture of inputs to the production system) are included in the calculation of an LCA. An LCA of bioreduction and other methods of storing and/or disposing of fallen stock (e.g. on-farm freezing, conventional collection and incineration or rendering) will indicate the respective impact of each method on the environment. This project will deduce if bioreduction offers any environmental benefit over other storage or disposal routes for fallen stock.

5.5 Survival and activity of pathogens within bioreduction

A laboratory-based analysis of the survival of a range of pathogens within a simulated bioreduction system will be performed. Samples of waste from the vessels will be inoculated and subsequent pathogen survival assessed thereafter. Furthermore, the activity (bioluminescence) of key pathogen species (*E. coli* O157 and *Pseudomonas* spp.) will be monitored within the system using segregationally stable, reporter constructs of both bacteria where reporter gene expression provides a measure of metabolic activity (Williams et al., 2007b). The findings from this work will provide critical information for comparative risk assessment purposes of bioreduction.

6 CONCLUSIONS

The concept of bioreduction is relatively simple, being based on containing fallen stock in a vessel which facilitates the microbial breakdown of carcasses in a biosecure environment. This reduces the volume of carcasses and hence the need for frequent disposal. This was the first trial to investigate the use of bioreduction as a containment method for dead sheep. The trial was managed so as to validate bioreduction under 'controlled' and 'on-farm' conditions.

Installation of the bioreduction system was relatively straightforward. However, the trial showed that the system should not be sited too close to households due to the possibility of undesirable odours. Other aspects to consider prior to installation include accessibility for the operator and for waste disposal by a suitable tanker, whether the area is liable to flooding, or whether it is particularly stony. Although some minor issues arose during the trial period, the system required minimal maintenance.

The volume of both the liquid and solid waste considerably reduced with time. The 'on-farm' vessel was half-emptied only once and was capable of handling all fallen stock generated on a farm with 1600 ewes over a twelve month period that included two lambing cycles. Two major advantages were that fallen stock could be immediately removed and placed in the vessel rather than having to store them whilst awaiting collection via the conventional system, and the vessel could be emptied by a waste disposal company at a convenient time for the operator. Using the bioreduction system was straightforward and required only minimal guidance at the onset.

The vessels used in this trial were specifically constructed for bioreduction. Although inherently well-designed, a number of alterations could further improve their efficacy. In particular, the issue of smell could be alleviated by adopting one or more of a range of options, e.g. altering the filtering system. Other issues could easily be rectified and it is expected that many of the suggestions have already been implemented by the manufacturer.

The trial involved monitoring basic chemical and microbiological properties of both the gaseous emissions and the liquor within the vessels. It was found that no harmful gases or pathogens were generated or dissipated during bioreduction.

In terms of Welsh agriculture, the system was evaluated on a farm with a relatively large flock of sheep. Based on current and projected animal disposal costs, our economical appraisal implies that bioreduction may offer significant financial savings to sheep farmers after approximately 7 years. However, the system would offer further financial gains and appeal to smaller farms should the initial outlay be reduced. Cost reductions are expected were the vessels would

be commercially-produced by a UK-based manufacturer or if such a system could be purchased and managed cooperatively between farmers' groups.

Whilst the findings of this study contribute greatly to our understanding of bioreduction, further in-depth information on some aspects of the system is required prior to making any formal application to the EC for its legislative approval. Much of this information will be acquired by a range of forthcoming projects. From this current trial, it can be concluded that bioreduction could potentially offer livestock farmers a practical, cost-effective, and biosecure method of containing fallen stock prior to disposal by an approved collector.

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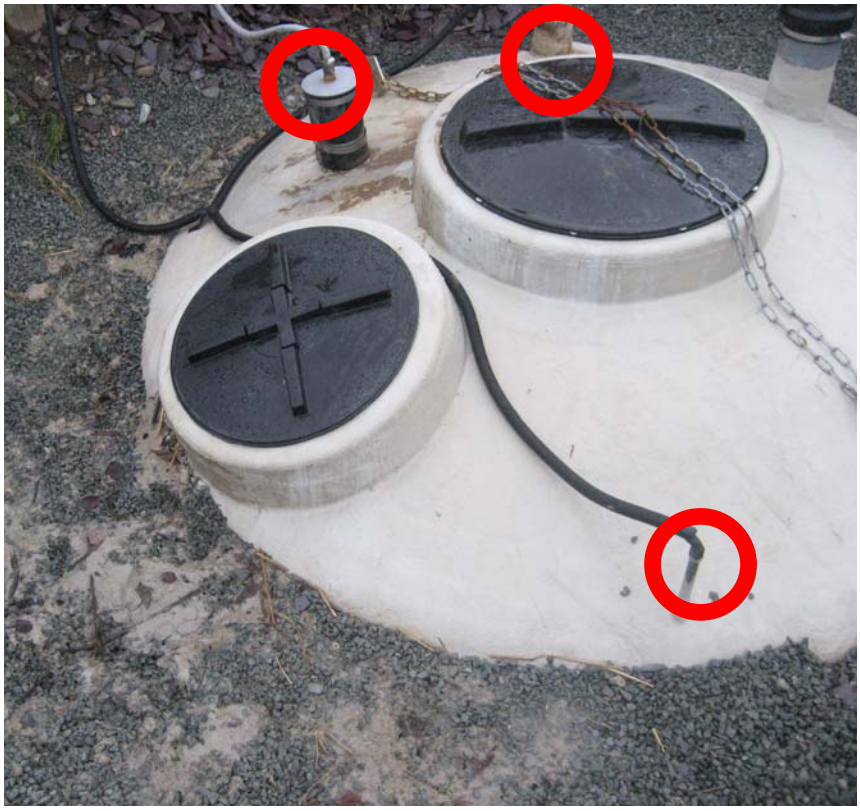
APPENDICES

APPENDIX 1. Location of both bioreduction vessels.

APPENDIX 2. Positioning of vessel piping and electrics (circled), and the chain placed across one lid as a safety measure.



APPENDIX 3. Methods employed

App. 3.1 Liquor analysis

App. 3.1.1 Sampling technique

At each sampling point, triplicate samples were collected in sterile, 500 ml polypropylene bottles. For collecting samples from the upper layer of the liquor, bottles were lowered into the vessel at the end of a rod, and liquor was collected from around the carcasses. For sampling from the lower depths of the vessels, bottles were lowered at the end of a rod with their caps closed. When the rod had reached the bottom of the vessel, the caps were removed via drawing on a long piece of string that was attached to the caps. This allowed the bottles to fill with liquor from the bottom of the vessel, and then the rod was bought back to the surface. Sample temperatures were measured immediately (Checktemp 1 Thermometer; Hanna Instruments Ltd., Leighton Buzzard, UK) and noted against the temperature reading on the instrument panel. Samples were transported to the laboratory within 30 min and stored at 4 ± 0.1 °C prior to further analysis.

App. 3.1.2 Physicochemical characterisation

All equipment were calibrated and used according to the manufacturer's instructions. Blanks and standards were included in all analyses to ensure accuracy, and all analyses were performed with 3 or more replicates. Dissolved oxygen concentration was measured (HQ10 Portable Oxygen Meter; Hach Lange Ltd., Salford, UK) within ten minutes of sampling. Nutrients were extracted using 1 M KCl (for nitrate and ammonium) or 0.5 M acetic acid (for phosphate and cations) at a 1:5 w/v ratio samples-to-1 M KCl/acetic acid. Samples were extracted by shaking (250 rev min^{-1}, 1 h, room temperature), centrifuging for 10 min (14,000 *g*), filtering (Whatman no. 42), and the supernatant recovered for analysis. NO_3^- and NH_4^+ were determined colorimetrically (Downes, 1978; Mulvaney, 1996) with a Skalar SAN^+ segmented flow analyzer (Skalar Analytical, Breda, The Netherlands). Phosphate was measured colorimetrically (Murphy & Riley, 1962), while K, Na and Ca were measured using a Sherwood Scientific 410 flame photometer (Sherwood Scientific, Cambridge, UK). Electrical conductivity (Jenway 4010 EC meter; Jenway Ltd., Dunmow, UK) and pH (Orion 410A pH meter; Thermo Scientific, Staffordshire, UK) were determined after a 1:1 (v/v) dilution of samples with distilled water. Dissolved organic carbon (DOC) and dissolved nitrogen (DN) were measured using a TC-TNV analyzer (Shimadzu Corp., Kyoto, Japan). Moisture content was determined by drying for 24 h at 105 °C.

App. 3.1.3 Microbiological characterisation

Samples were tested for a range of bacteria: *Salmonellae* spp., *Campylobacter* spp., *E. coli* O157, *E. coli*, coliforms, Enterobacteriacae and total viable counts (TVC).

Salmonellae spp.

A multi-step enrichment technique was utilised to test for the presence of *Salmonellae*. Twenty-five ml of each sample were added to 225 ml of sterile Buffered Peptone Water (Oxoid) in a stomacher bag, and stomached for 60 s to ensure effective homogenisation. The stomacher bag and its contents were subsequently incubated (37 °C, 18 h). For each separate pre-enrichment culture, 0.1 ml was inoculated into 10 ml of Rappaport-Vassiladis broth (RVB) and 1 ml into 10 ml of Muller Kaufmann tetrathionate novobiocin broth (MKTTN). These enrichment cultures were incubated (24 h, 41.5 °C for RVB; 24 h, 37 °C for MKTTN). One loop full was subsequently streaked onto XLD and BGA agar (Oxoid), and incubated at 37 °C for 25 h. Presumptive (circular red colonies with a black centre or translucent pink on XLD; pink/red colonies on BGA) colonies were confirmed as *Salmonellae* spp. by latex agglutination (Oxoid FT0203A); and positive samples were plated out in duplicate onto CT-SMAC agar followed by incubation and latex agglutination, as previously. Positive colonies were then enumerated.

Campylobacter spp.

An enrichment technique was utilised to test for the presence of *Campylobacter*. Twenty-five ml of each sample was placed in 75 g of sterile *Campylobacter* Enrichment Broth (CEB; Oxoid) in a stomacher bag, and stomached for 60 s to ensure effective homogenisation. The stomacher bag and its contents were subsequently incubated (37 °C, 4 h; followed by 42 °C for 44 h). One loopfull of broth was aseptically removed and streaked onto of Modified Charcoal Cefoperazone Desoxycholate agar (CCDA; Oxoid) to obtain isolated colonies. Plates were then incubated (48 h, 42 °C) in a microaerophilic atmosphere. Presumptive (small, grey, translucent) colonies were confirmed as *Campylobacter* spp. by latex agglutination (Oxoid DR0150).

E. coli O157

To test for the presence of *E. coli* O157, an enrichment technique was utilised by placing 5 ml of each sample into 15 ml modified Tryptone Soya Broth (mTSB; Oxoid), and shaking (150 rev min^{-1}, 6 h, 37 °C), before streaking onto sorbitol MacConkey agar plates supplemented with 0.05 mg l^{-1} cefixime and 2.5 mg l^{-1} potassium tellurite (CT-SMAC; Oxoid). Plates were then incubated at 37 °C for 18 h, then examined and scored for presence or absence of colonies with the characteristic appearance of *E. coli* O157:H7. Presumptive (non-sorbitol-fermenting; NSF) colonies were confirmed as *E. coli* O157:H7 by latex agglutination (Oxoid DR620); and positive samples were plated out in duplicate onto CT-SMAC agar followed by incubation and latex agglutination, as previously. Positive colonies were then enumerated.

E. coli and coliforms

Generic *E. coli* and coliform numbers were determined by plating serial dilutions of each sample onto Chromogenic *E. coli*/coliform Selective Agar (Oxoid) in triplicate, and enumerating colonies of characteristic *E. coli* and coliform appearance (purple and pink colonies, respectively) following incubation at 37 °C for 24 h.

Enterobacteriacae

Ten ml of each sample were placed aseptically in stomacher bags containing 90 ml of sterile Maximum Recovery Diluent (MRD; Oxoid), and stomached for 60 s to ensure effective homogenisation. One ml aliquots of serial dilutions were transferred onto sterile Petri dishes and 15 ml of molten Violet Red Bile glucose Agar (VRBG) (maintained at 48 °C) was poured into each plate, and subsequently agitated. After the medium had solidified, it was overlaid with 10 ml of the same medium and left to solidify. After setting, plates were placed in an incubator (18 h, 42 °C) and colonies counted.

Total viable counts (TVC)

Ten ml of each sample were placed aseptically in stomacher bags containing 90 ml of sterile MRD, and stomached for 60 s to ensure effective homogenisation. Serial dilutions were then plated on Petri dishes, and 15 ml of molten Plate Count Agar (Oxoid) maintained at 48 °C was poured into each plate, and subsequently agitated. After setting, plates were placed in an incubator (48 h, 37 °C) and colonies counted.

App. 3.2 Gaseous analysis

Gaseous emissions were analysed every three weeks from both vessels for the duration of the trial. Samples were taken at the opening of the vessels (i.e. near the carcasses), within the chimneys, and 5 m downwind of the vessels. For analysing gases within the chimney, a hole was made and sealed with a rubber bung in between sampling periods. Emissions were analysed for the following gases: CO_2, CO, O_2, H_2S, NH_3, CH_4, water vapour, and volatile organic compounds (VOC) (M40 Multi-gas monitor; Industrial Scientific UK, Turweston, England).

App. 3.2.1 Microbiological characterisation

Samples were tested for the same bacterial populations as above with an Andersen pump (Andersen Air Sampler 2000 INC, Atlanta, Georgia, USA) containing 5 agar plates selective for specific bacterial populations, as described previously. The orientation of the plates within the pump was randomised

between each sampling date. For sampling at the vessel opening, the extractor pipe was placed through the small opening on the main lid. For sampling within the chimneys, the extractor pipe was placed through the purpose-made hole on the horizontal section of the chimneys. For the sampling 5 m downwind, the pump was held at a height of approximately 1.5 m. The pump was switched on for a period of 2 min (approximate flow rate of 10 lire min^{-1}) at each sampling point, plates were removed and stored, and the process repeated twice again (*n* = 3). Plates were subsequently transported to the lab and incubated as described previously.

APPENDIX 4. Sequential carcass breakdown within V1.

Day 0.

Day 9.

Day 23.

Day 93 (pre-emtying).

Day 93 (post emptying).

APPENDIX 5. Breakdown of costs.

Running and waste disposal costs incurred during trial period for bioreduction system (based on the latest rates paid for utilities, Ingestor Product supplement, and for that paid for waste disposal during the trial):

- Water
  Total usage: 3.2 m^3
  @ £1.30 /m^3
  = £4.16

- Electricity
  Total usage: 1460 kWh
  @ £0.0686 /kWh
  = £100.15

- Ingestor Product supplement
  Total usage: 2.816 kg
  @ £16.50 /kg
  = £46.46

- Waste disposal
  Total weight disposed of: 2500 kg
  @ £90 /tonne
  = £225.00

Costs for disposal of fallen stock that would've been incurred during trial period via conventional route (based upon rates of the collection company that would normally collect from Henfaes Research Station):

NFSS member:
- Membership cost = £18
- 89 sheep @ £16.50 /head = £1468.50
- 11 bags of lambs @ £10.75 /bag = £118.25
Total cost = £1586.75

Non-member of NFSS:
- 89 sheep @ £21.00 /head = £1869.00
- 11 bags of lambs @ £12.50 /bag = £137.50
Total cost = £2006.50

(Note: All prices are subject to additional VAT costs. Minimum charge of £12.50 applies. Lambs may also be charged at £2.50 /head whether NFSS member or not. "Lamb" is defined as a sheep 0-2 months of age).

Calculation of time taken for bioreduction system to reimburse itself was based on figures derived from the current study and the number of fallen stock generated during the twelve month trial period.

Respective costs of both systems:

Bioreduction:
Running costs: £150.77
Waste disposal: £225
Total per year: £375.77
Total running and waste disposal costs over 7 years: £2630.39
Initial set-up costs: £8147.42
TOTAL OVER 7 YEARS: £10,777.81

Conventional system (NFSS members):
Carcass disposal: £1586.75
NFSS annual membership: £18
Total per year = £1604.75
TOTAL OVER 7 YEARS: £11,233.25

(For reasons previously discussed in depth (Sections 3.3 and 4.3), these figures cannot be extrapolated to all scenarios and are estimates of costs).

APPENDIX 6. Stakeholder opinion of bioreduction.

It is clear from the number of requests to present to farmers' groups and the attention the project received in the farming media and press that there is a genuine interest in bioreduction within the livestock industry. Over the course of the trial, sixteen different groups heard presentations on the bioreduction system and/or were given a tour of the site. Comments made and questions asked about the system during such events were noted. Oral feedback was gathered from staff using the bioreduction system during the trial period for a simple evaluation of stakeholder view. The following are some comments expressed on the system:

Regarding installing the vessel:

> "*Anyone capable of using a digger could effectively place it in the ground. The electrical work would probably require professionals which could turn out to be expensive and could put farmers off the system*".
>
> "*The pipes should be placed closer together and to one side as this slightly complicates installation in that you're not sure which direction they should point*".

Regarding operator usability:

> "*I thought I'd have to 'butcher' the carcasses, but only a small incision to the belly is needed at the last moment before you place them inside. Most farmers should be able to deal with that. However, weighing every carcass so you know how much supplement to put in can be a nuisance*".

Regarding maintenance of the system:

> "*It's easy to manage as it mainly looks after itself; you just need to top up the water level occasionally*".

General summary on the system:

> "*This is a great system in most ways, although there could be improvements to the vessel design. Certainly, something needs to be done about the issue of smell if they are to be installed on farms or they need to be carefully sited. However, on the whole it's got to be much better than the current option*".
>
> "*We prefer it to the conventional system as fallen stock can be removed from site and placed out of sight in the vessels as soon as possible. There were no problems with birds and animals attracted to eat any carcasses*".
>
> "*There is no need to phone a disposal company until it is necessary and convenient for you to do so. I hate the idea of lorries travelling across the country carrying mounds of dead stock from farm-to-farm; and this negates that*".

Some enquiries were also received by those in the ruminant abattoir industry. This industry generates vast volumes of waste which leads to a significant financial burden in terms of disposal. Both the poultry and fish industry are also faced with high costs of waste disposal (Bansback 2006). The pig industry has already agreed to fund relevant research into bioreduction (Section 5.3). Collectively, it is clear that bioreduction could play an important role in many sectors of the food and meat industry, from producers to processors. This may warrant the inclusion of such sectors in its future development.

APPENDIX 7. Peer-review

App. 7.1 Peer-reviewer

Prof. Ken Killham was nominated as a peer-reviewer for the project. He is a chair of soil science at the University of Aberdeen and is the Director for Research at the College of Life Sciences and Medicine at the university. He is the established Professor of Soil Science at the University of Aberdeen and is the Director for Research at the College of Life Sciences and Medicine at the university. He chairs the UK Soil Science Advisory Committee, is Past-President of the British Soil Science Society and sits on several UK Research Council committees and institute Boards. Prof. Killham has published several books on soil science and more than two hundred research papers, focussing on bioremediation, soil science and biodiversity, pathogen ecology, rhizosphere ecology, microbial sensors and ecotoxicity testing.

App. 7.2 Letters of approval by peer-reviewer

A letter was obtained by Prof. Killham validating the trial management and scientific procedures. The trial report and findings were also validated. The reporting letters follow.