FIRST-STAGE AND SINGLE-STAGE CONTINUOUSLY
STIRRED TANK ANAEROBIC DIGESTION OF SYNTHETIC
COMPLEX WASTEWATER AND PIGGERY WASTEWATER
(WITH EMPHASIS ON THERMOPHILIC TEMPERATURE)

By

Lily S.H. Ho

B.Sc. (Hons.) Biochemistry (Royal Holloway College, University of London)
Postgraduate Diploma Environmental Impact Assessment (Murdoch University)
M.Sc. Environmental Science (Murdoch University)
Graduate Diploma Adult and Tertiary Education (Murdoch University)

A dissertation submitted for the degree of Doctor of Philosophy

School of Environmental Science
Faculty of Sustainability, Environmental and Life Sciences
Murdoch University
Western Australia
January 2010
DECLARATION

I declare that this thesis is my own account of my research work undertaken which has not been previously submitted for a degree at any tertiary educational institution

________________
Lily S.H. Ho

The following paper has been presented and published from this research:

L. Ho, G. Ho, M. Kumar (2008). A comparative study of thermophilic (55°C) and mesophilic (37°C) CSTR acid-phase anaerobic digestion of synthetic complex wastewater under varying hydraulic retention times. Abstracts, IWA World Water Congress, Vienna 2008 (7th – 12th September)
ABSTRACT

Single-stage continuously stirred tank reactor (CSTR) is commonly used in the anaerobic treatment of animal manure slurry, municipal sewage sludge and concentrated wastewaters containing a high proportion of biodegradable particulate organic materials at relatively long hydraulic retention times (HRTs) of 12 to 24 days. It is also commonly used as a holding tank to equilise the big variations in wastewater flow or pollution strength as well as for pre-acidification of wastewater. Its simplicity, ease of operation, low capital and maintenance costs are appealing features that made it a natural choice of reactor configuration for the pilot-scale thermophilic first-stage acidogenic digester of a two-stage thermophilic-ambient anaerobic digestion system which is based at Roseworthy Campus of University of Adelaide, South Australia and operated by South Australian Research and Development Institute (SARDI).

As the first-stage acidogenic reactor plays a crucial role in the solubilisation of particulate organic matter in complex substrates to soluble organics and acidification to volatile fatty acids (VFAs) for enhancing pathogens destruction in wastewater treatment, the initial aim of this research study was to optimise the first-stage anaerobic CSTR to effectively convert particulate-containing complex organic wastewater to intermediate fermentation products for feed to the second-stage methane reactor. Pig feed pellets was used as the model substrate to prepare the complex synthetic wastewater to investigate the effects of temperature (37, 47 and 55°C) and pH (6, 7 and 8) on organics solubilisation and acidification in two sets of batch vial experiments while the effects of HRT (4- to 1-day) on organics conversion efficiency of the semi-continuous first-stage anaerobic CSTRs were investigated in two sets of experiments conducted at mesophilic (37°C) and thermophilic (55°C) conditions. Findings from the batch vial experiments with low organic strength (4 g/L TCOD) wastewater found mesophilic temperature at 37°C and pH 7-8 were optimum conditions for solubilisation (hydrolysis) and acidification than at thermophilic temperatures of 47°C and 55°C. Results from the semi-continuous CSTR anaerobic reactors confirmed that the mesophilic (37°C) reactor hydrolysed
and acidified significantly more particulate and soluble organic matter respectively than thermophilic (55°C) reactor, with 2-day HRT being the optimum for both the anaerobic acidogenic reactors. The lack of methane in the biogas which contained hydrogen and carbon dioxide confirmed that the methanogens present in the acidic reactor effluents were completely inhibited.

Following reports that the pilot-scale first-stage thermophilic (55°C) acidogenic reactors treating raw piggery wastewater was experiencing substantial lost of volatile fatty acids to methane formation at relatively short HRTs of 4 and 7-day, the complex synthetic wastewater was replaced with real piggery wastewater from Roseworthy Campus’s piggery holding sump to allow meaningful lab-scale reactor experiments to be conducted in order to provide realistic information for the pilot-scale acidogenic reactor. Semi-continuous first-stage anaerobic reactor experiments were carried out to examine the influence of piggery influent concentrations with and without pH reduction on the fermentation behaviour of the thermophilic acidogenic reactor at a shorter HRT of 2-day. The 2-day HRT was found to be optimum in previous acidogenic experiments with the synthetic complex wastewater.

The studies on the acidogenic treatment of piggery wastewater at 2-day HRT revealed that irrespective of the feed concentrations or organic loading rates, first-stage anaerobic treatment of the piggery wastewater without pH intervention could not completely inhibit the syntrophic acetogenic and methanogenic microorganisms because of the wastewater’s inherently high levels of alkalinity and ammonia-nitrogen which buffered the system against VFA souring. Some losses of total VFAs were observed at the highest TCOD feed concentration of 13 g TCOD/L and OLR of 6.5 g/L/d. The vast differences in the physico-chemical and microbiological characteristics of the raw piggery wastewater and synthetic complex wastewater, particularly with respect to their initial ammonia-nitrogen, soluble COD, volatile fatty acids (VFA), buffering capacity and anaerobic microorganisms, were the key determining factors for the contradictory outcomes in organics conversion performance of the thermophilic and mesophilic first-stage CSTRs.

Although the study on pH reduction of the piggery wastewater to pH 5.5 found the approach was successful in suppressing the activities of syntrophic consortia of acetogenic and methanogenic microbial populations while stimulating the acidogenic
bacteria, the operational inconvenience from foaming-related spillages and the anticipated need to re-adjust the acidic effluent pH to neutral for feed to the second-stage reactor far out-weighed the small gains in the increased hydrolysis and acidification of the piggery influent organic matter.

The observations that around 30% of the organics still remained as insoluble particulate form in the treated effluent and more than 60% of the organic carbon compounds in the raw piggery wastewater was already in soluble and acidified forms coupled with its high buffering capacity which protects the anaerobic system against failure from VFA souring, it was decided that single-stage thermophilic anaerobic digestion at longer HRT of 10- and 15-day might be more cost-effective for enhancing the solubilisation of the particulate organics and organic carbon conversion to methane in the undiluted piggery wastewater. Semi-continuous thermophilic CSTR experiments at 55°C were carried out to examine the extent of organic carbon conversion at 10- and 15-day HRT. Mesophilic CSTR experiment at 37°C was also carried out to compare its organics conversion performance with the thermophilic reactor at 15-day HRT.

The results show that while increasing the HRT of the thermophilic anaerobic CSTRs from 2- to 10- and 15-day saw a gradual increase in specific methane yields, the methane yield at the longer HRT of 15-day was considered low (26% of total COD fed) based on the COD material balance of the digested effluents. Around 30% of the organic matter still remained as non-biodegradable particulate organics while propionate (19%) and unidentified non-VFA soluble organic matter (17%) formed the two largest groups of unconverted soluble organics in the digested piggery effluent. The build-up of propionate at higher HRT of 10- and 15-day which correlated positively with increased free ammonia concentration implied that the syntrophic propionate-oxidising bacteria and hydrogenotrophic microorganisms were under increased stress. At 15-day HRT, although anaerobic thermophilic digestion at 55°C had significantly higher specific methane yield than mesophilic digestion at 37°C, the chemical quality of thermophilic digested effluent was poor with regards to its higher levels of free ammonia, propionate, total VFA and soluble COD compared to the mesophilic effluent. However, thermophilic digestion is universally recognised for its higher pathogens destruction efficiency than mesophilic digestion.
Five sets of thermophilic (55°C) batch vial experiments were conducted to investigate the single effect of pH reduction, chemical (zeolite, humic acid) and biological (piggery biomass, municipal biomass) supplements as well as the combined effects of pH reduction and chemical or biological supplements in enhancing methane production from thermophilic piggery effluent. Reduction of the piggery effluent pH from 8.1 to 6.5 alone and zeolite treatment (10 to 20 g/L) with or without pH reduction of the piggery effluent to pH 6.5 were found to be effective strategies for enhancing methane production yet not elevating the effluent COD level compared to its initial level.
I wish to express my sincere gratitude to all the people who have directly or indirectly contributed to the completion of my PhD research thesis:

- Professor Goen Ho, my supervisor, for giving me the opportunity to undertake this research study and providing valuable feedback on my draft thesis;
- Dr Ralf Cord-Rudwisch, for his generosity in giving me access to the use of his Labview software to automate my reactors and the use of his gas chromatograph equipment.
- Late Emeritus Chemistry Professor Doug Clarke, for his expert advice on gas-related matter.
- Gordon Thompson, for his generosity in sharing his lab resources and expert guidance on the use of the fluorescence microscope and digital camera as well as his helpful suggestions.
- Dr Wipa Charles, for her generosity in sharing her laboratory resources and useful suggestions on some aspects of my lab work.
- Dr Lucy Skillman, for her assistance in conducting the molecular real-time PCR and T-RFLP analysis as well as in our successful application of the EBCRC consumables grant for the supplementary thermophilic batch vial experiments.
- Lee Walker, for his help with Labview programming and providing instruction on the use and maintenance of the gas chromatograph equipment.
- Steven Goynich and Phil Good from the School of Environmental Science, for lending me some of the essential laboratory equipment and helping me with some minor equipment repair.
- John Snowball, Dan Hewitt, Kleber Claux and Murray Lindau for providing expert technical services in the construction and repair of reactors and associated lab equipment.
- Frank Salleo, Heather Gordon and Colin Ferguson from the School of Environmental Science, for providing invaluable general administrative services.
• Rajesh Sharma, Ottiele Bajsa, Robert Hughes, Ka Yu Cheng, Ying Law, Mitch Lever, Donny James and all my other fellow research students whose names are not mentioned here for their company and sharing of learning experiences along the way.

• Dr Sandra Hall (University of Queensland), for providing me some of the microbial FISH probes and general advice on FISH image interpretation.

• Dr Martin Kumar and his team (Babu Santhanam, Khalid Shamim, Sandy Wyatt, Belinda Rodda, Bennan Cheng, Andrew Ward, Phil Glatz, Paul Harris) from South Australian Research and Development Institute (SARDI) and University of Adelaide, for collecting and organising the delivery of raw piggery wastewater for my research project.

• Dr David Garman, Executive Director of Environmental Biotechnology Centre of Research Cooperative Pty Limited (EBCRC), for providing the PhD scholarship and research funds; as well as all past and present EBCRC staff in particular Claire Johnson, Jenny Campbell and Michaela Lauren who have been involved in organising the annual EBCRC conferences and training courses.

• Lastly, my husband Tony for his endless support and patience; my mother-in-law and late father-in-law for their unconditional help in taking care of our two young kids, Serena and Sebastian after school and during school holidays as well as when they were unwell.
## TABLE OF CONTENTS

DECLARATION........................................................................................................................i
ABSTRACT....................................................................................................................................ii
ACKNOWLEDGEMENTS ..............................................................................................................vi
TABLE OF CONTENTS .............................................................................................................viii
LIST OF TABLES ...................................................................................................................xvi
LIST OF FIGURES ................................................................................................................xxii
LIST OF ABBREVIATIONS ....................................................................................................xxxii

CHAPTER 1 .........................................................................................................................................1
INTRODUCTION ...........................................................................................................................1
1.1. BACKGROUND ....................................................................................................................1
1.2. OBJECTIVES OF THIS RESEARCH WORK ..................................................................6
1.3. ORGANISATION OF THIS THESIS .............................................................................7

CHAPTER 2 .........................................................................................................................................9
LITERATURE REVIEW..............................................................................................................9
2.1. INTRODUCTION ................................................................................................................9
2.2. ANAEROBIC DIGESTION PROCESS ........................................................................9
  2.2.1. Hydrolysis ..................................................................................................................12
  2.2.2. Acidogenesis (fermentation) .....................................................................................12
  2.2.3. Acetogenesis .............................................................................................................14
  2.2.4. Methanogenesis .......................................................................................................16
  2.2.5. Sulphidogenesis .....................................................................................................19
2.3. REACTOR CONFIGURATIONS AND THEIR APPLICATIONS ....................................20
2.4. FACTORS INFLUENCING THE PERFORMANCE OF ACID-PHASE
ANAEROBIC DIGESTION OF COMPLEX WASTEWATER .............................................23
  2.4.1. Wastewater type ......................................................................................................24
  2.4.2. pH ..........................................................................................................................24
  2.4.3. Temperature ...........................................................................................................25
  2.4.4. Hydraulic retention time (HRT) .............................................................................27
  2.4.5. Organic loading rate (OLR) or substrate concentration ........................................28
  2.4.6. Toxicants or inhibitors ..........................................................................................28
2.4.7. Seed types ................................................................................................... 29

2.5. FACTORS AFFECTING THE PERFORMANCE OF SINGLE-STAGE
ANAEROBIC DIGESTION OF COMPLEX WASTEWATER ............................... 30

2.5.1. pH and buffering capacity ................................................................. 30
2.5.2. Temperature ....................................................................................... 31
2.5.3. Hydrogen sulfide .............................................................................. 32
2.5.4. Ammonia .......................................................................................... 33
2.5.5. Long-chain fatty acids ...................................................................... 34
2.5.6. Organic overload ............................................................................. 34
2.5.7. Hydrogen ........................................................................................... 35
2.5.8. Macro-nutrients, trace and heavy metals ........................................... 35

2.6. NUCLEIC ACID-BASED MOLECULAR METHOD FOR MICROBIAL
ANALYSIS .......................................................................................................... 36

2.7. CONCLUSION ........................................................................................... 39

CHAPTER 3 .............................................................................................................. 41

GENERAL MATERIALS AND METHODS ............................................................. 41
3.1. INTRODUCTION ........................................................................................ 41
3.2. REACTORS DESIGN AND SYNTHETIC COMPLEX WASTEWATER .......... 41

3.2.1. Acidogenic culture reactors ............................................................... 41
3.2.2. Semi-continuous acid-phase anaerobic reactors ................................. 42
3.2.3. Synthetic complex wastewater ......................................................... 44

3.3. GENERAL ANALYTICAL METHODS ..................................................... 46
3.3.1. Mixed liquor analysis ........................................................................ 46

3.3.1.1. pH .................................................................................................. 46
3.3.1.2. Total alkalinity (APHA Standard Methods 2320 B) ....................... 46
3.3.1.3. Total solids, volatile solids, total suspended solids and volatile
suspended solids (APHA Standard Methods 2540 B) ............................... 47
3.3.1.4. Ortho-phosphate (Lachat automated flow injection analyser
QuickChem Method 31-115-01-3-A) ....................................................... 47
3.3.1.5. Sulphate (Lachat automated flow injection analyser QuickChem
Method 10-116-10-1-C) ........................................................................... 47
3.3.1.6. Chemical oxygen demand (APHA Standard Methods 5220 C closed
reflux titration method) ........................................................................... 47
3.3.1.7. Ammonia-nitrogen (Hach Nessler method)................................. 48
3.3.1.8. Volatile fatty acids (VFAs by Gas Chromatography method)...... 48
3.3.1.9. Carbohydrates (Morris, 1948)...................................................... 48
3.3.1.10. Proteins (Lowry et al., 1951) ...................................................... 48
3.3.1.11. Lipids (APHA Standard Methods 5520 B partition-gravimetric
          method) .......................................................................................... 49
3.3.1.12. Fluorescence *in situ* hybridisation or FISH (Advanced Wastewater
          Management Centre, University of Queensland)................................. 49
3.3.1.13. Real-time PCR (Skillman et al., 2009) ...................................... 50
3.3.2. Biogas volume and composition (CH₄, CO₂, H₂ by Gas Chromatography
          method) .......................................................................................... 52

CHAPTER 4 .............................................................................................................. 53
BATCH VIAL EXPERIMENTS ON ANAEROBIC ACID-PHASE DIGESTION OF
SYNTHETIC COMPLEX WASTEWATER ............................................................ 53
4.1. INTRODUCTION ...................................................................................... 53
4.2. MATERIALS AND METHODS ................................................................. 54
  4.2.1. Effect of temperature on the fermentation performance of acidogenic
          anaerobic bacteria ................................................................................. 54
    4.2.1.1. Objective .................................................................................. 54
    4.2.1.2. Method ..................................................................................... 54
  4.2.2. Effect of pH on solubilisation and acidification of organic matter .... 56
    4.2.2.1 Objectives ................................................................................ 56
    4.2.2.2. Method ..................................................................................... 56
4.3. RESULTS .................................................................................................... 57
  4.3.1. Effect of temperature on the acidogenic anaerobic bacteria cultivated at
          47°C (batch experiment 1 as described in section 4.2.1) ......................... 57
  4.3.2. Effect of pH on solubilisation and acidification of organic matter (batch
          experiment 2 in section 4.2.2) .............................................................. 59
    4.3.2.1. Batch vial acid-phase treatment at 37°C ................................. 59
    4.3.2.2. Batch vial acid-phase treatment at 55°C ................................. 64
    4.3.2.3. Comparison of batch acid-phase anaerobic treatment at 37°C and
              55°C.............................................................................................. 67
4.4. DISCUSSION .......................................................................................................................... 68
  4.4.1. Effect of temperatures on the acidogenic anaerobic bacteria cultivated at 47°C ................................................................. 68
  4.4.2. Effect of pH on solubilisation and acidification of organic matter at thermophilic and mesophilic temperature ........................................... 70
4.5. CONCLUSIONS ......................................................................................................................... 70

CHAPTER 5 ...................................................................................................................................... 72
SEMI-CONTINUOUS REACTOR EXPERIMENTS ON ANAEROBIC ACID-PHASE DIGESTION OF SYNTHETIC COMPLEX WASTEWATER .............. 72
  5.1. INTRODUCTION .................................................................................................................. 72
  5.2. MATERIALS AND METHODS ......................................................................................... 73
    5.2.1. Objective .................................................................................................................. 73
    5.2.2. Method .................................................................................................................... 73
  5.3. RESULTS ............................................................................................................................ 75
  5.4. DISCUSSION ....................................................................................................................... 87
  5.5. CONCLUSIONS ................................................................................................................... 92

CHAPTER 6 ...................................................................................................................................... 94
SEMI-CONTINUOUS FIRST-STAGE (PHASE) ANAEROBIC DIGESTION OF RAW PIGGERY WASTEWATER AT LOW HYDRAULIC RETENTION TIME 94
  6.1. INTRODUCTION .................................................................................................................. 94
  6.2. MATERIALS AND METHODS ......................................................................................... 96
    6.2.1. Semi-continuous first-stage CSTR anaerobic reactors treating low-strength piggery wastewater without pH reduction at thermophilic (55°C) and mesophilic (37°C) at 2-day hydraulic retention time ......................................... 96
      6.2.1.1. Objectives ........................................................................................................ 96
      6.2.1.2. Method ........................................................................................................... 96
    6.2.2. Semi-continuous thermophilic first-stage CSTR reactors (55°C) treating pH-unadjusted and pH-reduced medium- and high-strength piggery wastewater at 2-day hydraulic retention time ................................................. 98
      6.2.2.1. Objectives ........................................................................................................ 98
      6.2.2.2. Method ........................................................................................................... 98
6.3. RESULTS .......................................................................................................................... 99
  6.3.1. Semi-continuous first-stage thermophilic (55°C) and mesophilic (37°C) CSTR anaerobic reactors treating low-strength piggery wastewaters without pH reduction at 2-day HRT .................................................................................................................. 99
    6.3.1.1. Thermophilic and mesophilic anaerobic treatments of low-strength piggery wastewater ......................................................................................................................................................... 99
    6.3.1.2. Performance comparison of thermophilic (55°C) and mesophilic (37°C) first-stage anaerobic reactors treating low-strength piggery wastewaters with synthetic complex wastewaters at 2-day hydraulic retention time ................................................................................................................. 110
  6.3.2. Semi-continuous first-stage thermophilic (55°C) CSTR anaerobic reactors treating medium- and high-strength piggery wastewaters with and without pH reduction at 2-day HRT ................................................................................................................................. 115
    6.3.2.1. Piggery wastewater without pH reduction ................................................................................................................................. 115
    6.3.2.2. Piggery wastewater with pH reduction to 5.5 ......................................................................................................................... 127
    6.3.2.3. Performance comparison of the thermophilic (55°C) first-stage anaerobic reactors treating medium- and high-strength piggery wastewaters with and without pH-reduction ............................................................................. 135
6.4. DISCUSSION .................................................................................................................. 138
  6.4.1. Performance comparisons of the thermophilic (55°C) and mesophilic (37°C) first-stage anaerobic reactors treating low-strength piggery wastewaters with synthetic complex wastewaters at 2-day hydraulic retention time ......... 139
  6.4.2. Effects of piggery concentrations or organic loading loads (low, medium and high) without pH reduction on the performance of thermophilic (55°C) first-stage reactors at 2-day hydraulic retention time .......................... 142
  6.4.3. Effects of pH reduction on organics conversion performance of thermophilic (55°C) first-stage reactors treating diluted (medium-strength) and undiluted (high-strength) piggery wastewaters at 2-day hydraulic retention time ......................................................................................................................... 146
6.5. CONCLUSIONS .......................................................................................................... 148
CHAPTER 7 ............................................................................................................ 151
SEMI-CONTINUOUS SINGLE-STAGE ANAEROBIC DIGESTION OF RAW
PIGGERY WASTEWATER AT HIGH HYDRAULIC RETENTION TIMES .... 151
7.1. INTRODUCTION ............................................................................................ 151
7.2. MATERIALS AND METHODS ..................................................................... 152
  7.2.1. Thermophilic (55°C) single-stage anaerobic digestion of raw piggery
         wastewater at 10-day and 15-day HRT ............................................................ 152
  7.2.2. Mesophilic (37°C) single-stage anaerobic digestion of high-strength
         piggery wastewater at 15-day HRT ................................................................. 152
7.3. RESULTS ......................................................................................................... 153
  7.3.1. Thermophilic (55°C) anaerobic digestion of high-strength piggery
         wastewater at 2-day, 10-day and 15-day HRT ................................................. 153
  7.3.2. Thermophilic (55°C) and mesophilic (37°C) anaerobic single-stage
         digestion of high-strength piggery wastewater at 15-day HRT ....................... 164
7.4. DISCUSSION ................................................................................................... 172
  7.4.1. Performance comparisons of the thermophilic anaerobic reactors treating
         undiluted piggery wastewater at 2-, 10- and 15-day HRT ......................... 172
  7.4.2. Performance comparisons of the thermophilic and mesophilic single-
         stage anaerobic reactors treating undiluted piggery wastewater at 15-day HRT
         .................................................................................................................. 175
7.5. CONCLUSION ................................................................................................. 180

CHAPTER 8 ............................................................................................................ 181
THERMOPHILIC ANAEROBIC BATCH VIAL EXPERIMENTS TO MITIGATE
AMMONIA INHIBITION AND TO ENHANCE METHANE PRODUCTION
FROM PIGGERY EFFLUENT ............................................................................... 181
8.1. INTRODUCTION ............................................................................................ 181
8.2. MATERIALS AND METHODS ..................................................................... 184
  8.2.1. Effect of pH on methane production from thermophilic batch digestion of
         piggery wastewater ......................................................................................... 184
  8.2.2. Effect of biomass supplements on methane production from thermophilic
         batch anaerobic digestion of pH-unadjusted and pH-reduced piggery effluent
         .................................................................................................................. 185
8.2.3. Effect of zeolite treatment on methane production from thermophilic
batch anaerobic digestion of pH-unadjusted and pH-reduced piggery effluent ................................................................. 186
8.2.4. Effect of humic acid supplements on methane production from
thermophilic batch anaerobic digestion of pH-unadjusted and pH-reduced
piggery effluent ................................................................................................ 186
8.3. RESULTS ......................................................................................................... 186
8.3.1. Effect of pH on methane production from thermophilic batch digestion of
piggery effluent ................................................................................................ 186
8.3.2. Effect of biomass supplements on methane production from thermophilic
batch anaerobic digestion of pH-unadjusted and pH-adjusted piggery
wastewaters ........................................................................................................ 197
  8.3.2.1. Piggery biomass supplement ................................................................. 197
  8.3.2.2. DiCOM biomass supplement ............................................................ 205
  8.3.2.3. Comparison of the digestion efficiency between piggery biomass
  and DiCOM biomass .................................................................................. 213
8.3.3. Effect of zeolite treatment on methane production from thermophilic
batch anaerobic digestion of pH-unadjusted and pH-reduced piggery wastewater ................................................................. 218
8.3.4. Effect of humic acid supplements on methane production from
thermophilic batch anaerobic digestion of pH-unadjusted and pH-reduced
piggery effluent ................................................................................................ 231
8.4. DISCUSSION ................................................................................................... 235
8.4.1. Effect of pH on methane production from thermophilic batch digestion of
piggery effluent ................................................................................................ 235
8.4.2. Effect of biomass supplements on methane production from thermophilic
batch anaerobic digestion of pH-unadjusted and pH-reduced piggery effluent
.......................................................................................................................... 238
8.4.3. Effect of zeolite treatment on methane production from thermophilic
batch anaerobic digestion of pH-unadjusted and pH-reduced piggery effluent
.......................................................................................................................... 240
8.4.4. Effect of humic acid supplements on methane production from
thermophilic batch anaerobic digestion of pH-unadjusted and pH-reduced
piggery effluent ............................................................................................... 242
LIST OF TABLES

Table 2.1. Some examples of glucose and amino acids fermentation products...... 13
Table 2.2. Some examples of degradation reactions of fermentation products ...... 15
Table 2.3. Methane-producing reactions............................................................... 17
Table 2.4. Some examples of competitive oxidation reactions.............................. 20
Table 2.5. Advantages and disadvantages of completely mixed suspended-growth digesters........................................................................................................ 21
Table 2.6. Strengths and weaknesses of FISH method compared to traditional cultivation-based methods................................................................................. 40
Table 3.1. Typical analysis for weaner feed pellets................................................ 45
Table 3.2. Physico-chemical characteristics of commercial pig feed and synthetic complex wastewater for the culture reactors................................................. 46
Table 3.3. Group-specific oligonucleotide probes from ThermoHybaid, Germany. 50
Table 3.4. PCR primers selected for quantification of microbial populations.......... 51
Table 4.1. Total COD, total VFA-COD and hydrogen-COD concentrations at 37°C, 47°C and 55°C............................................................................................... 57
Table 4.2. pH (initial and final), COD (total and soluble), total VFA and hydrogen gas production rate at 37°C ................................................................. 61
Table 4.3. pH (initial and final), COD (total and soluble), total VFA-COD and hydrogen-COD concentrations at 55°C......................................................... 64
Table 5.1. pH, ammonium-nitrogen and soluble phosphorus concentrations of the synthetic wastewater influents and reactor effluents at 1- to 4-d HRT .............. 75
Table 5.2. Total and volatile suspended solids in the synthetic influents and effluents at 1- to 4-d HRT ....................................................................................... 76
Table 5.3. Solids removal data at 1- to 4-day HRT ................................................. 77
Table 5.4. Chemical oxygen demand (total and soluble) and total VFA concentrations of the synthetic influents and reactor effluents at 1- to 4-d HRT ...... 78
Table 5.5. Organic compounds in the synthetic wastewaters fed to the reactors..... 79
Table 5.6. Compositions of the volatile fatty acids in the influents and reactor effluents........................................................................................................ 80
Table 5.7. Biogas composition and hydrogen yields of the thermophilic (T) and mesophilic (M) acid-phase reactors ................................................................. 80
Table 5.8. Extent of net hydrolysis (solubilisation), net acidification and hydrogenogenesis in the thermophilic (T) and mesophilic (M) acidogenic anaerobic reactors .......................................................... 83
Table 5.9. Quantification of thermophilic (T) and mesophilic (M) anaerobic microorganisms in the acidogenic reactor effluents by FISH method ................. 84
Table 6.1. pH, total alkalinity, ammonium-nitrogen and free ammonia concentrations of the low-strength influents and effluents at 2-d HRT ......................................................... 99
Table 6.2. Total and volatile suspended solids in the low-strength influents and effluents at 2-d HRT .................................................................................................................. 100
Table 6.3. Chemical oxygen demand (total and soluble) and total VFA concentrations of the low-strength piggery influents and reactor effluents at 2-d HRT ........................................................................................................... 101
Table 6.4. Volatile fatty acid concentrations of the low-strength piggery influents and digested effluents at 2-d HRT .......................................................................................... 102
Table 6.5. Biogas composition and specific methane yields from thermophilic and mesophilic first stage reactors treating low-strength piggery wastewaters .......... 103
Table 6.6. Comparison of the extent of net hydrolysis, net acidification and methanogenesis in the first-stage anaerobic reactors treating low-strength piggery wastewaters .................................................................................................................. 106
Table 6.7. Quantification of anaerobic thermophilic and mesophilic microorganisms using 16S rRNA-domain specific FISH probes of ARC-915 for archaea and EUBMIX for bacteria .................................................................... 107
Table 6.8. T-RFLP molecular profile results of low-strength piggery influent and digested effluents from thermophilic (T) and mesophilic (M) anaerobic reactors at 2-d HRT .................................................................................................................. 108
Table 6.9. Comparison of some key chemical characteristics of the piggery wastewater and synthetic complex organic wastewater ........................................... 110
Table 6.10. pH, total alkalinity, ammonium-nitrogen, free ammonia, soluble phosphorus and sulphate concentrations of the low-, middle- and high-strength influents and effluents at 2-d HRT .................................................................................................................. 115
Table 6.11. Total and volatile suspended solids in the low- and high- strength influents and effluents at 2-d HRT .................................................................................................................. 116
Table 6.12. Chemical oxygen demand (total and soluble) and total VFA concentrations of low-, medium- and high-strength piggery influents and thermophilic digested effluents at 2-d HRT................................................................. 117
Table 6.13. Volatile fatty acid concentrations of the low-, medium- and high-strength piggery influents and thermophilic digested effluents at 2-d HRT................................................................. 118
Table 6.14. Biogas composition and specific methane yields from the thermophilic first-stage reactors treating low-, medium- and high-strength piggery wastewaters119
Table 6.15. Comparison of the degree of net hydrolysis, net acidification and methanogenesis in the thermophilic first-stage anaerobic reactors treating low-, medium- and high-strength piggery wastewaters .................................................... 121
Table 6.16. Quantification of viable bacteria and methanogen populations in the low-, medium- and high-strength pH-unadjusted piggery influents and thermophilic digested effluents using 16S rRNA-specific FISH probes of EUBMIX and ARC-915 respectively .............................................................................................................. 122
Table 6.17. Bacteria and methanogen populations estimated by real-time PCR method in the low-, medium- and high-strength pH-unadjusted piggery influents and thermophilic digested effluents.............................................................................................................. 122
Table 6.18. T-RFLP molecular profile results of pH-unadjusted piggery influents and thermophilic digested effluents at 2-d HRT............................................................................................... 122
Table 6.19. pH, total alkalinity, ammonium-nitrogen and free ammonia concentrations of the medium- and high-strength influents with pH-reduction and thermophilic digested effluents at 2-d HRT................................................................. 127
Table 6.20. Total and volatile suspended solids in the medium- and high-strength influents with pH-reduction and thermophilic digested effluents at 2-d HRT........... 128
Table 6.21. Chemical oxygen demand (total and soluble) and total VFA concentrations of pH-reduced piggery influents and thermophilic reactor effluents at 2-d HRT ................................................................................................................... 128
Table 6.22. Volatile fatty acid concentrations of the piggery influents and thermophilic reactor effluents at 2-d HRT ................................................................................................................... 129
Table 6.23. Biogas composition and methane yields from the thermophilic first-stage anaerobic reactors treating medium- and high-strength piggery wastewaters with pH-reduction........................................................................................................................................ 130
Table 6.24. Comparison of the degree of hydrolysis, acidification, methanogenesis and hydrogenogenesis in the first-stage anaerobic reactors treating medium- and high-strength piggery wastewaters with pH reduction............................................. 132
Table 6.25. Quantification of bacteria and methanogen populations in the medium and high-strength pH-adjusted piggery influents and thermophilic digested effluents using 16S rRNA-specific FISH probes of EUBMIX and ARC-915 respectively ... 132
Table 6.26. T-RFLP molecular profile results of piggery influents with pH reduction and thermophilic digested effluents at 2-d HRT ................................................................. 134
Table 7.1. pH, total alkalinity, ammonium-nitrogen and free ammonia concentrations of the piggery influents and thermophilic (T) effluents at 2-d, 10-d and 15-d HRT 153
Table 7.2. Solid concentrations in the undiluted influents and thermophilic (T) digested effluents at 2-d, 10-d and 15-d HRT ................................................................. 154
Table 7.3. Chemical oxygen demand (total and soluble) and total VFA concentrations of the undiluted piggery influents and thermophilic (T) digested effluents at 2-d, 10-d and 15-d HRT ................................................................. 155
Table 7.4. Volatile fatty acid concentrations of the undiluted piggery influents and thermophilic (T) digested effluents at 2-d, 10-d and 15-d HRT .................................................... 156
Table 7.5. Biogas composition and methane yield from the thermophilic reactors at 2-day, 10-day and 15-day HRTs ..................................................................................... 158
Table 7.6. Digestion performance data of the thermophilic reactors at 2-day, 10-day and 15-day HRT ........................................................................................................ 160
Table 7.7. Quantification of thermophilic (T) anaerobic microorganisms by domain oligonucleotide FISH probes of ARC-915 for archaea and EUBMIX for bacteria 161
Table 7.8. pH, total alkalinity, ammonium-nitrogen and free ammonia concentrations of the piggery influents and effluents at thermophilic (T) and mesophilic (M) temperatures ................................................................................... 164
Table 7.9. Solids concentrations in the undiluted influents and digested effluents at thermophilic (T) and mesophilic (M) temperatures ........................................................................ 165
Table 7.10. Chemical oxygen demand (total and soluble) and total VFA concentrations of the undiluted piggery influents and digested effluents at thermophilic (T) and mesophilic (M) temperatures ............................................................ 166
Table 7.11. Volatile fatty acid concentrations in the influents and digested effluents of the thermophilic (T) and mesophilic (M) reactors at 15-day HRT ........................................ 167
Table 7.12. Biogas composition and methane yields from the thermophilic (55°C) and mesophilic (37°C) reactors at 15-day HRT ................................................................. 169
Table 7.13. Digestion performance data of the thermophilic (T) and mesophilic (M) reactors at 15-day HRT ........................................................................................................ 171
Table 7.14. Quantification of anaerobic thermophilic (T) and mesophilic (M) microorganisms by domain oligonucleotide FISH probes of ARC-915 for archaea and EUBMIX for bacteria ............................................................................................ 171
Table 7.15. Redox reactions of the competing anaerobic microorganisms .......... 178
Table 8.1. Chemical oxygen demand (total and soluble) and total VFA of the thermophilic piggery wastewater at start and end of the batch serum vial digestion experiment .......................................................................................................................... 188
Table 8.2. Volatile fatty acid concentrations of the thermophilic piggery wastewater at the start and end of the test period ........................................................................ 190
Table 8.3. pH, ammonium-nitrogen and free ammonia in the thermophilic piggery wastewater at start and end of the batch serum vial digestion experiment .......... 192
Table 8.4. Wastewater volatile solids (VS) and total COD concentrations with and without piggery biomass (pb) supplements at the start of the experiment.............. 198
Table 8.5. Chemical oxygen demand (total and soluble) and total VFA concentrations of the pH-unadjusted (C1) and pH-adjusted (C2) piggery wastewaters .......................................................................................................................... 200
Table 8.6. Volatile fatty acid concentrations in the thermophilic piggery wastewater at the start and end of the test period ................................................................. 202
Table 8.7. pH, ammonium-nitrogen and dissolved free ammonia concentrations in pH-unadjusted (C1) and pH-reduced (C2) digested piggery wastewaters without and with piggery biomass (pb) supplement ................................................................. 203
Table 8.8. Wastewater volatile solids (VS) and total COD concentrations with and without DiCOM biomass (db) supplements at the start of the experiment .............. 206
Table 8.9. Chemical oxygen demand (total and soluble) and total volatile fatty acids at start and end of the batch serum vial digestion experiment with DiCOM biomass addition ........................................................................................................ 208
Table 8.10. Volatile fatty acid concentrations in the thermophilic piggery wastewater at the start and end of the test period ................................................................. 209
Table 8.11. pH, ammonium-nitrogen and dissolved free ammonia concentrations in pH-unadjusted (C1) and pH-reduced (C2) digested piggery wastewaters without and with DiCOM biomass (db) supplement ................................................................. 211
Table 8.12. Wastewater volatile solids (VS) and total COD concentrations with and without piggery biomass (pb) and DiCOM biomass (db) supplements at the start of the experiment ........................................................................................................... 213
Table 8.13. Chemical oxygen demand (total and soluble) and total volatile fatty acids at start and end of the batch serum vial digestion experiment with zeolite treatment ........................................................................................................ 219
Table 8.14. Effect of zeolite on volatile fatty acid concentrations in pH-unadjusted (C1) and pH–reduced (C2) thermophilic piggery wastewaters ......................................................... 222
Table 8.15. pH, ammonium-nitrogen and dissolved free ammonia concentrations in pH-unadjusted (C1) and pH-reduced (C2) digested piggery wastewaters without and with zeolite treatment ................................................................................................. 226
Table 8.16. Chemical oxygen demand (total and soluble) and total VFA of the thermophilic piggery wastewater at start and end of the batch serum vial digestion experiment ........................................................................................................ 232
Table 8.17. Effect of zeolite on volatile fatty acid concentrations in pH-unadjusted (C1) and pH–reduced (C2) thermophilic piggery wastewaters .............................................. 234
Table 8.18. Thermodynamic comparison of various anaerobic microbial groups using hydrogen as electron donors ........................................................................................................ 244
LIST OF FIGURES

Figure 2.1. The universal phylogenetic tree of life according to Carl R. Woese
(Source: Barton, 2005) .............................................................................................................. 10

Figure 2.2. Schematic representation of the biochemical processes operating during
the microbially-mediated anaerobic conversion of organic matter (adapted from de
Lemos Chernicharo, 2007; Gavala et al., 1996; Pavlostathis and Giraldo-Gomez,
1991; and Harper and Pohland, 1986) ................................................................................... 11

Figure 2.3. Generalised carbon flow in the anaerobic environment without
methanogenesis ......................................................................................................................... 23

Figure 2.4. Effects of pH and temperature on the dissociation of hydrogen sulfide and
ammonia (…… 25°C, ----- 37°C, —— 60°C) (Source: Stams et al., 2003) ......................... 33

Figure 3.1. Insulated acidogenic culture reactors at 37°C (left), 47°C (middle) and
55°C (right) ............................................................................................................................ 42

Figure 3.2. Experimental set-up of the two computer-controlled anaerobic acidogenic
continuously stirred tank reactors (CSTRs) ........................................................................... 43

Figure 3.3. Schematic diagram of the automated anaerobic acid-phase reactors
(CSTRs) set-up ......................................................................................................................... 44

Figures 4.1 (a), (b) and (c). Profiles of COD material balance at 37°C, 47°C and 55°C
as a function of incubation period ......................................................................................... 58

Figures 4.2 (a), (b) and (c). Volatile fatty acid production trends at 37°C, 47°C and
55°C as a function of incubation period (error bars indicate standard deviations) .... 59

Figures 4.3 (a), (b), (c) and (d). Profiles of COD material balance at pH 3.8 (initial),
pH 6, pH 7 and pH 8 respectively as a function of incubation day at 37°C ............... 62

Figures 4.4 (a), (b), (c) and (d). Volatile fatty acid production trends at pH 3.8
(initial), pH 6, pH 7 and pH 8 respectively as a function of incubation day (error bars
indicate standard deviations) ......................................................................................... 63

Figures 4.5 (a), (b), (c) and (d). Profiles of COD material balance at pH 4.9 (initial),
pH 6, pH 7 and pH 8 respectively as a function of incubation day at 55°C ............... 65

Figures 4.6 (a), (b), (c) and (d). Volatile fatty acid production trends at pH 4.9
(initial), pH 6, pH 7 and pH 8 respectively as a function of incubation day (error bars
indicate standard deviations) ......................................................................................... 67
Figures 5.1 (a), (b), (c) and (d). COD material balance of the feedwaters and reactor effluents plus biogas of the thermophilic and mesophilic reactors as a function of hydraulic retention time .................................................................................................................. 82

Figure 5.2. Comparison of the thermophilic (T) and mesophilic (M) organics conversion performance in relation to anaerobic bacteria population (error bars indicate standard deviations) .................................................................................. 85

Figures 5.3 (a), (b) and (c). Fluorescent images of archaea (green), archaea (green) plus low-GC bacteria (red) and fermentative bacteria (red) respectively in the thermophilic acidogenic reactor ................................................................................. 86

Figures 5.4 (a), (b) and (c). Fluorescent images of archaea (green), archaea (green) plus low-GC bacteria (red) and fermentative bacteria (red) respectively in mesophilic acid-phase reactor .............................................................................................................. 86

Figure 5.5. Distribution of phylogenetic domains of bacteria in the thermophilic (T) and mesophilic (M) efluents at 2-d HRT .................................................................................................................. 87

Figure 6.1. COD (total and soluble) and total VFA reductions of thermophilic (T) and mesophilic (M) digested effluents at 2-d HRT (error bars indicate standard deviations) .......................................................................................................... 102

Figure 6.2. Percentage removal of VFA-COD in the thermophilic (T) and mesophilic (M) digested effluents at 2-d HRT (error bars indicate standard deviations) .......................................................................................................... 103

Figure 6.3. COD material balance of the low-strength piggery influents and digested effluents of the thermophilic (T) and mesophilic (M) anaerobic reactors ................................................................. 105

Figures 6.4 (a), (b) and (c). Fluorescent images of archaea (green), archaea (green) plus low-GC bacteria (red) and total bacteria (red) in thermophilic reactor effluent respectively .............................................................................................................. 108

Figures 6.5 (a), (b) and (c). Fluorescent images of archaea (green), archaea (green) plus low-GC bacteria (red) and total bacteria (red) in mesophilic reactor effluent respectively .............................................................................................................. 108

Figure 6.6. Comparison of the distribution of phylogenetic groups of bacteria in the influents (FT, FM) and effluents of the thermophilic (T) and mesophilic (M) first-stage anaerobic reactors at 2-d HRT using 16S rRNA-group specific FISH probes .............................................................................................................. 109

Figures 6.7 (a) and (b). Comparison of anaerobic archaea and bacteria populations in the low-strength thermophilic (T) and mesophilic (M) piggery and synthetic influents as well as reactor effluents (error bars indicate standard deviations) ...... 111
Figures 6.8 (a) and (b). Comparison of anaerobic bacteria groups in the low-strength thermophilic (T) and mesophilic (M) piggery and synthetic influents (FT, FM) as well as reactor effluents (T, M) respectively .......................................................... 112
Figures 6.9 (a) and (b). COD material balance of low-strength thermophilic (T) and mesophilic (M) piggery and synthetic influents as well as reactor effluents respectively ............................................................................................................. 113
Figures 6.10 (a) and (b). Comparison of the extent of initial hydrolysis, net hydrolysis, initial acidification, net acidification, methane production and hydrogen production of the thermophilic (T) and mesophilic (M) first-stage anaerobic reactors treating piggery wastewaters and synthetic complex wastewater respectively (error bars indicate standard deviations) ............................................................................ 114
Figure 6.11. COD (total and soluble) and total VFA reductions of thermophilic digested effluents at 2-d HRT (error bars indicate standard deviations) .......................... 118
Figure 6.12. Percentage removal of VFA-COD in the thermophilic digested effluents at 2-d HRT (error bars indicate standard deviations) ........................................ 119
Figure 6.13. COD material balance of the low-, medium- and high-strength piggery influents and thermophilic digested effluents .......................................................... 120
Figures 6.14 (a) and (b). Comparisons of bacteria and methanogen numbers by molecular FISH and real-time PCR methods respectively (error bars indicate standard deviations) ................................................................................................. 123
Figure 6.15. Methanogen (archaea) FISH counts and specific methane yields as a function of the effluent organic carbon concentrations (error bars indicate standard deviations) ......................................................................................................... 124
Figures 6.16 (a) and (b). Fluorescent images of archaea (green) and total bacteria (red) in thermophilic reactor effluent (pH-unadjusted medium-strength) respectively .................................................................................................................. 124
Figures 6.17 (a), (b) and (c). Fluorescent images of archaea (green), archaea (green) plus low-GC bacteria (red) and total bacteria (red) in thermophilic reactor effluent (pH-unadjusted high-strength) respectively ........................................................ 125
Figure 6.18. Comparison of the distribution of phylogenetic groups of bacteria in the influents (FTu) and effluents (Tu) of the thermophilic (T) low- and high-strength anaerobic reactors at 2-d HRT using 16S rRNA-group specific FISH probes ....... 126
Figure 6.19. Percentage reduction or increase of volatile fatty acids in the thermophilic digested effluents relative to influent (error bars indicate standard deviations) ................................................................................................................ 129

Figure 6.20. COD material balance of the medium- (M) and high-strength (H) piggery influents with pH reduction and thermophilic reactor effluents (medium- and high-strength) .................................................................................................................................................................................. 131

Figures 6.21 (a) and (b). Fluorescent images of archaea (green) and archaea (green) plus total bacteria (red) in thermophilic reactor effluent (pH-reduced medium-strength) respectively ............................................................................................... 133

Figures 6.22 (a), (b) and (c). Fluorescent images of archaea (green), archaea (green) plus low-GC bacteria (red) and total bacteria (red) in thermophilic reactor effluent (pH-reduced high-strength) respectively .................................................................................................................. 133

Figure 6.23. Distribution of various phylogenetic groups of bacteria in the high-strength piggery influent with pH reduction (FTa) and digested effluent (Ta)........ 134

Figures 6.24 (a) and (b). COD material balance of mid-strength (M) and high-strength (H) piggery wastewaters respectively ........................................................ 135

Figure 6.25. Comparison of the extent of net hydrolysis, acidification and methanogenesis of the thermophilic anaerobic reactors treating pH-unadjusted and pH-reduced piggery wastewaters (error bars indicate standard deviations) .......... 136

Figures 6.26. (a) and (b). Anaerobic bacteria and archaea populations in the mid-strength and high-strength piggery wastewaters respectively (error bars indicate standard deviations) ................................................................................................. 137

Figure 6.27. Comparison of the distribution of phylogenetic bacteria groups in the pH-unadjusted (FTu) and pH-reduced (FTa) high-strength piggery influents as well as their thermophilic effluents (Tu and Ta) respectively at 2-d HRT using 16S rRNA-group specific FISH probes .................................................................................................................................................. 138

Figure 7.1. Solids reductions of thermophilic digested effluents at 2-d, 10-d and 15-d HRT (error bars indicate standard deviations) ......................................................... 154

Figure 7.2. COD (total and soluble) and total VFA reductions of the thermophilic digested effluents at 2-d, 10-d and 15-d HRT (error bars indicate standard deviations) .................................................................................................................................. 155

Figure 7.3. Percentage removal of VFA-COD in the digested effluents at 2-d, 10-d and 15-d HRT (error bars indicate standard deviations) ......................................................... 157
Figure 7.4. Relationships between acetate reduction, propionate increase and free ammonia concentration as a function of hydraulic retention time......................... 157
Figure 7.5. COD material balance of the undiluted piggery influents and thermophilic (T) reactor effluents at 2-, 10- and 15-day HRT .......................................................... 159
Figure 7.6. Relationship between archaea populations in the thermophilic reactor effluents and methane yields as a function of hydraulic retention time (error bars indicate standard deviations)....................................................................................... 162
Figures 7.7 (a), (b) and (c). Fluorescent images of thermophilic archaea (green), bacteria (red) and superimposed image of (a) and (b) at 10-d HRT………………… 162
Figures 7.8 (a), (b) and (c). Fluorescent images of thermophilic archaea (green), bacteria (red) and superimposed image of (a) and (b) at 15-d HRT………………… 163
Figure 7.9. T-RFLP distribution profiles of the bacteria group in the undiluted piggery feedwater and thermophilic digested effluents at 2-, 10- and 15-day HRT 163
Figure 7.10. Solids reductions of thermophilic (T) and mesophilic (M) digested effluents at 15-d HRT (error bars indicate standard deviations)…………………………………… 165
Figure 7.11. COD (total and soluble) and total VFA reductions of the thermophilic (T) and mesophilic (M) digested effluents at 15-d HRT (error bars indicate standard deviations)………………………………………………………………………………… 166
Figure 7.12. Percentage removal of VFA-COD in the thermophilic (T) and mesophilic (M) digested effluents at 15-d HRT (error bars indicate standard deviations)………………………………………………………………………………………… 167
Figure 7.13. Relationships between acetate, propionate and free ammonia concentrations in the mesophilic (37°C) and thermophilic (55°C) digested effluents (error bars indicate standard deviations)………………………………………………………… 168
Figure 7.14. COD material balance of the undiluted piggery influents, thermophilic (T) and mesophilic (M) digested effluents at 15-day HRT………………………………… 170
Figures 7.15 (a), (b) and (c). Fluorescent images of mesophilic archaea (green), bacteria (red) and superimposed image of (a) and (b) at 15-d HRT………………… 172
Figure 8.1. Effect of pH on methane production from thermophilic batch digestion of piggery wastewater (error bars indicate standard deviations)………………………… 187
Figure 8.2. Changes in methane production rate with time during thermophilic batch digestion of piggery wastewater………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………
Figure 8.3. COD (total and soluble) and total TVFA-COD reductions as a function of thermophilic piggery wastewater pH after 10 days of batch digestion (error bars indicate standard deviations) ................................................................. 188

Figure 8.4. Relationship between total VFA-COD degraded and methane production at different wastewater pH after 10 days of batch digestion ........................................ 189

Figure 8.5. VFA degradation as a function of thermophilic piggery wastewater pH (error bars indicate standard deviations) after 10 days of batch digestion .......... 190

Figures 8.6 (a) and (b). Relationships between acetate concentration and acetate degradation as well as acetate concentration and propionate degradation respectively .................................................................................................................................. 191

Figure 8.7 (a) and (b). Relationships between acetate concentration and propionate degradation as well as between propionate concentration and propionate degradation .................................................................................................................................. 192

Figure 8.8. pH and free ammonia concentrations of the thermophilic piggery wastewaters (error bars indicate standard deviations) ........................................... 193

Figure 8.9. Relationship between final free ammonia and initial pH of the thermophilic piggery wastewater .............................................................................. 194

Figure 8.10. Relationships between free ammonia, methane production and total VFA-COD reduction in the thermophilic piggery wastewater at different pH .... 194

Figure 8.11. Relationships between free ammonia, acetate and propionate degradation in the thermophilic piggery wastewater at different pH .................... 195

Figure 8.12. Approximate distribution of bacteria group in the thermophilic piggery wastewater at different pH at the end of the test (day 10) ......................... 196

Figure 8.13. Approximate estimations of methanogens and Clostridium perfringen populations (error bars indicate standard errors) ................................................. 197

Figure 8.14. Effect of supplementing piggery biomass (pb) on methane production from pH-unadjusted (C1) and pH-reduced (C2) thermophilic piggery wastewaters (error bars indicate standard deviations) ................................................. 198

Figures 8.15 (a) and (b). Effects of piggery biomass (pb) supplements on COD (total and soluble) and total TVFA reductions in pH-unadjusted (C1) and pH-reduced (C2) thermophilic piggery wastewaters respectively (error bars indicate standard deviations) ................................................................................................................................. 200
Figures 8.16 (a) and (b). Effect of piggery biomass (pb) supplements on VFA degradation in pH-unadjusted (C1) and pH-reduced (C2) thermophilic piggery wastewaters respectively (error bars indicate standard deviations) ........................................ 202
Figure 8.17. Relationships between total VFA-COD reduction, methane yield and initial free ammonia concentration in the pH-unadjusted (C1) piggery wastewater 204
Figure 8.18. Relationships between total VFA-COD reduction, methane yield and final free ammonia concentration in the pH-reduced (C2) piggery wastewater ...... 205
Figure 8.19. Effect of supplementing DiCOM biomass (db) on methane production from pH-unadjusted (C1) and pH-reduced (C2) thermophilic piggery wastewaters (error bars indicate standard deviations) ........................................................................ 206
Figures 8.20 (a) and (b). Effect of DiCOM biomass (db) supplements on COD (total and soluble) and total TVFA reductions in pH-unadjusted (C1) and pH-reduced (C2) thermophilic piggery wastewaters respectively (error bars indicate standard deviations) ........................................................................................................................................ 208
Figures 8.21 (a) and (b). Effect of DICOM biomass (db) supplements on VFA degradation in pH-unadjusted (C1) and pH-reduced (C2) thermophilic piggery wastewaters (error bars indicate standard deviations) .............................................................................................. 210
Figure 8.22. Relationships between total VFA-COD reduction, methane yield and initial free ammonia concentration in the pH-unadjusted (C1) piggery wastewater 212
Figure 8.23. Relationships between total VFA-COD reduction, methane yield and initial final ammonia concentration in the pH-reduced (C2) piggery wastewater ... 213
Figures 8.24 (a) and (b). Comparison of methane yields of 10% piggery biomass (pb) and DiCOM biomass (db)-supplemented wastewaters without pH reduction (C1), and comparison of methane yields of 19% piggery biomass (pb) and DiCOM biomass (db)-supplemented wastewaters with pH reduction (C2) (error bars indicate standard deviations) ........................................................................................................ 214
Figures 8.25 (a) Repeat and (b) Previous. Comparison of the methane yields of low (10%) and high (20%) piggery biomass- (pb) and DiCOM (db) biomass-supplemented piggery wastewaters with pH reduction (C2) (error bars indicate standard deviations) ............................................................................................................... 215
Figures 8.26 (a) and (b). pH-unadjusted (C1) and pH-reduced (C2) wastewaters’ key chemical characteristics at the end of the batch digestion period (error bars indicate standard deviations) ........................................................................................................ 216
Figure 8.27. Approximate distribution of bacteria group in the controls and biomass-supplemented pH-unadjusted and pH-reduced thermophilic piggery effluent at the end of the test period (day 11)........................................................................................................ 217
Figure 8.28. Effect of zeolite concentrations on cumulative methane production from thermophilic piggery reactor effluent (error bars indicate standard deviations)...... 218
Figure 8.29. COD (total and soluble) and total TVFA reductions in pH-unadjusted (pH 8.1) and pH-reduced (pH 6.6) thermophilic piggery effluent at varying zeolite concentrations (error bars indicate standard deviations) after 10 days of batch digestion................................................................................................................... 220
Figure 8.30. Relationship between apparent kinetic constant of TVFA-COD degradation and zeolite concentration at the end of the test period (day 10)........ 221
Figures 8.31 (a) and (b). Effect of zeolite concentrations on VFA degradation in thermophilic piggery effluents without and with pH reduction respectively (error bars indicate standard deviations) after 10 days of batch digestion ............................... 223
Figures 8.32 (a), (b) and (c). Relationships between acetate degradation, propionate degradation, n-butyrate degradation and zeolite concentrations in the piggery wastewater without pH reduction................................................................. 224
Figures 8.33 (a), (b), (c) and (d). Relationships between zeolite concentrations and acetate, propionate, i-valerate and caproate degradation in the pH-reduced piggery wastewater................................................................................................................ 225
Figures 8.34 (a) and (b). Relationships between ammonium-nitrogen and zeolite concentrations in the thermophilic piggery wastewater without pH reduction; and between free ammonia and zeolite concentrations in the thermophilic piggery wastewater without pH reduction respectively ......................................................... 227
Figures 8.35 (a) and (b). Relationships between ammonium-nitrogen and zeolite concentrations in the pH-reduced piggery wastewater; and between free ammonia and zeolite concentrations in the pH-reduced piggery wastewater respectively...... 228
Figures 8.36 (a) and (b). Relationships between acetate degradation and free ammonia concentrations in the thermophilic piggery wastewaters without pH-reduction (C1) and with pH-reduction (C2) respectively ................................................. 229
Figure 8.37. Approximate distribution of bacteria group in the controls and zeolite-treated pH-unadjusted and pH-reduced thermophilic piggery wastewaters at the end of the test period (day 10).................................................................................................................. 230
Figures 8.38 (a) and (b). Effect of humic acid (high) on methane production of thermophilic piggery effluents without pH-reduction (C1) and pH-reduction (C2); ad effect of humic acid (low) on methane production of pH-reduced (C2) thermophilic piggery effluent respectively (error bars indicate standard deviations) ................. 232
Figure 8.39. COD (total and soluble) and total TVFA reductions in pH-reduced (pH 6.5) thermophilic piggery effluent at low humic acid concentrations (error bars indicate standard deviations) .................................................................................................................. 233
Figure 8.40. Effect of humic acid concentrations on VFA degradation in pH-reduced thermophilic piggery effluent (error bars indicate standard deviations) ............... 234
Figure 8.41. Relationships between humic acid concentration and VFA degradation (acetate and propionate) in the pH-reduced piggery wastewater ............................... 235
LIST OF ABBREVIATIONS

APHA  American Public Health Association
atm   atmosphere
CSTR  Continuously Stirred Tank Reactor
CO₂   Carbon dioxide
d    day
FISH  Fluorescence In Situ Hybridisation
GC    Gas Chromatography
g    gram
HRT   Hydraulic Retention Time
hr   hour
H₂   Hydrogen
IBS   Integrated BioSystem
kg   kilogram
kJ   kiloJoule
L    Litre
mL   millilitre
μL   microlitre
mM   millimolar
mg   milligram
M    Molar
N    Normality
ng   nanogram
CH₄  Methane
OLR  Organic Loading Rate
PCR  Polymerase Chain Reaction
rRNA ribosomal RiboNucleic Acid
rDNA ribosomal DeoxyRiboNucleic Acid
stp  standard temperature and pressure
ΔG° standard Gibbs free energy
SRB  Sulphate Reducing Bacteria
SRT  Solid Retention Time
SCOD  Soluble Chemical Oxygen Demand
SARDI  South Australian Research and Development Institute
TPAD  Temperature-Phased Anaerobic Digestion
TVFAs  Total Volatile Fatty Acids
TSS  Total Volatile Solids
TS  Total Solids
T-RFLP  Terminal-Restriction Fragment Length Polymorphism
TCOD  Total Chemical Oxygen Demand
VSS  Volatile Suspended Solids
VS  Volatile Solids