final report

Project code: A.ENV.0155

Prepared by: Paul Jensen

Advanced Water Management Centre
The University of Queensland

Date published: October 2013

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Anaerobic Co-digestion of Paunch and DAF sludge

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

Executive Summary

This is the final report for A.ENV.0155 Anaerobic Digestion of Paunch and DAF sludge. Management of paunch and DAF sludge have been identified as key issues in the Australian red meat processing industry. Both paunch waste and DAF sludge are large byproduct streams from the red meat industry, which also represent a substantial potential source of energy, and nutrients.

The UQ Biosolids project has operated a paunch digester at Teys Beenleigh since 2010. The results had previously shown a high degree of degradability in the paunch solids (>60%), such that on the order of 20% of plant heating requirements can be generated from the paunch. While the demonstration plant has previously field-proven the technology, A.ENV.0155 was developed to make the technology more attractive, and increase its utility to processors, by maximising space loading (decreasing capital costs), evaluating the impact of anaerobic digestion on dewaterability and other properties (e.g. viscosity), and exploring co-digestion as a strategy to boost biogas yield.

The maximum sustainable organic loading rate for the demonstration plant was estimated at 1-1.3 kgVS/m^3/day using a feed solids concentration of approximately 3%. At this loading rate, organic solids destruction (60%) and methane production (220 L CH_4 kg^{-1} VS) in the demonstration plant were similar to levels predicted from independent batch tests. Results from the demonstration plant indicate that an average sized processing facility (processing 600 beef cattle per day) could reduce paunch waste from 15 tonnes of wet solid per day to around 5 wet tonnes per day using anaerobic digestion. At feed concentrations above 3% solids, the demonstration plant had significant problems with materials handling resulting in solids accumulation, blockages and process failure. This was an engineering limitation of the process and not a biological limitation. Loading rates could likely be improved by re-engineering the mixing systems in the process vessels.

Anaerobic co-digestion (AcoD) is a process where two or more substrates with complementary characteristics are mixed for combined treatment. Investigations of anaerobic co-digestion of slaughterhouse wastes demonstrated that co-digestion is a promising strategy to improve process performance. In all cases, the B_0 from AcoD mixtures was higher than the B_0 of the paunch-only digestions. Co-digestion of paunch and DAF sludge was the most promising with results suggesting synergistic effects where the microorganisms present in the paunch may have contributed to improved hydrolysis of the partially biodegradable fat conglomerates present in the DAF sludge.

Investigations of AcoD in this project were based on batch tests. The next major investigation in this area should focus on examining the outcomes of the batch co-digestion trials in a continuous process; particularly co-digestion of paunch solids and DAF sludge. While AcoD was identified as a suitable strategy to boost methane production from the paunch digester, the impact of substrate composition and AcoD ratios on nutrient release and recovery potential was not investigated in this project. This is another area recommended for future investigation.

Table of Contents

Executive Summary 2

Abbreviations 4

1 Introduction 5

1.1 Background 5
1.2 Project Objectives 6
2 Optimal Operation of Demonstration Scale Paunch Digester 6

2.1 Summary of Plant Design 6
2.2 Process Performance 7
2.2.1 Characterisation of Paunch 7

2.2.2 Biogas Production 8

2.2.3 Organic Solids Removal 9

2.2.4 Dewaterability 10

2.3 Summary 10
3 Anaerobic Co-digestion 11

3.1 Materials and Methods 11
3.1.1 Substrate 11

3.1.2 Biochemical Methane Potential test (BMP) 12

3.1.3 Model Implementation and Data Analysis 15

3.2 Results and Discussion 15
3.2.1 Methane Production from Synthetic Substrates 15

3.2.2 Methane Production from Meat Processing Wastes 17

3.2.3 Model-based Analysis 19

3.3 Summary 22
4 Conclusions and Recommendations 22

5 References 23

Abbreviations

AcoD	Anaerobic co-digestion
AD	Anaerobic Digestion
AL	Anaerobic Lagoon
BMP	Biochemical Methane Potential
B_0	Ultimate Modelled Biochemical Methane Potential
Ch	Carbohydrate
CAL	Covered Anaerobic Lagoon
COD	Chemical Oxygen Demand
DAF	Dissolved Air Flotation (tank)
EBCRC	Environmental Biotechnology Cooperative Research Centre
F_d	Fraction of Organic Material that is Degradable under Anaerobic Conditions
FOG	Fats, Oils and Grease
HRT	Hydraulic Residence Time
IC_{50}	Concentration where 50% inhibition occurs e.g. rate is reduced to 50%
K_{hyd}	First Order Hydrolysis Rate Constant: Speed of Degradation
LCFA	Long Chain Fatty Acids
Li	Lipid
NH_4-N	Ammonium nitrogen
PO_4-P	Phosphate Phosphorus
Pr	Protein
SRT	Sludge Retention Time
TKN	Total Kjehldahl Nitrogen
TKP	Total Kjehldahl Phosphorus
TPAD	Temperature Phased Anaerobic Digestion
TS	Total Solids
TSS	Total Suspended Solids
UQ	The University of Queensland
VFA	Volatile Fatty Acids
VS	Volatile Solids

1 Introduction

1.1 Background

There is strong ongoing interest in anaerobic digestion (AD) of waste solids, particularly paunch and DAF sludge. This is partly driven by an established need for an alternative to current waste sinks, which are largely composting, direct land application, and landfilling. Alternative waste sinks will address costs and risks associated with current disposal applications, which are increasing either due to implementation of policy (e.g., Qld Landfill Levy), regulations around pathogen control in land application and composting, and potential social impacts of direct disposal or land application. AMPC and MLA are actively exploring anaerobic digestion as a potential new sink, as well as other options such as thermal destruction or charring, which are likely to have specific strengths and weaknesses (e.g., complexity, capital cost); but are not addressed in this project.

The UQ Biosolids project has operated a paunch digester at Teys Beenleigh since 2010, with setup on paunch liquor in 2010/11, and operating on paunch solids in 2011/12. The results so far have shown a high degree of degradability in the paunch solids (>60%), such that on the order of 20% of plant heating requirements can be generated from the paunch. The demonstration has field proven the technology, and enough data and construction and operational experience now exists to build a full-scale digester. A.ENV.0155 was developed to make the technology more attractive, and increase its utility to processors, by maximising space loading (decreasing capital costs), evaluating the impact of anaerobic digestion on dewaterability and other properties (e.g., viscosity), and exploring co-digestion as a strategy to boost biogas yields.

Anaerobic co-digestion (AcoD) is a process where two or more substrates with complementary characteristics are mixed for combined treatment. AcoD often results in improved biogas production, however this improvement in methane production is generally a result of an increase in organic loading rate (*Astals et al. 2012*). When possible, co-substrates are selected and blended in ratios to: (i) favour positive interactions, i.e. synergisms, macro- and micro-nutrient equilibrium and moisture balance; (ii) dilute inhibitory or toxic compounds; (iii) optimise methane production and (iv) enhance stability of digested materials (*Astals et al. 2011, Mata-Alvarez et al. 2011*).

Cattle slaughterhouses generate multiple waste streams with highly variable compositions and methane yields ranging from 200 L CH_4 kg^{-1}VS to over 1000 L CH_4 kg^{-1}VS (findings taken from AMPC/MLA research projects A.ENV.0131, A.ENV.0151) (*Hejnfelt and Angelidaki 2009, Zhang and Banks 2012*). Anaerobic treatment of these wastes present risks associated with the high concentration of ammonium (NH_4+) and/or long chain fatty acids (LCFA) in some streams and the potential for inhibition of anaerobic microorganisms (*Cuetos et al. 2008*). Since ammonia is a by-product of protein acidification and LCFAs are intermediate products from the degradation of fat, oil and grease, inhibition may be directly linked to the macro composition of the substrate (carbohydrates, protein and lipids). At the present time, there is limited knowledge about the influence of macro composition on AcoD performance as well as on interactions between substrates that may enhance or attenuate inhibition thresholds, degradation rates, or biogas yields. The degradation of carbohydrates, protein and lipids are characterised by different metabolic pathways, rates and methane yields (*Angelidaki and Sanders 2004*); therefore knowledge about the influence of the substrate macro-composition would enhance the understanding and utility of potential and/or novel AcoD applications.

Reliable modelling of AcoD is required for clear and quantifiable predictions on the effect of mixing two or more wastes in a digester and remove potentially negative impacts from mixing based on random or heuristic decisions (*Astals et al. 2011, Mata-Alvarez et al. 2011*). In addition, a more detailed mechanistic understanding of how different substrates interact is expected to reduce the time and costs associated with laboratory experiments as well as improve co-substrate selection and dose rates (*Galí et al. 2009*). Models are also useful to estimate important biochemical parameters such as biodegradability, hydrolysis rate and inhibition characteristics, which are critical in AD process design, performance and troubleshooting (*Batstone et al. 2009, Jensen et al. 2011*).

1.2 Project Objectives

Anaerobic digestion has been successfully demonstrated on paunch solids and results are scalable. This project was developed in order to reduce risk and process costs and enhance feasibility through analysis of mixed stream co-digestion as well as quantify impact on dewaterability. Specific objectives were to:

- Identify the upper limit for paunch in terms of mass loading through increased feed concentration, monitoring, and viscosity testing;
- Test the feasibility of mixed paunch, DAF sludge, and red stream digestion through laboratory testing; and if possible extend to the consortium pilot plant;
- Assess the impact of digestion on dewaterability for feed and digestate using the Kopp drying test;
- Generate final solids reduction levels (volumetric destruction levels) for own or 3rd party cost benefit analysis.

2 Optimal Operation of Demonstration Scale Paunch Digester

2.1 Summary of Plant Design

The paunch digester used in this project was the EBCRC/UQ/MLA Biosolids Demonstration facility at Teys Aust, Beenleigh. The plant is based on a Temperature Phased Anaerobic Digestion (TPAD) process. TPAD is a two stage thermophilic-mesophilic treatment process. The first stage is operated at higher temperature (>50°C), with a 2-4 day retention time while the second stage is operated at moderate temperature (~35°C) with a 12-20 day retention time. The first stage is designed to destroy pathogens and enhance hydrolysis to condition the organic material and improve digestibility, while the second stage is designed to produce methane which can be used for renewable energy generation and stabilised organic residues (e.g. biosolids) which can be reused in agriculture. A process flow diagram for the demonstration plant is shown in Figure 1. The plant was commissioned as part of A.ENV.0099 and was operated previously at a solids feed concentration of 1-2% and organic loading rate of 0.5 kgCOD/m^3/d. Detailed design reports and process performance data are available as outputs from A.ENV.0099.

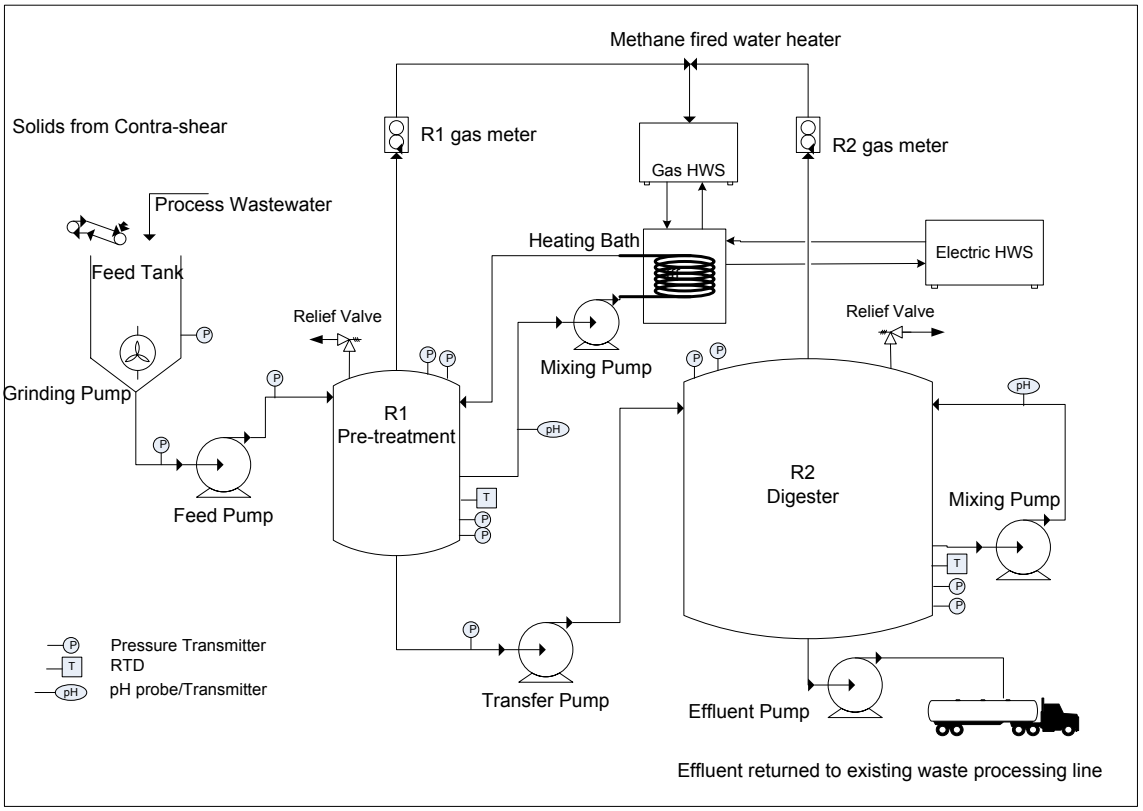

Figure 1: Process flow diagram of Biosolids Demonstration facility at Teys Aust.

2.2 Process Performance

2.2.1 Characterisation of Paunch

Paunch solids were used as the substrate for the Biosolids Demonstration Plant. The paunch solids were collected after screening using a contrashear rotating drum screen. The collection point was selected based on access limitations and screening is not a requirement of the process. The screened paunch had a solids content of approximately 12% and required dilution to approximately 3% solids using process water. Feed concentrations above 3% resulted in transportation issues. In addition, solids would accumulate in the thermophilic pre-treatment stage causing blockages of process equipment and subsequent process failures. The average composition of diluted paunch feed (and 95% confidence margin) is shown in Table 1.

Table 1: Characterisation of paunch solids used as process feed

Characteristics	Average
Total Solids (g/L)	29.6 ± 2.5
Volatile Solids (g/L)	26.2 ± 2.4
pH	6.8 ± 0.3
Chemical Oxygen Demand (g/L)	28.7 ± 2.5
Volatile Fatty Acid (g/L)	0.7 ± 0.3
NH_4^+-N (g/L N)	0.16 ± 0.02

The demonstration plant was operated successfully at an organic loading rate of 1-1.3 kgCOD/m^3/d. This is more than double the organic loading rate achieved in A.ENV.0099.

2.2.2 Biogas Production

Production of methane rich biogas is a primary performance indicator of anaerobic processes and indicates the potential for renewable energy production during the stabilisation of organic matter. Biogas production from the demonstration plant is shown in Figure 2. The biogas composition was typically 63% methane (CH_4) and 37% carbon dioxide (CO_2), during full and steady operation this corresponds to 15 m^3 of CH_4 per day or approximately 220 L CH_4 kg^{-1} VS.

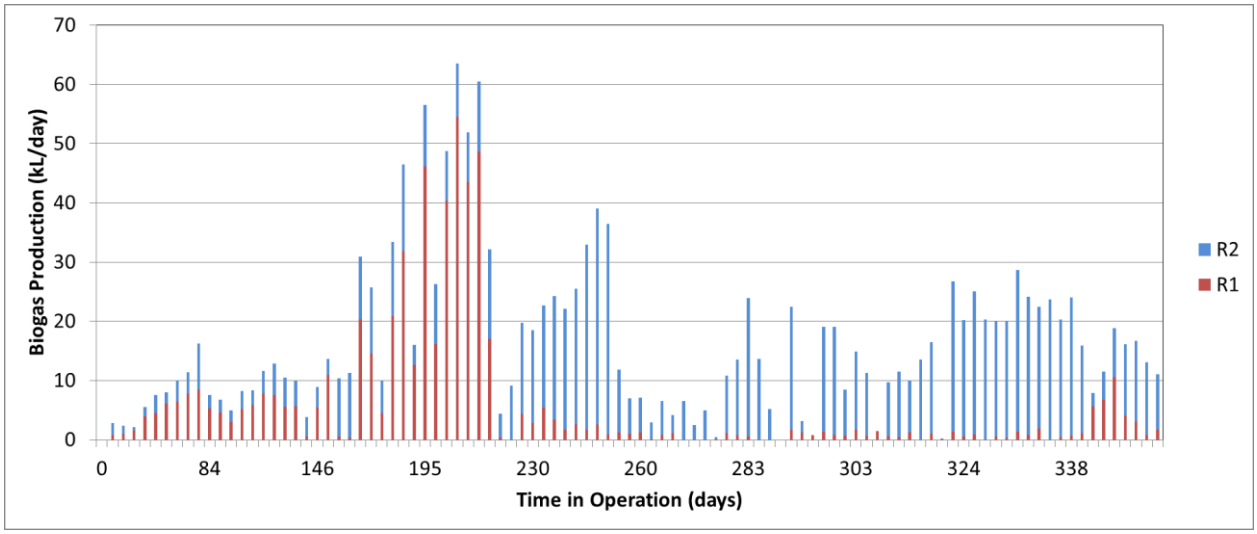

Figure 2: Biogas production from the Biosolids Demonstration facility (R1: Thermophilic Pre-treatment reactor; R2: Mesophilic Digester).

The biochemical methane potential of paunch solids at Teys Aust. (Beenleigh) was measured at 237 L CH_4 kg^{-1} VS using batch tests (Section 3.2.2). Comparisons of the demonstration plant performance and batch tests show that over 90% of the degradable material in paunch was converted to methane in the demonstration plant. The combination of biogas production and low VFA concentrations in the digester effluent were strong indications of a healthy and stable process.

Methane production from the demonstration plant appears low compared to methane production from anaerobic lagoons, however this is due to the structure of waste being treated. The biochemical methane potential of combined slaughterhouse wastewater is in the range of 600-800 L CH_4 kg^{-1} VS (A.ENV.0131 and A.ENV.0151) due to the higher protein and lipid content in the combined effluent. However, a CAL will typically only convert 60-80% of the organic matter to methane. Therefore, while methane production is higher in CALs, the actual conversion and yield is lower than the demonstration plant.

2.2.3 Organic Solids Removal

Volatile solids (VS) destruction is also a primary performance indicator of anaerobic processes and indicates the reduction and stabilisation of organic matter. VS destruction was calculated using two independent methods, the Van Kleeck equation, which assumes that the amount of fixed solids is conserved during digestion (*Switzenbaum et al. 2003*). It can be expressed as:

$$VS\ destruction\% = \frac{VS_{fraci} - VS_{fraco}}{VS_{fraci} - \left(VS_{fraci} \times VS_{fraco}\right)} \times 100 \qquad (1)$$

Where VS_{fraci} and VS_{frac0} are volatile fractions (VS/TS) in the influent and effluent solids.

VS destruction is also calculated using a mass balance equation:

$$VS\ destruction\% = \frac{VS_{conci} - VS_{conco}}{VS_{conci}} \times 100 \qquad (2)$$

Where VS_{conci} and VS_{conc0} are volatile solids concentrations (g/L VS) in the influent and effluent solids.

Volatile solids destruction levels in the demonstration plant were variable due to the variable composition of feed material (2-4% solids), however average VS destruction has remained high at approximately 60% (Figure 3). This is similar to results from laboratory tests showing VS destruction of 50-65% expected from paunch solids and confirms that the demonstration plant was operating successfully and removing a high fraction of the degradable material in paunch.

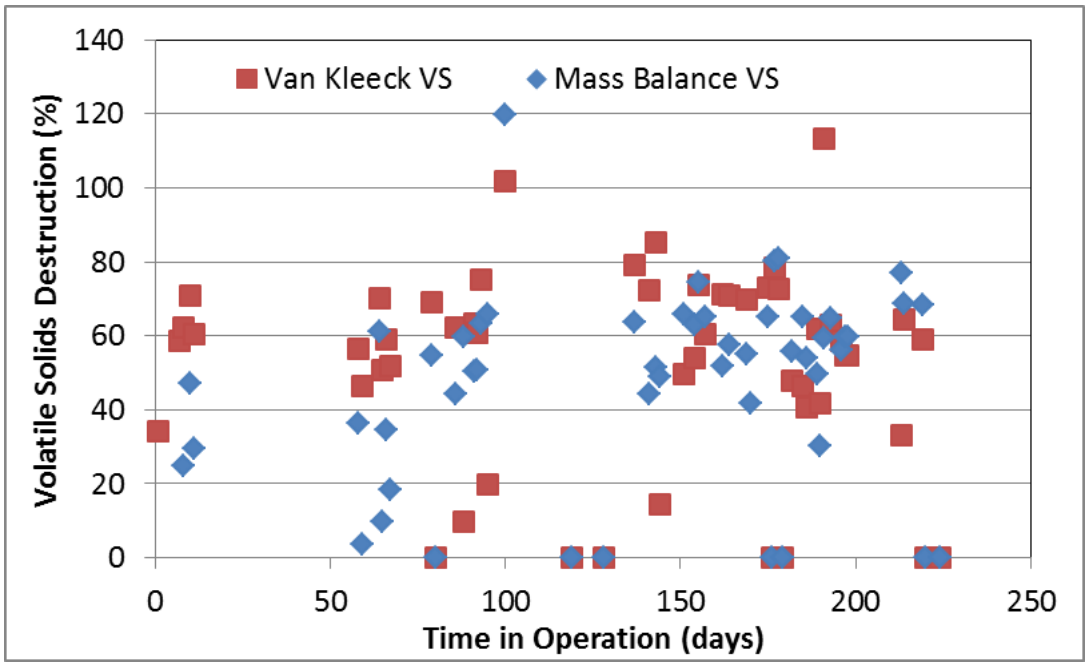

Figure 3: Volatile solids destruction across the Biosolids Demonstration facility as a percentage of feed material and assessed using the Van Kleeck equation and a mass balance equation.

2.2.4 Dewaterability

The Kopp method (*Kopp and Dichtl 2000*) was used to examine the dewatering properties of digested paunch. The Kopp method measures drying rate as the moisture changes in a controlled situation, and determines free and capillary moisture based on inflection points in the drying curve. The results are summarised in Table 2 and suggest digested paunch cake could be dewatered to approximately 24% solids in a full scale centrifuge or belt press. Actual cake solids may be influenced by paunch type (grass, grain etc.) and will be highly dependent on the actual dewatering unit. A recent MLA/AMPC project investigating paunch value-add options reported that the dewaterability of raw paunch solids varied from 15% solids to over 70% solids (A.ENV.0153). Therefore, the dewaterability of raw paunch in this project was low by industry standards. However, the digested paunch samples were very similar throughout the duration of this project and demonstrated that anaerobic digestion will increase the dewaterability of paunch.

Table 2: Summary of dewaterability analysis

Reactor	Dewaterability
Paunch	16±2%
Digested Paunch 1	23±2%
Digested Paunch 2	24±2%
Digested Paunch 3	24±3%

We concluded that AD will significantly reduce the volume of paunch requiring transport for disposal or beneficial re-use in agriculture. It is estimated that converting approximately 50% of solids to methane and increasing the solids content the digested cake using AD will reduce the solids transport load to 1/3 of the load from paunch without treatment. That is, instead of needing to truck over 15 m^3 of wet solid per day, an average sized processer (600 beef cattle per day) would need to truck about 5 m^3 per day.

Costs for paunch disposal are highly variable ranging from \$0/m^3 to \$30/m^3, with a median value of \$17.90/m^3 (as reported in MLA project A.ENV.0153). Therefore, while there is considerable room for cost reduction from paunch disposal, the actual savings will be highly site specific.

2.3 Summary

The Demonstration Plant was operated for over 200 days with automated feeding of paunch solids. Volatile solids destruction levels remain high averaging 60% with methane production corresponding to approximately 220 L CH_4 kg^{-1}VS added. The combination of biogas production and low VFA concentrations in the digester effluent are a good indication of a healthy and stable process.

In addition to generation of renewable energy, anaerobic digestion will significantly reduce the volume of paunch requiring transport for disposal or beneficial re-use in agriculture. It is estimated that converting approximately 50% of solids to methane and increasing the solids content the digested cake using AD will reduce the solids transport load to 1/3 of the load from paunch without treatment.

The maximum sustainable organic loading rate for the demonstration plant has now been estimated at 1-1.3 kgVS/m^3/d using a feed solids concentration of approximately 3%. At a feed concentration above 3%, solids accumulate in the thermophilic first stage causing materials handling issues, blockages of process equipment and subsequent process failures. Currently this is an engineering limitation of the system and could be addressed by re-designing the reactor mixing system. The biological limitation of the system has not been determined, but is estimated at 2.5-3 kgVS/m^3/d based on laboratory batch tests.

Organic loading rates for mesophilic digesters treating sewage are typically in the range of 1-2 kgVS/m^3/d. This is based on a feed concentration of 4-5% total solids and a solids retention time of 20-30 days (*Batstone and Jensen 2011*). While in the lower part of this range, the performance of the Biosolids Demonstration Plant was comparable to full scale digesters treating municipal wastewater sludge.

3 Anaerobic Co-digestion

The batch co-digestion experiments were conducted as a collaboration between the Advanced Water Management Centre at The University of Queensland and the Department of Chemical Engineering at The University of Barcelona.

The primary objective of the anaerobic co-digestion batch tests was to identify the interactions (synergisms and antagonisms) between carbohydrates, protein and lipids that take place during anaerobic co-digestion, focusing on process kinetics and the anaerobic biodegradability of the substrates for a mechanistic model-based understanding of AcoD. To achieve this, the project first examined synthetic substrates containing only a carbohydrate (Ch), protein (Pr) or lipid (Li). The project then investigated more complex slaughterhouse substrates representing a concentrated source of a carbohydrate, protein or lipid. The co-digestion tests were designed to identify AcoD opportunities within the red meat processing industry and consequently improve the performance of paunch digestion and AD of other slaughterhouse wastes.

3.1 Materials and Methods

3.1.1 Substrate

Pure substrates included analytical grade cellulose and casein purchased from Sigma-Aldrich® and white-label refined olive oil, which contained mainly palmitic, oleic and linoleic acid (*AOCS 2013*). Paunch solids, DAF sludge and blood wastewater were selected as slaughterhouse substrates to investigate co-digestion of carbohydrates (Ch), lipids (Li) and proteins (Pr) respectively. Table 3 shows a basic characterisation of the pure substrates, while Table 4 shows a complete physical-chemical characterisation of the slaughterhouse wastes used in this project. The slaughterhouse wastes were obtained from a Queensland slaughterhouse processing beef only (mixed grass and grain fed), wastes from mixed species plants, or beef only plants processing cattle with a different diet, may have different compositions.

Table 3: Characterisation of the pure substrates

	Units	Cellulose	Casein	Olive oil
TS	g kg^{-1}	918	946	1000
VS	g kg^{-1}	915	913	1000
COD_T	g O_2 kg^{-1}	976	1401	2890

Table 4: Characterisation of the slaughterhouse wastes

	Units	Paunch	Blood	DAF
TS	g kg^{-1}	117	187	360
VS	g kg^{-1}	106	178	353
COD_T	g O_2 kg^{-1}	106	266	1053
COD_S	g O_2 kg^{-1}	2.5	253	3.7
VFA	g L^{-1}	0.64	1.86	0.52
- Acetic acid	g L^{-1}	0.36	1.47	0.22
- Propionic acid	g L^{-1}	0.18	0.19	0.27
- Butyric acid	g L^{-1}	0.08	0.15	0.01
- Valeric acid	g L^{-1}	0.03	0.05	0.02
Ethanol	g L^{-1}	0.02	0.14	0.06
Oil and grease	g kg^{-1}	4.5	1.5	265
Total proteins	g kg^{-1}	10.2	129.5	11.8
Soluble proteins	g kg^{-1}	1.7	128.2	0.4
Total carbohydrates	g kg^{-1}	55.5	3.7	0.6
Soluble carbohydrates	g kg^{-1}	1.6	0.1	0.4
TKN	g kg^{-1}	0.60	26.7	1.2
TKP	g kg^{-1}	0.21	0.20	0.29
Chloride	mg L^{-1}	147	2617	84
Ammonium	mg N L^{-1}	143	391	49
Nitrite	mg N L^{-1}	0.2	1.1	0.5
Nitrate	mg N L^{-1}	0.05	0.97	0.01
Phosphate	mg P L^{-1}	161	164	162
Sulphate	mg S L^{-1}	9.3	38	19
Aluminium	mg g^{-1} TS	0.86	n.d.	n.d.
Calcium	mg g^{-1} TS	4.09	n.d.	7.48
Iron	mg g^{-1} TS	0.84	0.25	0.29
Lead	mg g^{-1} TS	0.003	0.004	0.011
Magnesium	mg g^{-1} TS	0.46	n.d.	n.d.
Phosphor	mg g^{-1} TS	2.13	0.13	2.53
Potassium	mg g^{-1} TS	1.39	n.d.	0.19
Silicon	mg g^{-1} TS	0.24	0.001	0.20
Zinc	mg g^{-1} TS	0.02	n.d.	0.01

There were significant analytical interferences when measuring the total COD of some substrates, therefore the total COD of cellulose and olive oil were estimated by multiplying the VS concentration by the theoretical oxygen demand of cellulose (1.07 g COD g^{-1} VS) and oleic acid (2.89 g COD g^{-1} VS) respectively. The total COD of DAF sludge was estimated by multiplying its VS concentration by 3.0 g COD g^{-1} VS.

3.1.2 Biochemical Methane Potential test (BMP)

Biochemical Methane Potential (BMP) tests were used to assess apparent first order hydrolysis rate (k_{hyd}), ultimate degradability (f_d), and ultimate biochemical methane potential (B_0) of organic wastes or waste mixtures. Batch BMP tests were set up in serum bottle reactors

(shown in Figure 4) using methods developed in conjunction with the IWA Anaerobic biodegradability, Activity and Inhibition Task Group (*Angelidaki et al. 2009*). Methanogenic inoculum used in the BMP tests was collected from a full-scale anaerobic digester (35 ±1°C) in the Luggage Point municipal waste water treatment plant. Specific methanogenic activity of the inoculum has been assessed previously and is approximately 0.2 gCOD-CH_4.gVS^{-1}.d^{-1}. All the BMP tests were conducted in triplicate in 240 mL serum bottles (approx. 160 g working volume) at 37°C. The ratio of inoculum and substrate (ISR) was designed at 2:1 (VS basis) in all the tests.

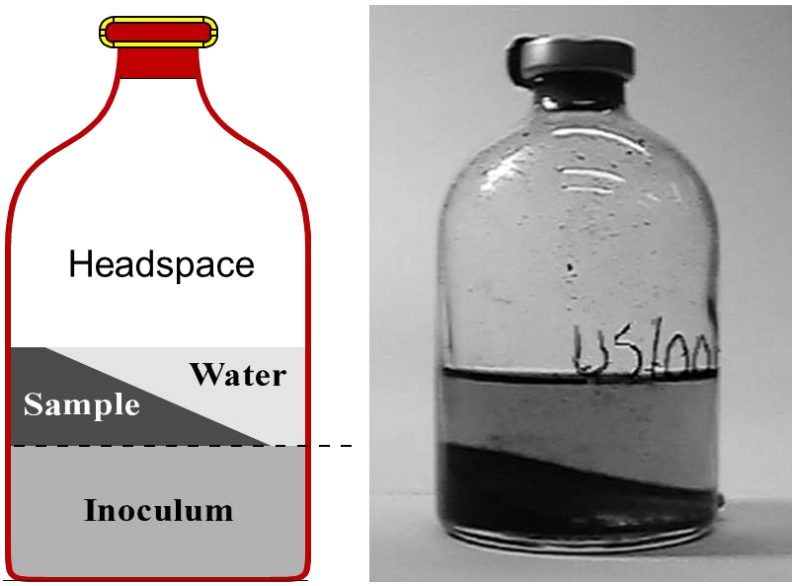

Figure 4: Typical biochemical methane potential test set-up.

During the BMP tests biogas volume was measured using 2 methods i) a precision gas tight syringe (SGE International Pty Ltd., Ringwood, Australia) and a water filled manometer or ii) a Druck PTX-1400 industrial pressure transmitter (-1 to 2.6 bar absolute). Biogas composition (H_2, CH_4, CO_2) was analysed using Gas Chromatography-Thermal Conductivity Detection (GC-TCD). The system was a Perkin Elmer auto system GC-TCD with a 2.44 m stainless steel column packed with Haysep (80/100 mesh). The GC was fitted with a GC Plus Data station, Model 1022 (Perkin Elmer, Waltham, MA, USA). High purity nitrogen (99.99%) was used as carrier gas at a flow rate of 24.3 mL min^{-1} and a pressure of 220 kPa. The injection port, oven and detector were operated at 75°C, 40°C and 100°C, respectively. The GC was calibrated using external gas standards from British Oxygen Company (Sydney, Australia).

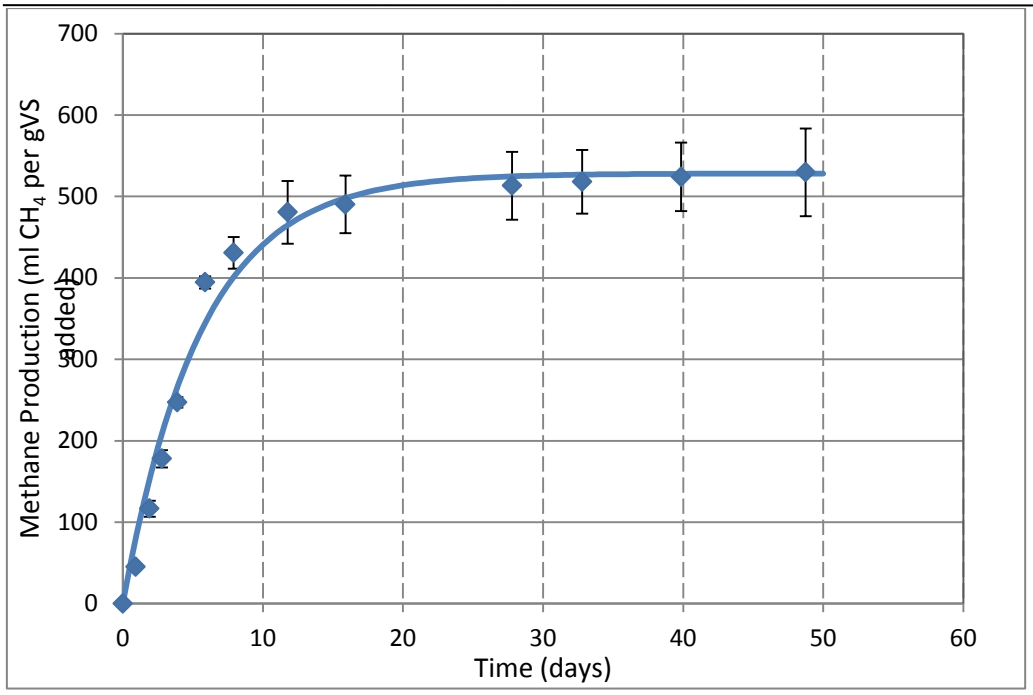

Figure 5: Example output from typical BMP test degrading municipal sludge with no inhibition. Error bars indicate 95% confidence errors from triplicate batches. The line indicates the model used to return key parameters.

Two sets of BMP tests were completed, the first set investigated pure substrates (i.e. cellulose, casein and olive oil), the second set investigated complex slaughterhouse residues (i.e. Paunch, DAF, Blood). Each set of BMP tests contained analysis of the 3 test substrates and 7 substrate mixtures. The experimental design is demonstrated in the triangular matrix shown in Figure 6.

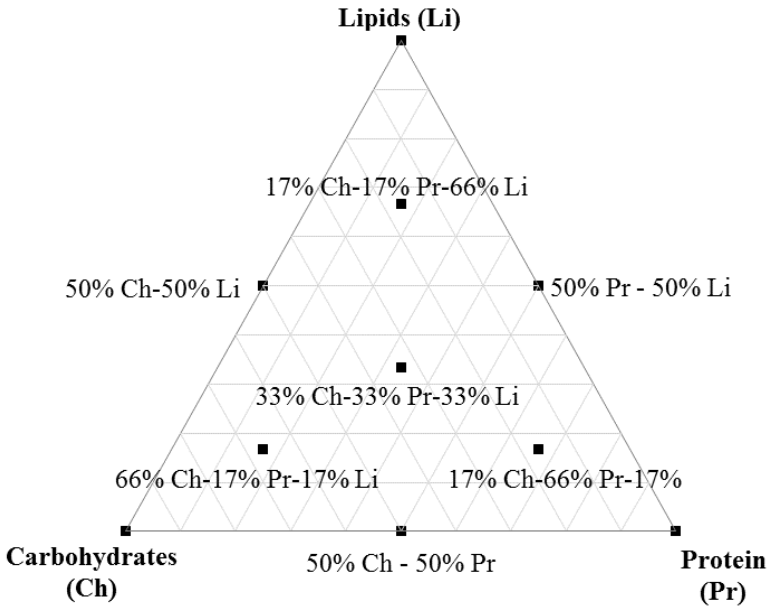

Figure 6: Design of the co-digestion mixtures, organic mass basis (VS), between carbohydrates, protein and lipids

3.1.3 Model Implementation and Data Analysis

Mathematical analysis of the BMPs was based on the IWA Anaerobic Digestion Model No. 1 (ADM1) (*Batstone et al. 2002*). Due to the high ISR, hydrolysis was considered the rate-limiting step during the batch tests (*Jensen et al. 2011*), therefore the BMPs were modelled using first order kinetics modified with an inhibition term (eq. 3) (*Pratt et al. 2012*).

$$r = \left(0_{t< t_{delay}} , \sum_i \left(f_i \cdot k_{hyd,i} \cdot S_i \cdot C_i \cdot I \right)_{t>t_{delay}} \right) \quad (3)$$

Where:

r is the process rate (mL CH_4 L^{-1} day^{-1}),
f_i is the substrate biodegradability (dimensionless),
$k_{hyd,i}$ is the first order hydrolysis rate constant of the substrate (day^{-1}),
S_i is the substrate concentration (g VS L^{-1}),
C_i is the COD-to-VS ratio of the substrate,
I is the inhibition factor and
t_{delay} is the lag-phase, which is global across all substrates.

The inhibition factor was included to model LCFA inhibition during AD of lipids or AD of co-digestion mixtures containing lipids. Inhibition was modelled as per Pratt et al. (2012) (eq. 4).

$$I = \left(\frac{K_{I,li}}{S_{li} + K_{I,li}} \right)^n \quad (4)$$

Where:

I is the LCFA inhibition factor, which range from 0 (total inhibition) to 1 (no inhibition),
S_{li} is the lipid concentration,
$K_{I,li}$ is the inhibition constant (g VS L^{-1}) and
n is the inhibition exponent. The exponent allows for an increase in inhibition progression rate compared with the standard non-competitive function.

The model was implemented in Aquasim 2.1d. Parameter estimation and uncertainty analysis were simultaneously estimated, with a 95% confidence limit, as for Batstone et al. (2009).

3.2 Results and Discussion

3.2.1 Methane Production from Synthetic Substrates

Methane production from BMP tests investigating AD and AcoD of the synthetic substrates is shown in Figure 7.

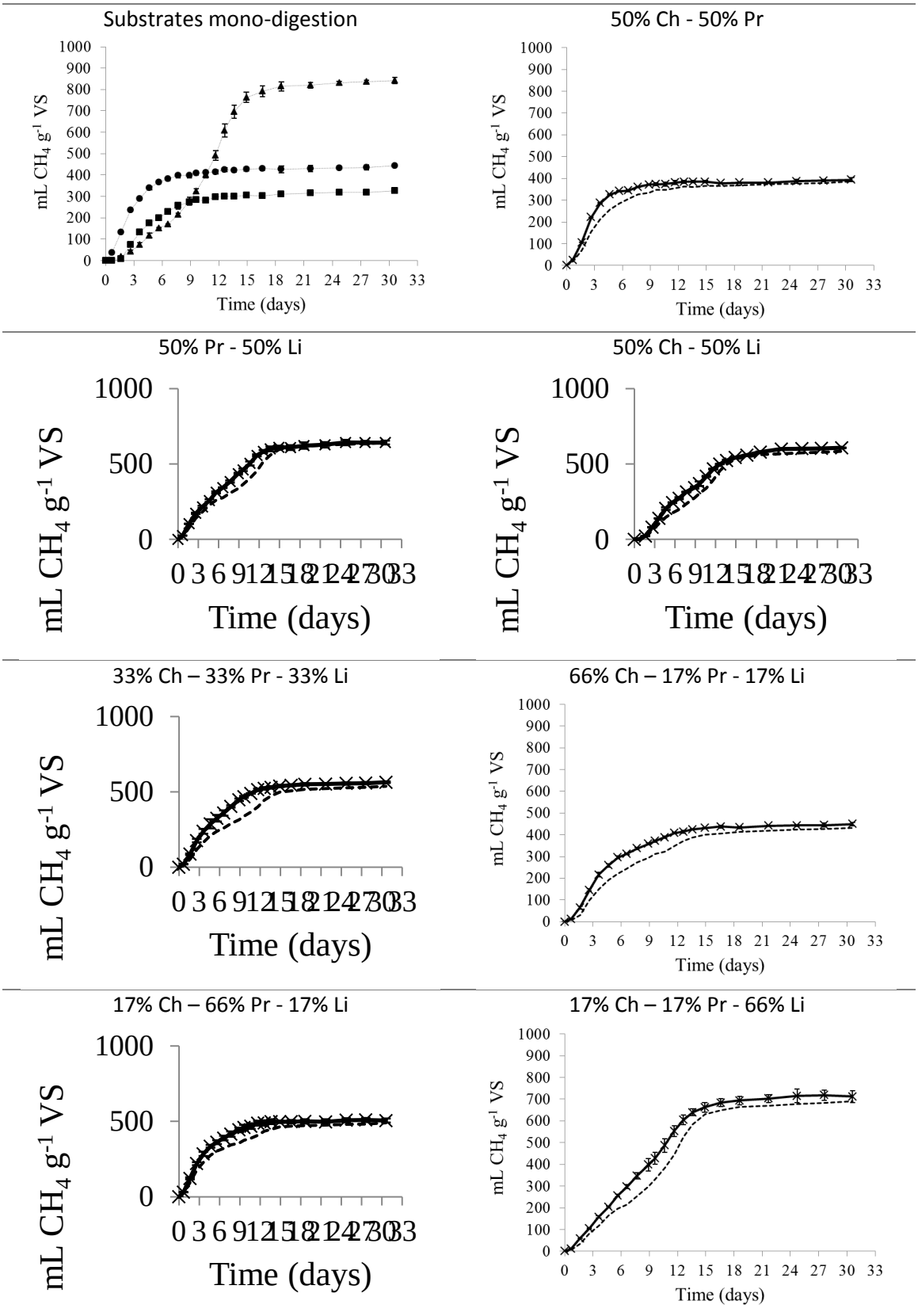

Figure 7: Cumulative methane production from digestion of synthetic substrates: mixture (×), theoretical profile of the mixture (dashed line), cellulose (■), casein (▲) and olive oil (●).

Methane production of cellulose and casein followed first order process kinetics with B_0 values of 319 L CH_4 kg^{-1} VS and 431 L CH_4 kg^{-1} VS, respectively. The B_0 of olive oil was higher at 816 L CH_4 kg^{-1} VS, however olive oil did not follow first order kinetics, the sigmoidal methane generation profile shown in Figure 7 is the result of LCFA inhibition. While the initial olive oil concentration (4800 mg L^{-1}) in our tests was far above reported inhibitory concentration (IC_{50}) values for LCFA, which range from 50 to 1500 mg L^{-1} (*Palatsi et al. 2009*) the relatively short lag phase (1.5 days) indicated that inhibition was overcome rapidly followed by successful production of methane. This is in contrast to the typical inhibition response and longer lag period (> 10 days) corresponding to a strong inhibition of methanogens (*Hwu et al. 1998, Palatsi et al. 2009, Salminen et al. 2000*). High ISR in the batch tests may have enhanced the communities' ability to mitigate substrate inhibition.

To assess synergistic and/or antagonistic effects of co-digestion, the methane production curves from the pure substrates were combined with the composition of each co-digestion mixture to predict methane production in each co-digestion trial (shown as dashed line in Figure 7). The AcoD batch tests demonstrate a clear advantage to process kinetics caused by mixing substrates, but with limited impact on ultimate methane yields (net B_0). Improvements in process kinetics where mixtures contained high concentrations of olive oil were clearly the result of inhibition mitigation. This could be a consequence of dilution and therefore lower LCFA concentrations in the mixture, or could be the result of synergy between substrates. We conclude that substrate diversification improves anaerobic digestion rates and reduced the inhibitory effect of LCFA.

3.2.2 Methane Production from Meat Processing Wastes

Paunch, blood and DAF sludge are high in carbohydrates, protein and lipids, respectively. Methane production from BMP tests investigating AD and AcoD of these slaughterhouse substrates is shown in Figure 8. When BMP results for these individual substrates were compared with the results obtained from the pure/synthetic substrates there was very strong overlap in methane profiles when comparing casein to blood, and when comparing olive oil to DAF sludge. DAF sludge showed LCFA inhibition similar to the olive oil test. In contrast, paunch digestion resulted in a flattened methane production profile and reduced B_0 compared to cellulose. The relatively poor degradation of paunch is due to the complex lingo-cellulosic structure; where lignin in particular is not degradable and reduces access to the degradable cellulose and hemicellulose.

The B_0 of paunch, blood and DAF sludge were 237 L CH_4 kg^{-1} VS, 410 L CH_4 kg^{-1} VS and 824 L CH_4 kg^{-1} VS, respectively. These values are largely consistent with the methane potential of slaughterhouse wastes reported in A.ENV.0131 and A.ENV.0151 and confirm that the source and composition of slaughterhouse wastes is the primary factor that will impact methane potential. Comparisons of the B_0 data with published literature are also largely consistent. The B_0 of paunch is in the range of values reported for paunch and lignocellulosic agricultural wastes (*Tong et al. 1990, Tritt and Kang 1991*). There was also a good agreement in the B_0 of blood (450 mL CH_4 g^{-1} VS), whereas the B_0 reported for fat (560 mL CH_4 g^{-1} VS) was much lower in some literature than in this project (*Hejnfelt and Angelidaki 2009*). Differences in the B_0 of DAF sludge and other lipid rich substrates can be related with the fat origin and structure.

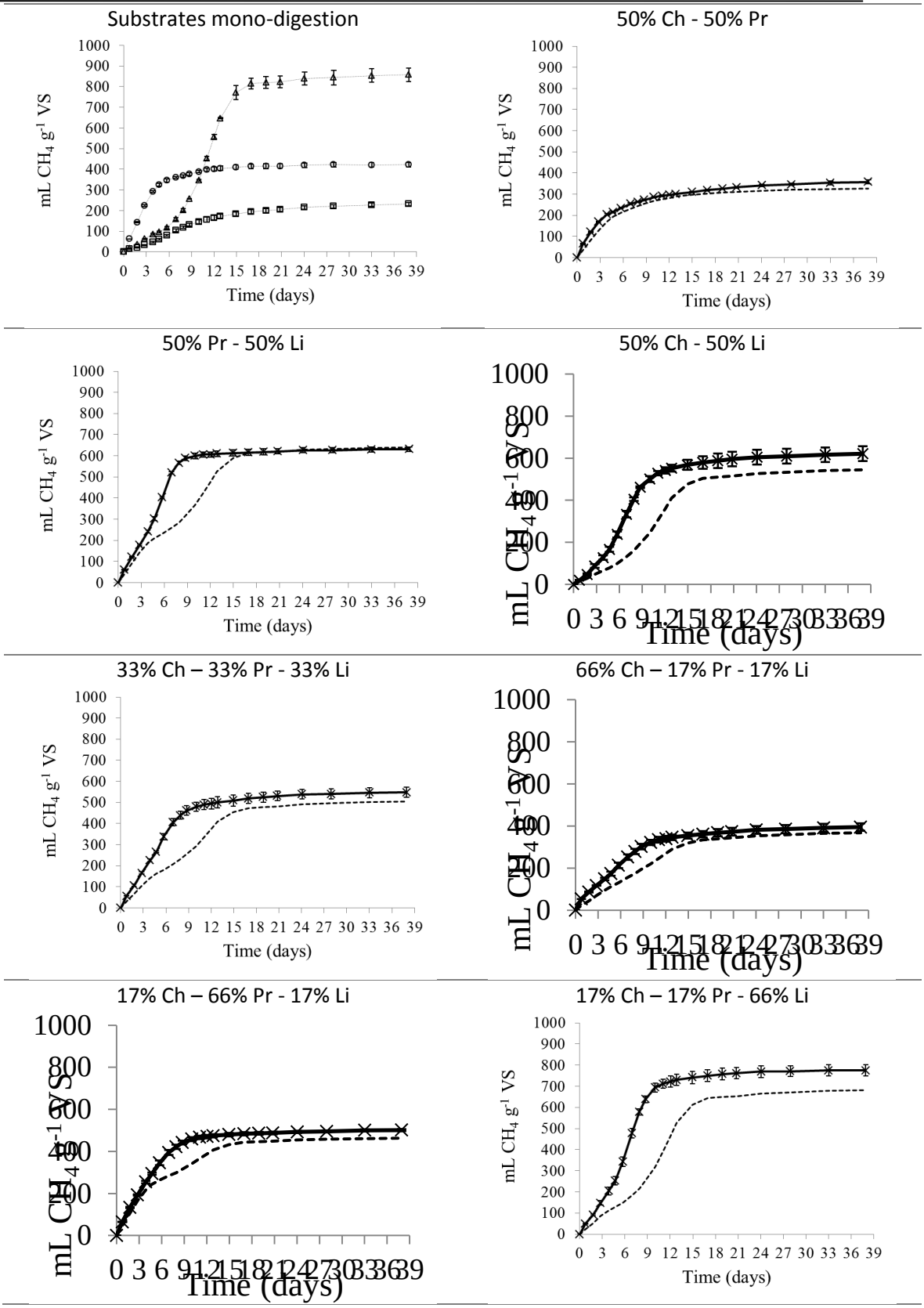

Figure 8: Cumulative methane production during digestion of each slaughterhouse waste mixture (×), theoretical profile (dashed line), paunch (□), blood (Δ) and DAF (○).

When considering substrate selection for the Biosolids Demonstration Plant, paunch had the lowest B_0 and therefore addition of either blood of DAF sludge would be expected to enhance methane yields (on a VS basis).

In all cases, the B_0 from the AcoD mixtures was higher than the B_0 of the paunch-only digestion. Two mixtures (50%Ch - 50%Li; 17%Ch - 17%Pr - 66%Li) resulted in a B_0 significantly higher than the theoretical prediction (>15% improvement). Non-degraded COD in the paunch-only and blood-only tests was very low and not enough to explain the increased B_0 in the AcoD trials. A COD balance showed that the increase in observed B_0 from these tests must have been the result of improved conversion of the DAF sludge. Paunch contains ruminant microorganisms with a range of metabolic capabilities including lipid hydrolysis (*Kim et al. 2009*) and it is possible that the increase in observed B_0 (compared to predicted) may be due to the hydrolytic capacity of these microorganisms to further degrade the DAF sludge (slurry with small fat conglomerates). Small improvements in B_0 values were recorded in other AcoD mixtures; however the improvements were less than 7% and were not considered significant.

All AcoD mixtures resulted in an improvement in the digestion kinetics when compared with the theoretical predictions (Figure 8). The lipid-rich mixtures (50%Ch - 50%Li; 50%Pr - 50%Li; 33%Ch - 33%Pr - 33%Li and 17%Ch - 17%Pr - 66%Li) showed greater improvements in process kinetics compared to pure substrates. In the lipid-rich mixtures, the increase of the slope in the cumulative methane production, signalling recovery from LCFA inhibition, was observed at Days 4-5 instead of Day 7. Again, we conclude that AcoD mitigated LCFA inhibition during digestion of slaughterhouse wastes.

3.2.3 Model-based Analysis

Kinetic parameters for the degradation of synthetic substrates and slaughterhouse wastes are presented in Table 5 and

Table 6 respectively. These parameters demonstrate the fraction of material that can be converted to methane (f), the speed of conversion (k_{hyd}) and the degree of LCFA inhibition. The high degradability (85% to 97%) obtained in all scenarios for cellulose, casein and olive oil are in agreement with the B_0 values obtained and confirm that there are no antagonistic effects associated with the intrinsic composition of organic matter. The degradability of blood and DAF sludge was also high (> 85 %), whereas the degradability of paunch was lower (~75 %) and was likely due to the complex lignocellulosic structure.

The improvement in process kinetics achieved using AcoD was reflected by the increase in hydrolysis rate of one or more compounds in the mixture (compared with the kinetics of single substrate digestion). However, the mitigation of LCFA inhibition is not well represented by the models. AcoD resulted in lower IC_{50} concentrations for the lipids mixtures and this would suggest that the lipids are inhibitory at lower levels; which is not consistent with the results of the BMP tests. Further investigations around inhibition modelling are recommended. These investigations could include analysis of the IC_{50} as a fraction of the initial lipid concentration, or modelling based on different modes of substrate inhibition (e.g. competitive vs non-competitive).

Table 5: Model parameters for anaerobic co-digestion of synthetic wastes

Parameter	Description	Units	Cellulose (Ch)	Casein (Pr)	Olive oil (Li)	50%Ch 50%Pr	50%Pr 50%Li	50%Ch 50%Li	33%Ch 33%Pr 33%Li	66%Ch 17%Pr 17%Li	17%Ch 66%Pr 17%Li	17%Ch 17%Pr 66%Li
f_{ch}	biodegradability of Ch	-	0.93 ± 0.02	-	-	0.90 ± 0.10	-	0.98 ± 0.02	0.97 ± 0.01	0.95 ± 0.05	0.95 ± 0.05	0.96 ± 0.04
f_{pr}	biodegradability of Pr	-	-	0.87 ± 0.01	-	0.91 ± 0.09	0.81 ± 0.02	-	0.97 ± 0.01	0.79 ± 0.04	0.95 ± 0.03	0.97 ± 0.03
f_{li}	biodegradability of Li	-	-	-	0.88 ± 0.03	-	0.93 ± 0.01	0.93 ± 0.01	0.91 ± 0.01	0.86 ± 0.05	0.90 ± 0.04	0.94 ± 0.03
$k_{hyd,ch}$	hydrolysis constant of Ch	day^{-1}	0.26 ± 0.02	-	-	0.33 ± 0.12	-	0.27 ± 0.03	0.34 ± 0.01	0.32 ± 0.07	0.20 ± 0.07	0.12 ± 0.05
$k_{hyd,pr}$	hydrolysis constant of Pr	day^{-1}	-	0.35 ± 0.03	-	0.75 ± 0.31	0.40 ± 0.05	-	0.36 ± 0.02	0.19 ± 0.05	0.26 ± 0.06	0.55 ± 0.10
$k_{hyd,li}$	hydrolysis constant of Li	day^{-1}	-	-	2.33 ± 0.52	-	0.79 ± 0.04	0.81 ± 0.16	0.89 ± 0.07	2.09 ± 0.39	2.71 ± 0.29	1.16 ± 0.28
$K_{I,li}$	inhibitor constant	g VS L^{-1}	-		5.78 ± 0.90	-	0.58 ± 0.14	0.73 ± 0.17	0.07 ± 0.03	0.46 ± 0.37	1.37 ± 0.45	1.44 ± 0.34
n	inhibitor exponent	-	-		3.50 ± 0.44	-	0.79 ± 0.12	0.94 ± 0.12	0.34 ± 0.05	0.66 ± 0.30	1.77 ± 0.39	1.47 ± 0.24
t_{dealy}	lag period	day	1.56 ± 0.19	0.45 ± 0.13	2.02 ± 0.39	1.00 ± 0.20	0.08 ± 0.05	0.68 ± 0.11	0.38 ± 0.04	1.01 ± 0.21	0.35 ± 0.23	0.43 ± 0.17
IC_{50}	50% lipids inhibitory concentration	g VS L^{-1}	-	-	1.27 ± 0.05	-	0.82 ± 0.02	0.79 ± 0.03	0.58 ± 0.07	0.70 ± 0.05	0.65 ± 0.04	0.85 ± 0.04

Table 6: Model parameters for anaerobic co-digestion of slaughterhouse wastes

Parameter	Description	Units	Cellulose (Ch)	Casein (Pr)	Olive oil (Li)	50%Ch 50%Pr	50%Pr 50%Li	50%Ch 50%Li	33%Ch 33%Pr 33%Li	66%Ch 17%Pr 17%Li	17%Ch 66%Pr 17%Li	17%Ch 17%Pr 66%Li
f_{ch}	biodegradability of Ch	-	0.74 ± 0.04	-	-	0.80 ± 0.17	-	0.87 ± 0.07	0.71 ± 0.07	0.76 ± 0.10	0.86 ± 0.04	0.64 ± 0.11
f_{pr}	biodegradability of Pr	-	-	0.87 ± 0.01	-	0.86 ± 0.14	0.87 ± 0.04	-	0.98 ± 0.02	0.93 ± 0.05	0.92 ± 0.02	0.97 ± 0.03
f_{li}	biodegradability of Li	-	-	-	0.85 ± 0.04	-	0.85 ± 0.03	0.98 ± 0.02	0.99 ± 0.01	0.95 ± 0.04	0.99 ± 0.01	0.97 ± 0.03
$k_{hyd,ch}$	hydrolysis constant of Ch	day^{-1}	0.11 ± 0.02	-	-	0.11 ± 0.05	-	0.14 ± 0.04	0.07 ± 0.04	0.15 ± 0.07	0.09 ± 0.04	0.15 ± 0.09
$k_{hyd,pr}$	hydrolysis constant of Pr	day^{-1}	-	0.31 ± 0.03	-	0.47 ± 0.18	0.55 ± 0.50	-	0.76 ± 0.13	0.50 ± 0.17	0.59 ± 0.07	0.62 ± 0.18
$k_{hyd,li}$	hydrolysis constant of Li	day^{-1}	-	-	2.65 ± 0.34	-	2.20 ± 0.45	2.47 ± 0.48	1.10 ± 0.32	0.77 ± 0.44	0.65 ± 0.21	2.02 ± 0.53
$K_{I,li}$	inhibitor constant	g VS L^{-1}	-		18.7 ± 0.7	-	2.82 ± 0.50	3.75 ± 0.31	0.88 ± 0.39	0.82 ± 0.54	0.70 ± 0.25	2.87 ± 0.64
n	inhibitor exponent	-	-		7.52 ± 0.46	-	2.30 ± 0.33	2.90 ± 0.38	0.99 ± 0.25	0.96 ± 0.49	0.79 ± 0.19	1.96 ± 0.37
t_{dealy}	lag period	day	1.47 ± 0.66	0.24 ± 0.20	0..31 ± 0.30	0.44 ± 0.12	0.11 ± 0.11	0.44 ± 0.29	0.15 ± 0.15	0.19 ± 0.19	0.09 ± 0.09	1.07 ± 032
IC_{50}	50% lipids inhibitory concentration	g VS L^{-1}	-	-	1.74 ± 0.05	-	0.99 ± 0.03	1.01 ± 0.02	0.86 ± 0.09	0.91 ± 0.08	0.97 ± 0.04	1.22 ± 0.02

3.3 Summary

The B_0 of paunch, blood and DAF sludge were 237 L CH_4 kg^{-1} VS, 410 L CH_4 kg^{-1} VS and 824 L CH_4 kg^{-1} VS, respectively. The high B_0 of DAF sludge relative to paunch indicates that methane production from the Demonstration Plant could be improved by substituting a portion of the organic loading rate as DAF sludge. However, DAF sludge also resulted in process inhibition. Therefore determination of an optimal mixture is required.

Investigations of anaerobic co-digestion of slaughterhouse wastes demonstrated in a clear and quantifiable manner that co-digestion will improve process kinetics and in some cases may improve overall substrate degradation and methane production. Improvements in process kinetics are expected to be related to the mitigation of LCFA inhibition, however inhibition modelling is ongoing.

Other conclusions are summarised as follows:

- In all cases, the B_0 from the AcoD mixtures was higher than the B_0 of the paunch-only digestion. Therefore AcoD would improve methane yields from the Biosolids Demonstration Plant.

- Substrate diversification improved process kinetics. The synergisms of mixing substrates led to an improvement in AD kinetics for all mixtures. However, as a general trend, the ultimate methane production was not affected.

- Mixing waste is a feasible option to reduce the impact of inhibitory compounds. The introduction of a carbohydrates and/or protein source to lipids reduced the LCFA inhibition present in lipid AD.

- AcoD of paunch and DAF sludge resulted in a higher methane yield than predicted. Results suggest that the microorganisms present in the paunch may contribute to improved hydrolysis of the partially biodegradable fat conglomerates present in the DAF sludge.

4 Conclusions and Recommendations

The Biosolids Demonstration Plant was operated for over 200 days with paunch solids as the sole substrate. The maximum sustainable organic loading rate for the Demonstration Plant was estimated at 1-1.3 kgVS/m^3/d using a feed solids concentration of approximately 3% and is comparable to full scale digesters treating municipal wastewater sludge. At solids concentrations above 3% the mixing system was not sufficient resulting in solids accumulation, blockages and subsequent process failures. Methane production (220 L CH_4 kg^{-1} VS) and organic solids destruction (60%) in the demonstration plant were similar to levels predicted from independent batch tests.

In addition to generation of renewable energy, anaerobic digestion will significantly reduce the volume of paunch requiring transport for disposal/beneficial re-use in agriculture. It is estimated that converting approximately 50% of solids to methane and increasing the solids content the digested cake using AD will reduce the solids transport load to 1/3 of the load from paunch without treatment. In addition to reduced transport costs, digested paunch is stable and may be suitable for beneficial re-use via land application. However, this would be subject to local environmental regulations. Performance data from the Demonstration Plant, including energy recovery and mitigation of paunch disposal costs will now be used in cost-benefit analysis undertaken as the next phase of research projects on paunch digestion and value-add options.

Investigations of anaerobic co-digestion of slaughterhouse wastes demonstrated that co-digestion is a promising strategy to improve process performance. In all cases, the B_0 from AcoD mixtures was higher than the B_0 of the paunch-only digestions. Generally, this was due to the higher B_0 of the individual substrates. However, AcoD of paunch and DAF sludge resulted in a higher methane yield than predicted and results suggest that the biomass present in the paunch may have contributed to improved hydrolysis of the partially biodegradable fat conglomerates present in the DAF sludge.

When considering the process kinetics of paunch digestion, paunch was the slowest degrading substrate. The addition of rapidly degrading substrates such as blood or DAF sludge would not increase the required treatment times. When considering the process kinetics of co-digestion mixtures, all AcoD mixtures resulted in an improvement in the digestion kinetics when compared with the theoretical predictions. These improvements are expected to be related to the mitigation of LCFA inhibition associated with digestion of DAF sludge; however this needs to be investigated further using inhibition modelling.

Investigations of AcoD in this project were based on batch tests. We recommend the next major investigation in this area focus on examining the outcomes of the batch co-digestion trials in a continuous process; particularly co-digestion of paunch solids and DAF sludge. While AcoD was identified as a suitable strategy to boost methane production from the paunch digester, the impact of substrate composition and AcoD ratios on nutrient release and recovery potential was not investigated in this study. This is another area recommended for future investigation.

5 References

1. Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., Kalyuzhnyi, S., Jenicek, P., and Van Lier, J. B. 2009 Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays, pp 927-934.
2. Angelidaki, I., and Sanders, W. 2004 Assessment of the anaerobic biodegradability of macropollutants, Reviews in Environmental Science and Biotechnology *3*, 117-129.
3. AOCS. 2013 American Oil Chemists' Society.
4. Astals, S., Ariso, M., Galí, A., and Mata-Alvarez, J. 2011 Co-digestion of pig manure and glycerine: Experimental and modelling study, Journal of Environmental Management *92*, 1091-1096.
5. Astals, S., Nolla-Ardèvol, V., and Mata-Alvarez, J. 2012 Anaerobic co-digestion of pig manure and crude glycerol at mesophilic conditions: Biogas and digestate, Bioresource Technology *110*, 63-70.
6. Batstone, D. J., and Jensen, P. D. 2011 Anaerobic processes., In *Treatise on Water Science* (Wilderer, P., Rogers, P., Uhlenbrook, S., Frimmel, F., and Hanaki, K., Eds.), pp 615-640, Academic Press, Oxford, U.K.
7. Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V., Pavlostathis, S. G., Rozzi, A., Sanders, W. T., Siegrist, H., and Vavilin, V. A. 2002 The IWA Anaerobic Digestion Model No 1 (ADM1), Water Science and Technology *45*, 65-73.
8. Batstone, D. J., Tait, S., and Starrenburg, D. 2009 Estimation of hydrolysis parameters in full-scale anerobic digesters, Biotechnology and Bioengineering *102*, 1513-1520.
9. Cuetos, M. J., Gómez, X., Otero, M., and Morán, 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*, 99-106.
10. Galí, A., Benabdallah, T., Astals, S., and Mata-Alvarez, J. 2009 Modified version of ADM1 model for agro-waste application, Bioresource Technology *100*, 2783-2790.

11. Hejnfelt, A., and Angelidaki, I. 2009 Anaerobic digestion of slaughterhouse by-products, Biomass and Bioenergy *33*, 1046-1054.
12. Hwu, C. S., Tseng, S. K., Yuan, C. Y., Kulik, Z., and Lettinga, G. 1998 Biosorption of long-chain fatty acids in UASB treatment process, Water Research *32*, 1571-1579.
13. Jensen, P. D., Ge, H., and Batstone, D. J. 2011 Assessing the role of biochemical methane potential tests in determining anaerobic degradability rate and extent, Water Science and Technology *64*, 880-886.
14. Kim, E. J., Huws, S. A., Lee, M. R. F., and Scollan, N. D. 2009 Dietary transformation of lipid in the rumen microbial ecosystem, Asian-Australasian Journal of Animal Sciences *22*, 1341-1350.
15. Kopp, J., and Dichtl, N. 2000 Prediction of full-scale dewatering results by determining the water distribution of sewage sludges, pp 141-149.
16. Mata-Alvarez, J., Dosta, J., Macé, S., and Astals, S. 2011 Codigestion of solid wastes: A review of its uses and perspectives including modeling, Critical Reviews in Biotechnology *31*, 99-111.
17. Palatsi, J., Laureni, M., Andrés, M. V., Flotats, X., Nielsen, H. B., and Angelidaki, I. 2009 Strategies for recovering inhibition caused by long chain fatty acids on anaerobic thermophilic biogas reactors, Bioresource Technology *100*, 4588-4596.
18. Pratt, S., Liew, D., Batstone, D. J., Werker, A. G., Morgan-Sagastume, F., and Lant, P. A. 2012 Inhibition by fatty acids during fermentation of pre-treated waste activated sludge, Journal of Biotechnology *159*, 38-43.
19. Salminen, E., Rintala, J., Lokshina, L. Y., and Vavilin, V. A. 2000 Anaerobic batch degradation of solid poultry slaughterhouse waste, pp 33-41.
20. Switzenbaum, M. S., Farrell, J. B., and Pincince, A. B. 2003 Relationship between the Van Kleeck and mass-balance calculation of volatile solids loss, Water Environment Research *75*, 377-380.
21. Tong, X., Smith, L. H., and McCarty, P. L. 1990 Methane fermentation of selected lignocellulosic materials, Biomass *21*, 239-255.
22. Tritt, W. P., and Kang, H. 1991 Ultimate biodegradibility and decay rates of cow paunch manure under anaerobic conditions, Bioresource Technology *36*, 161-165.
23. Zhang, Y., and Banks, C. J. 2012 Co-digestion of the mechanically recovered organic fraction of municipal solid waste with slaughterhouse wastes, Biochemical Engineering Journal *68*, 129-137.