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Esa Salminen

Anaerobic Digestion of Solid Poultry Slaughterhouse By-products and Wastes

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UNIVERSITY OF JYVÄSKYLÄ

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ABSTRACT

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Anaerobic digestion of solid poultry slaughterhouse by-products and wastes
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Diss.

The main objective of this work was to evaluate the viability of anaerobic digestion of solid poultry slaughterhouse by-products and wastes. In the biochemical methane potential batch assays blood, offal, and feather, showed methane yields of 0.6-0.7, 0.5, 0.7-0.9, and 0.2 m^3/kg volatile solids $(VS)_{added}$, respectively, and the mixture produced methane 0.6-0.7 m^3/kg VS_{added}. Combined thermal and enzymatic pre-treatments increased the methane yield of feather by 37 to 51 %, whereas thermal, chemical, and enzymatic treatments were less effective with methane yield increasing 5 to 32 %. The anaerobic degradation patterns of the mixture in batch assays indicated rapid hydrolysis/ acidogenesis, accumulation of long-chain fatty acids (LCFAs) and volatile fatty acids (VFAs), removal of LCFAs and subsequently that of VFAs, and methane production. The dynamic modelling of the results from the assays suggested that inhibited propionate degradation by LCFAs and inhibited hydrolysis by a high propionate concentration constituted the rate-limiting steps in the degradation. The anaerobic digestion of the mixture in semi-continuously fed laboratory-scale digesters appeared sustainable with a loading of up to 0.8 kg VS/m^3 d and a hydraulic retention time (HRT) of 50 days, showing a methane yield of up to 0.55 m^3 of methane/kg VS_{added}. At loadings from 1.0 to 2.1 kg VS/m^3 d, and HRTs from 12.5 to 25 days, the digester performance was inhibited. In the batch assays the accumulated LCFAs appeared the main factor affecting the slow recovery of the digester from inhibition. LCFAs floated on top of the digester, which could have affected their bioavailability and toxicity. The digested material was found to be rich in nitrogen, with ca. 20 % N of total solids (TS), mostly in the form of ammonia. Vascular plant growth assays showed the digested material to be potentially phytotoxic, apparently mainly because of VFAs and LCFAs present in it. Furthermore, aerobic post-treatment reduced the phytotoxicity. The review of the potential of and experiences with anaerobic digestion of solid poultry slaughterhouse by-products and wastes suggested that anaerobic digestion of these materials can be viable when operation conditions are carefully optimised.

Key words: Ammonia; anaerobic digestion; inhibition; long-chain fatty acids; nutrients; plant growth assays; poultry slaughterhouse; waste.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is a summary and discussion of the following articles and manuscripts, which are referred to by their Roman numerals in the text.

I Salminen, E., Einola, J. & Rintala, J. 2000. The methane production of poultry slaughtering residues and effects of pre-treatments on the methane production of poultry feather. J. Chem. Technol. Biotechnol. (submitted).

II Salminen, E., Rintala, J., Lokshina, L.Ya., & Vavilin, V. 2000. Anaerobic batch degradation of solid poultry slaughterhouse waste. Water Sci. Technol. 41(3): 33-41.

III Salminen, E. & Rintala, J. 1999. Semi-continuous anaerobic digestion of solid poultry slaughterhouse waste: effect of hydraulic retention time and loading. Water Res. (submitted).

IV Salminen, E., Einola, J. & Rintala, J. 2000. Characterisation and anaerobic batch degradation of materials accumulating in anaerobic digesters treating poultry slaughterhouse waste. Environ. Technol. (accepted for publication).

V Salminen, E., Rintala, J., Härkönen, J., Kuitunen, M., Högmander, H. & Oikari, A. 2000. Anaerobically digested solid poultry slaughterhouse wastes to be used as fertiliser on agricultural soil. Biores. Technol. (in press).

VI Salminen, E. & Rintala, J. 2000. Anaerobic digestion of solid poultry slaughterhouse by-products and wastes – a review. (manuscript).

ABBREVIATIONS

COD	chemical oxygen demand
HRT	hydraulic retention time
LCFAs	long-chain fatty acids
SS	suspended solids
TS	total solids
UASB	upflow anaerobic sludge blanket
VFAs	volatile fatty acids
VS	volatile solids
VSS	volatile suspended solids

1 INTRODUCTION

In the past few decades, poultry meat products have been gaining in popularity and presently constitute a significant part of all meat consumption (Finnish Food & Drink Industries' Federation 2000). The slaughtering of poultry has also changed significantly in the past decades as the industries have strived to improve processing efficiency. Today poultry are often processed in highly automated plants specially designed to slaughter and process poultry, which typically slaughter tens of thousands of birds per day. As a result, poultry slaughterhouses are producing increasing amounts of organic solid by-products and waste (Fig. 1). These materials are mostly used as a protein source for animal feed (Fig. 2, VI).

Now, because of legal restrictions, rising treatment costs, and environmentally conscious consumers, the treatment of some solid by-products and wastes has emerged as a major concern in poultry slaughterhouses. In addition, legislation has restricted the disposal of materials in landfill, while disposal costs have been constantly rising, reflecting the legislators' "polluter pays" principle. Feather, in particular, poses a major concern for poultry slaughterhouses because it is poorly degradable in its natural state and thus unsuitable for use in animal feed (Papadopoulos 1985; Boushy & van der Poel 1990; Onifade et al. 1998).

Anaerobic digestion is a promising alternative for the treatment of organic solid poultry slaughterhouse by-products and wastes, offering an attractive alternative for processing these materials into valuable products: methane, a combustible fuel, and digested material with potential use in agriculture. Anaerobic digestion also reduces pathogens and minimises odour while allowing most nutrients to remain in the digested material (reviewed by Shih 1987; 1993).

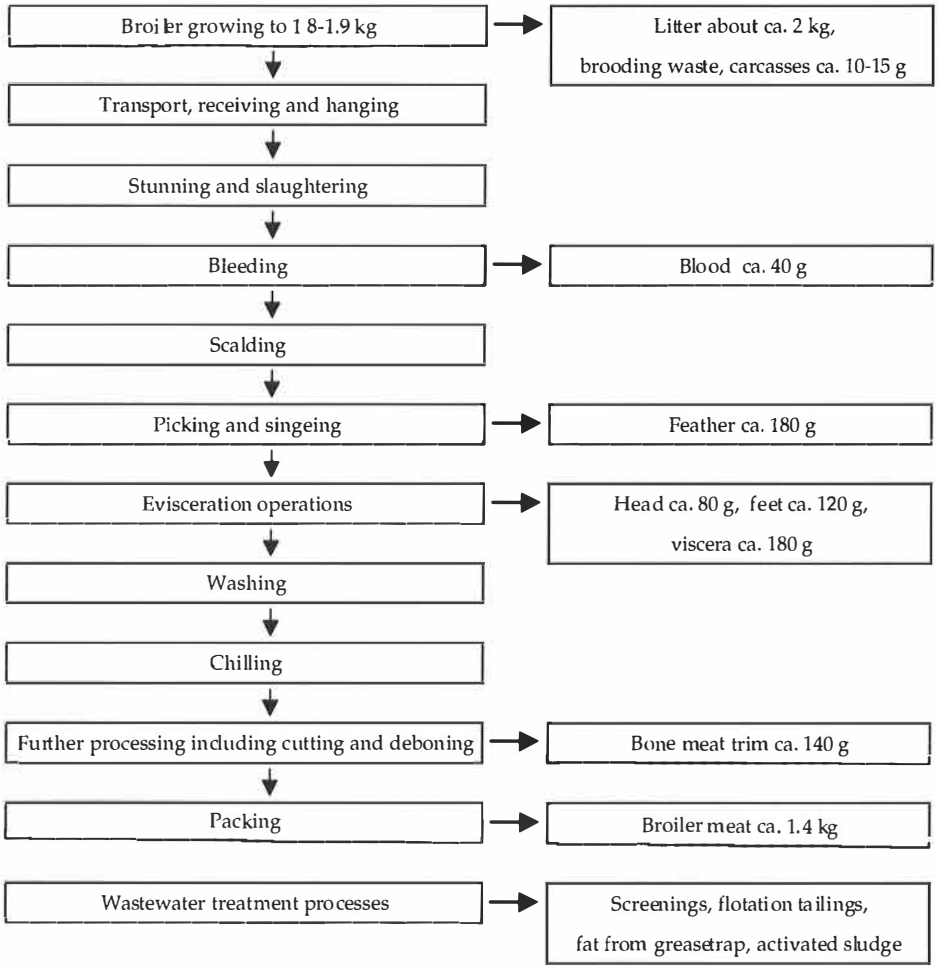

FIGURE 1 Organic solid materials (per broiler) produced in broiler growing and slaughtering (VI).

Little literature exists on the anaerobic degradability and methane yield of organic solid by-products and wastes produced in poultry slaughterhouses. Such information is crucial in the design and economic evaluation of anaerobic digestion treatment processes. Furthermore, as solid slaughterhouse by-products and wastes can differ greatly in both their chemical and physical characteristics, one has to be careful in generalising about their characteristics and methane production.

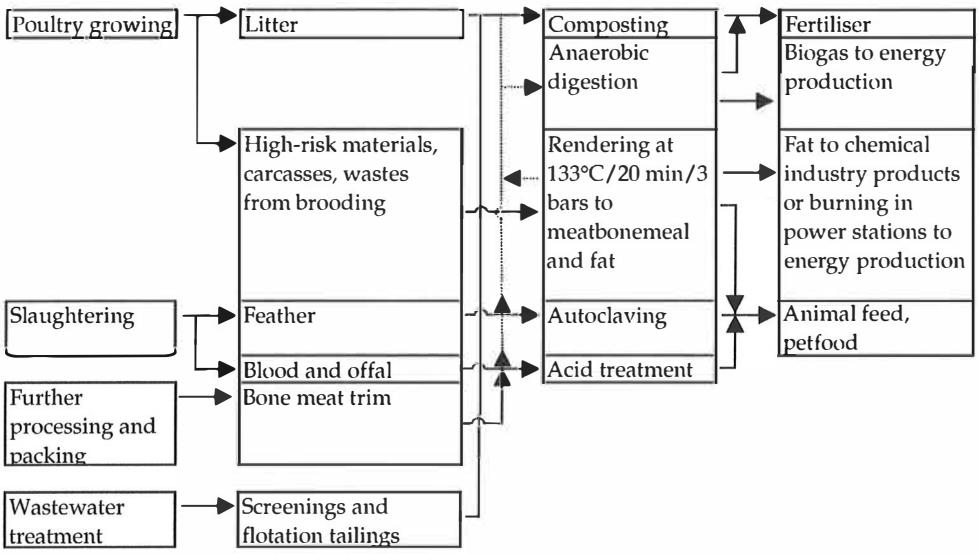

FIGURE 2 Current recovery and disposal of organic solid by-products and wastes produced in poultry growing and slaughterhouses in Finland and the option of anaerobic digestion for the recovery of these materials (dotted) (VI).

Feather is a challenge to anaerobic digestion due to its poor degradability under anaerobic conditions (Williams & Shih 1989). Various pre-treatments, which have been shown to improve the nutritive value of feather in animal feed (Papadopoulos 1985; Dalev 1994; Onifade et al. 1998), may also enhance its degradability by anaerobic micro-organisms, but according to our knowledge no studies have been reported on the subject.

Attempts to apply anaerobic digestion to treating solid slaughterhouse wastes alone have met with limited success (Banks 1994; Banks & Wang 1999). Success in treating these materials has been hampered mainly by the typically high protein and lipid content of the waste. Protein degradation produces ammonia, the unionized form of which is inhibitory to anaerobic microorganisms in high concentrations (e.g. Baere et al. 1984; Angelidaki & Ahring 1993; De Hansen et al. 1998). Lipids, on the other hand, may cause problems in anaerobic digestion because of their tendency to form floating scum and the accumulated LCFAs (Hanaki et al. 1981; Sayed 1987; Rinzema 1988; Broughton et al. 1998). LCFAs are intermediates of lipids degradation, the bacteriotoxicity of which has been well documented (Hanaki et al. 1981; Roy et al. 1985; Hwu et al. 1996; Hwu 1997). LCFA degradation (β-oxidation) is considered a limiting step in the anaerobic degradation of complex organic substrates (Novak & Carlson 1970; Hanaki et al. 1981; Rinzema 1988; Broughton et al. 1998), because of the slow growth of LCFAs consuming bacteria (reviewed

by Mackie et al. 1991) and because as syntrophic substrates, like VFAs, their anaerobic microbial degradation is limited by high H_2 partial pressure (reviewed by Zinder 1984; reviewed by Mackie et al. 1991). H_2 is produced in several steps in the anaerobic degradation of complex organic substrates and removed from the process mainly by H_2 consuming methanogens and some acetogenic bacteria (reviewed by Zinder 1984). Furthermore, in high concentrations LCFAs (Galbraith et al. 1971; Roy et al. 1986; Koster & Cramer 1987; Rinzema 1988; Hwu et al. 1996) and unionized VFAs (Lin et al. 1986; Fukuzaki et al. 1990) are inhibitory to anaerobic microorganisms. Consequently, to successfully prevent LCFAs and VFAs from accumulating in the anaerobic digestion of slaughterhouse wastes, the effect of loading, HRT, and feed TS in particular may be critical.

Furthermore, LCFAs may float on the top of the digester forming scum, and sediment may form on the bottom of the digester. Such stratification may result in a substrate gradient with mass transfer limitations affecting the bioavailibility and toxicity (Hobson & Wheatley 1988; Pagilla et al. 1997). In addition, when treating anaerobically potentially recalcitrant and toxic materials such as solid poultry slaughterhouse wastes screening for the most suitable inoculum and temperature conditions for treatment may be crucial. This is because inhibition by LCFAs correlates with the specific surface area of sludge (Hwu et al. 1996), whereas the pre-exposure of sludge to toxicants, including ammonia, may adapt sludge to tolerate higher toxicant concentrations (Hansen et al. 1998). Compared to mesophilic digestion, thermophilic digestion characteristically exhibits higher digestion rates and better pathogen destruction but requires more energy for heating; besides, the process is more sensitive to ammonia (Angelidaki & Ahring 1993) and LCFAs toxicity (Hwu 1997).

The possibility of an inhibited process makes the operation of an anaerobic digester treating poultry slaughterhouse waste both complex and demanding. It is therefore important to understand how the different operating parameters, conditions, and phenomena affect its performance and also how the digester recovers from a process failure. Studies on the subject will hence contribute particularly to understanding the actual treatment of slaughterhouse waste to ensure the feasibility of full-scale applications.

Experiments with anaerobic processes are time consuming, labour intensive, and expensive. In this regard, modelling can become a powerful tool for development and operation of such processes. <METHANE> is a generalised model of anaerobic digestion used successfully to describe the degradation of complex organic material (Vavilin et al. 1994).

The recovery of solid slaughterhouse by-products and wastes in agriculture conserves and recycles nutrients and reduces waste discharge and use of chemical fertilisers (Shih 1987; Marchaim et al. 1991). However, without sufficient treatment these materials may pose severe health risks, odour, and environmental pollution, or their use may be banned altogether by law. Treatment may also help improve the physical and chemical properties of these materials and reduce their phytotoxicity (Sudradjat 1990; Marchaim et al. 1991; Vermeulen et al. 1992).

Scanty information is available to assess the suitability of anaerobically

digested solid poultry slaughterhouse by-products and wastes as a fertiliser in agriculture. These could contain phytotoxic concentrations of potential plant growth inhibitors, VFAs and LCFAs (Lynch 1977; 1980; DeVleeschauwer et al. 1981; Manios et al. 1989; Marambe et al. 1993) or ammonia (Sudradjat 1990; Tiquia & Tam 1998). Aerobic post-treatment of anaerobically digested material may reduce the content of these inhibitors, but may also result in a loss of nitrogen through the volatilisation of ammonia (Sudradjat 1990; Rubæk et al. 1996). Phytotoxicity assays can strongly and holistically support chemical analysis in the evaluation of material (Baud-Grasset et al. 1993).

2 OBJECTIVES

The main objective of this work was to assess the viability of anaerobic digestion for the treatment of solid poultry slaughterhouse by-products and wastes.

The biological methane production yield and methane production rate of different poultry slaughterhouse by-products and wastes were investigated in batch assays at 55 °C with a thermophilic inoculum and at 35 °C with mesophilic granular and suspended inocula (I). Furthermore, the effects of different pre-treatments on feather methanation at 35 °C were investigated (I).

The anaerobic degradation patterns of the mixture of solid poultry slaughterhouse by-products and wastes (mixed in a ratio equivalent to that generated in the slaughterhouse) was investigated with different initial waste and inoculum concentrations and waste-to-inoculum ratios (II). Furthermore, the dynamics of the degradation process were simulated to assess the critical steps in degradation (II).

The anaerobic digestion of the by-product and waste mixture was studied in semi-continuously fed laboratory-scale digesters (III). Effects of hydraulic retention time and loading on performance of the process were investigated in order to optimize this process (III). Furthermore, materials accumulating in failed and stratified anaerobic digesters were characterized and the degradability of these materials was investigated to find factors limiting the recovery of the degradation (IV).

The suitability of digested poultry slaughterhouse by-products and wastes for fertiliser in agriculture was investigated by using chemical and physical analysis and vascular plant assays (V). Furthermore, the effects of aerobic post-treatment on the properties of the digested material were studied (V).

Information considered relevant in evaluating the potential of anaerobic digestion to material recovery and energy production from poultry slaughterhouse by-products and wastes was reviewed (VI).

3 MATERIALS AND METHODS

3.1 Wastes

The characteristics of the studied by-products and wastes are shown in Table 1. The solid poultry slaughterhouse by-product and waste mixture (see Table 1, I-III) was prepared as follows: The feather waste was autoclaved for about 5 minutes (120 °C). Materials were homogenised and mixed in the ratio generated in the slaughterhouse (bone and trimmings, 42; blood, 16; offal, 32, and feather, 10 % by weight) and frozen (-18 °C). Less than a week before its use the mixture was transferred to 4°C. A new feed in the digester studies was prepared daily by diluting the mixture with distilled water to the desired feed TS concentrations.

TABLE 1 Characteristics of poultry slaughterhouse by-products and wastes used in the studies.

By-product/waste	TS (%)	VS (%)	Kjeldahl-N (g-N/kg wet weight)	Protein (% of TS)	Lipids (% of TS)
Feather (I)	24	24	35	91	1-10
Blood (I)	22	20	17	48	2
Offal (I)	39	37	20	32	54
Bone and trimmings (I)	37	25	30	51	22
Mixture (I, II, III)	31	26	24	48	32

3.2 Pre- treatments of feather

Pre-treatments of feather (I) were carried out in 118-ml vials in triplicate with homogenised feather, placed 1.0 g/vial. The materials were treated and then stored at 4 °C until all treatments had been completed, i.e., for up to 24 h. Control vials with added feather were left untreated and kept at 4 °C for up to 24 h. One replicate of each treatment was then filled with distilled water to a volume of 60 ml (i.e., the liquid volume corresponding to that in methane production assays) and subsequently, after careful mixing, sacrificed for analysis, while the two other replicates were used immediately to assay methane production.

Thermal treatments were conducted by incubating homogenised feather, 1.0 g/vial, at 70 °C for 1 h or by autoclaving at 120 °C for 5 min.

Alkaline treatments were performed using sodium hydroxide in distilled water solution with a final volume of 10 ml. First, homogenised feather, 1.0 g/vial, was placed in vials. NaOH was solubilised in distilled water and added into the vials (final concentration in vial 2-10 g of NaOH/l, 8-40 g of NaOH/g feather TS) and mixed with the feather. The feather was then incubated statically in alkaline solution for 2 or 24 h at 35 °C. After that the pH was neutralised with 2 M HCl.

Enzymatic treatments were carried out using the Multifect P-3000® enzyme, a commercial alkaline endopeptidase from a genetically modified strain of *Bacillus subtilis*, used, for example, in protein processing and pet food production. The treatments were performed in distilled water solution with a final volume of 10 ml. First, homogenised feather, 1.0 g/vial, was placed in vials. An enzyme solution (activity of 2,750 GSU/g, Genencor, Finland) was then mixed in distilled water (2-10 g/l, 8-40% w/w feather TS) and added into the vials and mixed with the feather. Enzyme alone (without feather) was assayed to distinguish the performance of enzymes alone. The assays were then incubated statically for 2 or 24 h at 55 °C and at a pH of 8.5.

3.3 Inocula

Three different anaerobic sludges were used as inocula in the studies (I-V). A sludge from a mesophilic digester in the municipal sewage treatment plant (Viinikka, Tampere, Finland) was used as inoculum (I-V). In addition, a sludge from a thermophilic digester treating plant sorted municipal biowaste (Stormossen, Finland) and mesophilic granular sludge from a mesophilic UASB reactor in a starch sweetener plant (Jokioinen, Finland) were used (I).

An activated sludge from a municipal sewage treatment plant (Jyväskylä, Finland) was used as inoculum in the study to investigate the aerobic post-treatment of digested material (V).

3.4 Biochemical methane potential assays

The batch studies to assay the methane potential of different wastes (I) and to study the effect of initial waste and inoculum concentrations and waste-to-inoculum ratios on the waste mixture degradation (II) were conducted in triplicate in 118 ml vials with a liquid volume of 50-60 ml. The wastes as substrate were added to each vial. The VFA substrate (2 g/l, acetate 74% by weight, propionate 22% by weight, and butyrate 5% by weight) was added to control vials. Assays without added substrate were assayed to evaluate the performance of the inoculum alone. The inoculum was then transferred into the vials. $NaHCO_3$ (3 g/l) was added to the vials as buffer. Distilled water was added to complete the desired volume. The pH of the vials was adjusted to about 7.8. The vials were then flushed with N_2 / CO_2 (80%/20%) and sealed with butyl rubber stoppers and aluminium crimps. Finally, $Na_2S \cdot 9H_2O$ (0.25 g/l) was added to remove any residual O_2. The vials were then incubated in static (I) or shaken (II) cultures at 35 °C (I, II) or 55 °C (I). The gas samples were taken from the gas phase of the bottles by a pressure-lock syringe. Assays to study the batch degradation of the waste mixture (II) were run using 6 replicates with 4 vials sacrificed for analyses during incubation.

3.5 Semi-continuous digester experiments

The digester studies with the poultry slaughterhouse waste (III) were carried out in four identical, semi-continuously-stirred acrylic digesters (referred to as digesters 1, 2, 3, and 4), each with a total capacity of 3 l and a liquid volume of 2 l. The digesters were operated at 31°C and inoculated on day 0 with the mesophilic digested sewage sludge. Operation parameters are shown in Table 2.

All the digesters were usually fed every working day (normally 5 days a week) with the feed described above. Prior to feeding, digested material was removed with a pump from the digester to keep the digester volume constant. The content of digester 3 was diluted with water (50% of digester content) on day 100. The gas produced from the digesters was collected in aluminium gasbags from the gas phase of the digesters and its volume was measured by the displacement method.

TABLE 2 The operational parameters of semi-continuous poultry slaughterhouse waste digesters (see section 3.10 for the calculations, III).

Digester	Days	HRT (d)	Loading (kg VS/m^3 d)	Feed TS (%)
1	0-25	25	1.0	3.1
	26-95	13	2.1	3.1
2	0-25	50	1.0	6.2
	26-95	25	2.1	6.2
3	0-47	50	1.0	6.2
	51-59	50	0.5	3.1
	60-67	50	0.8	4.7
	68-137	50	0.5	3.1
	138-284	50	0.8	4.7
4	0-88	50	1.0	6.2
	89-137	100	0.5	6.2
	138-284	100	0.8	9.4

3.6 Batch experiments with digested materials

Materials (referred to as DM1 to DM4, Table 3) accumulating in the poultry slaughterhouse waste digesters (III) were characterised (IV) and the degradability of materials was investigated (IV). Materials were obtained from digesters 1-4 after they had been vigorously mixed manually for a minute. DM1 and DM2 were from digesters 1 and 2 operated prior to sampling for 72 days at an HRT of 13 days (feed TS 3.1%) and an HRT of 25 days (feed TS 6.2%), respectively, both at a loading of 2.1 kg VS/m^3 d. These digesters were considered failures in terms of their low methane production and high soluble COD values and LCFA and VFA concentrations. DM3 and DM4 were used as reference, and they originated from digesters 3 and 4 showing normal performance, as indicated by their stable soluble COD values and high methane production (III). Prior to sampling, the digesters were operated for 165 days at an HRT of 50 days (feed TS up to 4.7%) and an HRT of 100 days (feed TS 9.4%), respectively, both the digesters at a loading of 0.8 kg VS/m^3 d.

TABLE 3 Characteristics of digested materials from poultry slaughterhouse waste digesters (DM1 to DM9 and materials from different layers of digester, IV-V).

Parameter, unit	DM1	DM2	DM3	DM4	DM5	DM6	DM7	DM8	DM9	Whole material	Bottom layer	Middle layer	Top layer
TS (%)	2.1	2.5	1.2	2.3	0.9	1.0	1.4	0.9	1.2	2.8	2.6	0.9	6.1
VS (%)	1.8	1.9	1.0	1.9	0.7	0.7	1.2	0.7	0.9	2.1	2.0	0.7	4.9
Soluble COD (g/l)	10	15	4.8	9.3	0.6	1.4	4.3	0.7	2.2	6.2	6.0	6.0	6.3
Total COD (g/l)	42	42	Na[2]	Na	Na	Na	Na	Na	Na	33	28	8.7	29
Acetate (g/l)	4.3	11	2.7	3.7	0.08	0.5	1.8	0.01	1.1	3.0	3.2	2.9	3.9
Propionate (g/l)	1.8	1.7	0.25	0.64	<0.01	0.01	0.08	<0.01	0.04	0.12	0.13	0.12	0.16
Iso-butyrate (g/l)	0.45	0.82	0.10	0.20	<0.01	<0.01	0.02	<0.01	0.02	0.05	0.05	0.05	0.07
Butyrate (g/l)	0.86	0.89	0.02	0.74	<0.01	<0.01	0.01	<0.01	0.01	0.04	0.05	0.04	0.08
Iso-valerate (g/l)	0.82	1.4	0.09	0.22	<0.01	<0.01	0.05	0.01	0.02	0.12	0.12	0.11	0.15
Valerate (g/l)	0.55	0.38	0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
Caproate (g/l)	0.38	0.38	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.02
Myristate (g/l)	0.5	0.2	<0.1	<0.1	<0.1	<0.01	0.01	<0.01	0.01	<0.1	<0.1	<0.1	<0.1
Palmitate (g/l)	6	2	0.2	0.2	0.2	0.02	0.05	0.06	0.04	0.3	0.2	<0.1	2
Oleate (g/l)	0.1	0.4	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	0.5
Stearate (g/l)	2	0.6	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	0.1	0.1	<0.1	<0.1	0.2
Ammonia(g/l)	1.4	2.7	2.5	3.8	1.5	1.8	2.2	1.9	1.9	2.4	2.2	2.3	2.3
Total N (g/l)[1]	2.4	4.9	3.6	7.3	2.1	2.1	Na	Na	2.9	3.6	3.9	2.7	8.1
pH	6.2	6.9	7.4	7.6	7.8	7.5	7.3	7.4	7.7	7.4	7.4	7.4	7.4

[1]Calculated from digester feed values; [2]Na = not analyzed.

Materials accumulating and stratifying in various layers in digester were investigated (IV). Materials from the layers in the poultry slaughterhouse waste digester 4 operated prior to sampling for 91 days at an HRT of 50 days (feed TS 6.2%) at a loading of 1.0 kg VS/m^3 d were used. Before sampling, the digester was allowed to settle for ca. 20 hours, after which samples were taken from the top, middle, and bottom layers (Table 3). The digester content was then vigorously mixed for five minutes and sampled for the whole material (Table 3). The whole material was also centrifuged (3000 g, 15 min) to sample the cake (total COD 150 g/l) and the supernatant (total COD 6.5 g/l, soluble COD 6 g/l).

In all the assays, the pH was adjusted to 6.9-7.3 (1 M NaOH, 1 M HCl), if necessary. The vials were flushed with N_2/CO_2 (80%/20%) and sealed with butyl rubber stoppers and aluminium crimps. Finally, $Na_2S \cdot 9H_2O$ (0.25 g/l) was added to remove any residual O_2. The vials were then incubated in static cultures at 35°C or at 55 °C.

3.7 Anaerobically digested poultry slaughterhouse wastes as fertiliser in agriculture

3.7.1 Digested materials used in the plant assays and their aerobic post-treatment

Five different digested material samples (referred to as DM5 to DM9 (DM1-DM4 in article V, respectively), see Table 3) obtained from digesters 3 and 4 (III) were used in plant assays (VI). DM5, DM6, and DM9 were combinations of daily samples from digesters 3 and 4, stored in an anaerobic storage vessel (21±1°C) between operation days 56 to 120, 125 to 179, and 192 to 197, respectively. DM7 was material taken from digesters 3 and 4 on day 201, and DM8 was prepared by anaerobically incubating DM6 in batch-mode at 32±1°C for an additional 12 days. DM8 thus served as an anaerobic reference for aerobic treatments performed on DM6. The digested materials were used in plant assays as such (DM5, DM7 and DM8) or after aerobic post-treatment (DM6 and DM9). A commercial mineral fertiliser (total nitrogen content 160 g-N/kg, Kemira Agro Oy, Finland) was used as reference in 27-d plant growth assays.

The aerobic post-treatment of digested material samples DM6 and DM9 was conducted at 21±1°C in static Erlenmeyer flasks (250 ml), covered with aluminium foil. One hundred ml of DM6 was incubated both with and without 100 ml of added inoculum (activated sludge, 1.7 g/l of SS, 1.2 g/l of VSS). Incubations without DM6 were used to evaluate the decomposition of the inoculum alone, while those with DM6 and inoculum with 2 ml of added $HgSO_4$ (2 g/l) served as abiotic controls. One hundred ml of DM9 was incubated without inoculum and abiotic control. In all tests, distilled water was added to a volume of 202 ml. Air flow was created with a Rena Air 100 aquarium air pump (USA) and introduced into the media through Penn Plax

airstones (25 mm, USA) to maintain >2 mgO_2/l. DM6 and DM9 were incubated for 7 days and 6 h, respectively. The pH, drifting in the range between 6.5 and 8.7, was adjusted to 7.0 (1 M HCl, 1 M NaOH) in 7-d incubations on days 1, 2, and 4, and in 6-h incubations after 30 minutes.

3.7.2 Digested material as fertiliser (27-d plant growth assays)

The standard practice of the American Society for Testing and Materials (1994) was applied to compare digested material sample DM5, and the reference, a commercial fertiliser, to fertilise the soil for the growth of carrot (*Daucus carota*) and Chinese cabbage (*Brassica campestris* var. *chinensis*) (seeds purchased from Siemen Ltd., Finland). The soil substrate contained sieved and mixed sphagnum peat (Von Post humification index H 2-4% of humus 97%, pH 3.5-4.5, conductivity 2-4 mS/m, Kekkilä, Finland), the pH of which was raised to 7.0 by limestone ($CaCO_3$ + $MgCO_3$ + $CaCO_3$, Ca 30%, Mg 2%, Kekkilä, Finland). The seeds were then planted in the substrate in polyethene plant pots (6 l), 19 seeds per pot to run a test. DM5 (72 g DM5/kg substrate) and the fertiliser (690 mg fertiliser/kg substrate) were diluted with tap water and poured on the top of the substrate to obtain the desired concentration of soluble nitrogen (110 mg-N/kg substrate). The pots were placed in an environmental chamber at 20°C for 27 days and tap water was added daily to replace evaporation loss.

3.7.3 Germination assays

To study the phytotoxicity of DM7 and DM8 and the aerobically post-treated DM6 and DM9, germination assays were conducted with Chinese cabbage (*Brassica campestris* var. *chinensis*) and perennial ryegrass (*Lolium perenne*) (seeds purchased from Siemen Ltd. Finland), as described by Baud-Grasset et al. (1993) in triplicate 5-cm, sealable glass petri dishes, containing filter paper (Schleicher & Schuell). Test solutions were prepared using various dilutions of DM7 and DM8 (25 g/l and 50 g/l) and aerobically post-treated DM6 and DM9 (in g/l: 25, 50, 125, 250), diluted with deionised water. Deionised water was used as control. Each dish contained 4 ml of test solution adsorbed on filter paper with 10 seeds placed on the paper. The dishes were sealed and statically incubated at 20±1°C for 120 h in the dark. A 5-mm primary root was taken to define germination.

3.8 Model

The <METHANE> model used (II) was based on an earlier model (Vavilin et al. 1994). Slaughterhouse wastes were assumed to be a mixture of proteins, lipids, and carbohydrates. Hydrolysis, acidogenesis, acetogenesis, and methanogenesis as induced by different groups of microorganisms were described. Four groups of variables and equations were included in the model:

1. Suspended solid concentrations (X_k, k=1,2,3)

$$V \cdot \frac{dX_k}{dt} = q_f \cdot X_{Fk} - q_{BX} \cdot X_k - V \cdot \rho_{Xk}$$

where X_{Fk} =influent concentrations of suspended solids; q_f =feed flow rate; q_{BX} =discharge rate of excess suspended solids including biomass; ρ_{Xk} =rates of solids transformation; V =volume of liquid phase

2. Active biomass concentrations (B_i, i=1,2,...,10)

$$V \cdot \frac{dB_i}{dt} = q_f \cdot B_{Fi} - q_{BX} \cdot B_i + V \cdot \rho_{Bi},$$

where B_{Fi} =influent concentrations of active bacteria; ρ_{Bi} =growth rates of various subpopulations.

3. Soluble substrate concentrations (S_j, j=1,2,...,13)

$$V \cdot \frac{dS_j}{dt} = q_f \cdot (S_{Fj} - S_j) + V \cdot \rho_{Sj} + TRS_j$$

where S_{Fj} =influent concentrations of soluble substrates; ρ_{Sj} =rates of soluble substrate transformation; TRS_j =rate of mass exchange between gaseous and liquid phase. Note that $\rho_{Bi} = \rho_{Si}/Y_i$, where Y_i =yield coefficient.

4. Partial gas pressures (P_l, l=1,...,5)

$$\frac{dP_l}{dt} = \frac{RT}{V^g} \cdot \left[-TRS_l + \sum_n TRS_n \cdot \frac{P_l}{P_T} \right]$$

where R =universal gas constant; T =temperature (K); V^g =volume of gas phase; P_T =total gas pressure.

The rate of the main limiting substrate transformation by the i-th group of

micro-organisms (ρ_{Si}) was expressed as a product of several functions:

$$\rho_{Si} = \rho_{mi} \cdot FT_i \cdot FL_i \cdot FI_i \cdot B_i$$

where ρ_{mi} =maximum specific rate of limiting substrate consumption by *i*-th group of microorganisms under optimum conditions with biomass concentration B_i; FT_i, FL_i, FI_i =functions describing temperature dependence, a mechanism of substrate limitation, and inhibition, respectively. Inhibition processes by VFA (propionate and butyrate) and total LCFA were considered using the generalised function of non-competitive inhibition:

$$FI_i(I) = \frac{1}{1 + \left(I / K_{I1} \right)^{\ln(99)/\ln\left(\frac{K_{I2}}{K_{I1}}\right)}}$$

where I =inhibition agent; K_{I1}, K_{I2} =inhibition constants where the process rate decreases twice and 100 times, respectively. Experiment *pH* data was approximated in a stepwise manner to describe pH in the model. The inhibiting impact of hydrogen was given by the function

$$FI_i(H_2) = \frac{1}{1 + \left(P_{H_2} / K_{IHi} \right)^4}$$

where K_{Ihi} =constant of hydrogen inhibition. Inhibition processes by ammonia were not taken into account here.

The traditional Monod dependence, describing substrate limitation, was used for acidogenic, syntrophic, and acetotrophic micro-organisms. For hydrogen consuming methanogens, the hydrogen consumption rate was determined according to the principle of a minimum between the Monod functions for H_2 and CO_2 as the limiting substrates. First-order kinetics with inhibition by VFA was applied to describe the polymer hydrolysis of solids. The following stoichiometric equations were selected after model calibration:

Peptides → 0.1 H_2 + 1.7 CO_2 + 2.8 Acetate + 0.1 Propionate + 2.1 Butyrate + 4 NH_3
Lipids → 8.5 H_2 + 1.75 Acetate + 2.5 Propionate + 2.0 Stearate
Carbohydrates → 0.4 H_2 + 0.8 CO_2 + 1.3 Acetate + 0.2 Propionate + 0.5 Butyrate
Stearate → 1.0 Palmitate + 2.0 H_2 + 1.0 Acetate
Palmitate → 12.0 H_2 + 6.0 Acetate + 1.0 Butyrate

In the model, the stearate concentration was calculated, for simplicity, as the sum of stearate and oleate concentrations and the palmitate concentration as the sum of palmitate and myristate concentrations.

3.9 Analyses

pH was measured with a pH-meter immediately after sampling to avoid pH changes due to CO_2 losses. COD was determined according to the Finnish Standards (Finnish Standards Association 1988). Total Kjeldahl nitrogen was determined according to the Tecator application note (Perstorp Analytical/ Tecator AB 1995). Before distillation, the samples were digested with digester 2006 (Tecator AB).

Ammonia, TS, and VS were determined according to Standard Methods (American Public Health Association 1985). The ammonia and soluble COD samples were filtered with glass fibre filter papers (Schleicher & Schuell). Lipids were determined according to the Official Methods of Chemical Analysis (Association of Official Agricultural Chemists 1990).

Methane was analysed with a Perkin Elmer Autosystem XL gas chromatograph with a flame-ionisation detector as described elsewhere (Lepistö & Rintala 1995).

VFA (volatile straight and branched-chain fatty acids from C2 to C5) was analysed with the above Perkin Elmer Autosystem XL gas chromatograph equipped with a flame-ionisation detector and a PE FFAP column, as described elsewhere (Lepistö & Rintala 1995).

Samples for analysis of LCFAs were extracted as described by Bligh & Dyer (1959). 2 ml of chloroform extract was evaporated to dryness with nitrogen stream and the residue was dissolved in 2 ml of methyl *tert*-butyl ether. The samples were then methylated [with N-methyl-N-tolyl-sulphonyl-4 nitrosoamide] (II) or silylated [with N,O-bis(trimethylsilyl)-trifluoroacetamide with 1% trimethylchlorosilane (10% v/v) and subsequently derivatised at 70°C for 30 min] (I, III-V) and analysed with a Hewlett Packard HP 6890 series gas chromatograph, equipped with a 5973 mass selective detector and an HP-5 column (25 m, 0.2 mm i.d., 0.33 µm phase thickness). The analysis was performed in scan mode (mass range m/z 35-600, scan rate 1 scan/s) and quantified from the resulting total ion current. Heptadecanoic acid was used as an internal standard. The analysed LCFAs were saturated LCFAs of even-number carbon chain length in the range from C:10 to C:20 and unsaturated LCFAs, i.e., oleate (C18:1(n-9)), linoleate (C18:2), and linolenate (C18:3). However, only myristate (C14:0), palmitate (16:0), stearate (C18:0), and oleate were found in the samples and quantified, whereas the others were below the detection limit of 0.1 g/l.

In plant assays (V) the dry weights of roots and aboveground vegetation were determined upon drying for 18 h at 70°C.

Mo, Cd, Cr, Ni, Pb, and Hg were determined after acid digestion (HNO_3) and analysed with an atomic absorption spectrophotometer. S, P, K, Ca, Mg, Fe, Cu and Zn were determined after acid digestion (HNO_3) and analysed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

3.10 Calculations and data analyses

Loading ratio (kg VS/m^3 d) and HRT in the digester studies were calculated based on daily feed additions (III). Specific methane yields (m^3/kg VS_{added}) in the digester were calculated in weekly methane production and added VS values.

Protein content was calculated from the Kjeldahl-N content using a conversion factor of Kjeldahl-N x 6.25 (for meat). Insoluble nitrogen was calculated by subtracting ammonia concentration from Kjeldahl-N concentration.

The SPSS® for Windows 8.0 was used for the statistical procedures (V), except linear regression analysis, which was done using the Microsoft Excel 97 for Windows 98. The binomial test was used to study the equality of the samples as well as the samples and the control at α=0.05. The Kolmogorov-Smirnov test ($n \geq 50$) or the Shapiro-Wilk test ($n < 50$) was used to check the normality of the data with extreme observations excluded as outliers. The Levene test was used for the equality of variances at α=0.05 and one-way ANOVA was used to compare the equality of means of the samples against the control and to study the equality of means between the samples at α=0.05. Linear regression analysis was done to study the relationship between the phytotoxicity and chemical properties of the samples.

4 RESULTS AND DISCUSSION

4.1 Biochemical methane potential of solid poultry slaughterhouse by-products and wastes

The anaerobic degradability, methane production rate and yield of the major poultry slaughterhouse by-products and wastes was investigated in batch assays at 55 °C with thermophilic inoculum and at 35 °C with two different mesophilic inocula (I). The studied materials: bone and trimmings, blood, offal, and feather showed methane yields of 0.6-0.7, 0.5, 0.7-0.9, and 0.2 m^3/kg VS_{added} (Table 4), respectively, and the mixture produced methane 0.6-0.7 m^3/kg VS_{added}. In comparison, the methane yield of the source-sorted putrescible fraction of municipal solid waste is 0.4-0.6 m^3/kg VS_{added} (E.g. Cecchi et al. 1992; Rintala & Ahring 1994). The methane yield of poultry manure is somewhat lower, 0.2-0.3 m^3/kg VS_{added} (Huang & Shih 1981; Safley et al. 1987). The materials showed high VS reductions, 70-80 %, except for feather, which showed VS reduction of about 30-50 %. Neither incubation temperature nor inoculum affected the final methane yield or VS reduction of the different wastes.

The poor degradability of feather was not surprising, as it is considered recalcitrant to anaerobic microbial degradation. It is unlikely that keratin degraded in this study, whereas keratinous materials, such as feather, contain also small amounts of lipid components (1-10 %) called cuticle (Bourne 1993), which probably degraded to methane. The effects of thermal, chemical, and enzymatic pre-treatments to enhance the methanation of feather were investigated (see the next section, I).

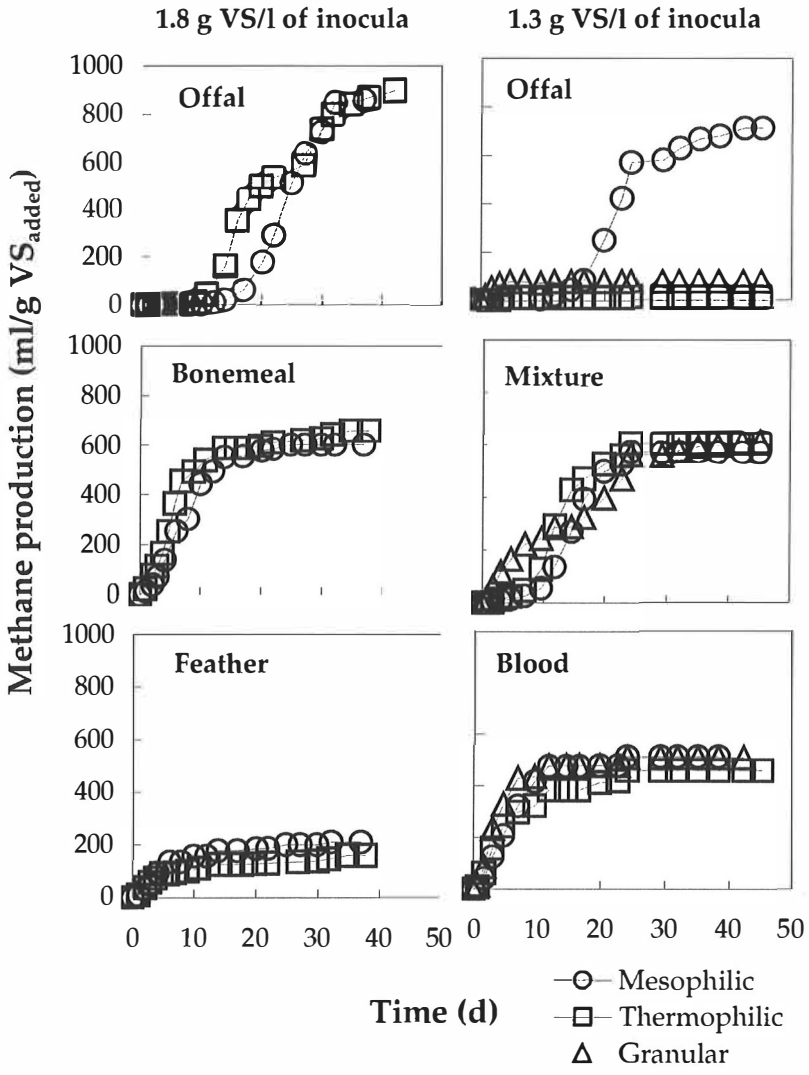

FIGURE 3 Batch methane production of poultry slaughterhouse by-products and wastes (all 3 g VS/l) with mesophilic digested sewage sludge and granular sludge at 35 °C and with a thermophilic digested plant sorted municipal biowaste at 55 °C with inocula concentrations of 1.3 g VS/l and 1.8 g VS/l, respectively (I).

The materials produced methane readily, except offal, which showed delayed methane production (Fig. 3). The delayed methane production of offal was probably due to LCFAs, produced as intermediates of lipid degradation. Offal had the highest lipid content of the materials, ca. 50-60 % of VS. The length of delay depended on the source and concentration of inoculum and incubation temperature, sewage sludge at 35 °C having the shortest delay of a few days,

while granular sludge did not produce methane within 94 days of incubation (Fig. 3). The fact that in this study digested sewage sludge was faster to produce methane than granular sludge, which is considered less sensitive to LCFA inhibition than suspended sludges (Hwu et al. 1996), may have been caused by the digested sewage sludge having a higher initial concentration of LCFA degrading micro-organisms which prevent the accumulation of LCFAs in toxic concentrations. On the other hand, methanogenesis is reportedly more susceptible to LCFA toxicity at 55 °C than at 35 °C (Hwu at el. 1996), which could explain the depressed methane production in the assays inoculated with suspended sludge at 55 °C with an inoculum concentration of 1.3 g VS/l. At 55 °C, an inoculum concentration more than 1.3 g VS/l was evidently needed to avoid accumulation of LCFAs. The final total of LCFAs in the assays inoculated with suspended sludge at 55 °C and granular sludge at 35 °C were 1.3 g/l and 1.2 g/l, respectively, while the LCFAs with suspended sludge at 35 °C were below the detection limit of 0.1 g/l.

The final soluble COD and VFAs in the assays were below 0.4 and 0.02 g VS/l, respectively, a result which verifies the high degradation of solublised material, except in the offal assays, which showed depressed methane yield and somewhat higher final soluble COD and VFA values (1.6 to 1.8 g/l and 1.0 to 1.2 g/l, respectively). The final pH in all the assays was in the range of 7.0 to 7.4.

TABLE 4 Methane yield of poultry slaughterhouse by-products and wastes in batch assays with different inocula at 35°C and 55°C (average values with standard deviation in parentheses, N = 3, l).

By-product/ waste	Methane yield with different inocula and at different temperatures[1]					
	Digested sewage sludge at 35 °C		Granular sludge at 35 °C		Digested putrescible fraction of municipal solid waste at 55 °C	
	(m^3/kg VS_{added})	(m^3/metric ton wet $weight_{added}$)	(m^3/kg VS_{added})	(m^3/metric ton wet $weight_{added}$)	(m^3/kg VS_{added})	(m^3/metric ton wet $weight_{added}$)
Feather	0.21 (0.02)[2]	49 (4)[2]	Na[4]	Na	0.21 (0.01)[2]	49 (2)[2]
Blood	0.51 (0.01)[3]	100 (3)[3]	0.51 (0.03)[3]	100 (6)[3]	0.46 (0.09)[3]	92 (18)[3]
Offal	0.91 (0.05)[2], 0.73 (0.03)[3]	340 (19)[2], 270 (96)[3]	Na	Na	0.89 (0.19)[2]	330 (70)[2]
Bone and trimmings	0.60 (0.05)[2]	150 (12)[2]	Na	Na	0.68 (0.05)[2]	170 (12)[2]
Mixture	0.57 (0.09)[3]	150 (24)[3]	0.61 (0.03)[3]	160 (8)[3]	0.61 (0.03)[3]	160 (8)[3]

[1] Blank excluded from methane productions; [2] Assays with 1.8 gVS/l of inoculum; [3] Assays with 1.3 gVS/l of inoculum; [4] Na = not analyzed.

4.2 Effect of pre-treatments on anaerobic degradation of feather

The feasibility of thermal, chemical, and enzymatic pre-treatments to solubilise feather and to enhance its methane production rate and yield was studied (I). Enzymatic treatments with a commercial alkaline endopeptidase (2-10 g VS/l, 2-24 h at 55 °C) and combined thermal (120 °C) and enzymatic treatments solubilised feather to a degree with soluble COD in the treated assays in the range of 0.7 to 0.9 g/l or produced soluble COD 0.2-0.4 g/g of feather (Table 5). In assays for the methane production of pre-treated feather, combined thermal and enzymatic treatment resulted also in the most increased methane yield, 37 to 51%, with an enzyme dose of 2 and 10 g/l, respectively. In comparison, enzymatic treatments alone increased the feather methane yield by 5 to 21% (Table 5), being slightly higher with a higher enzyme dose and a longer (24 h vs 2 h) incubation time. According to Papadopoulos (1985), feather treated with a commercial proteolytic enzyme showed increased amino acid digestibility, as determined in chick feeding assays. Furthermore, Dalev (1994) found that combined enzyme-alkaline treatment with a commercial alkaline protease fully solublised feather: the feather was first incubated for 30 min in alkali (2 g/l of NaOH) at 80 °C and then digested with 2 g of the enzyme at 55 °C while stirred.

The fact that thermal treatments did not significantly solublise the COD (<0.1 g/l, <0.1 g/g of added feather) and only slightly increased the methane yield of feather (120 °C treatment by 24% and 70 °C treatment negligibly) (Table 5) indicates that the treatment temperature in this study was too low, the treatments too short, or that they failed to enhance the anaerobic degradation of the feather with the inoculum used. In an earlier study, feather required a 2-min minimum autoclave pre-treatment (120 °C) for the aerobic growth of a feather-degrading bacterium isolated from a digester treating manure and poultry feather at 55 °C (Williams et al. 1990). Autoclaving for varying periods, typically 20 to 30 min at temperatures ranging from 120 to 142 °C, made feather protein more digestible as animal feed, while the major difference in the amino acid composition between the untreated and treated feather was a reduced cystine concentration, suggesting the breakdown of cystine disulphide linkages (Papadopoulos 1985).

Chemical treatments with NaOH (2-10 g/l, 2-24 h treatment) solublised feather only slightly (soluble COD 0.2-0.3 g/l, <0.1 g/g added feather), except for the treatment with NaOH at a concentration of 10 g/l and with an incubation time of 24 h, which produced a solublised COD of 1.2 g/l (0.3 g/g added feather) and resulted also in an increased methane yield of feather, by 32% (Table 5). Previously, treating feather with alkali was found to result in disulphide bond cleavage (Papadopoulos 1985).

The methane production rate was similar in all these assays with pretreated feather, except that the NaOH treated feather produced more slowly (Fig. 4). Increasing the NaOH dose and the incubation time reduced a methane production rate suggesting the formation of inhibitory toxicant concentrations. Nevertheless, it is unlikely that the inhibition was due to NaOH as such as it correlated also with the treatment period. However, NaOH (2-6 g/l) added

during thermal treatment had reportedly a negative effect on the digestibility of amino acids in feather meal with young chickens (Papadopoulos 1985).

The fact that after all the various pre-treatments both ammonia and VFA were below the detection limit of 0.01 g/l suggests that the solublised material, probably amino acids, did not degrade further. This accords with previous reports that different treatments of feather (thermal, chemical, and enzymatic) resulted only in limited amino acid degradation (Papadopoulos 1994) and had only a minor effect on the visual appearance of feather.

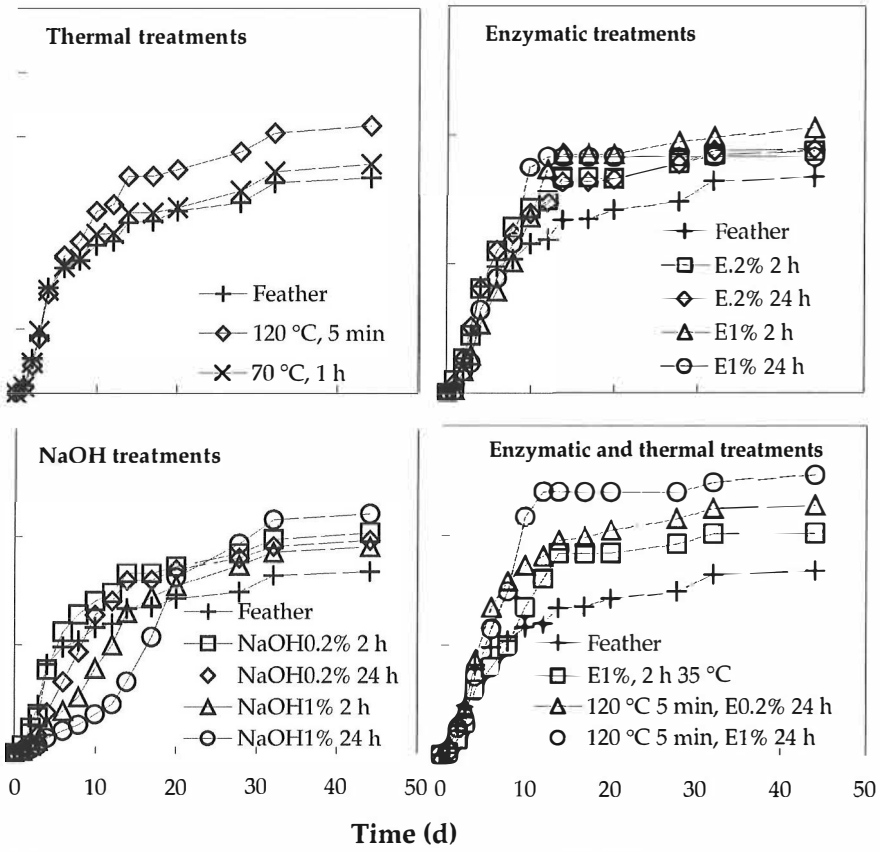

FIGURE 4 Specific cumulative methane production of pre-treated feather (For treatments see Table 3) in batch assays inoculated with digested sewage sludge at 35 °C. (Blank excluded from methane productions, enzyme excluded from methane production of enzymatically treated feather, l).

TABLE 5 Effects of thermal, chemical, and enzymatic pre-treatments on soluble COD and ammonia concentration in vials with 17 g of poultry feather/l (1.0 g of feather/vial) and specific methane yields of pre-treated feather waste in batch assays at 35 °C (I).

Treatment	After pre-treatment		Specific methane yield[2]	
	pH	Soluble COD (g/l)	(m^3/kg VS_{added})	(% of control)
Control, no treatment	7.1	0.2	0.164 (0.018)	
Thermal treatment (temperature, time)				
120 °C, 5 min	6.9	< 0.1	0.203 (0.033)	124
70 °C, 1 h	6.7	< 0.1	0.173 (0.005)	105
Enzymatic treatment (dose per volume, time, and temperature)				
0.2% w enzyme/v, 2 h 55 °C	6.9	0.8[1]	0.185[3] (0.004)	113
0.2% w enzyme/v, 24 h 55 °C	6.7	0.8[1]	0.188[3] (0.013)	115
1% w enzyme/v, 2 h 55 °C	6.8	0.9[1]	0.198[3] (0.008)	121
1% w enzyme/v, 24 h 55 °C	7.0	0.7[1]	0.172[3] (0.005)	105
Combined thermal (temperature, time) and enzymatic treatment (dose per volume, time, and temperature)				
120 °C, 5 min; 0.2% w enzyme/v, 24 h 55 °C	6.7	0.7[1]	0.225[3] (0.011)	137
120 °C, 5 min; 1% w enzyme/v, 24 h 55 °C	6.5	0.8[1]	0.248[3] (0.023)	151
Chemical treatment (dose per volume, time, and temperature)				
0.2% w NaOH/v, 2 h 35 °C	Na[4]	0.2	0.198 (0.012)	121
0.2% w NaOH/v, 24 h 35 °C	Na	0.2	0.191 (0.009)	116
1% w NaOH/v, 2 h 35 °C	Na	0.3	0.186 (0.021)	113
1% w NaOH/v, 24 h 35 °C	Na	1.2	0.216 (0.040)	132

[1] Enzymatic control excluded; [2] Blank excluded from methane production, standard deviation in parentheses; [3] Enzyme methane production excluded from methane production; [4] Na = not analyzed.

4.3 Anaerobic batch degradation of solid poultry slaughterhouse waste mixture

Anaerobic batch degradation of solid poultry slaughterhouse waste mixture (Table 3) was investigated with different initial waste and inoculum concentrations and waste-to-inoculum ratios (II). Furthermore, the dynamics of the degradation process were modelled.

The assays indicated that during the degradation of solid poultry slaughterhouse wastes, the different factors did, to a degree, affect the concentrations of the individual intermediate compounds and the onset and rate of methane production. In general, however, the degradation patterns resembled each other and indicated rapid hydrolysis / acidogenesis, accumulation of LCFA and VFA, removal of LCFA and subsequently that of VFAs, and methane production (Table 6, Fig. 5).

TABLE 6 Performed assays, methane yields, LCFA and VFA concentrations, and methane production during anaerobic batch degradation of solid poultry slaughterhouse waste mixture (II).

Assay	Inoculum (gVS/l)	Waste (gVS/l)	Methane yield[2] (m^3/kg VS_{added})	VFA, LCFA, Cumulative methane (mM)[3]	Time (d)				
					0[1]	3	6	12	27
1	8.4	3.9	0.62 (0.03)	VFA	0.4	20.6	21.2	14.4	<0.1
				LCFA	1.3	2.1	2.7	4.1	<0.1
				Methane	0.0	2.7	5.3	35.4	111.9
2	16.8	3.9	0.67 (0.02)	VFA	0.5	24.0	22.2	27.2	<0.1
				LCFA	1.3	6.3	3.7	0.4	<0.1
				Methane	0.0	7.1	15.1	80.2	141.3
3	8.4	7.8	0.55 (0.04)	VFA	0.6	34.4	49.1	17.0	<0.1
				LCFA	2.7	4.2	3.6	5.9	<0.1
				Methane	0.0	2.3	5.3	55.2	185.5
4	16.8	7.8	0.61 (0.06)	VFA	0.7	36.0	41.8	19.1	<0.1
				LCFA	2.7	12	11	6.2	<0.1
				Methane	0.0	4.9	10.7	91.1	226.5

[1] Calculated values, [2] Blank excluded, methane yield on day 27, standard deviation in parentheses, [3] Methane production presented per litre of medium.

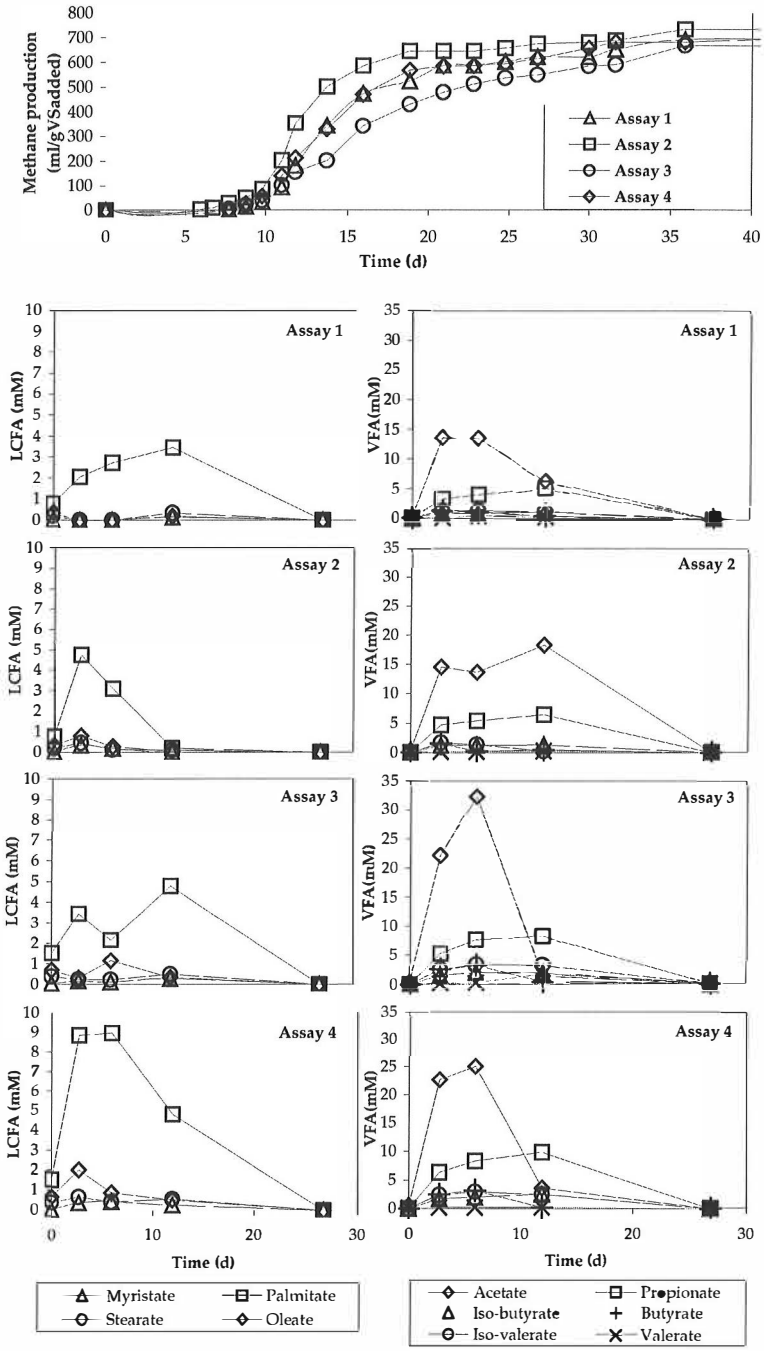

FIGURE 5 Specific cumulative methane productions (above) and LCFA (left) and VFA (right) concentrations during anaerobic batch degradation of solid poultry slaughterhouse waste mixture (Assays 1-4 see Table 6, II).

In all the assays, methane production started after a 6 to 9 day lag. Lower waste-to-inoculum ratios exhibited a slightly faster onset and a higher methane production rate (Fig. 5). The average methane yields within 27 days ranged from 0.55 to 0.67 $m^3/kg\ VS_{added}$. The highest waste-to-inoculum ratio exhibited a slightly lower methane yield by day 27, while the yields were similar after 35 days of incubation (Fig. 5). The methane yield equalled that determined in other batch assays (I) and in semi-continuously fed laboratory-scale digesters (III).

Acetate was the most abundant VFA in all the assays and was consumed before propionate (Fig. 5). In the different assays, the highest total LCFA ranged from 4.1 mM to 12.0 mM, the concentrations being below the detection limit after 27 days of incubation (Table 6). Palmitate was the most abundant LCFA (up to 9.0 mM) in the assays, whereas oleate, stearate, and myristate were present up to 2.1, 0.7 and 0.5 mM, respectively (Fig. 5). The results showed complete degradation of waste-derived LCFA and VFA to methane, as also found in anaerobic batch treatment of tallow (Broughton et al. 1998).

In all the assays, 50 to 60 % of nitrogen was ammonificated within 3 to 6 days of incubation, indicating that ammonification did not significantly depend on the different factors. The resulting ammonia concentration ranged from 0.6 to 1.4 g-N/l in the assays.

TABLE 7 Model parameters for simulation of anaerobic batch degradation of solid poultry slaughterhouse waste mixture presented in Fig 6 (II).

Process	ρ_m, mM/mM/d	Y, mM/mM	K_s, mM, bar[1]	K_{I1}, K_{I2}, mM, bar[1]
Hydrolysis	0.73			12.03; 12.13 (VFA)
Acidogenesis	100.0	0.05	0.94	
Acetogenesis (butyrate)	7.0	0.04	0.23	4.0; $9.0 \cdot 10^5$ (LCFA)
Acetogenesis (propionate)	8.5	0.05	1.0	2.0; $9.0 \cdot 10^5$ (LCFA)
Acetogenesis (palmitate)	44.0	0.02	0.39	
Acetogenesis (stearate)	70.0	0.11	0.35	0.086 (hydrogen)
Methanogenesis (H_2/CO_2)	70.0	0.02	0.0012, 0.0001[2]	
Methanogenesis (acetate)	36.4	0.01	1.33	

ρ_m =specific maximum rate of substrate utilisation; Y =yield coefficient; K_s =half-saturation coefficient; K_{I1} and K_{I2} =inhibition constants; [1] gas components are expressed in bars; [2] two values corresponded to H_2 and CO_2 concentrations, respectively; [3] first-order rate constant is expressed in $days^{-1}$.

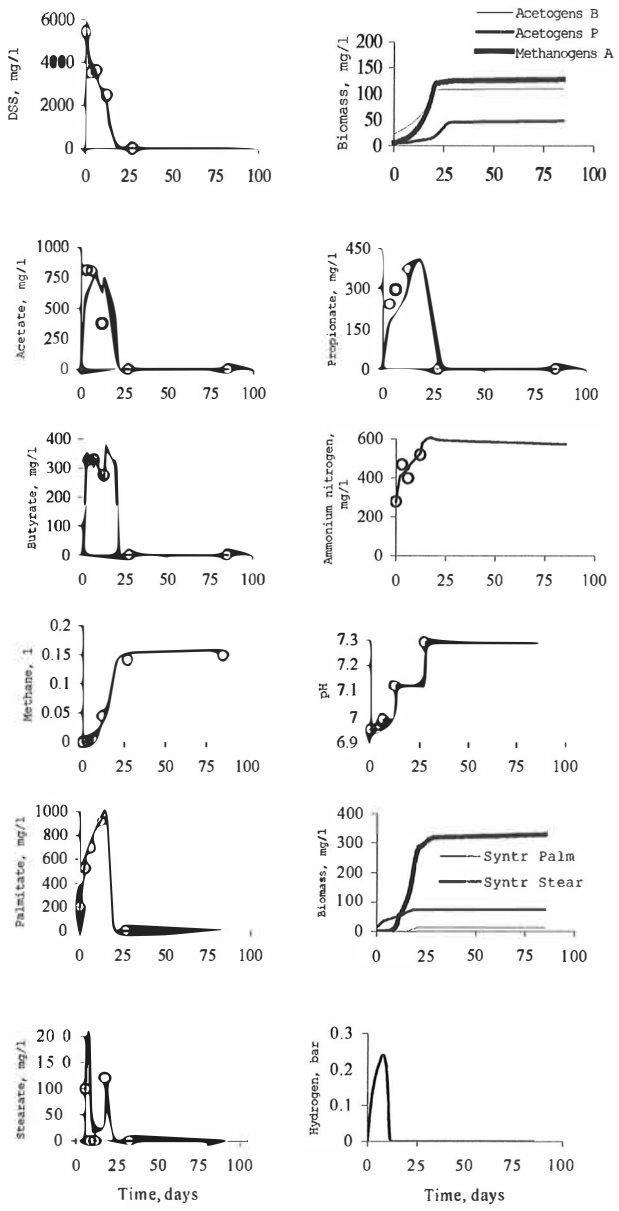

FIGURE 6 Time profiles of main model variables for simulation of anaerobic batch degradation of solid poultry slaughterhouse waste mixture (II). Symbols: experimental data in assay 2; lines: model predictions (DSS - degradable suspended solids, B - butyrate, P - propionate, A - acetoclastic, Syntr - syntrophs, Palm - palmitate, Stear - stearate).

The degradation patterns were simulated with kinetic constants, obtained by model calibration with data from all the assays (Table 7). The model, assuming an inhibition of hydrolysis by VFA, an inhibition of acetogenesis by LCFA, and a stearate transformation by H_2, fitted the experiments reasonably well, as shown for the assay with the lowest waste-to-inoculum ratio (assay 2, Fig. 6). The model suggested that the rate-limiting step, indicated by delayed methane production, rather than being the result of inhibited aceticlastic methanogenesis, was caused by LCFAs inhibiting propionate degradation, followed by high propionate inhibiting hydrolysis. During modelling it was shown that aceticlastic methanogenesis was not inhibited. In the model, acetogenesis was assumed to be inhibited by the total LCFA concentration, whereas in practice inhibition is caused by various LCFAs.

All in all, the model suggested that syntrophic LCFA degradation was the rate-limiting step at the beginning of all the assays, whereas in the final stage of the degradation, polymer hydrolysis turned out to be the rate limiter. Hence LCFA degradation is most likely the critical area in continuous digestion processes, an assumption which was verified with digesters treating poultry wastes on a semi-continuous basis (see the next section, III).

4.4 Semi-continuous anaerobic digestion of solid poultry slaughterhouse waste: effect of hydraulic retention time and loading

Effects of hydraulic retention time and loading on semi-continuous anaerobic treatment of poultry slaughterhouse wastes was investigated, using semi-continuously fed, laboratory-scale digesters at 31 °C (III). The effect on process performance was highly significant: Anaerobic digestion was feasible with a loading of up to 0.8 kg VS/m^3 d and an HRT of 50 to 100 days. The specific methane yield in the studies was high, from 0.52 to 0.55 m^3/kg VS_{added} (Fig. 8, Table 8), which was comparable to the methane yield of the waste mixture in batch assays (See sections 4.1 and 4.3, I, II). On the other hand, at a higher loading, in the range from 1.0 to 2.1 kg VS/m^3 d, and a shorter HRT, in the range from 25 to 13 days, the process appeared inhibited and/or overloaded, as indicated by the accumulation of VFAs and LCFAs and the decline in the methane yield (Figs. 7, 9, and 10). For comparison, Banks (1994) reported mesophilic (31 °C) anaerobic digestion of solid slaughterhouse wastes with a loading of 3.6 kg COD/m^3 d and an HRT of 43 days.

Compared to the effects of HRT and loading, the TS concentration seemed to have considerably less effect on process performance under the conditions studied.

VFAs and LCFAs accumulated easily (Figs. 9 and 10), as was also observed in anaerobic batch degradation assays of the studied waste (II).

Acetate was the most abundant of VFAs (Fig. 9, Table 8). LCFAs followed the same general pattern as total VFA in the digesters (Figs. 9, 10), though the concentrations and profiles of LCFAs varied under different conditions (Fig. 10, Table 8). In general, palmitate was the most abundant LCFA (Fig. 10, Table 8) as was found in anaerobic batch degradation assays of the studied waste (see section 4.3, III). The modelling of batch assays with the waste mixture (see section 4.3, III) indicated that LCFAs inhibited propionate degradation, and a high propionate concentration inhibited hydrolysis, these being rate-limiting steps in waste degradation. High propionate concentrations were measured particularly in digester 1 (Fig. 9, Table 8), where recovery from process failure appeared also the slowest (IV). The materials accumulating in the digesters were chracterised to find their anaerobic degradability and the factors affecting the degradation (IV).

About 50 to 70 % of waste nitrogen was ammonificated during the anaerobic digestion treatment. The TS and VS removals amounted to about 60 to 80 %, respectively.

TABLE 8 The effect of loading and HRT on methane yield and digested material characteristics in semi-continuous anaerobic digestion of solid poultry slaughterhouse waste (III).

Parameter, unit	Digester 1 (day 88-98)	Digester 2 (day 88-98)	Digester 3 (day 268-284)	Digester 4 (day 121-136)	Digester 4 (day 268-284)
HRT (d)	13	25	50	100	100
Loading (kg VS/m^3d)	2.1	2.1	0.8	0.5	0.8
Feed TS (%)	3.1	6.2	4.7	6.2	9.4
Effluent TS (%)	2.1 (0, 1)[2]	2.5 (0, 1)	1.2 (0, 1)	Na[1]	2.3 (0, 1)
TS removal (%)	32	55	74	Na	76
Feed VS (%)	2.6	5.2	3.9	5.2	7.8
Effluent VS (%)	1.8 (0, 1)	1.9 (0, 1)	1.0 (0, 1)	Na	1.9 (0, 1)
VS removal (%)	31	63	74	Na	76
Soluble COD (g/l)	10.1 (0.7, 5)	14.9 (0.8, 5)	4.8 (0, 1)	1.0 (0.3, 2)	9.3 (0, 1)
Acetate (mg/l)	3.8 (0.5, 3)	9.1 (1.5, 3)	2.3 (0.1, 2)	0.22 (0.01, 2)	4.1 (0.3, 2)
Propionate (mg/l)	1.5 (0.3, 3)	1.2 (0.4, 3)	0.17 (0.01, 2)	0.01 (0.002, 2)	0.57 (0.01, 2)
Iso-butyrate (mg/l)	0.40 (0.11, 3)	0.63 (0.18, 3)	0.066 (0.005, 2)	<0.001	0.20 (0.03, 2)
Butyrate (mg/l)	0.61 (0.22, 3)	0.63 (0.22, 3)	0.022 (0.002, 2)	0.014 (0.019, 2)	0.088 (0.09, 2)
Iso-valerate (mg/l)	0.74 (0.16, 3)	1.1 (0.3, 3)	0.066 (0.004,2)	0.004 (0.001, 2)	0.35 (0.11, 2)
Valerate (mg/l)	0.38 (0.17, 3)	0.25 (0.16,3)	0.003 (0.004, 2)	<0.001	0.009 (0.012, 2)
Caproate (mg/l)	0.25 (0.17, 3)	0.25 (0.17,3)	0.003 (0.004, 2)	<0.001	0.04 (0.02,2)
Myristate (g/l)	0.5 (0, 1)	0.1 (0, 1)	<0.1 (0, 1)	<0.01 (0, 1)	<0.01 (0, 1)
Palmitate (g/l)	5.5 (0, 1)	2.4 (0, 1)	0.2 (0, 1)	<0.01 (0, 1)	0.3 (0, 1)
Oleate (g/l)	0.1 (0, 1)	0.4 (0, 1)	1.8 (0, 1)	<0.01 (0, 1)	0.1 (0, 1)
Stearate (g/l)	1.9 (0, 1)	0.6 (0, 1)	<0.1 (0, 1)	<0.01 (0, 1)	<0.1 (0, 1)
Total LCFA (g/l)	8.1 (0, 1)	3.5 (0, 1)	2.0 (0, 1)	<0.01 (0, 1)	0.4 (0, 1)
Ammonia (g-N/l)	1.38 (0, 1)	2.73 (0.02, 2)	2.45 (0, 1)	Na	3.78 (0, 1)
pH	6.2 (0.1, 8)	6.9 (0.1, 8)	7.4 (0, 1)	7.5 (0.1, 3)	7.6 (0, 1)
Methane yield (m^3/kg VS_{added})	0.09 (0.09, 2)	0.31 (0.04, 2)	0.55 (0.06, 2)	0.51 (0, 1)	0.52 (0.06, 3)

[1] Na = not analyzed; [2] Standard deviation and number of analyses in parentheses.

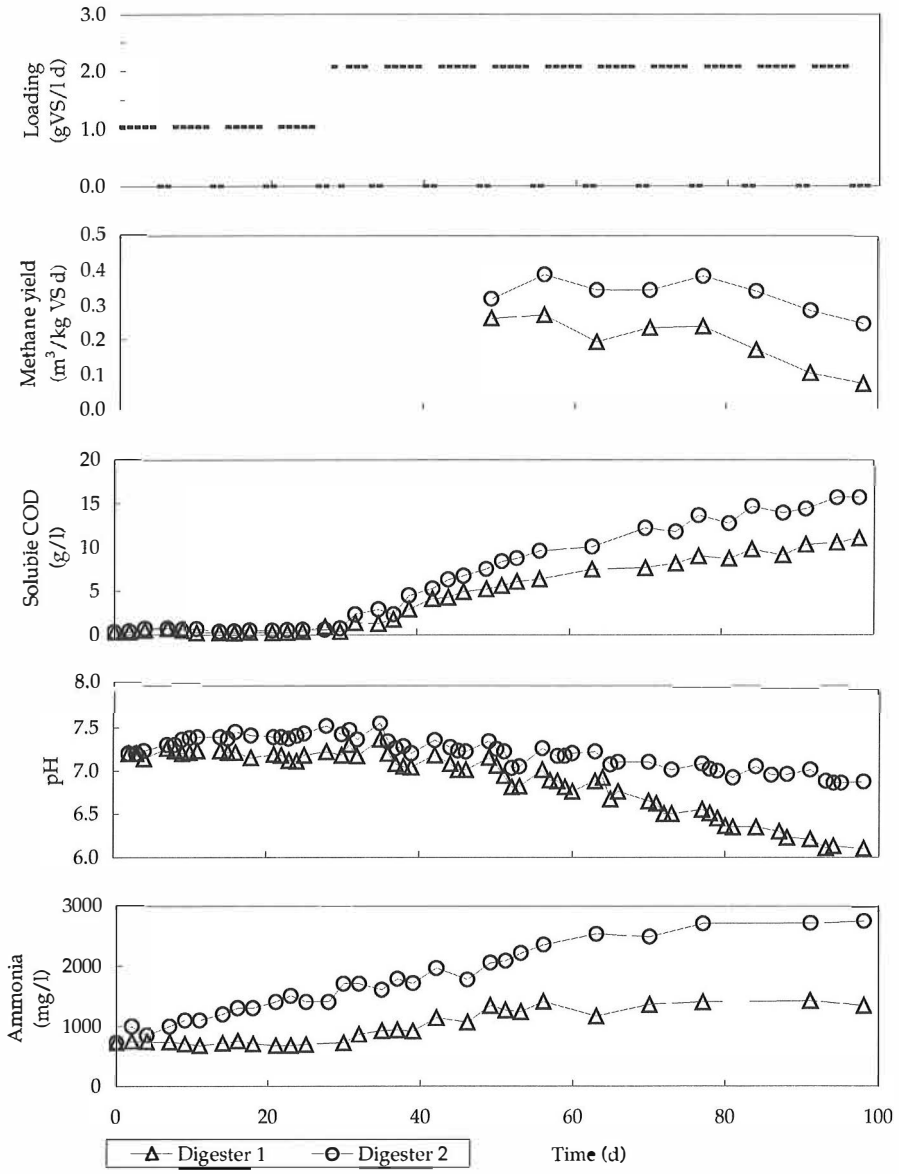

FIGURE 7 Loading, methane yield, soluble COD, pH and ammonia in poultry slaughterhouse waste digesters 1 and 2 (III).

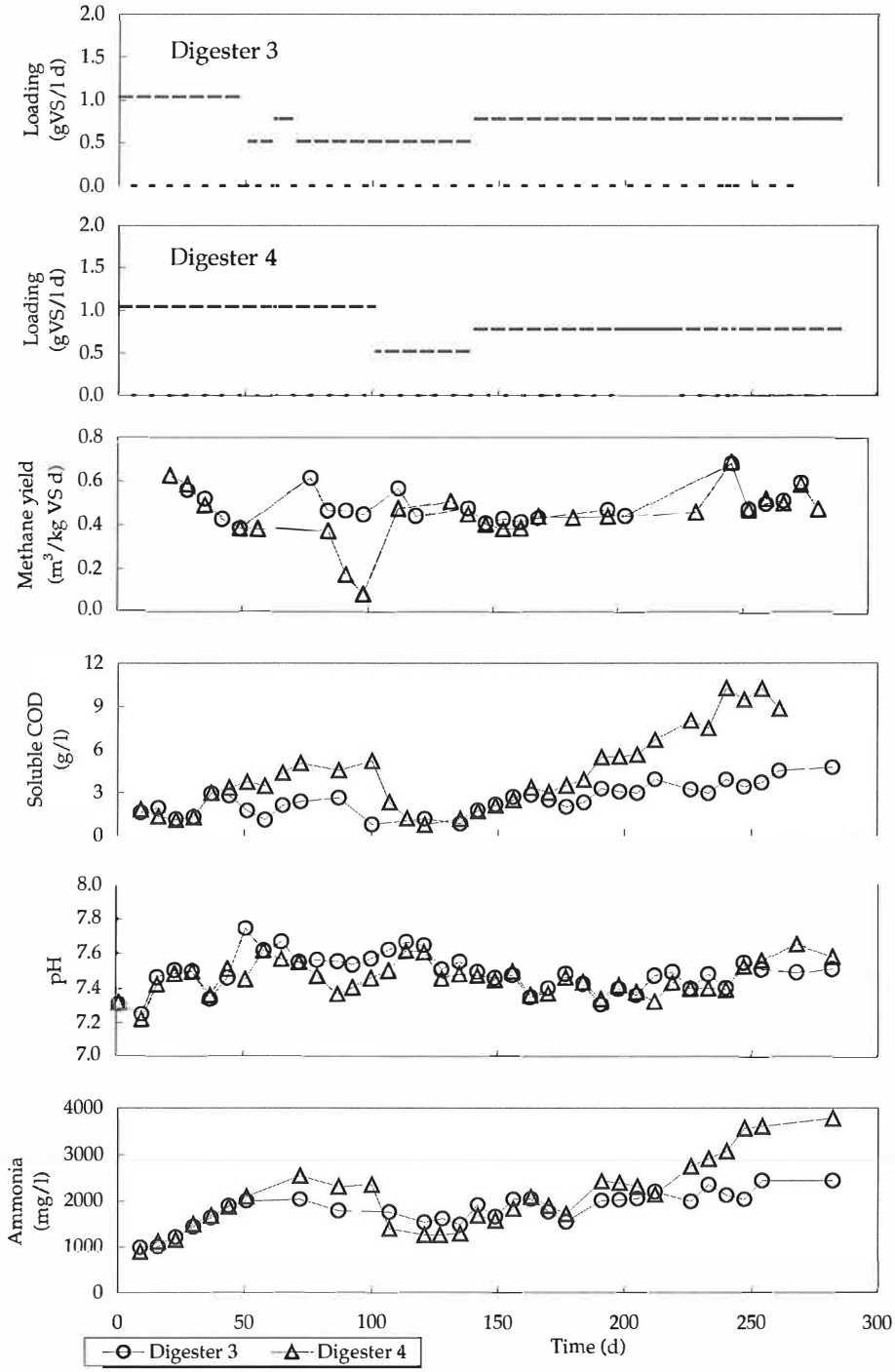

FIGURE 8 Loading, methane yield, soluble COD, pH and ammonia in poultry slaughterhouse waste digesters 3 and 4 (III).

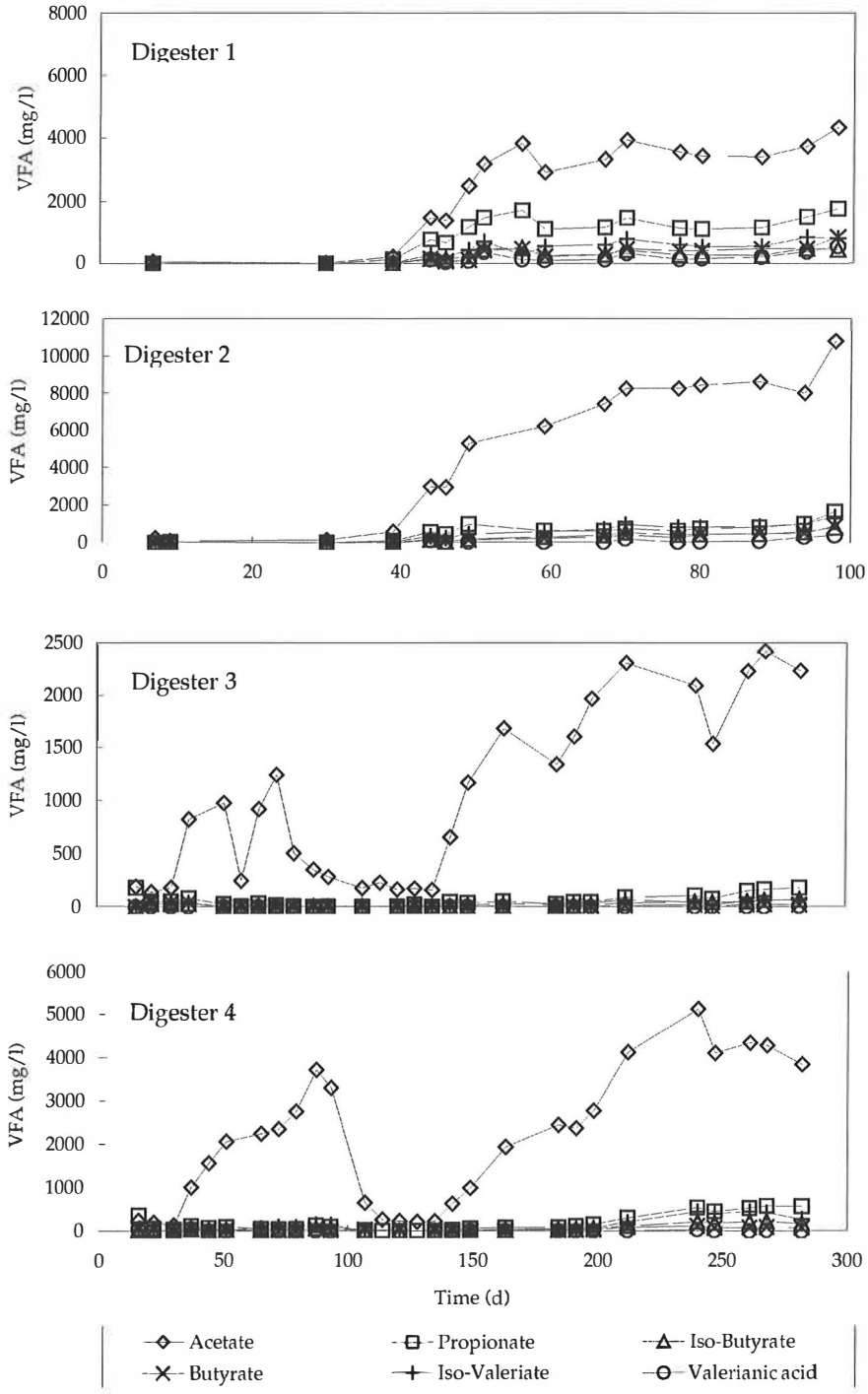

FIGURE 9 VFA in poultry slaughterhouse waste digesters (III).

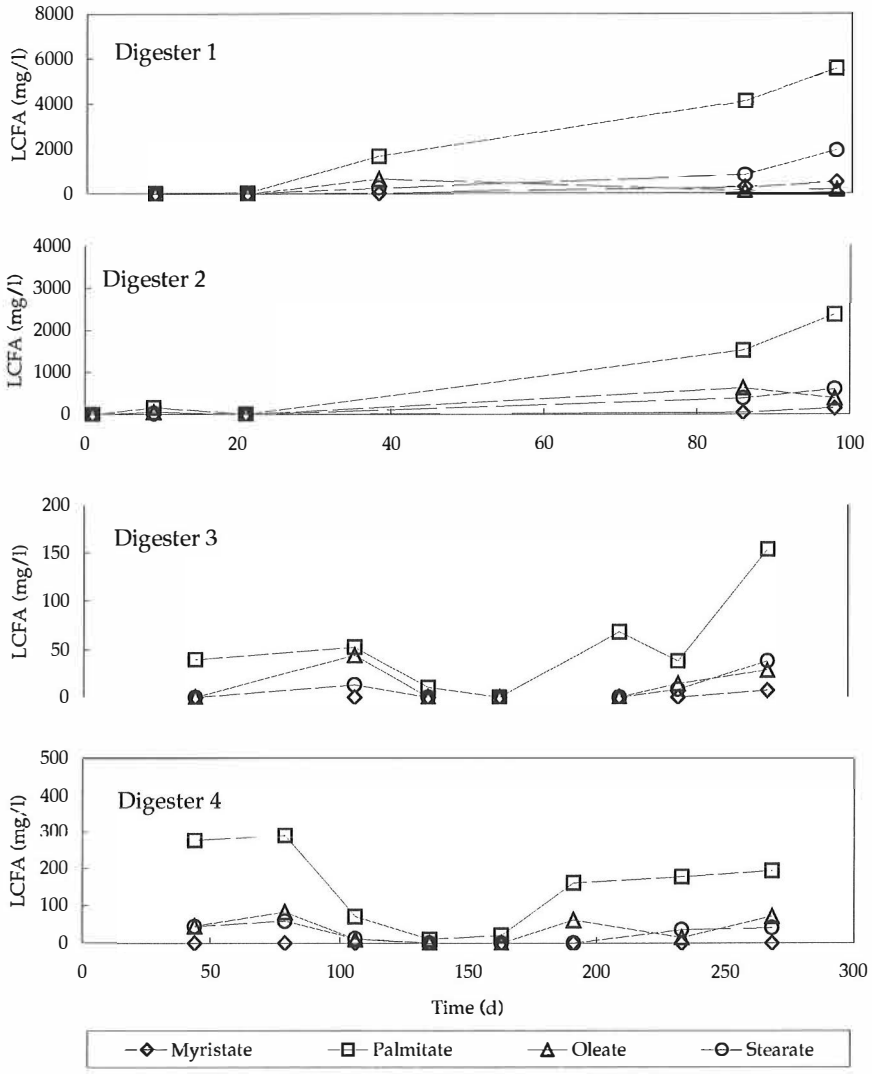

FIGURE 10 LCFA in poultry slaughterhouse waste digesters (III).

4.5 Characterisation and anaerobic batch degradation of materials accumulating in anaerobic digesters treating poultry slaughterhouse waste

Materials accumulating in failed and stratified mesophilic semi-continuous anaerobic digesters treating poultry slaughterhouse waste (digesters 1-4, III) were characterised (DM1-4, see Table 3) to find its anaerobic degradability and the factors affecting the degradation (IV). Methane production from the material from digester 1 (DM1) was slow and was not significantly enhanced even with 33 percent dilution (Fig. 11), indicating that recovery from process failure due to too short hydraulic retention time (13 days) can be very slow. On the other hand, the failure was still reversible, and methane was finally produced. DM2 (HRT of 25 days), too, originated from a digester considered a failure (digester 2), the methane production of which, however, was comparable to that of DM3 and DM4, which originated from digesters showing normal performance (digesters 3 and 4). Hence, recovery from process failure appears to depend on the degree of inhibition.

In DM1, the initial concentration of total LCFAs was the highest in the studied materials, 8.1 g/l (31 mM) (Table 3, Fig. 12), whereas other potential inhibitors, unionised VFAs and ammonium, were not higher than in other materials, which showed considerably faster methane production (Table 3, Fig. 11). Thus the slow methane production with DM1 (see Fig. 11) was apparently mainly due to accumulated LCFAs, suggesting that LCFAs are probably the critical part of the solid poultry slaughterhouse waste degradation process in a semi-continuous anaerobic digester. The initial concentration of total LCFAs was considerably higher than that found in the batch assays used to study the anaerobic degradation of the poultry slaughterhouse waste (up to 12 mM) (see section 4.3, II).

On the other hand, in DM2 the initial concentration of total LCFAs was somewhat higher than in the DM1 diluted to 33% (Table 3, Fig. 13), the methane production of which was considerably more severely inhibited compared to DM2 (Fig. 11). Furthermore, even though the LCFAs were apparently the main inhibitor the allowable concentration of LCFAs in digesters cannot be reliably determined as depending, in addition to concentration (Koster & Cramer 1987; Rinzema 1988), also on several other factors, including the cell wall structure of micro-organisms (Roy et al. 1985), the specific surface area of sludge (Hwu et al. 1996), and the carbon chain length and saturation of LCFAs (Koster & Cramer 1987; Galbraith et al. 1971). In addition, the synergistic toxicity of LCFAs, i.e., increased toxicity by the presence of another LCFA, was found for methanogenic bacteria (Koster & Cramer 1987).

The digested poultry slaughtering waste from the disturbed digesters (short HRTs: DM1 and DM2 of 13 and 25 days, respectively), with initially high VFAs and LCFAs, registered methane yields of up to 0.70 to 1.32 $m^3/kg\ VS_{added}$, a result which was significantly higher than the depressed methane yield (0.07-0.24 $m^3/kg\ VS_{added}$) of the digesters (III). LCFAs and VFAs contributed

significantly to the methane produced in the assays, as indicated by significant removals (Fig. 12). In comparison, a stable methane production of 0.52-0.55 $m^3/kg\ VS_{added}$ was obtained in semi-continuous anaerobic digestion of poultry slaughterhouse waste with HRTs of 50 to 100 days (III). The present results also show that digested poultry slaughtering waste from a digester which functioned well, and had low initial VFAs and LCFAs, could still produce up to 0.28 to 0.35 $m^3/kg\ VS_{added}$ (0.1 to 0.2 $m^3/kg\ VS_{added}$ in digester feed) of methane.

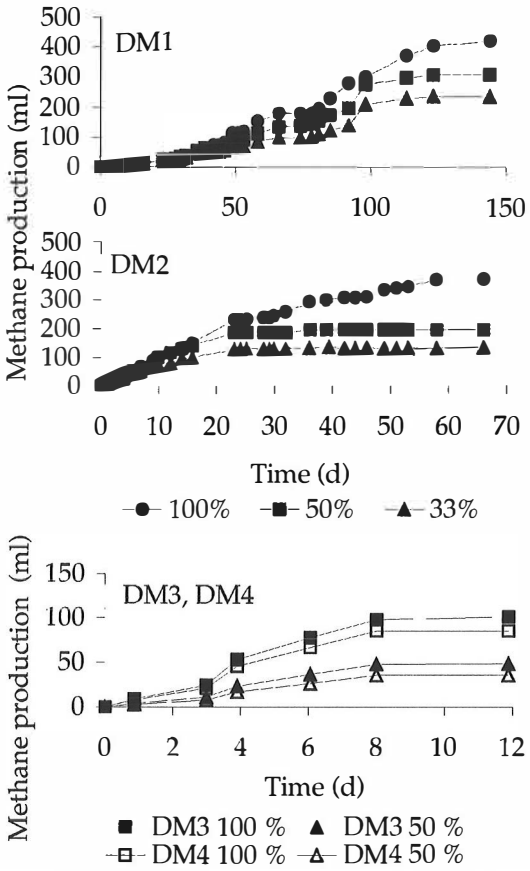

FIGURE 11 Methane production (note different x- and y-scales) of digested materials in assays with materials from different poultry slaughterhouse waste digesters (referred to as DM1 to DM4) with and without dilution (indicated in percent of material in assay) (IV).

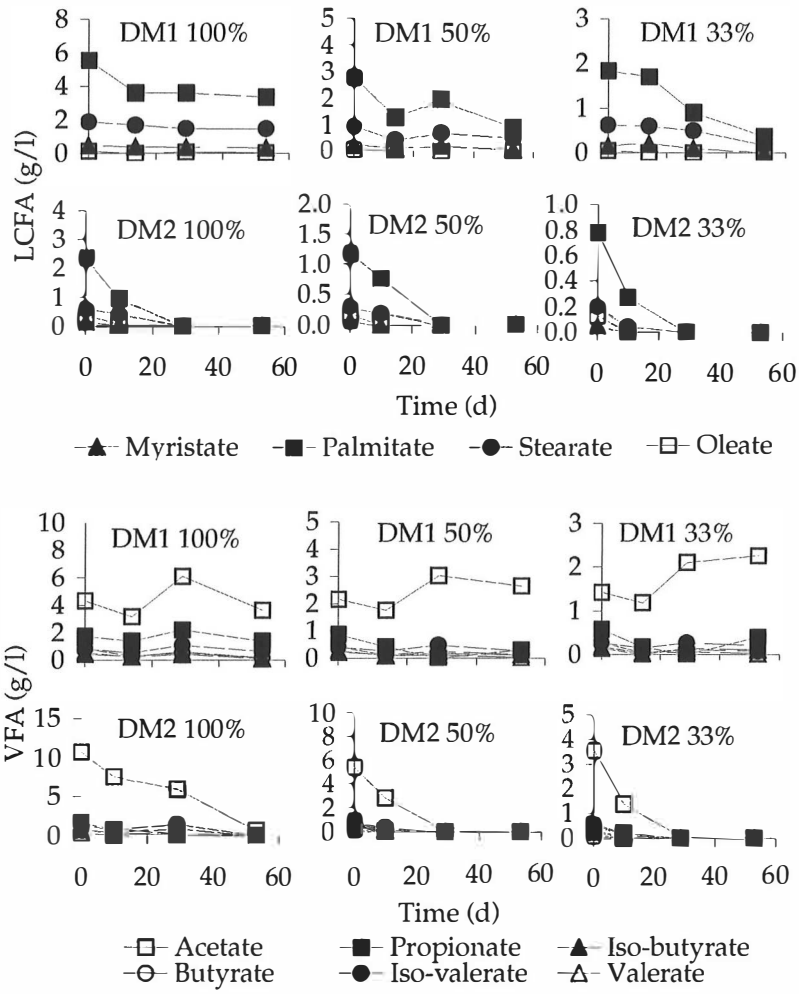

FIGURE 12 VFA and LCFA concentrations in assays with materials from different poultry slaughterhouse waste digesters (referred to as DM4 to DM7) with and without dilution (indicated in percent of material in assay) (IV).

Materials from the different layers of the poultry slaughterhouse waste digester 4 (III) showing normal performance were characterised (Table 3) and assayed to study their methane production (IV). Furthermore, the whole digested material, the centrifuged cake and supernatant, were assayed with reference to layer degradation (IV). All materials were assayed as such and with additional inocula to examine whether adding active biomass may have enhanced their methane production.

The highly non-uniform distribution of different materials in the different layers of the digester 4 (see Tables 3 and 9) suggests the high tendency of the

poultry slaughterhouse waste to stratification in anaerobic digestion. About 85 percent of total LCFAs floated on top of the digester (Table 9), a fact which may have affected their bioavailability and toxicity. In the batch assays the methanation of the materials in any of the layers did not appear severely inhibited, as the microbial populations in the different layers were readily capable of using the layers for considerable methane production and as additional inocula did not considerably enhance methane production (Fig. 13, Table 10). However, the layers in the assays originated from a well functioning digester with a low concentration of LCFAs, and these results could well have been different with materials from the layers of a disturbed digester, for most of the LCFAs would undoubtedly have appeared in the top layer, inhibiting the degradation. On the other hand, because LCFAs were proven to be floating, they could easily be removed from the top of the digester, which would probably relieve the inhibition. This, however, would also lower the methane yield in the digester.

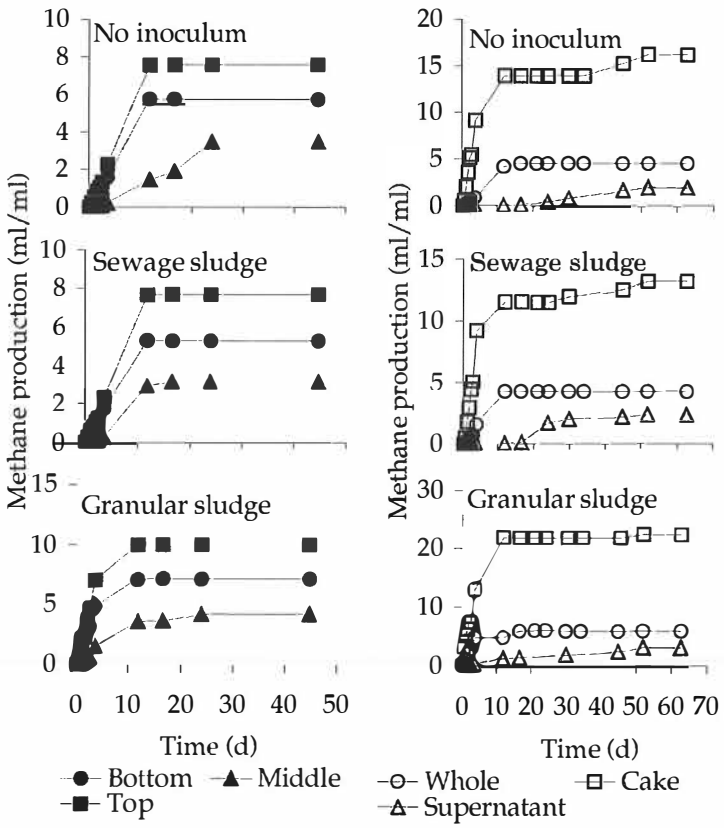

FIGURE 13 Methane production (note different x- and y-scales) of digested materials in assays with materials from different poultry slaughterhouse waste digester layers assayed with different inocula (IV).

TABLE 9 Comparison of contribution (%) of different layers to total mass with different layers of poultry slaughterhouse waste digester (IV).

Layer	Volume	TS	VS	LCFAs	Kjeldahl-N	Insoluble N	Methane yield
Bottom layer	24	34	33	15	27	32	31
Middle layer	65	31	31	0	49	20	51
Top layer	11	35	36	85	24	48	18

TABLE 10 Methane yields and specific methane yields in assays with different poultry slaughterhouse waste digester layers in 63 days assayed with different inocula (IV).

Material	Methane yield (l/kg sample)[1]			Specific methane yield (m^3/kg VS_{added})[1]		
	No Inoculum	DS	G	No Inoculum	DS	G
Bottom layer	5.7 (0.5)	5.3 (0.4)	7.1 (0.9)	0.28 (0.01)	0.27 (0.04)	0.36 (0.10)
Middle layer	3.5 (0.2)	3.2 (0.6)	4.2 (0.8)	0.50 (0.02)	0.46 (0.03)	0.52 (0.10)
Top layer	7.6 (0.9)	7.7 (1.7)	10 (1)	0.16 (0.02)	0.16 (0.03)	0.20 (0.02)
Whole material	4.5 (0.1)	4.2 (0.5)	5.8 (0.7)	0.22 (0.05)	0.20 (0.03)	0.28
Centrifuged cake	16 (1)	13 (1)	22.6 (0.8)	Na	Na	Na
Centrifuged supernatant	2.0 (0.1)	2.4 (0.2)	3.1 (0.4)	Na	Na	Na

DS = Digested sewage sludge; G = Granular sludge; [1] Inoculum methane yield excluded, average values with standard deviation in parentheses, N =3.

4.6 Anaerobically digested poultry slaughterhouse by-products and wastes as fertiliser in agriculture

Chemical and physical analysis and plant assays were used to investigate the suitability of anaerobically digested poultry slaughterhouse by-products and wastes for fertiliser in agriculture and the effect of aerobic post-treatment on its material properties (V).

The digested material from the semi-continuously fed, laboratory-scale digesters treating poultry slaughterhouse by-products and wastes (See section 4.3, III) was rich in nitrogen (ca. 20% N of TS) when compared to digested biowaste (ca. 7% N of TS; Vermeulen et al. 1992) or digested municipal sewage sludge (typically 2.5% N of TS; Metcalf & Eddy 1991). The growth of carrot in 27-day growth assays with the material as nitrogen source was almost comparable to that in the reference fertiliser, a commercial mineral fertiliser. However, the material inhibited the growth of Chinese cabbage (Table 11).

TABLE 11 Effect of anaerobically digested poultry slaughterhouse waste (72 g DM1/kg substrate) on carrot and Chinese cabbage root and aboveground lengths and dry weights as compared to reference fertiliser (690 g/kg) (110 mg soluble N/kg substrate, N=30) (V).

Material	Carrot (*Daucus carota*)		Chinese cabbage (*Brassica campestris var. chinensis*)	
	DM1[1]	Fertiliser	DM1[1]	Fertiliser
Aboveground vegetation length (mm)	9.3 (1.6)	10.3 (1.5)	4.0 (1.7)[2]	13.4 (0.9)
Root length (mm)	4.9 (2.2)	6.8 (1.9)	3.5 (1.8)[2]	11.8 (2.3)
Aboveground vegetation dry weight (mg)	12.6 (4.1)[2]	20.9 (10.2)	29.2 (22.1)[2]	340.5 (134.0)
Root dry weight (mg)	1.8 (1.1)[3]	1.3 (0.5)[3]	3.4 (1.9)[3]	24.2 (10.3)[3]

[1] Standard deviation in parentheses; [2] Significantly different from fertiliser at $\alpha = 0.05$ by one-way ANOVA; [3] Ln transformation applied to equalise variances.

In further 5-d phytotoxicity assays, the digested material inhibited the germination (Fig. 14) and root growth (Fig. 15) of ryegrass and Chinese cabbage, apparently because of organic acids present in it. Regression analysis suggested that the inhibition was related to organic acids, which have previously been shown to inhibit root growth and cause ion loss from roots (Lee 1977; Lynch 1980). However, in these tests, organic acid concentrations were lower than those previously found inhibitory (Lynch 1977; 1980; DeVleeschauwer et al. 1981; Manios et al. 1989; Marambe et al. 1993).

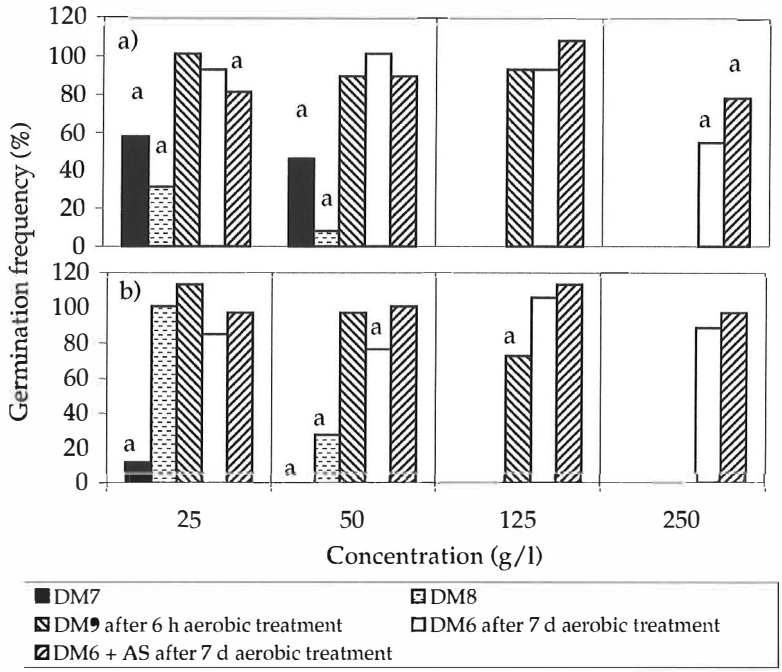

FIGURE 14 Effect of anaerobically digested poultry slaughterhouse waste samples DM7 and DM8 and aerobically post-treated digested material samples DM6 and DM9 on a) ryegrass (*Lolium perenne*) and b) Chinese cabbage (*Brassica campestris* var. *chinensis*) germination frequency at different material concentrations (in range of 25 g/l to 250 g/l), diluted with deionised water, deionised water as control. The control germination frequency of ryegrass and Chinese cabbage was 86% and 82%, respectively (N=19); AS = activated sludge; Significantly different from the control at $\alpha = 0.05$ by binomial test (V).

Regression analysis suggested a slight ammonia inhibition as well. Both the unionised and ionised form of ammonia may affect plant growth, but the inhibition mechanisms are different (Sudradjat 1990). The concentration of both, however, were very much lower than those reported earlier to be inhibitory (Hill et al. 1997; Tiquia & Tam 1998; Wong et al. 1983). Ammonia concentration followed the same general trend as the organic acid concentrations and soluble COD. Thus no single inhibitory factor emerged in the study.

As a whole, aerobic post-treatment of the digested material considerably reduced the phytotoxicity of the material, suggesting that the inhibiting compounds in the material were readily degraded aerobically or volatilised during the treatment. Previous studies confirm that aerobic post-treatment is capable of significantly reducing the phytotoxicity of anaerobically digested biowaste (Vermeulen et al. 1992).

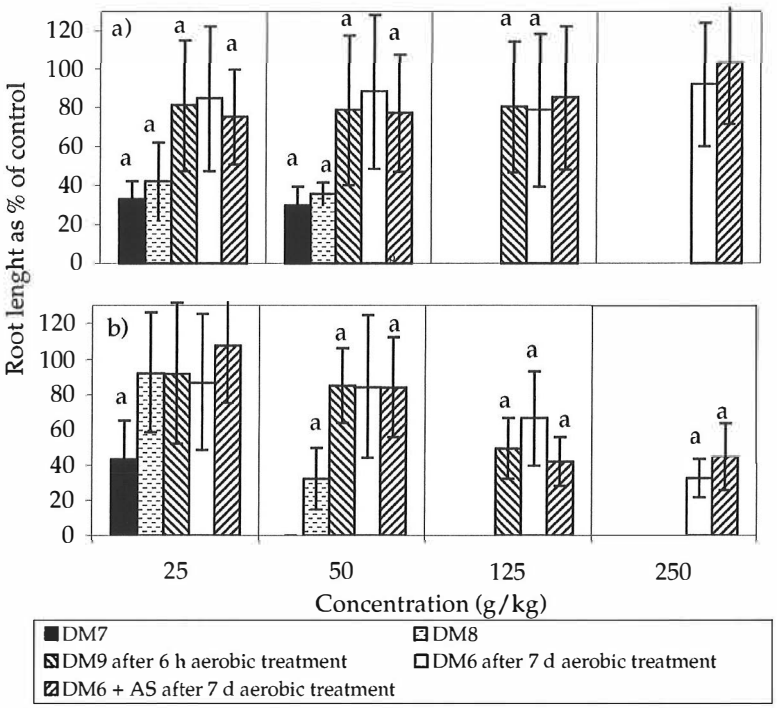

FIGURE 15 Effect of anaerobically digested poultry slaughterhouse waste samples DM7 and DM8 and aerobically post-treated digested material samples DM6 and DM9 on a) ryegrass (*Lolium perenne*) and b) Chinese cabbage (*Brassica campestris* var. *chinensis*) root lengths at different material concentrations (in range of 25 g/l to 250 g/l), diluted with deionised water, deionised water as control. Mean of control root length of ryegrass and Chinese cabbage was 25.1 mm, with standard deviation of 10.1 mm, and 26.9 mm, with standard deviation of 9.1, respectively (N=19); AS = activated sludge; Significantly different from the control at $\alpha = 0.05$ by one-way ANOVA (V).

In this study, the aerobic post-treatment of the digested material removed significant amounts of organic matter and ammonia, with the length of the post-treatment duration playing a key role, while the effect of the inoculum was less significant (Table 12). Apparently both non-biological (volatilisation) and biological (assimilation, dissimilation and nitrification) processes were involved, as indicated by the soluble COD and ammonia removals in biotic assays and abiotic controls. Efficient pH control up to moderate acidity might have reduced the significant nitrogen loss during the treatment, caused by the volatilisation of ammonia. To minimise emissions of volatile compounds and loss of ammonia during storage and spreading of treated materials, the storage tank and handling systems should be covered, and emissions should be recovered.

TABLE 12 Effects of aerobic post-treatment on characteristics of anaerobically digested poultry slaughterhouse waste samples DM6 and DM7. DM6 was treated as such and with activated sludge (AS). Abiotic tests (with $HgSO_4$ additions) and activated sludge alone were incubated as controls (all units mg/kg, except pH) (V).

Analysis	6-h treatment of DM9		7-day treatment of DM6 and controls							
			DM6		DM6+ $HgSO_4$	DM6 AS		DM6 +AS+ $HgSO_4$	AS	
	Initial	Final[1]	Initial	Final[1]	Final[1]	Initial	Final[1]	Final[1]	Initial	Final[1]
pH	7.7	8.5	7.1	6.7	6.6	7.0	6.8	6.5	7.0	6.8
Soluble COD	1100	840	720	190	390	740	210	460	20	20
Acetic acid	568	226	223	2	Na[2]	224	4	Na	1	2
Propionic acid	19	8	3	<1	Na	4	<1	Na	1	<1
Butyric acid	10	<1	<1	<1	Na	<1	<1	Na	<1	<1
Iso-butyric acid	3	6	<1	<1	Na	<1	<1	Na	<1	<1
Valeric acid	<1	<1	<1	<1	Na	<1	<1	Na	<1	<1
Iso-valeric acid	10	8	2	1	Na	2	<1	Na	<1	<1
Caproic acid	4	<1	<1	<1	Na	<1	<1	Na	<1	<1
Myristic acid	<1	<1	<1	<1	Na	<1	Na	Na	<1	Na
Palmitic acid	20	17	8	<1	Na	8	Na	Na	<1	Na
Stearic acid	5	3	<1	<1	Na	<1	Na	Na	<1	Na
Oleic acid	<1	<1	<1	<1	Na	<1	Na	Na	<1	Na
TS	5400	4900	5100	3200	Na	6300	2900	Na	1200	800
VS	4100	3900	3700	2400	Na	4400	2100	Na	700	600
Ammonia-N	970	680	920	230	200	920	20	220	<10	< 10
Kjeldahl-N	1450	1130	1040	580	460	1400	490	600	360	Na

[1] Water evaporation during the assays considered in calculated values; [2] Na = not analysed.

5 CONCLUSIONS

The anaerobic digestion of solid poultry slaughterhouse by-products and wastes rich in proteins and lipids is possible, but operation conditions need careful optimisation to avoid inhibition by LCFAs/ammonia.

In the biochemical methane potential batch assays at 35 and 55 °C, the different solid poultry slaughterhouse by-products and wastes: bone and trimmings, blood, offal, feather, and the mixture of the materials showed methane yields of 0.6-0.7, 0.5, 0.7-0.9, 0.2, and 0.6-0.7 m^3/kg VS_{added}, respectively. Probably LCFA inhibition delayed the methane production of offal, depending on the type and concentration of inoculum and on incubation temperature. Pre-treatments of feather increased its methane yield: combined thermal and enzymatic treatments increased methane yield by 37 to 51 %, whereas thermal, chemical, and enzymatic pre-treatments were less effective with methane yield increasing in the range of 5 to 32 %.

The anaerobic batch degradation patterns of the mixture of solid poultry slaughterhouse by-products and wastes indicated rapid hydrolysis/ acidogenesis, accumulation of LCFAs and VFAs, removal of LCFAs and subsequently that of VFAs, and methane production. The dynamic modelling of the results from the assays suggested that inhibited propionate degradation by LCFAs and inhibited hydrolysis by a high propionate concentration constituted the rate-limiting step in the degradation. The model also suggested that syntrophic LCFA degradation was the rate-limiting step at the beginning of all the assays, whereas in the final stage of the degradation, polymer hydrolysis turned out to be the rate limiter.

The anaerobic digestion of the slaughterhouse by-product and waste mixture was possible in semi-continuously fed laboratory-scale digesters, which, with a loading of up to 0.8 kg VS/m^3 d and a HRT of 50 days, showed a methane yield of up to 0.55 m^3 of methane/kg VS_{added}. The effect of HRT and loading on digester performance were found highly significant as digester performance was found inhibited at loadings from 1.0 to 2.1 kg VS/m^3 d, and HRTs from 12.5 to 25 days. The accumulated LCFAs were shown to be the main factor affecting the slow recovery of the digester from inhibition. LCFAs floated

considerably on top of the digester, which could have affected their bioavailability and toxicity.

The anaerobically digested poultry slaughterhouse by-products and wastes mixture was found rich in nitrogen, with ca. 20 % N of TS, mostly in the form of ammonia as a result of ammonification of organic nitrogenous compounds during anaerobic digestion. TS and VS reductions in the semi-continuous digester studies amounted to 50-70 %. In plant assays with the digested material as nitrogen source, carrots grew almost as well as those fertilised with a commercial mineral fertiliser used as reference, wheras the growth of Chinese gabbage was inhibited. The digested material was potentially phytotoxic probably because of VFAs and LCFAs present in it, whereas aerobic post-treatments considerably reduced the phytotoxicity, indicating that the inhibiting compounds in material were readily degraded aerobically or volatilised during the treatments.

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YHTEENVETO

Siipikarjateurastuksen sivutuotteiden ja jätteiden anaerobinen käsittely

Siipikarjan lihan kulutus on viimeisten vuosikymmenten aikana kasvanut huomattavasti. Siipikarjateurastamot tuottavat myös suuria määriä orgaanisia jätteitä ja sivutuotteita, joista huomattava osa hyödynnetään rehun tuotannossa. Rehukäyttöä ollaan enenevässä määrin rajoittamassa. Lainsäädännön kiristyessä myös jätteiden loppusijoittaminen kaatopaikoille kallistuu ja käsittelemättömän orgaanisen jätteen sijoittamista kaatopaikoille ollaan kieltämässä. Mahdollinen rehukäytön estyminen tai käsittelykustannusten kohoaminen voisi merkittävästi heikentää siipikarjateurastamoiden toimintaedellytyksiä.

Kiinnostus kiinteiden jätteiden anaerobikäsittelyä kohtaan on lisääntynyt viime vuosina. Anaerobikäsittelyssä jätteestä tuotetaan metaania, jota voidaan käyttää energiantuotantoon, sekä maanparannusaineeksi tai lannoitteeksi soveltuvaa ainesta. Anaerobikäsittelyssä ravinnehäviöt ovat vähäiset. Käsittelyssä jätemassa stabiloituu. Anaerobinen käsittely tuhoaa lisäksi patogeenejä.

Tämän tutkimuksen tavoitteena on ollut kehittää luotettavia ja kilpailukykyisiä menetelmiä siipikarjateurastuksen orgaanisten sivutuotteiden ja jätteiden käsittelyyn. Nämä materiaalit sisältävät tyypillisesti runsaasti typpeä ja rasvoja, jotka voivat anaerobikäsittelyssä aiheuttaa ongelmia. Orgaanisen typen ammonifikaation seurauksena muodostuu ammoniakkia, joka voi korkeissa pitoisuuksissa hidastaa tai estää anaerobisten mikro-organismien toimintaa. Rasvojen hajotessa muodostuvat pitkäketjuiset rasvahapot voivat myös hidastaa tai estää anaerobisten mikro-organismien toimintaa.

Tutkittu siipikarjateurastamon jäte osoittautui anaerobisesti hyvin hajoavaksi. Runsaasti rasvoja sisältävien teurastamojätteiden anaerobikäsittelyn mitoituksessa ja operoinnissa on kuitenkin kiinnitettävä erityistä huomiota pitkäketjuisten rasvahappojen muodostumiseen ja edelleen hajoamiseen.

Tutkituista siipikarjateurastamon jätteistä broilerin sisäelimet tuottivat eniten metaania, 0,7-0,9 m^3/kg VS. Hajoamisen alkuvaiheessa todennäköisimmin pitkäketjuiset rasvahapot kuitenkin inhiboivat metaanintuottoa. Siementietteellä näyttäisi olevan ratkaiseva merkitys inhibition suhteen. Siipikarjateurastamon jätteiden seos tuotti panoskokeissa metaania 0,55-0,67 m^3/kg VS. Höyhenen metaanintuotto oli tutkituista jätejakeista alhaisin, 0,21 m^3/kg VS. Höyhenen keratiini on anaerobisesti vaikeasti hajoavaa. Höyhenen hajoaminen kokeissa olikin vähäistä. Lämpö-, emäs- ja entsyymikäsittelyiden vaikutusta höyhenen anaerobiseen hajoamiseen tutkittiin. Yhdistetyllä lämpö- ja entsyymikäsittelyllä höyhenen metaanintuottoa voitiin lisätä jopa 50 prosentilla.

Jatkuvatoimisissa mesofiilisissa täyssekoitusreaktoreissa prosessi oli stabiili kuormituksella 0,8 kg VS/ m^3 d, mikä vastasi n. 50 päivän käsittelyviipymää. Metaanintuotto oli 0,52 to 0,55 m^3/kg VS. Korkeammilla kuormituksilla 1,0-2,1 kg VS/ m^3 d ja lyhyemmällä käsittelyviipymällä 12,5-25 d prosessin toiminta oli epästabiilia. Akkumuloituneet pitkäketjuiset rasvahapot todennäköisesti inhiboivat metaanintuoton. Panoskokeet reaktorista otetuilla näytteillä osoittivat, että inhiboituneen reaktorin toipuminen oli hidasta samoin kuin pitkäketjuisten rasvahappojen hajoaminenkin. Näytteiden laimentaminen

vedellä ei nopeuttanut metaanintuottoa.

Matemaattisella mallinnuksella pystyttiin osoittamaan kriittisiä hajoamisen osavaiheita ja arvioimaan eri osatekijöiden, kuten pitkäketjuisten rasvahappojen pitoisuuden vaikutusta muihin osatekijöihin ja hajoamisen eri vaiheisiin, kuten metaanintuottoon. Anaerobiprosessi on monimutkainen, itseään säätelevä järjestelmä, johon vaikuttavat useat toisistaan riippuvat ja riippumattomat, yleiset ja tapauskohtaiset tekijät. Mallinnuksen tulosten perusteella siipikarjateurastuksen jätteiden hajoamisessa tutkituissa olosuhteissa pitkäketjuiset rasvahapot inhiboivat propionaatin hajotuksen. Akkumuloitunut propionaatti puolestaan inhiboi hydrolyysivaiheen. Pitkäketjuisten rasvahappojen hajoaminen osoittautui hajoamista rajoittavaksi vaiheeksi kokeen alussa. Kokeen lopussa polymeerien hydrolysoitumisesta tuli hajoamista rajoittava vaihe.

Alustavien kasvatuskokeiden perusteella anaerobisesti käsitelty siipikarjateurastuksen jäte näyttää soveltuvan eräin edellytyksin lannoitteeksi tai lannoitteen raaka-aineeksi. Materiaalin typpipitoisuus oli huomattavan korkea (noin 20 %/TS). 30 päivän kasvatuskokeessa porkkanalla ei havaittu typen lähteenä merkittävää eroa (verson ja juuren pituus ja kuivapaino) kaupalliseen mineraalilannoitteeseen verrattuna. Sen sijaan käsitellyn jätteen todettiin jonkin verran heikentävän kiinankaalin kasvua. Viiden päivän itävyyskokeissa kiinankaalilla ja rairuoholla lyhytketjuisten ja pitkäketjuisten rasvahappojen todettiin inhiboivan itävyyttä. Jo lyhytaikainen ilmastus vähensi merkittävästi rasvahappojen pitoisuuksia ja kasvitoksisuutta.

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Original papers

I

The methane production of poultry slaughtering residues and effects of pre-treatments on the methane production of poultry feather

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II

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IV

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V

Anaerobically digested poultry slaughterhouse wastes as fertiliser in agriculture

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VI

Anaerobic digestion of organic solid poultry slaughterhouse waste – a review

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