General Enquiries on the form should be made to:
Defra, Strategic Evidence and Analysis
E-mail: StrategicEvidence@defra.gsi.gov.uk

Department for Environment Food & Rural Affairs

Evidence Project Final Report

- **Note**
  In line with the Freedom of Information Act 2000, Defra aims to place the results of its completed research projects in the public domain wherever possible.
  The Evidence Project Final Report is designed to capture the information on the results and outputs of Defra-funded research in a format that is easily publishable through the Defra website
  An Evidence Project Final Report must be completed for all projects.
- This form is in Word format and the boxes may be expanded, as appropriate.
- **ACCESS TO INFORMATION**
  The information collected on this form will be stored electronically and may be sent to any part of Defra, or to individual researchers or organisations outside Defra for the purposes of reviewing the project. Defra may also disclose the information to any outside organisation acting as an agent authorised by Defra to process final research reports on its behalf. Defra intends to publish this form on its website, unless there are strong reasons not to, which fully comply with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.
  Defra may be required to release information, including personal data and commercial information, on request under the Environmental Information Regulations or the Freedom of Information Act 2000. However, Defra will not permit any unwarranted breach of confidentiality or act in contravention of its obligations under the Data Protection Act 1998. Defra or its appointed agents may use the name, address or other details on your form to contact you in connection with occasional customer research aimed at improving the processes through which Defra works with its contractors.

Project identification

1. Defra Project code: SE4404

2. Project title: Anaerobic digestion of porcine carcase material

3. Contractor organisation(s): Harper Adams University
   Edgmond
   Newport
   Shropshire
   TF10 8NB

4. Total Defra project costs (agreed fixed price): £ 123,881

5. Project: start date ..... 1st October 2013

   end date ..... 30th June 2015

6. It is Defra's intention to publish this form.

   Please confirm your agreement to do so..... **YES** ✓ **NO** ☐

   (a) When preparing Evidence Project Final Reports contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.

   Defra recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (not to be published) so that the Evidence Project Final Report can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and section (b) completed. NB: only in exceptional circumstances will Defra expect contractors to give a "No" answer.

   In all cases, reasons for withholding information must be fully in line with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.

   (b) If you have answered NO, please explain why the Final report should not be released into public domain

N/A

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

1. The UK pig industry currently produces over 210,000 tonnes of category 2 and 3 animal by-products (ABP) per annum that must be disposed of by either incineration or collection and disposal by rendering, at considerable cost to the farmer. In addition, movements between farms to facilitate ABP collection represents a significant risk to biosecurity. European legislation provides scope for consideration of alternative methods of ABP disposal if it can be demonstrated that they are equivalent to accepted methods and present no risk to public or animal health.

2. Anaerobic digestion (AD) is the transformation of organic matter to volatile fatty acids (VFAs) and biogas (CO_2 and CH_4) by a mixed microbial ecosystem in the absence of oxygen. Animal by-products potentially provide ideal substrates for us in AD systems because of their high protein and fat content, which has a high energy recovery potential. The advantages of on-farm AD are that it may reduce the energy use and increase the biosecurity of ABP disposal, and provide a more cost effective and sustainable alternative to currently accepted methods. In addition, assuming acceptable levels of pathogen destruction the residue produced may potentially be used as a source of plant nutrients in agricultural systems.

3. The objective of the project was to develop protocols for AD of porcine carcase material (PCM) to optimise biogas production, carcase reduction and pathogen destruction. In this context, two experiments were carried out at Harper Adams University using 10 litre bench top reactors between October 2013 and March 2015. In experiment 1, the effects of reactor temperature and PCM concentration were investigated. The experiment consisted of two temperatures (35 °C and 45 °C) and three PCM concentrations (100, 125 and 150 g/kg DM). Following adaptation to the feedstock, reactors were loaded with PCM over a 10 day period, before sealing and digestion using a batch process for 40 days. All reactors were spiked at the end of the loading period (day 10) with *Salmonella typhimurium*, *E. coli (K88)*, *Clostridium pefringens*, *Porcine parvovirus (PPV)* and *Ascaris suum* eggs. At the end of the experiment the residue was subjected to tyndallisation at 70 and 80 °C for 30 minutes.

4. The results indicated that biogas and CH_4 production, and PCM reduction were maximised at 35 °C with a PCM concentration of 100 g/kg DM. A >5 $\log^{10}$ reduction in *S. typhimurium* and *E. coli (K88)* was achieved on all treatments. Similarly *PPV* and *A. suum* were undetectable or not-viable at the end of the experiment. However, the reduction in *C. perfringens* was <3 $\log^{10}$. It was concluded that AD of

PCM was most effective at 35 °C with a PCM concentration of less than 100 g/kg DM. However, although acceptable levels of pathogen destruction were achieved for *S. typhimurium, E.coli, PPV* and *A. suum*, it proved difficult to destroy *C. perfringens,* even following tyndallisation at 60 or 70° C. Some form of pre-treatment by sterilisation (133 C at 300 kPa for 20 minutes) will be necessary.

5. The advantages of pre-treatment by sterilisation are that, AD of sterilised PCM is currently approved within existing EU legislation, and that it opens up the possibility of using continuous as opposed to batch AD processes. This would allow higher PCM loading rates, and hence the design of smaller scale on-farm systems. In addition, the use of pre-treatment by sterilisation potentially reduces the need for post-treatment by pasteurisation. In experiment 2, the effect on AD process and feedstock composition on the digestion of PCM pre-treated by sterilisation were investigated. The experiment consisted of three processes (batch, continuous (1-stage) and continuous (2-stage)) and two feedstock's (PCM + water) and PCM + slurry). All reactors were operated at 35 °C, and the PCM was pre-treated by sterilisation at 133 °C and 300 kPa for 20 minutes using ohmic heating (C-Tech Innovations). The optimum organic matter loading rates (OLR) were calculated from experiment 1 to be 1.2 g/litre/day for the batch, and 2.2 g/litre/day for the continuous processes respectively. The hydrolytic retention time was 40 days.

6. The results indicated that biogas gas and CH_4 production, and PCM reduction were maximised in the continuous (1 stage) process and that sterilisation and feedstock composition had no significant effect on the digestion of PCM. However, there was considerable variation in reactor performance between periods 1 and 2, suggesting that source material and adaptation to feedstock has a major effect on fermentation of PCM. Overall, it was concluded that the optimum protocol for AD of PCM should consist of a one stage continuous process operated at 35 °C with an OLR of <2.2 g/litre/day and a HRT of approximately 40 days. Some form of pre-treatment by sterilisation is required to ensure adequate levels of pathogen destruction, and pig slurry can be effectively utilised as a co-substrate.

7. The results of this project confirm that PCM can be effectively digested by AD with high levels of biogas and CH_4 production, and carcase reduction. However, further research is required to test the stability of the protocol over a longer period of time (3-4 HRTs), and to evaluate the residue produced as a potential fertiliser or soil improver. In addition, the data obtained can be used to inform a desk based study to design and evaluate the cost effectiveness of an on-farm pilot scale system.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
   - the objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Exchange).

1.0 Introduction:

The UK pig industry currently consists of a breeding herd of 422,000 sows with approximately 8,200,000 pigs being slaughtered per annum (BPEX, 2011). Assuming average levels of performance and typical on-farm mortality rates, pig production produces approximately 10,000 tonnes of category 2 ABP per annum. In addition, assuming an average slaughter weight of 100 kg and killing out proportion of 0.75, pig processing produces a further 200,000 tonnes of category 2 and 3 ABP. Category 2 animal by-products (ABP) must be disposed of by either incineration or collection and disposal by rendering (EC, 2009) at a cost to the pig industry of up to £20 million. In addition, movement between farms to facilitate ABP collection represents a serious risk to bio-security (Masse *et al.,* 2008). European legislation provides scope for the consideration of alternative methods of ABP use or disposal if it can be demonstrated that they are equivalent to accepted methods and present no risk to public or animal health (EC, 2009). Anaerobic digestion (AD) is the transformation of organic matter to volatile fatty acids and biogas (CO_2 and CH_4) by a mixed bacterial ecosystem in the absence of oxygen. Animal by-products potentially provide ideal substrates for use in AD systems because of their high protein and fat content, which has a high energy recovery potential. The advantages of AD are that it may reduce energy use and increase the bio-security associated with ABP disposal, and provide a more cost effective and sustainable alternative to currently accepted methods. In addition, assuming acceptable levels of pathogen destruction the digestate produced may potentially be used as a source of plant nutrients in agricultural systems. Previous work at Harper Adams University suggests that AD can be used for storage and bio-reduction of pig carcase material (PCM) with high levels of biogas and carcase reduction (Kirby, 2012). In addition, it has been shown to significantly reduce pathogen numbers. However, further work is required to develop protocols to optimise digester performance and destroy pathogens. The aim of the project is to quantify the risks associated with on-farm anaerobic digestion of fallen pigs and develop protocols to optimise biogas production, bio-reduction and pathogen destruction of PCM.

2.0 Objectives:

1. To develop protocols for anaerobic digestion of PCM to optimise biogas production, bio-reduction and pathogen destruction.

2. To prepare a report on the potential efficacy and safety of anaerobic digestion of fallen pigs to prepare the groundwork for a submission to EFSA for an opinion and to inform further research.

3.0 Material and methods:

Two experiments were carried out at Harper Adams University between October 2013 and March 2015 in the category 2 bio-fermentation laboratory using 16 bench top anaerobic reactors of 10 litres capacity. The reactors were designed to operate at temperatures of 35-55 °C and were heated by surface electric heaters covered by insulated jackets. Each reactors was fitted with a mechanical stirrer to ensure that the contents were mixed both horizontally and vertically, with the speed and frequency of stirring being controlled by a variable speed rheostat and a time switch. The reactors were designed for use either in batch mode or linked together to simulate a continuous flow system. Loading and sample collection were facilitated by ports in both the head plate and sides of each reactor.

4.0 Experiment 1: Effect of loading rate and reactor temperature on biogas production and pathogen destruction during anaerobic digestion of porcine carcase material.

4.1 Materials and methods:

4.1.1 Feedstock:

Prior to the experiment PCM was prepared by slaughtering a 60 kg pig from the Harper Adams herd, The whole carcase, including gut contents was then transported to the university of Nottingham and minced twice using a carcase mincer (Wolf King) through a 5.0 mm end plate. The minced PCM was then thoroughly mixed and stored at -20 °C in 2.0 kg bags prior to use. The chemical composition of the PCM used is presented in Table 1.

Table 1:
Chemical composition (g/kg DM) of the porcine carcase material (PCM) used during the experiment.

	DM (g/kg)	OM	CP	EE	Ash	pH	N	C	S
PCM	324	923	538	437	76	6.08	86	523	4.29

4.1.2 Preparation and adaptation of seed material:

Seed material was obtained from the Harper Adams commercial AD plant treating food waste and farm slurry at 35 °C. Seed material was collected under anaerobic conditions in sealed containers and transported to the laboratory. Prior to the experiment starting 500 ml of seed material was introduced to each reactor and incubated for 7 days to remove any remaining biodegradable material from the previous feedstock.

4.1.3 Experimental design:

Twelve reactors were used to investigate the effect of feedstock loading rate and reactor temperature on the fermentation of PCM. The three loading rates were 100, 125 and 150 g DM PCM/kg w/w and the two temperatures were 35 and 45 °C, in a 3 x 2 factorial design as follows:

1. 100 g DM PCM at 35 °C
2. 125 g DM PCM at 35 °C
3. 150 g DM PCM at 35 °C
4. 100 g DM PCM at 45 °C
5. 125 g DM PCM at 45 °C
6. 150 g DM PCM at 45 °C

Each treatment was replicated twice over two periods giving a total of 4 replicates per treatment. Each period consisted of an 10 days loading period during which each reactor was loaded with 500 g/day of feedstock (Table 2) to give a final working volume of 10 litres. They were then allowed to digest for 40 days.

Table 2:
Feedstock composition and loading rates (g/day) for each treatment used during the 10 day loading period.

	100 g DM	**125 g DM**	**150 g DM**
Fresh carcase material	154	193	231
Water	346	307	269
Total	**500**	**500**	**500**

4.1.4 Experimental routine:

Throughout the experiment biogas was collected in 3.0 litre gas bags (Fenton Packaging Ltd) and the volume recorded daily using a dry gas meter, prior to correction for standard temperature and pressure (STP). Gas composition (CH_4, CO_2) was also monitored daily using a portable gas analyser (Gas Data GFM416, Coventry, England). At the end of the loading period (day 10), and at weekly intervals thereafter, 50 ml of digestate was removed from each reactor for pH, volatile fatty acid (VFA), ammonium nitrogen (NH_4) and alkalinity analysis until the end of the experiment. At the end of the experiment each reactor and its contents were weighted, after which the contents were manually mixed and samples collected for DM and OM analysis.

At the end of the loading period (day 10) all reactors were spiked with *Clostridium perfringens*, *Salmonella typhimurium, Escherichia coli (K88)* and *Porcine parvo-virus* in 100 ml of nutrient broth to provide a concentration of $>10^6$ CFU/ml of reactor contents, together with a chamber containing 100,000 *Ascaris suum* eggs. Following addition of pathogens each reactor was stirred for 15 minutes prior to withdrawal of 20 ml of digestate for initial pathogen enumeration. Further samples were collected for *C. perfringens, S. typhimurium* and *E. coli* enumeration after 24 hours, 10, 20 and 30 days. The chambers containing *A. suum* eggs were retrieved at the end of the experiment.

At the end of the experiment digestate samples were subjected to tyndallisation at 70 and 80 °C. Tyndallisation was investigated as a potential option to destroy resistant micro-organisms such as *C. perfringens* that form spores in a hostile environment. A cycle of heating, cooling and incubation over a 3 day period may encourage spores to germinate followed by heating to destroy the vegetative forms. Digestate samples were placed in Hungate tubes and placed in a water bath at either 70 or 80 °C for 30 minutes followed by cooling and incubation at 35 °C for 24 hours, with the process being repeated 3 times.

4.1.5 Chemical analysis:

Porcine carcase material was freeze dried and analysed for, organic matter (OM), crude protein (CP) and ether extract (MAFF 1986). In addition, total nitrogen (N), carbon (C) and sulphur (S) were determined using the Dumas method (LECO FP-528).

Digestate pH was measured using a pH meter (Jenway 3505, England) and alkalinity was measured by potentiometric titration of the sample with 0.05 M sulphuric acid using a burette and beaker with a magnetic stirrer. Total alkalinity (TA) was measured by titrating the sample to pH 4.30, with partial alkalinity (PA) being measured by titrating to a pH of 5.75. Intermediate alkalinity (IA) was calculated as the difference between the two (Escudero 2014, Zhang 2012). Partial alkalinity measures the alkalinity due to bicarbonates, whereas IA measures the alkalinity due to volatile fatty acids (VFA). The total VFA concentration was also determined by titration using 0.05 M sulphuric acid. The sample pH was determined, prior to titration through to pH 5.10, followed by titration from pH 5.10 to pH 3.50 (Feitkenhauer, 2002). The total VFA concentration was then calculated using two sets of simultaneous equations as described by Anderson, 1992).

The DM content of digestate samples was determined by oven drying at 105 °C, with the OM being subsequently determined by ashing at 550 °C (MAFF, 1986). Digestate samples were also analysed for ammonium nitrogen by centrifuging at 4000 rpm for 10 minutes, prior to decanting the supernatant and analysed for ammonium nitrogen using a Kjeltec 8400 Auto-Analyser (FOSS, UK).

4.1.6 Microbial analysis:

Clostridium perfringens was obtained as Selectrol® discs from TCS Biosciences, Buckingham, England, with all other reagent being obtained from Thermo-Scientific, Basingstoke, England. Cooked meat media (OXOID-CM0081) was prepared in accordance with the manufacturer's instructions and incubated with two Selectrol® discs for 48 hours at 35 °C. After incubation the initial count of *C. perfringens* was carried out by performing serial dilutions of the culture in maximum recovery diluent (MRD) (OXOID-CM0733), prepared in accordance with the manufacturer's instructions. Perfringens TSC Agar (OXIOD-CM0587) was prepared in accordance with the manufacturer's instructions and poured into petri dishes. An aliquot (0.1 ml) of each serial dilution was spread onto each petri dish followed by a further overlay of 10 ml TSC Agar. The TSC Agar plates were then placed in anaerobic jars with anaerobic sachets (AnaeroGen AN0035) to maintain an anaerobic environment. The jars were then sealed and placed in an incubator at 35 °C for 18-24 hours. After incubation, all black and grey colonies were counted as *C. perfringens*. During period 1, *C. perfringens* enumeration in digestate samples was carried out by ALS Laboratories, Mirfield, West Yorkshire using technique ESGM-M310 based on BS EN ISO 7937:2004. During period 2 enumeration of *C. perfringens* was carried out as described above.

Salmonella typhimurium (NCTC 12023) was obtained from the Animal Health and Veterinary Laboratories Agency (AHVLA, Weybridge, England). *S. typhimurium* was streaked onto Nutrient Agar (OXOID-CM003), and incubated at 35 °C for 24 hours. After incubation, the culture was swabbed and inoculated into nutrient broth (OXOID-CM001) and placed in a shaking incubator at 35 °C for 24 hours. *S. typhimurium* was enumerated using Brilliant Green Agar (mBGA) was as a selective medium. Agar was prepared in accordance with the manufacturer's (LABM Limited Lancashire, England) instructions and poured into petri dishes. An aliquot (0.1 ml) of each serial dilution was spread onto each petri dish and incubated at 35 °C for 18-24 h. After incubation, red colonies were counted as *S. typhimurium*. Enumeration of *S. typhimurium* in digestate samples was carried out after serial dilution in MRD as described above.

Escherichia coli (K88) was obtained from AHVLA (Weybridge, England) and steaked onto Nutrient Agar (OXOID-CM003) and incubated at 35 °C for 18-24 hours. After incubation, the culture was swabbed and inoculated into nutrient broth (OXOID-CM001) and placed in a shaking incubator at 35 °C for 24 hours. *E. coli* was enumerated after serial dilution in MRD using Eosine Methylene Blue Agar (EMB) as a selective medium. Agar was prepared in accordance with the manufacturers (LABM Limited, Lancashire, England) instructions and poured into petri dishes. An aliquot (0.1 ml) of each serial dilution was spread onto each petri dish and incubated at 35 °C for 18-24 h. After incubation blue-black colonies with a metallic sheen were counted as *E. coli (K88). E. coli (K88)* in digestate samples was carried out after serial dilution in MRD as described above.

Porcine parvovirus (PPV) containing seed material containing 10_8 $TCID_{50}$/ml was obtained from AHVLA (Weybridge, England). This was added to each reactor with no further preparation. PPV analysis of digestate samples was performed by AHVLA (Weybridge, England). Digestate samples were centrifuged at 1000 g for 10 minutes followed by removal of the supernatant and diluting 1/10 in GM. A preparation of 200 ml of LLC-PK1 cell suspension at 1 x 10^5 cells/ml was made in a flask, to which 10 ml of cell

suspension was added. The AD digestate sample (0.5 ml) was added and incubated at 37°C for 6 days and observed for cytopathic effects (CPE) twice during this period. For *PPV* titration, samples with no CPE were centrifuged at 100 g for 10 minutes and the supernatant removed and diluted 1/10 in GM, followed by the addition of 25 ul of each sample to 8 wells of one column of a 96-well micro-titre plate. LLC-PK1 cell suspension at 1 x 10^5 cells/ml was prepared and 200 ul of cell suspension added to each well. Flasks with CPE were centrifuged at 100 g for 10 minutes, with the supernatant being removed and diluted from 1/10 to 1/100,000 in GM. 25 ul of each dilution was added to 8 wells of one column of a 96-well micro-titre plate. LLC–PK1 cell suspension at 1 x 10^5 cells/ml was prepared and 200 ul of cell suspension added to each well. All micro-titre plates were incubated at 37 °C for 6 days and observed for CPE during the period. After 6 days incubation, each micro-titre plate was fixed using 80% acetone, air dried and stored at -20 °C. Fluorescent anti-body test (FAT) was performed on micro-titre plates using VMRD *PPV* direct FA conjugate and the titre calculated for PPV in each sample.

Ascaris suum eggs were obtained from Excelsior Sentinel Inc. (New York, USA). The eggs were contained in 25 mm x 25 mm polycarbonate "Californian" chambers (US#400 SS mesh, encased in a 0.45 um nylon filter) containing 100,000 viable *A. suum* eggs. One chamber was added to each reactor. At the end of the experiment chambers were retrieved from each reactor, washed and emptied into a petri dish. The eggs were then counted using a binocular microscope. The eggs were then transferred into T-25 flasks with loosely fitting caps (to ensure aerobic conditions) and placed in a rocker for 30 days. They were then placed back in petri dishes and the presence of hatch or larva development checked using a binocular microscope.

4.1.7 Mass balance

A mass balance calculation was performed based on the volume of dry CH_4 and CO_2 produced at STP. Conversion of gas volumes to STP was performed according to Richards *et al.* (1991).
Dry biogas is assumed to behave as an ideal gas consisting entirely of CH_4 and CO_2.The mass of biogas was calculated using the molecular mass of CH_4 and CO_2 (16 and 44 g/mol respectively), the molar volume of an ideal gas at STP (22.413 mol/g) and the average individual gas composition (vol %) using the relationship of Rao, (2004).

$$M_b = [V (16 \times CH_4/100) + (44 \times CO_2/100] / 22.413$$

Where: M_b = mass of biogas (g), V = volume of dry biogas (STP) and CH_4 and CO_2 are their concentrations (% age) in the biogas.

The mass balance was calculated as the mass of DM input against the mass a dry biogas produced and the mass of DM remaining in each reactor.

4.1.8 Statistical analysis and calculations:

The experiment was analysed by ANOVA as a 2 x 3 factorial design using Genstat 16, with the main effects being PCM concentration and reactor temperature. Dry matter reduction was calculated as:

$$DM_{red}\ (\%) = (DM_{in} - DM_{rem}) / DM_{in} \times 1000$$

Where: DM_{red} is the reduction in DM, DM_{in} is the initial DM loaded into each reactor and DM_{rem} is the DM remaining in each reactor.

A similar calculation was performed for OM reduction.

Specific CH_4 yield (SMY) was calculated as:

$$SMY = \text{Methane yield (l)} / \text{OM added (kg)}$$

4.2 Results

4.2.1 Reactor performance:

Table 3
Effect of reactor temperature and porcine carcase material concentration (PCM, g DM/kg w/w) on total biogas production and composition, CH_4 and CO_2 production and specific gas yields.

	35 °C			45 °C			SED	Probability		
	100	**125**	**150**	**100**	**125**	**150**		**Temp**	**Conc**	**Int**
Total biogas (l)	284	93	71	225	143	108	37.9	NS	***	NS
CH_4 (%)	67.9	46.5	35.6	65.1	53.2	45.1	5.82	NS	***	NS
CO_2 (%)	32.1	53.5	64.4	34.9	46.8	55.0	5.82	NS	***	NS
Total CH_4 (l)	195	48	28	140	66	42	30.5	NS	***	NS
Total CO_2 (l)	90	45	43	85	76	67	15.0	NS	*	NS
Biogas (l/g OM)	0.615	0.161	0.102	0.491	0.248	0.155	0.081	NS	***	NS
CH_4 (l/g OM)	0.421	0.083	0.041	0.307	0.114	0.090	0.067	NS	***	NS

Table 4

Effect of reactor temperature and porcine carcase material concentration (PCM, g DM/kg w/w) on mass balance and PCM reduction.

	35 °C			45 °C			SED	Probability		
	100	**125**	**150**	**100**	**125**	**150**		**Temp**	**Conc**	**Int**
DM input (g)	500	625	750	500	625	750				
DM remaining (g)	182	449	618	261	432	594				
DM CH_4 (g)	139	42	21	112	66	41				
DM CO_2 (g)	176	93	84	145	116	112				
Balance	0.994	0.934	0.964	1.036	0.982	0.996				
DM loss (g/kg)	636	296	219	476	308	208	58.8	NS	***	NS
OM loss (g/kg)	739	371	287	614	380	280	64.6	NS	***	NS

The effects of reactor temperature and PCM concentration on reactor performance are presented in tables 3 and 4. There were no significant interactions or effects of temperature on reactor performance. However, reactors loaded at 100 g DM/kg w/w produced significantly higher ($P<0.001$) gas yields and levels of PCM reduction than those loaded at 125 or 150 g DM/kg w/w. The highest gas yield and levels of PCM being in reactors operated at 35 °C with 100 g DM/kg w/w. The performance of reactors operated at 35 °C with 100 g DM/kg w/w was similar to that reported in the literature. Hejnfelt and Angelidaki (2009) reported a methane yield of 0.62 l/g OM during a biochemical methane potential (BMP) analysis of mixed pork waste whereas, Cuetos *et al.,* (2008) observed an OM reduction of 767 g/kg during anaerobic digestion (35 °C for 50 days) of poultry slaughterhouse waste. The levels of gas production and PCM reduction observed in the current study suggest that 35 °C and 100 g DM/kg w/w is the optimum for AD of PCM.

4.2.2 Reactor stability:

Table 5:

Effect of reactor temperature and porcine carcase material concentration (PCM, g DM/kg w/w) on reactor pH.

	35 °C			45 °C			SED	Probability		
	100	**125**	**150**	**100**	**125**	**150**		**Temp**	**Conc**	**Int**
Day10	7.49	7.29	7.29	7.33	7.26	7.30	0.076	NS	*	NS
Day 17	7.70	7.35	7.34	7.69	7.41	7.53	0.171	NS	*	NS
Day 50	8.17	7.92	7.64	8.28	8.09	7.89	0.165	NS	**	NS

Table 6:
Effect of reactor temperature and porcine carcase material concentration (PCM, g DM/kg w/w) on partial alkalinity (PA), intermediate alkalinity (IA) and alkalinity ratio (AR).

	100			125			150		
	PA	**IA**	**AR**	**PA**	**IA**	**AR**	**PA**	**IA**	**AR**
Reactors 35 °C									
Day 10	7420	8357	1.13	7181	13229	1.84	7420	13414	1.86
Day 17	7555	7940	1.05	7222	13176	1.82	7699	15551	2.02
Day 24	9728	7596	0.78	7475	14423	1.93	7742	15766	2.04
Day 31	13187	5446	0.41	7259	13934	1.92	7749	15599	2.01
Day 38	15837	4051	0.26	6896	13850	2.01	7597	15791	2.08
Day 45	15076	3094	0.21	7869	13347	1.70	7659	15358	2.01
Reactors 45 °C									
Day 10	6568	12596	1.93	6807	14917	2.19	7826	15961	2.04
Day 17	7693	10811	1.41	9062	20841	2.30	8555	19093	2.23
Day 24	10636	10281	0.97	10411	22033	2.12	10630	20238	1.90
Day 31	13475	8830	0.66	11366	21282	1.87	10906	19458	1.78
Day 38	14945	7698	0.52	12946	19857	1.53	11689	18933	1.62
Day 45	15889	6796	0.43	15518	19238	1.24	11855	18508	1.56

Table 7:
Effect of reactor temperature and porcine carcase material concentration (PCM, g DM/kg w/w) on reactor volatile fatty acid (VFA, mmols/l) and ammonium nitrogen (NH_4, g/l)) concentration.

	35 °C			45 °C			SED	Probability		
	100	**125**	**150**	**100**	**125**	**150**		**Temp**	**Conc**	**Int**
VFA										
Day 10	127	153	194	144	179	200	1.5	***	***	***
Day 17	213	245	295	173	271	290	1.2	***	***	***
Day 50	65	228	279	86	192	262	1.6	***	***	***
NH_4										
Day 10	8.7	12.0	13.1	9.0	11.7	13.9	0.97	NS	***	NS
Day 17	9.1	11.3	11.9	9.7	11.8	13.2	0.57	NS	***	NS
Day 50	10.0	12.4	12.8	9.8	12.5	13.4	0.68	NS	***	NS

The effects of reactor temperature and PCM concentration on reactor stability are presented in tables 5, 6 and 7. There were no significant interactions or effects of temperature on reactor pH. However, throughout the experiment reactors loaded with 100 g DM/kg w/w maintained a significantly higher ($P<0.05$) pH than those loaded at either 125 or 150 g DM/kg w/w. There was a highly significant effect ($P<0.001$) of both temperature and PCM concentration on total VFA production, with VFA production being generally higher at 35 than 45 °C, and higher at the higher PCM concentrations. There were no significant interactions or effects of reactor temperature on NH_4 concentration. However, reactor NH_4 concentration was significantly higher ($P<0.001$) at higher PCM concentrations. The AR provides a measure of stability of the fermentation process. A ratio >1.0 indicates that VFA concentration is greater than buffering capacity and could result in failure of the digestion process. Throughout the experiment the AR was influenced by both reactor temperature and PCM concentration with the AR being higher at 45 than 35 °C and higher at higher PCM concentrations. At PCM concentrations of 125 and 150 g DM/kg w/w the AR was >1.0 throughout the experiment. At 100 g DM/kg w/w the AR initially increased during the hydrolysis phase, but reduced to <1.0 by day 24, indicating recovery and conversion of VFA to CH_4. The accumulation of NH_4 during anaerobic digestion of protein in slaughterhouse waste is thought to inhibit methanogenic bacteria (Escudero, 2014, Cuetos, 2008 & 2010). However, the threshold considered to be inhibitory varies between 1.7-14.0 g/l depending on a variety of factors (Chen, 2008). In the current study, the reactor stability data suggests that fermentation was more stable at 35 than 45 °C and that fermentation was inhibited at PCM concentrations of 125 and 150 g DM/kg w/w.

4.2.3 Pathogen destruction:

Table 8:
Effect of reactor temperature and porcine carcase material concentration (PCM, g DM/kg w/w) on pathogen numbers (Log^{10}) throughout the experiment.

	35 °C			45 °C			SED	Probability		
	100	125	150	100	125	150		Temp	Conc	Int
C. perfringens										
15 minutes	4.86	3.86	4.73	4.84	3.46	3.26	1.140	NS	NS	NS
24 hours	5.74	3.46	4.74	4.50	3.41	3.13	0.807	0.052	*	*
Day 10	3.24	3.26	2.96	3.03	2.73	3.33	0.459	NS	NS	NS
Day 20	3.15	3.20	2.93	2.96	2.94	2.82	0.405	NS	NS	NS
Day 30	3.13	2.97	3.10	2.93	2	2.92	0.486	NS	NS	NS
Tyndallisation 70°	3.15	2.98	3.66	2.80	2.77	2.65	0.270	*	NS	NS
Tyndallisation 80°	2.88	2.92	3.00	2.67	2.18	2.38	0.400	*	NS	NS
S. Typhimurium										
15 minutes	6.29	6.12	6.15	3.12	0	0	1.408	***	0.062	0.098
24 hours	6.21	5.08	3.45	2.96	0	0	1.224	***	*	NS
10 days	1.00	1.89	0.82	0	0	0	0.983	*	NS	NS
20 days	0	1.27	0.47	0	0	0	0.506	0.062	NS	NS
30 days	0	0	0	0	0	0	---	---	---	---
E. coli (K88)										
15 minutes	6.32	6.17	5.94	3.00	0	0	1.002	***	0.051	NS
24 hours	6.08	4.57	2.76	2.72	0	0	1.067	***	**	NS
10 days	1.57	1.88	0.88	0	0	0	1.218	0.055	NS	NS
20 days	0	0.975	0	0	0	0	0.325	NS	0.075	0.075
30 days	0	0	0	0	0	0	---	---	---	---
PPV										
15 minutes	4.51	4.16	4.20	4.10	3.08	3.72	0.886	NS	NS	NS
30 days	1.58	3.48	3.07	0	0	0	0.704	***	NS	NS
50 days	0	1.36	2.51	0	0	0	0.550	***	*	*
A suum (egg)										
Day 50	4.42	4.33	4.31	3.82	4.20	4.19	---	NS	NS	NS

The effects of reactor temperature and PCM concentration on pathogen destruction are presented in tables 8. The concentration of all pathogens reduced during the course of the experiment, with the rate of pathogen reduction being significantly higher at 45 than 35 °C. In all reactors the concentration of *S. typhimurium* and *E. coli* had reduced to zero by day 30, with a >5 log^{10} reduction being achieved. Similarly, PPV was largely destroyed by day 50, although the reduction was <5 log^{10} owing to the lower initial concentrations. Whilst all the *A. suum* larva were not destroyed, the remaining eggs showed no viability or indication of hatch after incubation for 30 days. The optimum temperature for growth and survival of most pathogens is 30-40 °C, hence mesophilic temperatures do not destroy pathogens directly (Smith, 2005). Factors other than temperature and time may be important in the reduction in pathogens during anaerobic digestion. In particular, pH, VFA, NH_4 and sulphide concentrations (Ahring, 2003). Volatile fatty acid concentration is considered to one of the most important factors affecting pathogen destruction in during anaerobic digestion (Kunte, 2004, Salsali *et al.* 2008).

In all of the reactors the concentration of *C. perfringens* reduced during the course of the experiment. However, there was no significant effects of either temperature or PCM concentration. The log reduction achieved was approximately 2 log^{10}, and tyndallisation at 70 or 80 °C did not significantly increase the level of *C. perfringens* destruction. Similar levels of *C. perfringens* destruction were reported by Salsali *et al.* (2008) during mesophilic and thermophilic anaerobic digestion of primary and thickened waste activated sludge. The low levels of *C. perfringens* destruction may be due to the fact that when under stress the bacteria produces resistant spores that are difficult to destroy and suggest that some form of pre-treatment by sterilisation (133 °C at 300 kPa for 20 minutes) will be required prior to anaerobic digestion of PCM.

4.3 Conclusions:

The results of experiment 1 suggest that biogas and CH_4 production, and PCM reduction were maximised at 35 °C with a PCM concentration of 100 g DM/kg w/w. A >5 log^{10} reduction in *S. typhimurium* and *E. coli (K88)* was achieved on all treatments. Similarly, *PPV* and *A. suum* were undetectable or non-viable at the end of the experiment. However the reduction in *C. perfringens* was <3 Log^{10}. It can be concluded that AD of PCM was most effective at 35 °C with a PCM concentration of less than 100 g DM/kg w/w. However, although acceptable levels of pathogen destruction were achieved for *S. typhimurium, E.coli, PPV* and *A. suum*, it proved difficult to destroy *C. perfringens*, even following tyndallisation at 70 or 80 °C. Some form of pre-treatment by sterilisation (133 °C at 300 kPa for 20 minutes) will be required.

5.0 Experiment 2: Effect of anaerobic digestion process on the fermentation of porcine carcase material pre-treated by sterilisation.

5.1 Introduction:

From experiment 1 it can be concluded that AD of PCM was most effective at 35 °C with a PCM concentration of <100 g DM/kg w/w. However, pre-treatment of PCM by sterilisation (133 °C at 300 kPa for 20 minutes) will be required prior to fermentation to destroy *C. perfringens.* The introduction of a pre-fermentation sterilisation stage opens up the possibility of using either continuous or two phase processes (instead of batch), which could significantly increase OM loading rates and reduce hydrolytic retention times (HRT), resulting in smaller and therefore cheaper on-farm systems. Pre-sterilisation of PCM could be achieved using a variety of processes. However, the technology used needs to be quick, efficient, controllable, and able to cope with liquids and solids.

The fermentation of PCM requires the addition of significant quantities of water, which represents a potential cost to farmers. Pig slurry is another co-product arising from pig production that requires appropriate disposal. The DM content of pig slurry ranges from 2.6-6.9% (Hejnfelt and Angelidiki, 2009). Consequently, pig slurry could potentially provide the water required for co-digestion of PCM. However, although pig slurry contains some OM, it has a low C:N ratio, which could lead to high levels of NH4 and reduce fermentation efficiency. The objectives of experiment 2 were to investigate the effects of batch, continuous and two stage AD processes on the fermentation characteristics, biogas production and bio-reduction of PCM pre-treated by sterilisation.

5.2 Materials and methods:

5.2.1 Feedstock:

Prior to the experiment PCM was prepared by slaughtering a 60 kg pig from the Harper Adams herd, The whole carcase, including gut contents was then transported to the university of Nottingham and minced twice using a carcase mincer (Wolf King) through a 5.0 mm end plate. The minced PCM was then thoroughly mixed and transported back to Harper Adams University. The minced PCM was then sterilised (133 °C at 300 kPa for 20 mins) using a bench scale Ohmic heater (C-Tech Innovations, Cheshire, England). The minced PCM (1.0 – 1.2 kg) was placed in the heating chamber and sealed. Power was applied by controlling the voltage between two surface electrodes. The resistance provided by the PCM generates heat and pressure, which was monitored using a digital thermometer and pressure gauge respectively. When the required values were reached the voltage was held constant for 20 minutes. The equipment was then allowed to cool and the pressure valve gradually opened. After further cooling the heating chamber was fully opened and the sterilised PCM extracted. Sterilised PCM was stored at -20 °C prior to use.

Table 9:
Chemical composition (g/kg DM) of the porcine carcase material (PCM) and pig slurry used during the experiment.

	DM (g/kg)	OM	CP	EE	Ash	pH	N	C	S
PCM	334	901	536	448	99	6.13	85	519	4
Pig slurry	32	692	---	---	---	---	---	---	---

5.2.2 Preparation and adaptation of seed material:

During period 1, seed material was obtained from the Harper Adams University small scale AD plant processing food waste at 35 °C. Due to unforeseen circumstances in period 2, seed material was collected from a local commercial AD plant processing food waste. Seed material was collected under anaerobic conditions in sealed containers and transported to the laboratory. For the batch treatments 4500 ml seed material was introduced into each 10.0 litre bench-top reactor, and for the continuous treatments 9500 ml of seed material was introduced into each 10.0 litre reactor. For the two phase treatments, 4500 ml of seed material was introduced into the primary reactor and 9500 ml into the secondary reactor. Each reactor was then adapted to the PCM over an 11 day period by loading with 50 g/d of feedstock containing a PCM concentration of 50 g DM/kg w/w to give a total volume of 5050 ml in the batch, 10050 ml in the continuous, and 5050 ml and 10050 ml in the primary and secondary reactors of the two phase process respectively. On day 11, 50 ml of adapted feedstock was withdrawn for subsequent chemical analysis.

5.2.3 Experimental design:

Sixteen reactors were used to investigate the effect of AD process and feedstock composition on the fermentation of PCM. The two feed stocks used were PCM + water and PCM + pig slurry, and the three AD processes were batch, continuous and two phase, and in a 3 x 2 factorial design as follows:

1. PCM + Water – Batch (PWB)
2. PCM + Water – Continuous (PWC)
3. PCM + Water – Two phase (PWT)
4. PCM + Slurry – Batch (PSB)
5. PCM + Slurry – Continuous (PSC)
6. PCM + Slurry – Two Phase (PST)

Each treatment was replicated twice over two periods giving a total of 4 replicates per treatment. In each period, reactors were loaded with the experimental feed stocks as follows:

Batch treatments:

The results of experiment 1 indicated that in batch mode 94% of total biogas production had been produced by day 40 Following adaption, the batch reactors were loaded with 500 g/day of each feedstock containing 100 g DM/kg w/w (Table 10) over a 10 day loading period. Following the loading period, the reactors were sealed and left to ferment for 40 days. Overall, the OM loading rate for the batch reactors was calculated to be approximately 1.2 g/litre/day.

Continuous treatments:

Following adaption, the continuous reactors were fed 250 g/day of each feedstock containing 100 g DM/kg w/w (Table 10). Prior to loading, 250 ml of digestate will be removed from each reactor and discarded so that the total volume of digestate remains constant at 10000 ml. The OM loading rate for the continuous treatments was calculated to be 2.2 g/litre/day and the HRT was 40 days.

Two phase treatments:

The two phase treatments consisted of two reactors. The primary reactor had a volume of 5000 ml and the secondary reactor ha a volume of 10000 ml, giving a total working volume of 15000 ml. Following adaption, the primary reactor will be fed 375 g/day of each feedstock containing 100 g DM/kg w/w (Table 10). Prior to feeding 375 ml of digestate will be removed from the secondary reactor and discarded. Similarly, 375 ml of digestate will be removed from the primary reactor and introduced into the secondary reactor, so that the total volume of digestate in each reactor remained constant. The OM loading rate for the two phase treatments was calculated to be 2.2 g/litre/day and the HRT in the primary and secondary reactors was 13.3 and 26.6 days respectively.

Table 10:
Composition and total daily amounts of the two feedstock used in the experiment.

	C OM (g)	S OM (g)	C DM (g)	S DM (g)	Fresh PCM (g)	Fresh Slurry g	Water (g)	Total (g)
Batch reactors								
PCM + Water	45.0	---	50.0	---	149.7	---	350.3	500
PCM + Slurry	38.2	6.8	42.4	9.8	126.9	306.2	66.9	500
Continuous								
PCM + Water	22.5	---	25.0	---	74.8	---	175.2	250
PCM + Slurry	19.1	3.4	21.2	4.9	82.3	153.1	14.6	250
Two phase								
PCM + Water	33.8	---	37.5	---	112.3	---	262.7	375
PCM + Slurry	28.7	4.9	31.8	7.1	95.2	221.8	58	375

C OM = PCM organic matter, S OM = slurry organic matter, C DM = PCM dry matter and S DM = slurry dry matter
The proportion of OM supplied from PCM in the PCM + slurry was 0.85.

5.2.4 Experimental routine:

Throughout the experiment biogas was collected in 3.0 litre gas bags (Fenton Packaging Ltd) and the volume recorded daily using a dry gas meter, prior to correction for standard temperature and pressure

(STP). Gas composition (CH_4, CO_2) was also monitored daily using a portable gas analyser (Gas Data GFM416, Coventry, England). At the end of the adaptation period (day 11), and at weekly intervals thereafter, 50 ml of digestate was removed from each reactor for pH, volatile fatty acid (VFA), ammonium nitrogen (NH_4) and alkalinity analysis until the end of the experiment. At the end of the experiment each reactor and its contents were weighted, after which the contents were manually mixed and samples collected for DM and OM analysis.

5.2.5 Chemical analysis:

Porcine carcase material was freeze dried and analysed for, organic matter (OM), crude protein (CP) and ether extract (MAFF, 1986). In addition, total nitrogen (N), carbon (C) and sulphur (S) were determined using the Dumas method (LECO FP-528).

Digestate pH was measured using a pH meter (Jenway 3505, England) and alkalinity was measured by potentiometric titration of the sample with 0.05 M sulphuric acid using a burette and beaker with a magnetic stirrer. Total alkalinity (TA) was measured by titrating the sample to pH 4.30, with partial alkalinity (PA) being measured by titrating to a pH of 5.75. Intermediate alkalinity (IA) was calculated as the difference between the two (Escudero 2014, Zhang 2012). Partial alkalinity measures the alkalinity due to bicarbonates, whereas IA measures the alkalinity due to volatile fatty acids (VFA). The total VFA concentration was also determined by titration using 0.05 M sulphuric acid. The sample pH was determined, prior to titration through to pH 5.10, followed by titration from pH 5.10 to pH 3.50 (Feitkenhauer, 2002). The total VFA concentration was then calculated using two sets of simultaneous equations as described by Anderson, 1992).

The DM content of digestate samples was determine by oven drying at 105 °C, with the OM being subsequently determined by ashing at 550 °C (MAFF, 1986). Digestate samples were also analysed for ammonium nitrogen by centrifuging at 4000 rpm for 10 minutes, prior to decanting the supernatant and analysed for ammonium nitrogen using a Kjeltec 8400 Auto-Analyser (FOSS, UK).

5.2.6 Mass balance:

A mass balance calculation was performed based on the volume of dry CH_4 and CO_2 produced at STP. Conversion of gas volumes to STP was performed according to Richards *et al.* (1991).
Dry biogas is assumed to behave as an ideal gas consisting entirely of CH_4 and CO_2.The mass of biogas was calculated using the molecular mass of CH_4 and CO_2 (16 and 44 g/mol respectively), the molar volume of an ideal gas at STP (22.413 mol/g) and the average individual gas composition (vol %) using the relationship of Rao, (2004).

$$M_b = [V (16 \times CH_4/100) + (44 \times CO_2/100] / 22.413$$

Where: M_b = mass of biogas (g), V = volume of dry biogas (STP) and CH_4 and CO_2 are their concentrations (% age) in the biogas.

The mass balance was calculated as the mass of DM input against the mass a dry biogas produced and the mass of DM remaining in each reactor.

5.2.7 Statistical analysis and calculations:

The experiment was analysed by ANOVA as a 2 x 3 factorial design using Genstat 16, with the main effects being PCM concentration and reactor temperature. Dry matter reduction was calculated as:

$$DM_{red} (\%) = (DM_{in} – DM_{rem}) / DM_{in} \times 1000$$

Where: DM_{red} is the reduction in DM, DM_{in} is the initial DM loaded into each reactor and DM_{rem} is the DM remaining in each reactor.

A similar calculation was performed for OM reduction.

Specific CH_4 yield (SMY) was calculated as:

$$SMY = \text{Methane yield (l)} / \text{OM added (kg)}$$

5.3 Results:

5.3.1 Reactor performance:

Table 11:
Effect of feedstock composition and AD process on total biogas production and composition, CH_4 and CO_2 production and specific gas yields.

	PCM + Water			PCM + Slurry			SED	Probability		
	B	C	T	B	C	T		Feed	AD	Int
Total biogas (l)	189	444	517	163	460	437	101	NS	***	NS
CH_4 (%)	67.6	67.7	69.0	68.5	67.8	68.2	4.64	NS	0.074	NS
CO_2 (%)	32.4	32.3	31.0	31.5	32.2	31.8	4.64	NS	0.074	NS
Total CH_4 (l)	128	300	357	112	312	298	71.3	NS	***	NS
Total CO_2 (l)	61	143	160	51	148	139	30.4	NS	***	NS
Biogas (l/g OM)	0.418	0.517	0.402	0.360	0.536	0.340	0.161	NS	NS	NS
CH_4 (l/g OM)	0.282	0.350	0.277	0.246	0.364	0.232	0.116	NS	NS	NS

Table 12:
Effect of feedstock composition and AD process on mass balance and PCM reduction during the experiment.

	PCM + Water			PCM + Slurry			SED	Probability		
	B	C	T	B	C	T		Feed	AD	Int
DM input (g)	500	976	1464	500	976	1464				
DM remaining (g)	284	460	792	301	416	912				
DM biogas (g)	212	508	584	181	526	498				
Balance	0.992	0.992	0.934	0.964	0.965	0.963				
DM loss (g/kg)	0.123	0.521	0.398	0.361	0.539	0.340	0.1552	NS	NS	NS
OM loss (g/kg)	0.470	0.578	0.442	0.401	0.598	0.378	0.1724	NS	NS	NS

The effects of feedstock composition and AD process on reactor performance are presented in Tables 11 and 12. There were no significant interactions or effects of feedstock composition on reactor performance. However, reactors operating the continuous and two phase AD processes produced significantly higher ($P<0.001$) gas yields than those running the batch process. There were no significant effects of AD process on specific gas production. Similarly, there were no significant effects of AD process on PCM reduction. Numerically, reactors operated continuously had the highest specific yields and levels of PCM reduction. Considerable variation in gas production was observed between periods 1 and 2, with higher levels of gas production being observed in period 2. This was attributed to the different source materials used and highlights the importance of source material and thorough adaptation to feed stock for effective AD (Novak *et al*. 2011, Foster-Carneiro, 2007). The specific gas yields obtained in the current experiment were similar to the values of 0.52-0.55 l/g OM reported by Salminen and Rintala (2002) during AD of untreated poultry slaughterhouse waste, and the value of 0.62 l/g OM reported by Hejnfelt and Angelidaki (2009) for untreated pork waste. Pre-treatment by sterilisation has been reported to have no effect on AD of animal by-products (Hejnfelt and Angelidaki 2009, HAU-BPEX, 2004).

5.3.2 Reactor stability:

Table13:
Effect of feedstock composition and AD process on reactor pH during the experiment.

	PCM + Water			PCM + Slurry			SED	Probability		
	B	C	T	B	C	T		Feed	AD	int
Day10	7.46	7.96	7.73	7.54	8.03	7.87	0.213	NS	*	NS

Day 23	7.69	8.03	7.46	7.87	8.14	7.62	0.170	NS	***	NS
Day 40	7.85	7.90	7.52	7.78	7.98	7.65	0.147	NS	**	NS

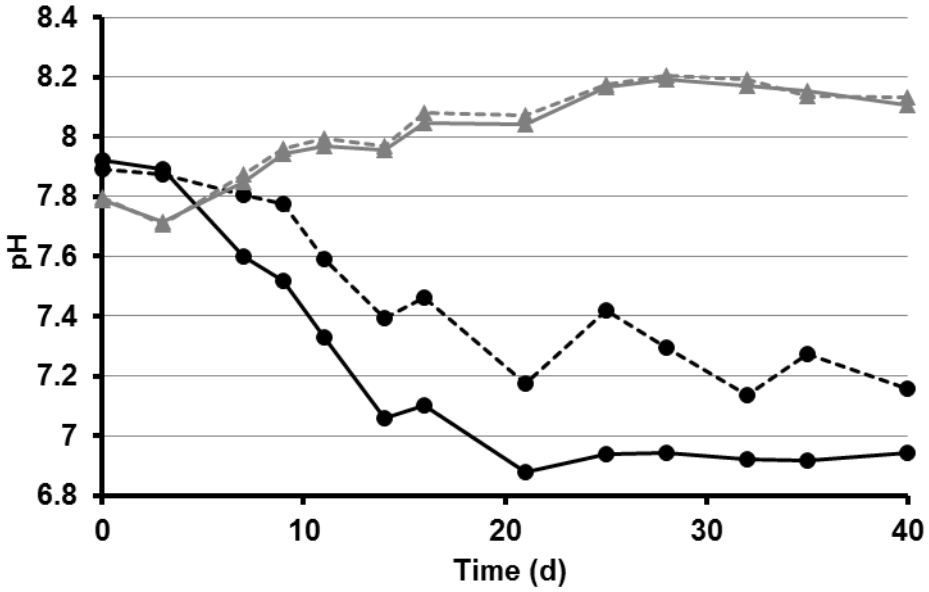

Figure 1:
Effect of feedstock and AD process on reactor pH in the primary and secondary reactors of the two phase treatments (PTW primary = black solid, PTW secondary = grey solid, PTS primary = black dashed, PTS secondary = grey dashed)

Table 14:
Effect of feedstock and AD process on partial alkalinity (PA), intermediate alkalinity (IA) and alkalinity ratio (AR) during the experiment.

	Batch			Continuous		
	PA	**IA**	**AR**	**PA**	**IA**	**AR**
PCM + Water						
Day 1	9345	1816	0.19	8969	1957	0.22
Day 10	6511	7588	1.23	10080	2789	0.28
Day 17	8015	7794	1.14	10896	3719	0.36
Day 22	9602	6799	1.14	12589	3507	0.28
Day 31	10459	6950	0.99	10449	5822	0.64
Day 40	11057	6282	0.86	9682	5395	0.69
PCM + Slurry						
Day 1	9414	1769	0.18	8301	1552	0.19
Day 10	8088	8765	1.12	11255	2555	0.22
Day 17	9232	8830	1.07	13965	3098	0.22
Day 22	11663	7147	0.89	16487	3833	0.23
Day 31	12135	7668	0.86	13556	6669	0.50
Day 40	11529	7475	1.02	13859	7406	0.54

	Two phase (primary reactor)			Two phase (secondary reactor)		
	PA	**IA**	**AR**	**PA**	**IA**	**AR**
PCM + Water						
Day 1	8714	1789	0.22	8695	1968	0.22
Day 10	8495	6454	1.30	9551	1754	0.17
Day 17	4774	9084	2.00	11065	2522	0.23
Day 22	5635	10962	1.94	13285	2763	0.21
Day 31	4522	10367	2.34	11458	4669	0.42
Day 40	4477	11575	2.76	10785	6984	0.67
PCM + Slurry						
Day 1	9372	1740	0.19	8892	1823	0.19
Day 10	8518	6379	0.75	10212	1822	0.18

Day 17	7161	11310	1.59	11715	2629	0.23
Day 22	7399	13075	1.77	14316	3532	0.25
Day 31	6205	12026	1.93	12335	6254	0.52
Day 40	6539	13825	2.15	12617	7332	0.58

Table 15:
Effect of feedstock and AD process on reactor volatile fatty acid (VFA, mmols/l) and ammonium nitrogen (NH_4, g/l)) concentration.

	PCM + Water			PCM + Slurry			SED	Probability		
	B	C	T	B	C	T		Feed	AD	Int
VFA										
Day 10	235	35	89	254	18	208	16.6	**	***	**
Day 24	171	72	153	194	83	146	79.1	NS	NS	NS
Day 40	158	196	216	200	194	303	107.2	NS	NS	NS
NH_4										
Day 10	5.6	4.1	4.6	7.2	4.4	4.8	0.59	0.066	**	NS
Day 24	7.0	5.4	5.7	8.5	7.1	6.0	0.89	*	*	NS
Day 40	8.0	6.8	7.3	9.6	8.1	8.1	0.84	*	NS	NS

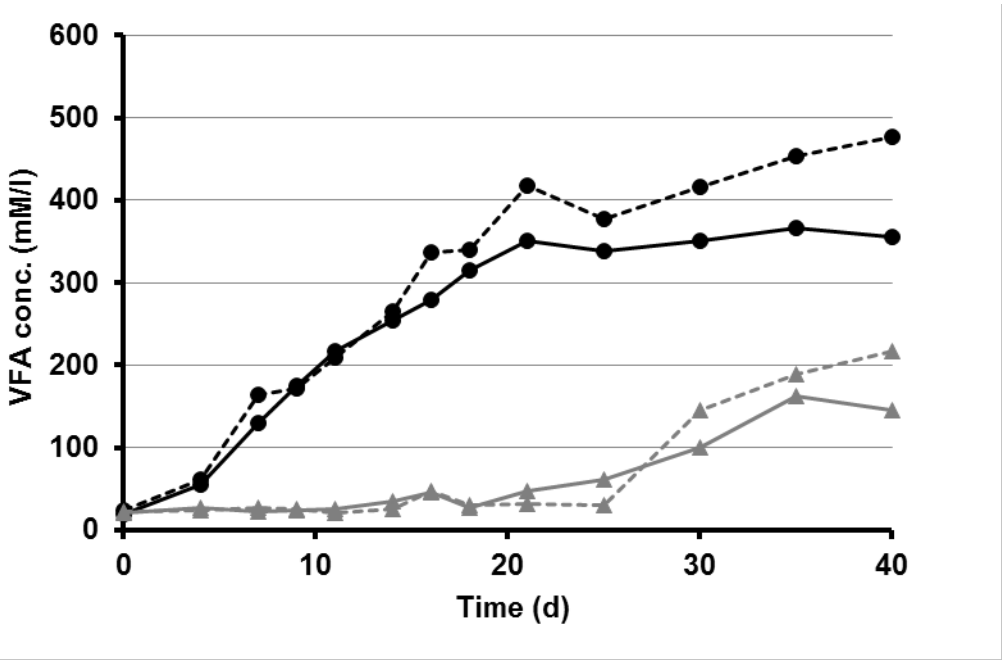

Figure 2:
Effect of feedstock and AD process on reactor VFA concentration in the primary and secondary reactors of the two phase treatments (PTW primary = black solid, PTW secondary = grey solid, PTS primary = black dashed, PTS secondary = grey dashed).

The effects of feedstock composition and AD process on reactor stability and presented in tables 13, 14 and 15, and figures 1 and 2. There were no significant interactions or effects of feedstock composition on reactor pH. However, throughout the experiment reactors operating the continuous process maintained a higher pH than those operating either the batch or two phase processes. In the two phase reactors, pH dropped quickly in the primary reactors, but was maintained at a higher and relatively constant level in the secondary reactors. The VFA concentration in reactors operating the batch process increased quickly during the loading period (days 1-10), such that by 10 the batch reactors had a significantly higher ($P<0.001$) VFA concentration than those operating the continuous or two stage processes. However, during the experiment, VFA concentration increased in the continuous and two phase reactors, such that by day 40 there was no significant difference between treatments. There was a significant effect ($P<0.05$) of feedstock composition on reactor NH_4, with reactors offered PCM + slurry having a higher NH_4 concentration than reactors offered PCM + water. Similarly, there was a significant effect ($P<0.01$) of AD process on reactor NH_4 concentration, with reactors operating the batch process having a higher NH_4 concentration than those operating the continuous or two phase processes. The AR provides a measure of stability of the fermentation process. A ratio >1.0 indicates that VFA production is greater than buffering capacity and could result in failure of the fermentation process. Throughout the experiment AR was influence by AD process, but there were no real effects of feedstock composition. Reactors operating the

continuous and two phase AD processes had an AR <1.0 throughout the experiment. As expected, in the two phase reactors, the AR was >1.0 in the primary reactors, but <1.0 in the secondary reactors. As with reactor performance (section 5.3.1), considerable variation in reactor stability was observed between periods 1 and 2, with higher stability being observed in period 2. This was attributed to the different source materials used and highlights the importance of source material in AD. The gradual increase in VFA concentrations and AR in the continuous and two stage processes indicates that the loading rate of 2.2 g/l/day may be too high and could lead to process instability. A similar observation was reported by Bayr et al. (2012) during mesophilic co-digestion of rendering a slaughterhouse waste at a loading rate of 2.0 g/l/day.

5.4 Conclusion:

The results of experiment 2 indicate that PCM pre-treated by sterilisation can be effectively fermented by AD with significant levels of gas production. Feedstock composition had no effect on reactor performance, but biogas production and PCM reduction were maximised in reactors operating the continuous AD process. Reactor stability was high in both the continuous and two phase processes, but the gradual increase in VFA concentration and AR suggest that at a loading rate of 2.2 g/l/day these reactors could potentially become unstable over a longer period of time. There was considerable variation in reactor performance between periods 1 and 2 suggesting that source material and adaption to feed stock has a major effect on fermentation of PCM.

6.0 Overall conclusions:

The results of the current project indicate that PCM can be effectively digested by AD with high levels of biogas and CH_4 production, and carcase reduction. The optimum protocol for AD of PCM consisted of a one stage continuous process operated at 35 °C with an OM loading rate of <2.2 g/litre/day and a retention time of approximately 40 days. Some form of pre-treatment by sterilisation will be required to ensure adequate levels of pathogen destruction. Pig slurry can be used as a co-substrate. Prior to submission to EFSA for consideration of AD as an alternative system for disposal of category 2 PCM further work is required to test long term stability proposed protocol in longer term bench top studies (3-4 hydrolytic retention times). In addition, the results of the current study can be used to design a pilot scale system, which can then undergo full economic and environmental evaluation in a desk top modelling exercise. If the proposed systems proved to be economically and environmentally viable the next stage would be development and testing of an on-farm pilot scale system and the generation of data and a HACCP plan required to inform an EFSA submission.

7.0 Literature review on alternative methods of ABP disposal:

7.1 Current methods:

Following the Bovine Spongiform Encephalopathy (BSE) outbreak in cattle, animal by-products (ABP) were classified by European legislation (EC, 2009) according to risk. Category 1 materials include animals, or parts of animals infected with, or potentially infected with transmissible spongiform encephalopathy, and include skulls, brains, tonsils, spinal cord, intestines, entire heads and the vertebral column of bovine animals. Category 1 material must be disposed of by incineration, either directly, or following rendering (133 °C at 300 kPa for 20 minutes). Category 2 materials mainly include fallen stock, slaughterhouse waste and digestive tract content. Following rendering, category 2 material may be used in composting, biogas production (following pressure sterilisation) and the oleo-chemical industry. Category 3 materials represent material that has been derived from carcases which have been passed as fit for human consumption, or which do not show signs of communicable disease. Category 3 material may be used for a wide range of purposes including pet food production.

Article 20 of the ABP Regulations (EC1069/2009) provides scope for the consideration of alternative processes for ABP disposal if there is sufficient evidence that the process is equivalent to currently accepted methods in relation to reduction of risks to public and animal health. Alternative methods can either be methods that prevent pathogen proliferation, prior to disposal by currently accepted methods, or methods where the degree of risk reduction is equivalent to currently approved methods. However, there is currently considerable uncertainty in relation to the meaning of ‘equivalence’. To be accepted as an alternative process for ABP disposal a dossier of evidence needs to be submitted by a competent authority for consideration by the European Food Standards Agency (EFSA) Panel on Biological Hazards. The alternative system must be a closed system to minimise the risk of infection. In addition, all the risks agents in the category 2 material must be identified and controlled, and the submission must include a full hazard analysis and critical control point (HACCP) plan. Over the last few years a number of submissions by EU member states have been made to EFSA for approval as alternative methods for ABP disposal with various outcomes as follows:

7.2 Approved alternative containment methods:

7.2.1 Aerobic maturation and storage of dead pigs:

This process has recently been approved for use in France, Ireland, Latvia, Portugal and the United Kingdom, where authorised by the competent authority for containment of category 2 and 3 porcine material (EU 2015/9). The process must be carried out in in a closed system consisting of several cells, with a waterproof floor, delimited by solid walls and consists of two phases. During the filling phase, a 30 cm layer of sawdust is placed on the floor of a cell. A layer of porcine material is then placed on the sawdust, followed by successive layers of sawdust (30 cm) and porcine material until the cell is full. During the maturation phase, which must last 3 months, the temperature rise facilitates the degradation of porcine material. During the maturation phase the temperature must be monitored using an automatic recording device and reach 55 C for a minimum of three consecutive days. The resulting material can be disposed of by incineration or co-incineration.

7.2.2 Hydrolysis and subsequent disposal:

This process has recently been approved for use in Ireland, Spain, Latvia, Portugal and the United Kingdom, where authorised by the competent authority for containment of category 2 and 3 porcine material (EU 2015/9). The process must be carried out in a closed, waterproof, corrosion proof and hermetically sealed container, fitted with appropriate filters to prevent the transmission of diseases to humans and animals. The container must have be sited on a separate site to the holding, must have dedicated access and constructed in accordance with EU legislation for protection of the environment. The container must be operated in batch mode and closed for a period of a least three months, such that unauthorised opening is prevented. Following hydrolysis, the material must be collected and disposed of in accordance with EC 1069/2009.

7.3 Approved alternative disposal methods:

7.3.1 High temperature and pressure alkaline hydrolysis:

Alkaline hydrolysis (AH) is a process that uses sodium or potassium hydroxide to catalyse the hydrolysis of organic compounds at a temperature of 150 °C to produce sterile simple sugars, peptides, amino acids and soaps (CAST, 2008). The solid residue (approximately 2%) consists of sterile inorganic material that is easily crushed and can be used as a soil additive. The liquid residue can be disposed of in accordance with local regulations in relation to pH and temperature of effluents. Alkaline hydrolysis is carried out in a pressure vessel with a tight lid. The vessel is loaded with carcase material and the lid shut. The required alkali is added together with process water, followed by the application of heat via a steam jacket and steam heating coils (Bio-safe Life Sciences, 2015). The time required for AH of carcase material is 3-6 hours. The protein coat of viruses are destroyed and prions are deactivated due to the combination of high temperature and alkaline conditions (Taylor, 2011).

An application for consideration of AH by Waste Reduction Europe Ltd was approved for category 2 and 3 ABP in June 2002 (ESSC, 2003a) with the following conditions. Either sodium hydroxide (NaOH) or potassium hydroxide (KOH) in an amount that assumes approximate molar equivalency to the weight, type and composition of ABP digested is used. The ABP are heated to a core temperature of 150°C for at least 3 hours without interruption at a pressure of at least 400 kPa. The process is carried out in batch and the material in the vessel is constantly mixed, such that the requirements in relation to time, temperature and pressure are achieved simultaneously. In a single experiment AH was shown to reduce TSE/BSE infectivity by $10^{3.5}$-$10.^{4.5}$. However, further studies on the combination of heat, pH and time were required before any final assurance could be given in respect of the safety of the process with respect to TSE risks.

7.3.2 High pressure, high temperature hydrolysis (APHTH)

High pressure, high temperature hydrolysis is a process used to catalyse the hydrolysis of whole animal carcases, meat and bone meal, food processing waste and other compostable materials. The process is carried out in a reactor heater to 180 °C at 1200 kPa for 40 minutes, with heating being applied by indirect steam application to the reactor. The process is carried out in a batch mode with the material being constantly mixed, such that requirements in relation to time, temperature and pressure are achieved simultaneously.

An application for consideration of HPHTH submitted by the Biosphere Refinery Cooperation was approved for treatment of category 2 and 3 ABP in April 2003 (ESSC, 2003b) as the process ensures that temperature and pressures in excess of 133 °C and 300 kPa are achieved. However, it was not

considered safe for treatment of category 1 ABP.

7.3.3 High pressure hydrolysis biogas (HPHB)

High pressure hydrolysis biogas is a multi-stage process involving conventional rendering and ABP (133 C at 300 kPa for 20 minutes) followed by high pressure hydrolysis (220 °C at 2500 kPa for 20 minutes). The resulting solid products are heated in a two-stage process, first by direct steam injection, and secondly using a co-axial heat exchanger. The process is carried out in batch or continuous mode with the material being constantly mixed such that the requirements in relation to time, temperature and pressure are achieved at the same time. The dissolved fraction of the process is then fermented in an aerobic reactor, with the biogas being used to produce electricity.

An application for consideration of HPHB submitted by the Fraunhofer Institute for Environmental Safety and Energy Technology was approved for treatment of category 2 and 3 ABP in April 2003 (EFSA BIOHAZ Panel, 2003). However, the solid and liquid waste arising from the process must be burnt, or disposed of in way that is already approved for conventional rendering, such that combination of rendering with the HPHB process presents no additional risks when disposing of category 1 ABP.

7.3.4 Biodiesel production:

This process involves conventional rendering, followed by the use of rendered fat to produce biodiesel. An application for consideration was submitted by Saria Bio-Industries GnbH & Co KG in April 2003 (ESSC, 2003b). The process was considered to be a sound approach, but the submission did not clearly show a reduction in BSE infectivity. It was concluded that the process was considered safe for category 2 and 3 rendered fat, but could not be considered safe for category 1 rendered fat until more reliable and precise data on the capability of the process to effectively reduce BSE infectivity was available.

7.4 Non-approved alternatives:

7.4.1 On-farm treatment of pig carcases:

This on-farm process was designed to reduce the costs associated with disposal of pig farm mortalities, reduce the hazards associated with transporting potentially infected carcases and provide a useful product that would otherwise have been destroyed. The process is a batch process consisting of pre-heating pig carcase material in a boiling tank. The material is then minced to a particle size of <150 mm and heated to 100 °C for 10-12 hours. The process is conducted in 'dissipaters' (boilers) heated by circulation of oil within the wall of the equipment. The intention was that the end product would be mixed with slurry prior to application to land as a fertiliser, or being used in biogas production.

An application for consideration of the process submitted by the Italian Competent Authority was rejected for category 2 pig material (EFSA BIOHAZ Panel, 2011). The Panel noted problems in the dossier relating to the experimental approach, the level of pathogen reduction and the absence of a formal HACCP plan. In conclusion, the Panel noted some deficiencies in the level of risk reduction, and that the process had not been validated under real scale conditions. The proposed process cannot be considered equivalent to pressure sterilisation. This would be particularly relevant in the case where more heat resistant spores than *C. perfringens may be present*. The panel recommended that to assess alternative methods, the relevant hazards and their level of inactivation to be targeted should be specified in a more precise and detailed way. In addition, test organisms with defined resistance patterns should be specified and the required level of quantitative risk reduction of such organisms should also be provided.

7.4.2 Compositing and incineration of pig carcases:

This process is carried out in cells with a waterproof floor delimited on three sides by a brick wall under a water proof roof and protected by a wildlife proof fence. Four cells are intended. Two primary cells, where the first phase of carcase degradation takes place, and two secondary cells, where the material is moved after the first and second turning of the pile. All the cells are connected by a drainpipe to collect any effluent. A 30 cm layer of sawdust is placed in a primary cell followed by PCM, which is then covered by a 20 cm layer of sawdust. The cell is filled with 5-6 alternate layers of sawdust and PCM and left for 3 months. During this period the temperature is allowed to rise to facilitate the decomposition of soft tissue. At the end of three months the compost pile is turned and moved to a secondary cell 1 for period of five weeks. At the end of this period the compost is turned again and moved to secondary cell 2 for a further five weeks. At the end of the process the resulting compost is considered to be a stable product, which does not emit odour that can be stored prior to incineration either on or of the farm.

An application for consideration of the process submitted by the French Competent Authority was rejected for category 2 pig material (EFSA BIOHAZ Panel 2012a). The Panel noted concerns in relation to risk containment during composting and storage, bio-security and the fact that no HACCP plan was presented. In particular, the temperatures reached during composting were not sufficient to inactivate the relevant pathogens, thus the final compost must be considered to be category 2 ABP and stored accordingly. However, since the compost was intended for incineration the final step of the process would destroy all pathogens. In addition, as there are no solid walls, doors or floors around the buildings the process cannot be considered a closed system. In conclusion, the Panel stated that provided that the mentioned concerns where addressed and that the composting and processing steps take place in a closed system under the supervision of the competent authority, this alternative method would not present an additional risk compared to currently approved processes.

7.4.3 'Biomation':

Biomation is based on alkaline hydrolysis. The process is a batch process involved mincing ABP to a <5.0 mm and transferring the minced material to a refrigerated holding tank. Sodium hydroxide (15% w/w) is then added until a pH of 12.5 is reached, followed by heating to a temperature of 70 °C for 20 minutes. The resultant product is then neutralised by the addition of acrylic acid to decrease the pH to 7.0-7.7 C for 20-30 minutes, followed by polymerisation by the addition of ammonium persulfate (0.5% w/w) for a further 10 minutes, all at 70 °C. The resultant product is then tunnel dried at 200-220 °C and could potentially be used as a fertiliser or soil improver, with the waste water being disposed of according to current legislation.

An application for consideration of Biomation submitted by the UK Competent Authority was rejected for category 2 and 3 ABP (EFSA BIOHAZ Panel 2012b). The panel noted concerns in relation to risk reduction, biosecurity and the HACCP plan. In particular, information on the level of risk reduction was only based on the literature and two laboratory scale experiments. In addition, the fate of exhaust gases and water generated during the process was not specified and the HACCP plan did not specify critical parameters for the process, or procedures for cleaning and disinfection. In conclusion, the Panel stated that to assess alternative methods, the relevant hazards and their level of inactivation to be targeted should be specified in a more precise and detailed way. In addition, test organisms with defined resistance patterns should be specified and the required level of quantitative risk reduction of such organisms should also be provided.

7.4.4 Aerobic bio-reduction:

Aerobic bio-reduction consists of the aerobic degradation of ABP in a vented, leak proof vessel containing water, which is buried in the ground (bio-reducer). The contents of the vessel are heated to 30-42 °C and aerated at 40 – 55 kPa to produce mesophilic aerated environment. The moist and warm conditions create a favourable environment for bacterial degradation of ABP, resulting in their partial breakdown and volume reduction, through the loss of water vapour. The gas generated within the bio-reducer are vented to the atmosphere through a bio-filter consisting of woodchips and compost. The resulting liquor is collected and disposed of as category 1 ABP, with the frequency of collection being dependent on usage of the bio-reducer.

An application for consideration of aerobic bio-reduction submitted by the UK Competent Authority was rejected for treatment of category 2 and 3 sheep ABP (EFSA BIOHAZ Panel, 2013). The Panels noted a number of concerns relating to the extent of pathogen reduction, biosecurity of the systems and the HAACP plan. In conclusion, the Panel noted that the process could reduce the risks of pathogens having similar survival patterns to the test organisms. However, it is highly improbable that the risks relating to more resistant agents (e.g. bacterial spores and TSE agents would be reduced using this method. The proposed bio-reduction system cannot be considered as a safe alternative method for on-farm containment of ABP.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

1. Ahring, B. K. (2003). Perspectives for anaerobic digestion: Fatty acids inhibit anaerobic growth of Escherichia coli. *Biotechnology Letters* **7 (2)**: 125-128.
2. Anderson, G.K. (1992). Determination of bicarbonate and total volatile acid concentration in anaerobic digestors using a simple titration. *Water Environment Research* **64 (1)**: 53-59.
3. Bayr, S. Pakarinen, O., Korppoo, A., Liuksia, S., Vaisanen, A., Kaparaju, P. and Rintala, J. (2012a). Effect of additives on process stability of mesophilic anaerobic mono-digestion of pig slaughterhouse waste. *Bioresource Technology* **120**: 106-113.
4. Bayr, S., Rantanen, M., Kaparaju, P. and Rintala, J. (2012b). Mesophilic and thermophilic anaerobic co-digestion of rendering plant and slaughterhouse waste. *Bioresource Technology* **104**: 28-36.
5. Bio-safe Life Sciences (2015). *Alkaline Hydrolysis* [on-Line]. Available from http://www.biosafelifesciences.com/alkaline_hydrolysis
6. BPEX, (2011). *Industry statistics annual technical report 2010-11* [on-line]. Available from http://pork.ahdb.org.uk/.
7. CAST (Council for Agricultural Science and Technology). (2008). *Swine carcase disposal options for routine and catastrophic mortality* [on-line]. Available from http://www.cast-science.org/download.cfm?PublicationID=2938&File=1030fbf4cb23eee81c1a3347e60173855e60TR
8. Chen, Y., Cheng, J.J. and Creamer, K.S. (2008). Inhibition of anaerobic digestion process: A review. *Bioresource Technology* **99 (10)**: 4044-4064.
9. Cuetos, M.J., Gomes, X., Otero, M. and Moran, A. (2008). Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale: Influence of co-digestion with the organic fraction of municipal solid waste (OFMSW). *Biochemical Engineering Journal* **40 (1)**: 99-106.
10. Cuetos, M.J., Gomez, X., Otero, M. and Moran, A. (2010). Anaerobic digestion and co-digestion of slaughterhouse waste. Influence of heat and pressure pre-treatment on biogas yield. *Waste Management* **30 (10)**: 1780-1789.
11. EC Commission Regulation (EC) No 1069/2009 of 21st Oct 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). *Off J. Eur Communities 2009*.
12. EC Commission Regulation (EU) 2015/9. Amending Regulation (EU) No 142/2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive. *Official Journal of European Union 2015*
13. Escudero, A., Lacelle, A., Blanco, F., Pinto, M., Diaz, I. and Dominguez, A. (2014). Semi-continuous anaerobic digestion of solid slaughterhouse waste. *Journal of Environmental Chemical Engineering* **2 (2)**: 819-825.
14. EFSA BIOHAZ Panel. (2003). Opinion of the Panel on Biological Hazards of the European Food Safety Authority on the process of high pressure hydrolysis biogas (HPHB) as a method for disposal of category 1 animal by-products (ABP) not intended for human consumption. *EFSA Journal* **11**: 1-4.
15. EFSA BIOHAZ Panel. (2011). Scientific opinion on on-site treatment of pig carcasses. *EFSA Journal* **9 (11:2425)**: 1-11.
16. EFSA BIOHAZ Panel. (2012a). Scientific opinion on compositing and incineration of dead on-farm pigs. *EFSA Journal* **10 (2:2559)**: 1-11.
17. EFSA BIOHAZ Panel. (2012b). Scientific opinion on the biomation application for for an alternative method for the treatment of animal by-products. *EFSA Journal* **10 (3-2614)**: 1-12
18. EFSA BIOHAZ Panel (2013). Scientific opinion on the bioreduction application. *EFSA Journal* **11 (12:3503):** 1-13.
19. ESSC. (2003a). Final opinion and report on the treatment of animal waste by means of high temperature (150 °C, 3 hours) and high pressure alkaline hydrolysis. *Journal European Scientific Steering Committee*: 1-26.
20. ESSC (2003b). Opinion on six alternative methods for safe disposal of animal-by-products. *Journal European Scientific Committee*: 1-30.
21. Foster-Carneiro, T., Perez, M., Romero, L.I. and Sales, D. (2007). Dry thermophilic anaerobic digestion of organic fraction of municipal solid waste: Focusing on the inoculum source. *Bioresource Technology* **98 (17)**: 3195-3203.
22. Feitkenhauer, H., von Sachs, J. and Meyer, U. (2002). On-line titration of volatile fatty acids for the

process control of anaerobic digestion plants. *Water Research* **36**: 212-218.
23. HAU-BPEX. (2004). *Anaerobic digestion of porcine carcase material*. BPEX report.
24. Hejnfelt, A. and Angelidaki, I. (2009). Anaerobic digestion of slaughterhouse by-products. *Biomass and Bioenergy* **33 (8)**: 1046-1054.
25. Kirby, M. (2012). *Evaluation of the disposal methods of fallen stock, with the development of anaerobic digestion as a novel on-farm storage and bio-reduction process*. Ph.D thesis submitted to Harper Adams University.
26. Kunte, D. P., Yorle, T.Y. and Ranade, D.R. (2004). Two stage anaerobic digestion process for complete inactivation of enteric bacterial pathogens in human night soil. *Water Science and Technology* **50 (6)**: 103-108.
27. MAFF, (1986). *The Analysis of Agricultural Materials*. MAFF Agricultural Development Advisory Service, reference book 427. Her Majesty's Stationary Office, London.
28. Masse, D.I., Masse, L., Hince, J.F. and Pomar, C. (2008). Psychrophilic anaerobic digestion biotechnology for swine mortality disposal. *Bioresource Technology* **99**: 7307-7311.
29. Novak, D., Stres, B., Osojnik, G., Skrjanec, I and Logar, M.R. (2011). General microbial community flexibility in biochemical methane potential assay is highly correlated to initial biogas production rates. *Acta Chim Slov* **58**: 171-175.
30. Richards, B.K., Cummings, R.J., White, T.E. and Jewell, W.J. (1991). Methods for kinetic analysis of methane fermentation in high solids biomass digesters. *Biomass and Bioenergy* **1(2)**: 65-73.
31. Salsali, H., Parker, W. and Satter, S. (2008). The effect of volatile fatty acids on the inactivation of clostridium perfringens in anaerobic digestion. *World Journal of Microbiology and Biotechnology* **24 (5):** 659-665.
32. Smith, S.R., Lang, N.L., Cheung, K.H.M. and Spanoudaki, K. (2005). Factors controlling pathogen destruction during anaerobic digestion of biowastes. *Waste Management* **25 (4)**: 417-425.
33. Salminen, E. and Rintala, J. (2002). Anaerobic digestion of organic solid poultry slaughterhouse waste – a review. *Bioresource Technology* **83**: 13-26.
34. Taylor, D.M. (2011). *Equipment marketed by Waste Reduction Europe Ltd to destroy animal carcasses and tissues by alkaline hydrolysis as reported in NABCC-USDA (2004), carcase disposal a comprehensive review.* USDA, USA.
35. Zhang, Y. and Banks, C.J. (2012, Co-digestion of the mechanically recovered organic fraction of municipal solid waste with slaughterhouse waste. *Biochemical Engineering Journal* **68**: 129-137.