Anaerobic Co-digestion of Chicken Litter with Food and Agro-industrial Wastes/Residues, an Australian Case Study: Use of Carbon-to-Nitrogen Ratio for Substrate Mixing and Semi-Solids versus Wet Anaerobic Digestion

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and, ethics procedures and guidelines have been followed. I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

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Abstract

In Victoria, Australia, around 0.45 million tonnes of chicken litter (CL) are produced every year. CL has high potential for bioenergy production, mainly in the form of biogas (methane), however, it has not been widely practiced because it has high levels of protein and uric acid which are known to cause inhibition, during anaerobic digestion (AD), in the form of ammonia. The current CL management practice is to transport CL to a composting facility which has limited benefit to the broiler industries. This has triggered research to assess the feasibility of biogas production from broiler farms' CL co-digested with other substrates. Among the agro-industrial wastes usually available in the proximity of chicken farms are agro-industry processing wastes and agricultural residues such as yoghurt whey (YW), wheat straw (WS) and hay grass (HG). Municipal waste i.e. the food wastes (FW) in the household bins is also available to use. These wastes have high methane potential; but varying characteristics and composition, e.g. carbon-to-nitrogen (C/N) ratio, pH, alkalinity, ammonia, structural recalcitrance. Therefore, these wastes need to be characterised, require understanding if they are suitable for co-digestion and what ratios they can be mixed together.

Also, most of the literature concerning co-digestion of different wastes focused on mixing one or two wastes; but little research has been done on the co-digestion of a wide variety of solid waste streams. Therefore, an efficient and economic energy recovery from those wastes through anaerobic co-digestion, an investigation into optimum process conditions is required.

Wet anaerobic digestion (W-AD) is a well-established technology operating at solids concentration of<10% total solids (TS), whereas, high solids AD (HS-AD), (10-15% TS) and dry AD (D-AD), <15% TS) are not a common practice. This is due to long retention times and types of infrastructure required, but the most important factors are the lack of knowledge concerning treatment operating conditions, loading and composition. However, as most of the agro-industrial wastes have high TS, the research has focused on both conventional wet and high solids AD.

The aims of this research are to assess the potential of anaerobic co-digestion (ACoD) of CL and co-substrates and to determine the optimum AD process

conditions where the process is stabilised and show no inhibition, for different AD configurations, and to assess the performance of HS-AD and W-AD fed with a range of co-substrates, under continuous feeding conditions for different organic loading rates (OLRs). The research focused on developing a method that can be used to determine the optimum mixing ratio of agro-industrial wastes of varying characteristics, optimum running conditions for continuous AD, pre-treatment of waste to improve bio-degradation and use of the optimum conditions to develop a high solid AD process.

The wastes have been characterised thoroughly before doing their biochemical-methane potential (BMP) under conventional W-AD solids concentrations (2-4% volatile solids (VS)). Four different kinetic models i.e. the first order kinetic model, modified Gompertz model, transfer function model and the cone model were used to fit the biogas yield of the single substrates and the modified Gompertz model (R2: 0.93-0.99) showed better fit among all the models which explains the variation in lag phase and methane production rate depending upon the substrate characteristics.

The second batch of BMP tests assessed the co-digestion of a range of different wastes mixed using C/N ratio as the control parameters. The batch experiments were designed according the response surface model (RSM). All the BMP tests were carried out in batch assays under mesophilic conditions in duplicates with 1:2 g/g VS of substrate to inoculum. Analysis of the BMP results using Matlab indicated that the maximum methane production could be achieved for a feedstock of 30-35% VS of CL and 65-70% VS of agro-industrial waste (i.e. YW, FW, HG and WS) that have a total C/N ratio of 26-27.5.

In the second phase of the experimental work, semi-continuous anaerobic co-digestion were performed at 4-6% TS based on the predictions and conditions from the batch assesses to observe process performances. The AD reactors were operated at organic loading rates (OLRs) of 2.0-3.0 g TS/L. d and hydraulic retention time (HRT) of 20 days. The optimum feedstock (substrates mixture) was CL: FW:WS of 60:20:20, where 73%, 167% and 117% increase in total biogas production at OLR of 2.0, 2.5, 3.0 g TS/L. d, respectively, compared to that from CL, was achieved. Principal component analysis (PCA) was applied with the characteristics parameter and 68.1% of data variability was explained with second principal component. During

semi-continuous work, a new concept, that C/N ratio and lignocellulosic structure degradation might be related during AD Digestate, is applied and found that carbohydrate degradation i.e. cellulose and water soluble contents plays a major role in explaining the variation in performance and produced biogas for different feedstocks of balanced C/N ratio.

As most of the agro-industrial wastes are lignocellulosic in nature which resists biodegradability, therefore, the next phase of experimental work focused on selective fractionation of lignocellulosic biomass using sequential alkaline (AKP) and dilute acid (DAP) pre-treatments to increase biodegradability. NaOH pre-treatment was applied both on CL (as it had included bedding material in it) and WS under independent factors of NaOH concentration (1-5% w/v), reaction time (30-90 min) and temperature (60-120°C), and experiment design using RSM. The optimum conditions were analyses with Minitab and the optimum conditions are NaOH concentration 1% (W/V) for 30 min at 120ºC and NaOH concentration 5% (W/V) for 90 min at 120ºC for AKP of WS and CL, respectively. Sequential DAP with H_2SO_4 (1%-3% w/v) was applied with the best conditions found for the AKP using RSM 2 with same conditions as AKP i.e. same temperatures and times. With sequential DAP+AKP, higher removal of lignin and hemicellulose and an increment of 25% in biogas was obtained compared to single pre-treatment.

Finally, High solids AD were performed in sequential batch assays to give it enough time to degrade and to observe the long-time process performances. sequential ACoD at 15% TS was performed and reported with the optimum conditions from the batch assays (W-AD) i.e. CL: FW: WS mixed at a ratio of 35:32.5:32.5 to have C/N ratio of 26.5 over 215 days in six cycles. The reactors that were fed with untreated substrates produced 321.6±13.4 mL_N biogas/g VS_{added}, which increased by 88%, when CL and WS were sequentially pre-treated using AKP and DAP. The VS removal in the reactors that received pre-treated substrates showed improved biogas production compared to those that received untreated feedstock. A VS removal of 55% were observed with AKP+DAP pre-treated substrates fed reactors, compared with only 36% VS removal from untreated substrate fed reactors. A reduction in ammonia and cellulose with an increase in water soluble contents was also observed in the reactors that received these pre-treated substrates. Additionally, it was also

noted that biogas production using sequential SS-AD at 15% was almost 38% less than W-AD, however this was negated with the pre-treatment of substrates, indicating that co-digestion at high TS of 15% is achievable.

List of Publications

Journal Articles:

1. **Zahan, Z.,** Othman, M.Z. and Muster, T.H., 2018. Anaerobic digestion/co-digestion kinetic potentials of different agro-industrial wastes: A comparative batch study for C/N optimisation. Waste Management, 71, pp.663-674. https://doi.org/10.1016/j.wasman.2017.08.014

2. **Zahan, Z.,** Stelios, G., Muster, T.H. and Othman, M.Z., 2018. Semi-continuous anaerobic co-digestion of chicken litter with agricultural and food wastes: A case study on the effect of carbon/nitrogen ratio, substrate mixing ratio and organic loading. Bioresource technology, 270, pp. 245-254. https://doi.org/10.1016/j.biortech.2018.09.010

3. **Zahan, Z.,** Stelios, G. and Othman, M.Z., 2019. Degradation of structural carbohydrates of agro-industrial wastes through sequential pre-treatment strategies. Biomass and bioenergy (Drafted)

4. **Zahan, Z.** and Othman, M.Z., 2019. Effect of pre-treatment on sequential anaerobic co-digestion of chicken litter with agricultural and food wastes under semi-solid conditions and comparison with wet anaerobic digestion. Bioresource technology, 281, pp. 286-295. https://doi.org/10.1016/j.biortech.2019.01.129

   Appendix: Separate Case Study

5. **Zahan, Z.,** Othman, M.Z. and Rajendram, W., 2016. Anaerobic Codigestion of Municipal Wastewater Treatment Plant Sludge with Food Waste: A Case Study. BioMed Research International, 2016. https://doi.org/10.1155/2016/8462928

Conference Papers:

1. **Zahan, Z.,** Othman, M.Z. and Muster, T.H., 2016. Characterisation of agro-industrial wastes and their anaerobic digestion/co-digestion kinetic potential: A comparative batch study. Sixth International Symposium on Energy from Biomass and Waste (pp. 1-9), 14 - 17 November 2016, CISA Publisher, Italy.

2. **Zahan, Z.,** Othman, M.Z. and Muster, T.H., 2017. Bioreactor performances in continuous anaerobic co-digestion of chicken litter with agro-industrial wastes: effect of C/N ratio, organic loading and lignocellulose fractionation. 15th IWA World Congress conference on Anaerobic Digestion (AD-15), 17-20 October 2017, Beijing, China.

3. **Zahan, Z.** and Othman, M.Z., 2018. Sequential anaerobic co-digestion of chicken litter with agro-industrial wastes under high total solids and comparison with their wet anaerobic digestion. 26th European Biomass Conference & Exhibition, 14-17 May 2018, Copenhagen, Denmark.

Conference Paper (Abstract):

1. **Zahan, Z.** and Othman, M.Z., 2015. Characterisation and anaerobic degradation kinetic potential of chicken litter. WETT HDR Symposium, 25th November, RMIT University.

2. **Zahan, Z.** and Othman, M.Z., 2017. Characterisation of agro-industrial wastes and their anaerobic digestion/ Co-digestion potential. WETT HDR Symposium, 22th November, RMIT University.

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List of Abbreviations

AD	Anaerobic digestion
W-AD	Wet anaerobic digestion
HS-AD	High solids anaerobic digestion
D-AD	Dry anaerobic digestion
BMP	Biochemical methane potential
ACoD	Anaerobic co-digestion
CL	Chicken litter
YW	Yoghurt whey
FW	Food waste
OFMSW	Organic fraction of municipal solid waste
I	Inoculum
SFW	Synthetic food waste
WS	Wheat straw
HG	Hay grass
SV	Sustainability Victoria
WWTP	Waste water treatment plant
MWTP	Municipal waste water treatment plant
TS	Total solids
VS	Volatile solids
I: S ratio	Inoculum: substrate ratio
COD	Chemical oxygen demand
tCOD	Total COD
$_{s}$COD	Soluble COD
TOC	Total organic carbon
IC	Inorganic carbon
TC	Total carbon
C/N ratio	Carbon/ Nitrogen ratio
TN	Total nitrogen
TAN	Total ammonia nitrogen
TKN	Total kjeldhal nitrogen
NH_4	Ammonium

FA	Free or unionised ammonia
Anammox	Anaerobic ammonia oxidization
TP	Total phosphorous
VA	Volatile acids
VFAs	Volatile fatty acids
CH_4	Methane
GC	Gas chromatography
HPLC	High performance liquid chromatography
SEM	Scanning electron microscope
TCI	Total crystallinity index
CI	Crystallinity index
GW	Garden waste
Na	Sodium
Ce	Cerium
NaOH	Sodium hydroxide
H_2SO_4	Sulphuric acid
AKP	Alkaline pre-treatment
DAP	Dilute acid pre-treatment
DoE	Design of experiment
CO_2	Carbon dioxide
SBP	Specific biogas potential
SS	Sewage sludge
WAS	Waste activated sludge
PS	Primary sludge
FOG	Fat, oil and grease
PF	Processed food
GT	Grease trap waste
EPA	Environment protection agency
W/V	Weight/ volume
W/W	Weight/weight
V/V	Volume/volume
OLR	Organic loading rate
HRT	Hydraulic retention time

SRT	Solids retention time
OL	Organic loading
CM	Chicken manure
H_2	Hydrogen
MWS	Municipal solid waste
APHA	American public health association
NREL	National renewable energy laboratory
FOS/TAC	Ratio of volatile acids to alkaline buffer capacity
AIL	Acid insoluble lignin
ASL	Acid soluble lignin
VS_{added}	Volatile solids added
DM	Dry matter
DW	Dry weight
FTIR	Fourier transform infrared
ATR	Attenuated total reflection
rMSPE	Root mean square prediction error
K	Rate constant
λ	Lag phase
Rm	Maximum methane production rate
n	Shape factor
PCA	Principal component analysis
PCs	Principal components
PC1	First principal component
PC2	Second principal component
$CaCO_3$	Calcium carbonate
ANOVA	Analysis of variance
CA	Cluster analysis
HMF	Hydroxyl methyl furfural
RSM	Response surface methodology
BBD	Box- Behnken design
CCD	Central composite design
Zn	Zinc
Cu	Copper

Ni	Nickel
Cd	Cadmium
Pb	Lead
Cr	Chromium
Hg	Mercury
He	Helium
Ca	Calcium
Mg	Magnesium
Al	Aluminium
Si	Silicon
AFEX	Ammonia fibre-expansion
CSTR	Continuous stirred tank reactor
GHG	Greenhouse gas

Introduction

1.1 Background

The total chicken meat consumption in Australia has been estimated at 580 million tonnes of birds (Agriculture Victoria, 2014), which is estimated to increase by 4% annually with the increase in population (Scott et al., 2009). According to the report of World Food and Agriculture (2013), the chicken consumption in the world was nearly 20 billion (FAOSTAT and CommoditiesProduction, 2013). In Victoria, the chicken production is around 128 million tonnes of birds which is around 28% of total meat production in Australia (Agriculture Victoria, 2014). This has been associated with the production of large quantities of chicken litter. For example: the production of chicken litter (CL) in a single four shed broiler is approximately 600 tonnes every two months which includes bedding materials. State-wide 450,000 tonnes of chicken litter produces every year (Scott et al., 2009). Figure 1-1 shows the main chicken farming areas in Victoria where chicken meat production localities refers to the areas where most of the chicken farms are located and developing production localities are the areas where new chicken farms are being built. There are 3 major areas namely Geelong, south east of Melbourne and Bendigo where these chicken farms are concentrated. For example, in Mornington, Peninsula, there are 68 broiler farms. The waste from these farms is usually transferred to a composting facility. But these wastes, have high potential for bioenergy production, especially in the form of biogas (Bujoczek et al., 2000; Rahman et al., 2017; Rodriguez-Verde et al., 2018). The potential of using the chicken farm wastes as a resource to produce energy that can be utilised onsite offers a more cost-effective operation of the broilers compared to the production of compost. Therefore, this has raised the interest of chicken farmers to investigate the feasibility of using the CL as a feedstock for bioenergy generation using an AD technology. This triggered this study, with the aim to focus on AD of CL from the broilers.

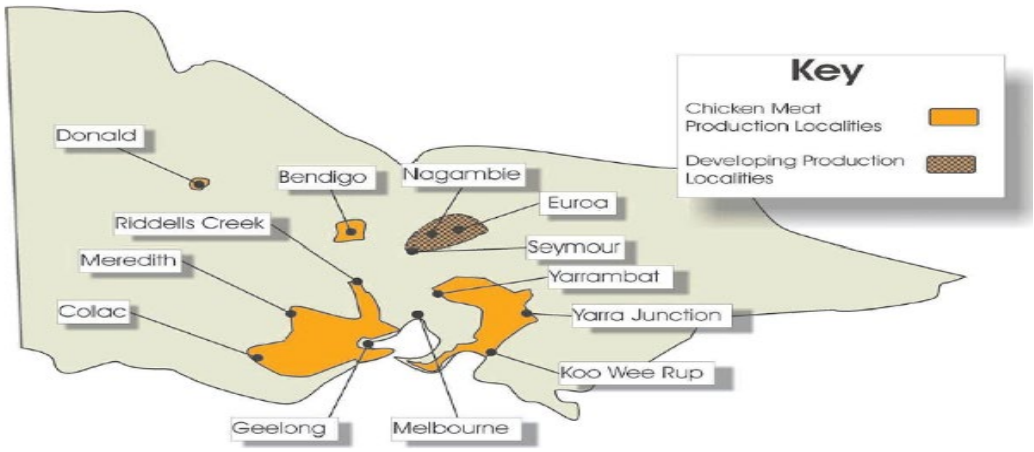

Figure 1- 1 : Locations of Broiler farms in Victoria

The main challenge and drawback for the anaerobic digestion (AD) of CL as a single substrate, is the production of high concentrations of total ammonia nitrogen (TAN) which have inhibition effects on the AD process. This is mainly, because CL contains high amounts of uric acid (Nie et al., 2015; Sun et al., 2016; Zahan and Othman, 2018). Although, there are ammonia removal technologies that have been researched and practiced, e.g. air stripping (Nie et al., 2015) of the feedstock and precipitation (Krylova et al., 1997), requires huge cost and time. Anaerobic co-digestion (ACoD), the process of combining two or more substrates, was found to be effective in reducing/ eliminating inhibition, compared to other ammonia removal processes. ACoD addresses the challenges of single substrate AD by controlling the carbon-to-nitrogen (C/N) ratio in the feedstock to be within a designated range (Rico et al., 2015; Uludag-Demirer et al., 2008; Yilmazel and Demirer, 2013).

During the selection of the co-substrates, the availability and transportation cost are major factors that need to be considered. For this reason, one of the main factors that affect the selection of substrates for ACoD is their proximity to the AD plant. The substrates that are usually available in the proximity of chicken farms include food wastes (FW), yoghurt whey (YW), wheat straw (WS) and hay grass (HG).

According to the study of Randell, Picking & Grant (2014) on waste generation and resource recovery reported that during the year 2010/2011 approximately 800,000 tonnes of food wastes (FW) were produced in Victoria, Australia, from household & commercial sources and almost all of the wastes are disposed of to landfills (Randell et al., 2014).

Among other possible co-substrates, a yogurt industry in greater Dandenong area produces 24,000 tonnes of whey per year. This whey has high COD, low TS and showed high bio-chemical methane potential (BMP). From the farming sectors, hay grass, wheat straw, silages are produced in a considerable quantity. These are herbaceous plants that are usually cut, dried and stored as animal fodder or animal bedding (Stephenson et al., 1990). The spent substrates are usually spoiled and considered as waste streams. According to the energy consultant interested in this research (Renewable future, VIC, Australia), these are very low-cost wastes that can be better used as a substrate for co-digestion.

Australia, is still in infancy stage with only 78 AD plants in total compared to 9545 in Germany and 1497 in USA (Edwards et al., 2015). Among this AD plants, almost 46 are used at wastewater treatment plants for sludge digestion; 13 are at industrial sites and 10 are agricultural AD plants just started in last five years (Edwards et al., 2015). Currently, AD provides only 0.17% of total electricity production. The government of Australia has introduced strategic policy to promote renewable energy; with the aim to increase the renewable energy. A goal is set of 2413 and 55,815 GWh for bioelectricity, predominantly from on-farm AD and AD systems fed bio-waste and industrial organics, for 2020 and 2050 respectively (Edwards et al., 2015). The amount of CL and other agro-industrial and food wastes generated annually can potentially be utilised in fulfilling the goals.

Wet AD (W-AD) is mostly used in wastewater treatment plant (Angelonidi and Smith, 2015; Jain et al., 2015; Kothari et al., 2014). Although W-AD is classified as <10% TS, in practice, most W-AD digesters are operated at 2-6% TS with WTTPs. W-AD has some advantages as quick starting and low retention time, complete mixing of substrates (Angelonidi and Smith, 2015). W-AD, D-AD and HS-AD each has its own pro and cons, for example, D-AD and HS-AD are advantageous because it requires smaller reactor volume, low amount of water, little nutrient loss and effluent; however, D-AD is limitedly practiced. In USA and Europe, there are a few large-scale D-AD plants, for example- there is a full-scale D-AD facility both in North America (USA) and Toronto (Canada) that treat organic fraction of municipal solid waste (OFMSW) or SS-OFMSW. One commercial scale D-AD in San Jose, USA has been established which is operated with food wastes of 90000 tonnes per yr. In Australia there are no D-AD or HS-AD facilities on small or commercial scale.

Therefore, this study focused on the ACoD of CL with co-substrates (FW and agro-industrial wastes) how feedstocks of varying substrates ratios affect biogas yield and biogas composition (CH_4 and CO_2) at different AD configurations; find the optimum substrate balance and conditions. Further, this research has been undertaken using the facilities currently operating in Victoria, Australia.

1.2 Aim and Objectives

The aims of the research were to investigate biogas production potential from chicken farm wastes and potential enhancement in biogas production by co-digestion with different co-substrates, and to investigate the potential of using substrates characteristics, e.g. C/N ratio, as one of the main control parameters for substrates mixing in both conventional W-AD and HS-AD.

The associated research objectives for this study are as follows:

- Investigate the characteristics (physical, chemical and structural) of different wastes/ residues streams.
- Predict the biogas potential through biomethane potential tests from the agro-industrial waste streams.
- Determine the optimum co-digestion mixing ratios and C/N ratios for co-digestion of CL with different agro-industrial wastes using surface optimisation methodology to design the experiments and determine the optimum ACoD conditions at W-AD.
- Investigate or assess the performance of W-AD under semi-continuous conditions using the conditions predicted form batch ACoD.
- Investigate the effect of sequential diluted acid (DAP) and alkaline (AKP) pre-treatments for lignocellulosic substrates on enhancing biogas yield using statistical based methods for experimental design e.g. Response surface methodology.
- Assess the feasibility of HS-AD using the optimum condition found at W-AD.
- Investigate the feasibility of W-AD versus HS-AD for the co-digestion of a wide variety of agro-industrial wastes.

1.3 Research Question

The dissertation aimed to answer the following questions

I. What are the composition, characteristics and biogas potential of different agro-industrial wastes investigated as potential co-substrates with CL?

II. Can C/N ratio of the feedstock be used as a parameter to determine the optimum co-substrate mixture ratio and what is the effect of C/N ratio during co-digestion?

III. How the reactors perform under semi-continuous AD with CL and co-substrates?

IV. Why pre-treatment is necessary and what is the effect of pre-treatment on lignocellulosic agro-industrial wastes during AD?

V. How HS-AD system performs for untreated and pre-treated agro-industrial substrates and how comparable the biogas yield of HS-AD with W-AD?

1.4 Research Design and Contribution

The research activities that have been undertaken to answer the research questions can be divided in six different phases. Table 1-1 list the phases and the activities undertaken under each phase and contribution of each phase to the research study.

Table 1- 1: Research phases, activities and contribution to the study

Phase	Activities
Phase 1	-Collection of samples from different farms across Victoria - Sample preparation, size reduction and characterisation **Contribution:** assess variation in composition
Phase 2	- BMP tests for the different substrates for single substrates AD. -Determination of kinetics of the AD of the different substrates at different TS loadings, for batch test of the wastes at same AD conditions. **Contribution:** assess the biogas production kinetic potential at different TS loading.
Phase 3	- BMP tests for the co-digestion of different waste streams at different C/N ratio and organic loading (OL). -Surface modelling to determine the optimum conditions of co-digestion **Contribution:** understand the effect of C/N ratio and OL on co-digestion of different agro-industrial wastes.
Phase 4	-Design the experimental program based on phase 2 & 3 results for bench scale continuous AD. -Carry out continuous ACoD under different organic loading rates (ORLs), substrate mixing ratio, feedstock total solids. -Biogas measurement, characterisation, observation of process performance and stabilisation on a regular basis **Contribution:** the performance of semi-continuous AD in terms of stabilization and inhibition
Phase 5	- alkaline (AKP) and dilute acid (DAP) pre-treatment of wastes -pre-treatment experiments and fractionation analysis for degradation **Contribution:** optimisation of pre-treatment for maximising the degradation during AD
Phase 6	- BMP tests for the co-digestion of different waste streams in H-AD in batch conditions

- BMP tests to improve the degradation rates considering pre-treatment and improved conditions.
- characterisation for changes in compositions

Contribution: enhancement in degradation and BMP of HS-AD

1.5 Research Significance

In this research, the feasibility of biogas production from CL was studied exhaustively and co-digestion potential with agro-industrial wastes, food wastes from neighbouring sources were examined. The ACoD of CL with these wastes have not been reported before.

This study had utilised compositional analysis to determine how combining agro-industrial wastes with CL results in substrates that are better balanced in terms of nutrients (i.e. C/N) and degradation. The relationship between lignocellulosic properties at balances C/N has been explored which was not studied before. The kinetic parameters that could be used to predict the substrates combination for ACoD were also explained.

There is no literature on the chemical pre-treatments of CL especially which include the effect of pre-treatment on the digestate fractions and degradability. Pre-treatment is well suited for CL and WS with high degradation. No published paper focuses on using combinations of AKP+DAP pre-treatment of CL and WS in an integrated system (e.g. sequential chemical pre-treatments and ACoD) and these methods exert a positive effect on solid removals. There is little research, if any, on the ACoD of CL with FW and agro-industrial wastes for different total solids loadings, C/N ratios and their effect on methane yield especially in terms wastes available in Australian context.

From engineering point of view, this research has contributed in developing models for waste optimisation as well as pre-treatment of lignocellulosic wastes. It has also developed a new method that has shown correlation between C/N ratio and lignocellulosic fractionation. It also developed a new way for successful semi solid

anaerobic co-digestion from CL using pre-treatment of lignocellulosic wastes and acclimatisation of inoculum.

AD and biogas production is one of the most promising areas in the field of environmental engineering. Although, CL has high biogas yield, currently there is no successful biogas plant from CL in large scale. Therefore, this research will contribute in this area where agro-industrial wastes and food wastes would be utilised with CL. In a broader context, advance resource recovery technologies will be researched which will be directly applicable to Australian agro-industrial wastes management and resource recovery.

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Chapter 2 Thesis Overview

The aim of this chapter is to give an overview of how the research study was developed and how the content of all chapters is related to each other. This thesis is organised into nine chapters. The major research contributions are presented in chapter 4 to chapter 7 and are predominantly composed of published papers in Q1 journals, except chapter 6 which is a paper submitted for reviewing for a Q1 ranked journals (the most influential 10% in their field according to SCImago). Each journal paper is presented as an individual chapter, being preceded by a literature review in chapter 3. The last chapter, chapter 8 is a rap up of the thesis by presenting concluding remarks and recommendations for future works.

The literature review chapter (Chapter 3) provides an overview of the recent developments and research work carried out regarding the study that has paved the way for this research. The experimental work is reported in chapters (Chapter 4 to Chapter 7), where each chapter provides highlight in completing the research work at the beginning of the chapter. After that, it is structured as introduction, materials, methods and experimental design, results and discussion and finally the conclusions as the same way as published in the journal articles.

The research works were designed, and experiments were conducted by me as well wrote up the papers and the thesis. Dr Maazuza Othman was my Primary supervisor who provided guidance and advice concerning the development of the experiments and reviewing & editing of the manuscripts. Dr Tim Muster, my associate supervisor contributed to discussions concerning data analysis to and reviewing chapters 4 and 5. Dr Stelios Georgiou was co-authors of chapter 5 and 6 by providing guidance in developing the response surface methodology design, factorial design and principal component analysis as well as providing feedback on the relevant sections in the papers.

The structure of this thesis and an overview of the content of each chapter are provided in the section:

• **Chapter 1** presents an introduction to the thesis which includes the problem and the rationale for carrying out this study; states the scope of the research as well as

details the research aims and objectives, design of the thesis and significance and the research questions.

• **Chapter 2** highlights on the thesis structure which overviews about how the thesis has been presented. This chapter also provides a brief discussion of what each chapter contains and its significance. It also explains how the chapters are linked and how they answer to the research questions.

• **Chapter 3** is a literature review that discusses the background of the work, along with a review of key studies that have paved the way for this research. It provides critical review of the recent developments and research work carried on anaerobic digestion of chicken litter, agricultural wastes, food wastes as well as other alternative sources to be paired with. It presents the literature under five categories.

First, it gives an idea on chicken farm wastes, their availably and current waste management scenario mostly in Australia detailing with concerns and potential for renewable energy such as anaerobic digestion. This section will also review on the research advancements achieved so far with this type of waste.

Second, it overviews on anaerobic digestion process along with different types and conditions of anaerobic digestion exists. It also discusses the problem associated with single substrates and advantages with co-digestion. The wastes available for co-digestion and their characteristics and potential for anaerobic digestion were also provided. A detailed review listing the research work done in literature with these types of wastes was also included.

Third, as lignocellulosic wastes contributed a major part in the process and it also helped to improve the C/N ratio to mitigate ammonia inhibition; this section discusses the structure of lignocellulosic wastes and the degradability of lignocellulosic biomass during AD. The necessity of pre-treatment as well as the effectiveness of pre-treatment in unlocking the lignocellulosic structural bonding are also presented here. Different types of pre-treatment and their advantages and disadvantages are discussed in detail which assists in the study to choose the pre-treatment works necessary for the wastes.

Four, from all the literature review, a list of key research work and finding along with the research gaps that need to be addressed are presented in this section.

Lastly, the process parameters and analytical technique that are very important to carry out the research work are enlightened here. The significances of using the techniques along with the methods used for the techniques are also discussed. The process parameter that indicates the digester stability or inhibition are also reviewed and presented in this section.

• **Chapter 4** presents a through characterisation and AD potentials of agro-industrial wastes and food wastes of Victoria, Australia. Among the agro-industrial wastes, chicken litter (CL), yoghurt whey (YW), hay grass (HG), and wheat straw (WS) were selected along with household food wastes for their availability in the neighbouring area of proposed AD facility and different characteristics parameter were analysed to understand their nature of the wastes. This chapter then focuses on two major experiments at wet AD conditions.

In the first experiment, anaerobic digestion of the wastes as single substrates was carried out in batch assays at different solids loadings (2-4% TS) to figure out the inhibition limit and the biogas production from each substrate. Then comparison of the characteristics parameters before and after digestion were analysed to explain the variation in biogas production. The work also presents the kinetic potential of methane production with four different kinetic models which predicted the rate constant, lag phase and the methane production rate that helped in co-substrate section for co-digestion.

In the second experiment, the ACoD experiments were performed as two, three and four substrate mixtures considering the C/N ratio as well as substrate mixture ratio. The study then provides analysis for synergistic effects and improvement in biogas production as well as process performances with improved C/N ratio at various mixture ratios of CL and agro-industrial. The comparison of lag phase and maximum daily methane production results with the predicted kinetic model values after single digestion are provided. Finally, the optimum co-substrate mixture ratio with optimum C/N ratio is also presented using surface optimisation modelling.

Therefore, this work is mainly a screening experiment of the substrates; provides significant knowledge for the understanding of the characteristics of different waste streams and their kinetic parameters as well as the bio-methane potentials. The co-digestion potential and the optimum conditions & parameters for AD are also be highlighted.

The content that appears in this chapter has been published in the journal article as

Zahan, Z., Othman, M.Z. and Muster, T.H., 2018. Anaerobic digestion/co-digestion kinetic potentials of different agro-industrial wastes: A comparative batch study for C/N optimisation. Waste Management, 71, pp.663-674.

• **Chapter 5** describes the improvement of continuous AD efficiency of CL by co-digesting with agro-industrial wastes and food wastes (households) at different organic loading rate and reports their process performances. In the previous section, the experiments were carried in batch assays, however, in commercial establishments, most of the AD facilities run in continuous modes. This work, therefore, presents here the effect of co-digestion and efficiency of digestion in continuous ACoD at 4-6% TS by using the conditions that has been predicted to have high digestion and biogas production in batch mode with a C/N ratio of higher than 20.

The effect of organic loading rate and feed concentration of the wastes on biogas production during continuous AD are observed and reported here. It also presents a new concept that C/N ratio and lignocellulosic structure degradation through fractionation are two important factors for agro-industrial substrate during AD and with these two parameters, it is possible to understand any variation in the process stability and performance. The lignocellulosic fractionation analysis was done at the start and end of the experiment and the effect of the lignocellulosic degradation of the substrates with similar C/N ratio on biogas production are presented as well.

Although different performance parameters were determined in literature to explain the variation in performances, how these parameters are correlated with each other has not been focused before. In this section, this gap has been considered and the relationship between different processes parameters along with correlation

coefficients were analysed with principal component analysis (PCA). The variability of the parameters with their magnitude, highlighting the inter dependency are explained. The main effect and interaction of the performance parameters is observed, and cluster analysis is presented with the experimental conditions tested.

Therefore, this study provides significant knowledge for the understanding of the potential of an ACoD facility of different agro-industrial and conveys sufficient technical details to inform the design and operation of such.

The content that appears in this chapter has been published in the journal article as

Zahan, Z., Stelios, G., Muster, T.H. and Othman, M.Z., 2018. Semi-continuous anaerobic co-digestion of chicken litter with agricultural and food wastes: A case study on the effect of carbon/nitrogen ratio, substrate mixing ratio and organic loading. Bioresource technology, 270, pp. 245-254

• **Chapter 6** details the degradation of structural carbohydrates of agro-industrial wastes through sequential pre-treatment with four different response optimisation models. After observing from previous chapter that lignocellulosic structure of the substrates has major effect on biogas production, the pre-treatment of the lignocellulosic agro-industrial wastes are reported in this chapter. The main objective of this chapter is to find the optimum conditions that required for breakdown of the structural bondage of lignocellulosic substrates for maximum biogas production.

The study focuses on the pre-treatment of agro-industrial wastes i.e. chicken litter (CL) and wheat straw (WS) with dilute sulfuric acid (DAP) (1.0 - 3.0 %, v/v), dilute sodium hydroxide (AKP) (1.0–5.0 %, w/v) and sequential execution of these pre-treatments at temperatures (60-120°C) and reaction times (30–90 mins) for maximizing sugar release (cellulose and hemicellulose from lignin). The pre-treatment effectiveness is presented by maximum recovery of cellulose with response surface models (RSM). The optimum conditions and recovery obtained from RSMs are also reported.

Degradable structural compounds i.e. cellulose, hemicellulose, lignin and water soluble contents are reported after quantifying for all the conditions. Mass balance calculations were carried out for best pre-treatment sequence to verify the correct

account of the input agro-industrial wastes and output products. Analysis of variance, effect and interaction of time, temperature and chemical concentration on pre-treatment are also presented here. AKP removed more than half of the lignin from the agro-industrial wastes in all cases. It also shows that acid and alkali concentrations and temperature played a relevant role in all pre-treatment sequences, while reaction time was less important with an almost-linear effect on the total carbohydrates yields.

Therefore, this study provides significant knowledge on the structural breakdown of the lignocellulosic agro-industrial wastes as well as informs of the optimum conditions of the pre-treatment which will enhance the biogas production.

The content that appears in this chapter has been submitted in the journal article as

Zahan, Z. and Othman, M.Z., 2019. Degradation of structural carbohydrates of agro-industrial wastes through sequential pre-treatment with response optimisation. (Drafted for Biomass and Bioenergy)

• **Chapter 7** provides an overview on the effect of pre-treatment on the sequential anaerobic co-digestion of chicken litter with agro-industrial wastes under semi-solids condition and comparison with their wet anaerobic digestion performance. As most of the agro-industrial wastes are solid substrates, performing high solid AD is the goal. In the previous chapters i.e., Chapter 6 enlightened with pre-treatment of agro-industrial wastes and chapter 4 presented the optimum substrate mixture ratio for ACoD with optimum C/N ratio under wet AD (4% TS), this chapter utilised the finding from previous chapters to develop and design an AD process at high solid conditions.

The chapter presents the results obtained using sequential co-digestion of the substrates, chicken litter (CL), food waste (FW) and wheat straw (WS) mixed to achieve a C/N ratio of 26.5 at 15% TS over 215 days in six cycles under four different conditions in triplicates. The first two cycles were operated at the same conditions with untreated wastes and then pre-treated wastes were applied from cycle 4 to 6. Two of the AD reactors were fed with a feedstock that contained either of the pre-treated substrates of lignocellulosic characteristics; i.e. the CL and WS,

and one received both the pre-treated CL and WS. The effects of pre-treatment on biogas production as well as the performance parameters (ammonia, VA, pH, TS and VS removal) are analysed and presented. Fractional analysis of digestate for cellulose, hemicellulose and lignin were also monitored and explained for biogas production.

The chapter further provides a comparison of biogas production at 15% TS for untreated and pre-treated wastes with batch and continuous AD at 4% TS. The results showed that with the pre-treated substrate co-digestion at high TS of 15% is achievable. These results do not only have the potential to reduce the reactor volume by almost 75% but also reduce the wastewater, heat and energy requirements.

Therefore, this chapter provides significant knowledge on high-solid AD and the reactors performances with pre-treated substrates. Furthermore, the comparison of wet and high solid AD would be helpful in the decision-making process of which AD process to consider and further investigate, given the substrates of interest.

The content that appears in this chapter has been accepted in the journal article listed below.

Zahan, Z. and Othman, M.Z., 2018. Effect of pre-treatment on sequential anaerobic co-digestion of chicken litter with agricultural and food wastes under semi-solid conditions and comparison with wet anaerobic digestion. Bioresearch Technology, 281, pp. 286-295.

• The final chapter, **Chapter 8**, presents the overall concluding remarks of the research along with limitations, this chapter also provides possible interesting research trends for continuation and recommendations for future work.

The diagram of the structure of the thesis, and how the thesis chapters relate to the objectives, is shown in Figure 2-1.

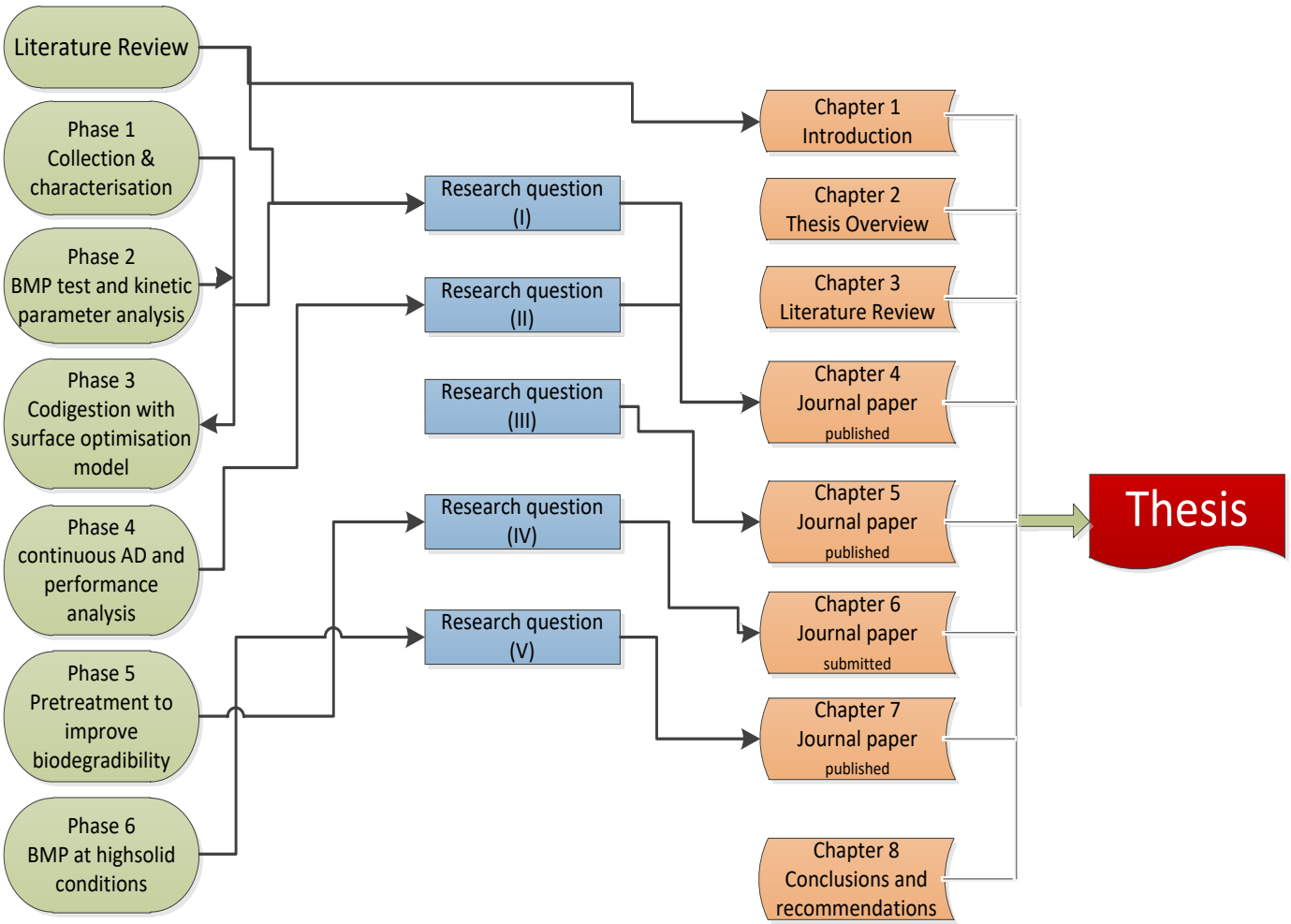

Figure 2- 1: Overview of thesis

Chapter 3 Literature Review

This literature review chapter focuses on the topic relevant to the dissertation including, CL current waste management practices, co-digestion of food and agro-industrial wastes with CL, anaerobic digestion systems e.g. wet, high solid and dry, pre-treatment of CL and agro-industrial wastes, various experimental methods and techniques of AD. The discussion begins with the production and waste management systems of chicken litter, the rationale behind this work, the research that has had been done so far and the problems or alternatives can be used for anaerobic digestion of chicken litter. Secondly, an overview of what is anaerobic digestion, different types of anaerobic digestion process and the operation conditions is discussed. After that, benefits of co-digestion and the co-substrate available as well as overview of those substrates are also discussed. A table summarising the key studies on those topics is provided and after research gaps were identified. Finally, analytic techniques used for sample characterisation, anaerobic digestion, biogas analysis, performance analysis have been reviewed with importance of performing the analysis.

3.1 Chicken Litter Production

The consumption of chicken meat in Australia was estimated 811591 tonnes which is equivalent to 454 million birds during 2006-2007 (Scott et al., 2009). This is equivalent to approximately 37.4 kg meat consumption per capita or the processing of around 8.73 million broiler chickens per week. Chicken manure production is approximately 1 ton per 10,000 hens (Liu et al., 2012). If improperly managed, these poultry wastes can cause serious damage to the environment by polluting water and air; for example, ground and surface water contamination, odour, dust, contamination of food and land from inappropriate application, vermin and fly infestation which can seriously impact human and animal health ((Chen and Jiang, 2014). There is little benefit for the broiler industries through composting of chicken litter and other wastes generated by the industry. In the meantime, these wastes are mainly organics which means they have high potential for

Figure 3- 1: A poultry farm in Victoria, Australia (photo by Zubayeda Zahan)

bio-methane production. Therefore, these wastes need to be collected and used as a useful resource for renewable energy.

3.1.1 Current poultry waste management systems

Animal waste may be defined as carcasses or parts of animals, including products of animal origin not intended for direct human consumption (Sakar et al., 2009; Scott et al., 2009). Animal waste is classified either as high-risk material, if it is suspected to present serious health risks to people or animals, or as low-risk material, if it does not (Chen and Jiang, 2014). High-risk material includes animals died on the farm, stillborn and unborn animals, and spoiled and condemned materials (Salminen and Rintala, 2002). The most common management for poultry wastes are rendering, incineration, use for animal food, burial or controlled land filling, composting.

- Rendering is a heating process for the extraction of usable elements, for example, protein, meats and fats. It mainly uses slaughterhouse meat or meat meal, bone and feather meal which are all highly valued feed ingredients.
- Composting is mainly attractive for chicken carcass waste where it places in layers with carbon-source and manure to allow the natural decomposition and

reduction of mass. It is done both onsite and offsite; and requires biosecurity and pathogen control. Negative impact on environment and health are associated with the composting site operations and loss of amenity to the adjoining land owners also occurs.

- Disposal pits and trenches are the typical methods for on-farm disposal of chicken wastes, however, is not environmentally preferred and strongly discouraged ((EPA), November 1999).
- Sometimes the waste litter and carcass are collected and taken to an offsite disposal such as landfill. This is though being very limited.
- Direct transfer of litter and manure substrates from chicken farms to local markets are common practice in some parts of Australia ((EPA), November 1999). Inappropriate application of the litter/manure material could cause pathogen contamination on food produce, and the potential spread of disease.

Recently, there has been a growing interest for the management of chicken farm wastes using thermal e.g. pyrolysis and biological processes e.g. AD. The thermal processes had some limitations whereas AD especially ACoD have shown potential, but more research needed to establish the feasibility of ACoD. Figure 3- 2 shows the possible pathways for recovery and disposal of poultry wastes adapted from (Salminen and Rintala, 2002).

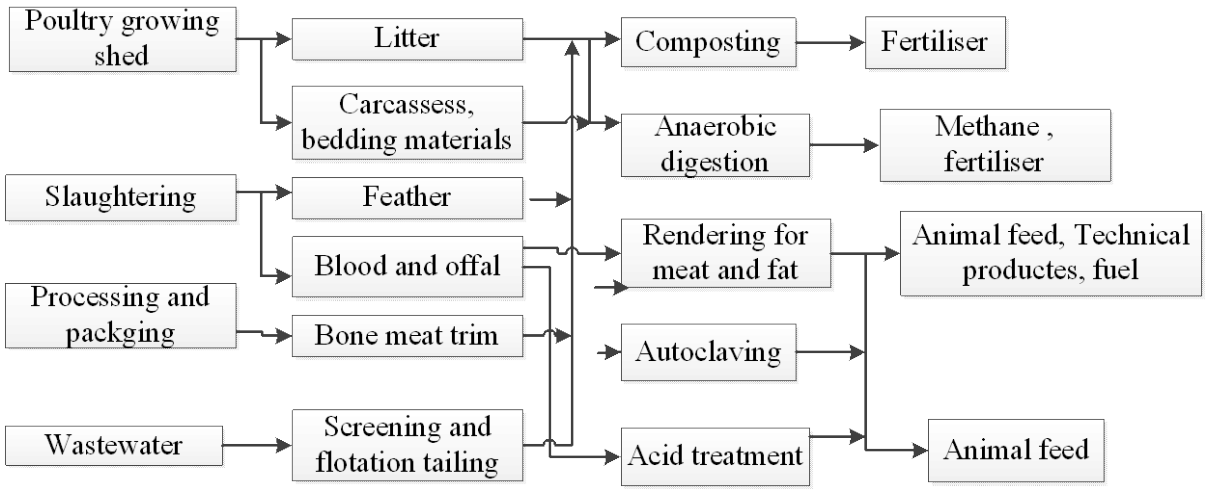

Figure 3- 2: Current recovery and disposal of solid poultry wastes

3.1.2 Characteristics and anaerobic digestion of chicken manure

The characteristics of chicken manure samples vary from region to region depending on weather conditions, feeding practice and nurturing ways. The chicken litter from broilers usually contain large proportion of bedding material (such as straw, hull or wood shaving), which is also typical of CL from broilers in Australia. This makes the CL very solid substrates having TS of 70-90%. On the other hand, the literature studies found on CL have TS content of nearly 20-47%. Table 3-1 shows a comparison of characteristics of the studied samples with literatures.

In literature, different studies have been done to assess or enhance the potential of energy production from poultry wastes as a single substrate (Nie et al., 2015; Salminen and Rintala, 2002). In table 3-2, the characteristics and biogas potential are shown from literature.

Table 3- 1: Comparison of chicken litter characteristics reported in literature

parameters	(Zahan et al., 2018c)	(Bhatnagar et al., 2018)	(Rodriguez-Verde et al., 2018)	(Li et al., 2013b)
pH	8.15	7.4-7.9	7.8	9.3
TS	90.10%	44.7-54%	806 g/kg	24.90%
VS(%TS)	52.30%	70-89%		78.10%
VS	47.10%	31.24-50%	490 g/kg	19.40%
CODt	150.68 g/ kg	0.89-1.40 g/gVS	783 g/kg	
CODs	21.15 g/kg		154 g/kg	
Total N	4.12 g/kg	31.4-55.2 g/kg		
NH_3	1.18 g/kg	2.96g/kg		2.8 g/kg
TP	0.60 g/kg	0.45 g/kg	9.5-15.3 g/kg	
Total C	25.94%	19.20%		36.2
Total N	1.93%	1.47%		3.6
C:N	13.43	13.1		10.1
volatile acids	6.26	3.56		
Alkalinity	13.5 g/kg	207mg/L	15.2 g/kg	10 g/kg

Table 3- 2: Characteristics of poultry wastes and their biomethane potential (Salminen and Rintala, 2002)

Different poultry industries waste	TS (%)	Vs (%)	TKN (% of TS)	Protein (% of TS)	Lipids (% of TS)	Methane potential (m^3/kg VS_{added})	Methane potential (m^3/kg wet weight)
Carcass	37	Na	Na	Na	Na	Na	0.20-0.25
Poultry litter*	52-81	61-65	3.2-57	Na	Na	0.14-0.22	0.10-0.15
Manure	20-47	60-76	4.6-6.7	Na	1.5-2.1	0.2-0.3	0.04-0.06
Feather	24.3	96.7	15	91	1-10	0.2	0.05
Blood	22	91	7.6	48	2	0.5	0.1
Offal, feet, and head	39	95	5.3	32	54	0.7-0.9	0.3
Trimming and bone	22.4	68	68.6	51	22	0.6-0.7	0.15-0.17

*Poultry litter includes manure and bedding material, Na- not included.

Due to good biological degradability of CL, AD with CL is a good choice to minimize waste and recover bioenergy. However, because of the high content of organic nitrogen and low C/N ratio in CL, ammonia inhibition has been the main challenge for using CL as a substrate for AD (Neeteson, 2000; Rodriguez-Verde et al., 2018). The two main nitrogen sources in CL, undigested protein and uric acid, decompose into ammonia during the anaerobic fermentation process (Neeteson, 2000; Nie et al., 2015; Niu et al., 2013). Nitrogen is an essential nutrient for the growth of anaerobic bacteria involved in AD, and ammonia is a major nitrogen source in the digester. However, excess ammonia will inhibit methanogenesis (Hansen et al., 1998; Nie et al., 2015; Rao et al., 2008). Ammonia inhibition is a common problem in the anaerobic digestion of substrates rich in nitrogen such as poultry litter. Many studies have investigated ammonia inhibition with an aim to identify the mechanism of inhibition and develop strategies to recover the ammonia inhibition from happening (Nie et al., 2015). However, when it comes to the threshold, the levels at which ammonia inhibition occur that are reported in the literature are conflicting. The discrepancy in reported literature can be due to the nature of substrates, the acclimation of inoculum, and other operating parameters such as hydraulic retention time. In addition, the criteria used to identify the threshold are not consistent.

3.1.3 Digestate from the AD of CL

Due to the higher rates of mineralization during AD, digestate has an improved fertilizer quality compared with the raw CL and is usually used as bio fertiliser (Nie et al., 2015; Wanqin et al., 2012). Digestates have higher ammonium nitrogen, decrease organic contents, decreased COD, elevated pH, reduced viscosity (Möller and Müller, 2012). Furthermore, volatile acids contents were also reduced with improved N and P availability (Möller and Müller, 2012). A study on poultry manure and agricultural residue showed that the heavy metal in the digestate was mainly sourced from poultry manure (Demirel et al., 2013). Ni, Zn, Cu, Pb, Cr, Cd, and Hg were detected in the digestate where Zn being the major content among them; however, concentration of all of the heavy metal was below the inhibitory guideline (Demirel et al., 2013).

During AD, digestate will be produced throughout the year and it needs to be stored before field application during periods in which it cannot be applied to the field – this also the case for raw manure (Nie et al., 2015; Wanqin et al., 2012). High moisture content of the digestate makes its storage, transportation and application expensive and uncontrolled anaerobic digestion can cause greenhouse gas emissions from open storage facilities (Nie et al., 2015). However, AD is advantageous that any other management options as it provides not only the heat and biogas that can be the energy source for the farms but also to sell digestate as fertilizer to be benefited.

3.1.4 Effect of ammonia

3.1.4.1 Ammonia inhibition

Various drugs, metals and other compounds mixed to poultry feed for nutrients and pharmaceutical purposes (Liu et al., 2012; Salminen and Rintala, 2002). These pollutants may accumulate in poultry litter and wastewater. Poultry litter is mainly a mixture of manure or faeces, wasted feeds, bedding materials and feathers (Bhatnagar et al., 2018; Chen and Jiang, 2014; Kim et al., 2012). Poultry litter is rich in nitrogen because of the presence of high level of protein and amino acid (Chen and Jiang, 2014; Zahan et al., 2018b). Ammonia which is an important indicator of

AD produces by the biological degradation of proteins and urea (Niu et al., 2013; Uludag-Demirer et al., 2008) and accumulates in the AD process(Liu et al., 2012). In literature, different threshold concentration of inhibition has been reported on factors such as pH, temperature and adaptation of AD sludge, which directly affect the form of ammonia (Calli et al., 2005; Chen et al., 2008; Liu et al., 2012; Meneses-Reyes et al., 2018). Therefore it is difficult to control ammonia inhibition in actual AD operation because of the complexity and fluctuation of effluent characteristics, biodegradability and COD removal efficiency of CL in AD (Markou, 2015; Meneses-Reyes et al., 2018). The change in influent characteristics is an important factor in AD treatment of semi-digested effluent(Liu et al., 2012).

The decomposition of urea and undigested proteins in the CL during AD process produces large amounts of unionised ammonia (NH_3) or free ammonia (FA) and ammonium ions (NH_4^+) (Zahan et al., 2017). Unlike ammonium ion, FA can diffuse across the cell membrane (Salminen and Rintala, 2002; Zahan et al., 2016a). Thus, FA has been observed as an actual toxic agent (Borja et al., 1996; Liu et al., 2012) and inhibits anaerobic microorganisms, particularly methanogens (Markou, 2015; Salminen and Rintala, 2002). The increase in FA shifts the FA to ionised ammonia (NH_4^+) ratio that increases pH. An increase in pH may result in increased toxicity (Chen et al., 2008; Liu et al., 2012). It has been found that FA becomes inhibitory when it ranges between 1500 and 3000 mg/L and the pH is greater than 7.4 (Liu et al., 2012). The sCOD/TAN ratio was found an important controlling factor in indicating ammonia inhibition with careful consideration (Liu et al., 2012). An ammonia inhibition experiment was designed with biogas yield, sCOD removal efficiency, pH change, at different value of sCOD/ TAN ratio and corresponding TAN concentration by Liu et al. (2012) and confirmed that the sCOD/TAN ratio better reflects the effect of ammonia inhibition on methanogens than the absolute TAN concentration (Liu et al., 2012).

3.1.4.2 Stoichiometry of ammonia production from CL

CL contains high amount of biodegradable matter such as lipids, carbohydrates, proteins and uric acid (Niu et al., 2013). Ammonia is produced from the biological degradation of protein and uric acids(Zahan et al., 2018c). One of the study found

the elemental composition of CL as C (35.16%), H (4.83%), O (30.12%), S (0.84%) and N (5.44%) (Niu et al., 2013).

The stoichiometric biochemical formula (Niu et al., 2013; Richards et al., 1991),

$$C_nH_aO_bN_c + (n - 0.25a - 0.5b + 1.75c)\ H_2O \rightarrow (0.5n + 0.12a - 0.25b - 0.375c)\ CH_4 + (0.5n - 0.125a + 0.25b - 0.625c)\ CO_2 + cNH_4^+ + c\ HCO3^- \quad (3\text{-}1)$$

The CM AD was identified according to the equation as:

$$C_{7.5}H_{12.4}O_{4.8}N + 3.89H_2O \rightarrow 3.7\ CH_4 + 2.8CO_2 + NH_4^+ + HCO3^- \quad (3\text{-}2)$$

From this formula, the degradation of 1kg of VS of CL produces 0.74m^3 biogas, 0.42 m3 methane (56%) and 70.9 g of ammonia nitrogen (Niu et al., 2013).

3.1.4.3 Ammonia removal technology

In literature, several studies have been attempted to avoid ammonia accumulation and inhibition during AD of CL. A most common approach is the dilution of the substrate with fresh water (Nie et al., 2015). Fresh CL usually has a high a concentration of total solids ranging from 20% to 85 %. Before adding it the AD, it is diluted to very low concentration for wet digestion (0.5-3%) (Bujoczek et al., 2000; Nie et al., 2015) of semi-solids 10-11.5% (Bujoczek et al., 2000; Zahan and Othman, 2018) which requires huge amount of waters. It has several disadvantages including: decrease in biogas production per unit of digester volume, increase in water consumption and processing cost of the slurry, significantly larger digester volume and space and finally increase the cost of storage and transportation. Co-digestion with other cattle manure, sludge or organic wastes is another option. However, most of these is done in wet AD which results in large volume of wastes with increase cost of transportation and storage (Abouelenien et al., 2010).

Physical, chemical and biological removal of ammonia from the sludge after anaerobic digestion was studied by several researchers to reduce ammonia inhibition. Physical method includes ammonia stripping, chemical method includes chemical precipitation, zeolite and clay process, phosphorate ore addition, biological process. Table 3-3 summarises different ammonia removal technologies studied in the literature.

Table 3- 3: Processes for ammonia removal from CL

Approach	Method	process	Reference
Physical	Air stripping/ Ammonia stripping	-do not require any chemicals -dependent on operation temperature, pressure, high pH (8.5 and more) and length of the operation time. -multi-step process which requires additional time and cost -60-85% removal efficiency	(Abouelenien et al., 2010; Abouelenien et al., 2009; Belostotskiy et al., 2013; Nie et al., 2015; Rao et al., 2008)
	Biogas recirculation	-Ammonia stripping and biogas recirculation as stripper gas -single reactor system and possible application on dry methane fermentation (25%) -80-88% removal efficiency. -Only one study found. Process needs optimisation.	(Nie et al., 2015)
Chemical	Chemical precipitation of digestate	-Magnesium ammonium phosphate (MAP) process or powdered phosphorite ore -for the cost of chemical and effectiveness at low TS level of CL	(Krylova et al., 1997)
	Zeolite and clay process	-addition of clay mineral compounds such as andesite, bentonite and natural zeolite. -used for wet AD up to 5% TS -only effective with ammonia acclimatized inoculum	(Fotidis et al., 2014)
Biological		- Ammonia is oxidized to nitrogen gas using nitrite as the electron acceptor	(Dong and Tollner, 2003)
Co-digestion	Cattle manure Wastewater sludge Food waste Lignocellulosic	-effective in control C/N ratio -Require some pre-treatment to be accessible to microbes	(Bhatnagar et al., 2018; Meneses-Reyes et al., 2018; Paul and Dutta, 2018; Rico et al., 2015; Uludag-Demirer et al., 2008; Ye et al., 2015; Yilmazel and Demirer, 2013; Zahan and Othman, 2018)

3.2 Anaerobic Digestion

Anaerobic digestion (AD), a highly recognised technology, involves the degradation and stabilization of organic materials under anaerobic conditions (in the absence of oxygen) by microbial organisms and leads to the formation of biogas (a mixture of CH_4 and CO_2, a renewable energy source) and microbial biomass (Chen et al., 2008; Mao et al., 2015). Anaerobic digestion offers numerous significant advantages, such as low sludge production, low energy requirement, and possible high-quality energy and fertiliser and finally converts a waste management issue into a profit centre. Despite these benefits, however, poor operational stability still prevents anaerobic digestion from being widely commercialized and research study on the improve of the process is continuing.

The anaerobic digestion process comprises of four stages, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis in which the organic substrates are converted to methane and CO_2. Figure 3-3 shows the main phases of AD.

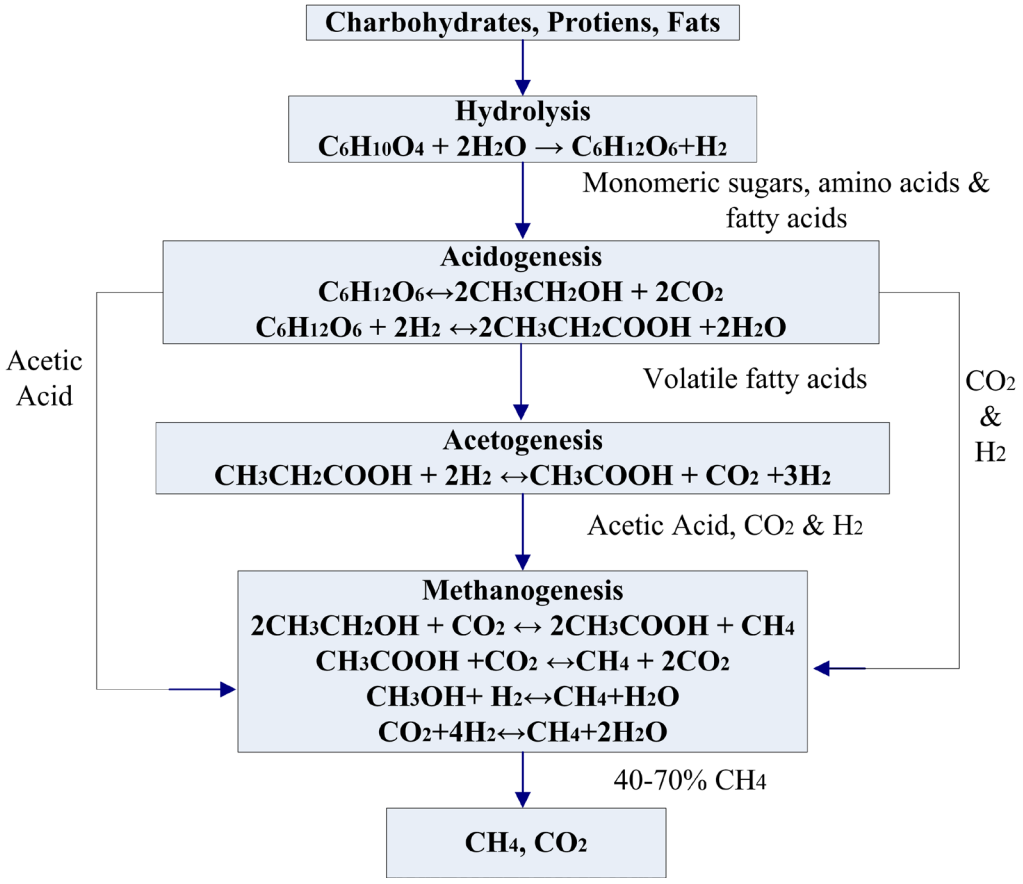

Figure 3- 3: Main stages of anaerobic digestion (Li et al., 2011)

3.2.1 Classification of anaerobic digestion

Anaerobic digestion can be classified according to the total solids concentration, digester feeding process, steps involved or numbers of substrates. Table 3-4 summarises all the process. All the classified AD systems work on the same mechanism as four basic stages (Kothari et al., 2014), which is shown in Figure 3-3. In the present study, AD focused on TS concentration inside the digester, feeding frequencies and number of substrates for single stage AD process under mesophilic conditions.

Table 3- 4: Classification of anaerobic digestion system

AD system	characteristics
1. Total solids basis(Karthikeyan and Visvanathan, 2013; Kothari et al., 2014)	
(a) Wet AD	Total solids inside the reactors <10%
(b) High solid AD	Total solids inside the reactors <10-20%
(c) Dry AD	Total solids inside the reactors <20-40%
2. Feeding frequency basis (Kothari et al., 2014)	
(a) batch	The reactors feed with substrates and inoculum before sealing the reactors for biogas production. At the end of the process, the reactors were emptied for new feed.
(b) continuous	The digester is continuously feed and wasted
3. Process steps basis (Mata-Alvarez et al., 2000)	
(a) Single stage	All digestion steps occur in one digester
(b) Multi stage	AD process consists of several reactors for hydrolysis and methanogenesis
4. Temperature basis (Saratale et al., 2018)	
(a) Psycophilic	Digestion temperature <20°C
(b) Mesophilic	Digestion temperature 20-45°C, usually 37°C
(c) Thermophilic	Digestion temperature >45°C, usually 55°C
5. No of feedstock basis	
(a) Single-substrates digestion	Only single substrates digested with inoculum
(b) Co-digestion	Two or more substrates are mixed to improve the digestion process by maintaining mixture ratio, C/N ratio, composition

3.2.2 Wet and dry anaerobic digestion

TS concentration is one of the most important parameters during AD which determines the efficiency assessment (Zhang et al., 2018). It is widely defined and practiced including wet, semi-dry, and dry anaerobic digestion, when TS of substrate are < 10, 10–15, or > 15%, respectively (Li et al., 2011; Liotta et al., 2015; Zhang et al., 2018). Wet anaerobic digestion (W-AD) is applied mainly to wastewater treatment plants, livestock and poultry breeding wastewater, food waste and energy crop due to high methane yield per unit substrate, low level of sludge generation and convenient operation and maintenance (Zhang et al., 2018).However, for feedstock with low moisture content, such as straw, dry manure, and agricultural wastes, dry anaerobic digestion (D-AD) is a better choice because of low consumption of water, small reactor requirement and high volumetric methane production (Shahbaz et al., 2018). However, the microbial source that accelerates the start-up of D-AD reactors has aroused the concern of researchers (Li et al., 2014b). Different parameter and conditions for dry and wet anaerobic digestion is shown in Table 3-5.

There are several AD companies, successfully treated municipal wastes. Most AD facilities incorporate the four stages described in figure 3.3 with some differences in the pre-treatment processes and to a lesser extent in the post-treatment of the products. Structurally, the AD chambers are mostly similar, though operating parameters also vary between treatment processes. Some of the technologies are BTA, Valorga, BRV, DRANCO, Kompogas, WASSA, Maltin, HIMET, ArrowBio.The WAASA process, developed by Citec in 1984, has operations in Finland, Sweden, Japan, Spain, France and the Netherlands (Citec, 2004). In this process, digestion occurs in a vertical digester at either mesophilic or thermophilic temperatures with 10-15% TS content. The digester is a single vessel which is subdivided to two separate chambers for two stages. Mixing is done through biogas injection at the base and sometimes through top mixing for household waste. The digestate is dewatered and aerobically composted. The WASSA process reduces 60% waste volume and 50-60% weight (Citec 2004).

Organic Waste Systems of Belgium developed an AD demonstration plant (DRANCO) in 1984 in Gent, Belgium.The DRANCO process is employed as part of the SORDISEP process (SORting, DIgestion and SEParation) of municipal and

industrial waste for a maximum recovery of recyclables and energy. The feedstock is mixed with digested material, to form a mix of 15-40% TS content. DRANCO is a single stage, vertical gravity driven plug flow system, where the waste fed at the top of the chamber and digested were collected at the bottom with no mixing. The system is run at low pressures and thermophilic temperatures for 15-30 day. Biogas production ranges from 100 to 200 m3 /ton of waste. The solid digestate is dewatered to about 50% and then aerobically processed for two weeks to stabilize the material (Organic Waste Systems 2004).

Table 3- 5: Parameters for dry and wet AD(Karthikeyan and Visvanathan, 2012b; Kwietniewska and Tys, 2014)

Parameter	Wet Anaerobic Digestion	Dry Anaerobic Digestion
Total Solids (TS)	<15%	20-40%
Feedstock Type	e.g. Wastewater sludge	e.g. OFMSW
Water Requirement	Med-High	Low
Operational Mode	Single, two, multi-	Single, two, multi-
Volatile Solids (VS) Loss	High	Minimal
Organic Loading Rate	2-5 kg VS / m^3 day	5-12 kg VS / m^3 day
Max Biogas Yield	0.417 (WASSA process)	0.622 (DRANCO process)
Volume/Heating Requirement	Large volume, high heating	Smaller volume, less heating
Dispersion of Inhibitors	Shock loads, more dispersion	Less mixing, less dispersion
Digestate Dewatering	High requirements	Low requirements
Digestate Characteristics	Less stable with high VS	More stable than wet process
Wastewater/Compost	More wastewater, less compost	Less wastewater, more compost
Maintenance	Abrasion from sand/grit, clogging and deposition, mixers lead to short circuiting	Not susceptible to abrasion, less moving parts limits short circuiting

3.3 Co-digestion

Anaerobic co-digestion (ACoD) is the process where two or more substrates were digested to overcome the drawbacks of mono-digestion and to improve the production of biogas as well as the digestate quality. At the starting of AD period, ACoD was done focusing on mixing substrates which favour positive interactions, i.e. macro- and micronutrient equilibrium, moisture balance to dilute toxic compounds for mostly two substrates for synergistic effects (Mata-Alvarez et al., 2014). Nowadays, because of the industrial outlook and globalisation, multi-substrates i.e. all kind of mixtures substrates is considered for co-digestion. To this the concern is the distance, availability and transportation cost of the co-substrates to the AD plant (Zahan et al., 2018a). This is also one of the key selection criteria. However, it is still important to choose the best co-substrate and mixture ratio with the aim of synergisms by diluting harmful compounds, optimize methane production with improve digestate quality.

Biogas production from mixed substrates is shown to be higher (up to 200% times) than the sum of the biogas production from substrates digested separately which is known to as synergistic effects (Zahan et al., 2016b). Co-digestion study that has been published are mostly on animal manures (54%), SS (22%) and the OFMSW (11%),industrial waste (41%), agricultural waste (23%) and municipal waste (20%) (Mata-Alvarez et al., 2014). The co-substrates that are used for co-digestion in different researches are shown in Table 3-6. The wastes that have been found in the area for this case study are food wastes, yoghurt whey and agricultural residue which could be used as possible co-substrates. An overview of these wastes is given below.

Table 3- 6: Various feedstock used for co-digestion (Kothari et al., 2014)

Feedstock		
Agricultural	**Industrial**	**Communities**
• Manure (poultry, pig, dairy/cattle) • Energy crop • Harvest residue (Straw, hull, saving) • Algal biomass	• Food processing • Dairy • Sugar and starch industry • Pharmaceutical • Cosmetic • Pulp and paper industry • Rendering and slaughter house	• MSW • OFMSW • Sewage sludge • Food remains from household bins • Grass clipping • Garden wastes

3.3.1 Food wastes

Food wastes (FW) are the leftovers which are inedible and produced during preparing, processing, cooking, washing and after meals. FW characteristics vary seasonally, geographically and also by collection method, leading to wide variation of biogas production among different research studies (Bong et al., 2018; Opatokun et al., 2015; Shahbaz et al., 2018; Tampio et al., 2014). The relationship among the variation of food waste characteristic, its effect on the operational parameters & inhibition, and its effect on the efficiency of the methods required further study (Brown and Li, 2013; Koch et al., 2015). Food waste has carbohydrates to be around 11.8–74%, protein 13.8–18.1% and lipid 3.78–33.72%; and the biogas yield of 0.27–0.642 m^3 CH_4/kg VS for mono-digestion and 0.272–0.859 m^3 CH_4/kg VS for ACoD with other substrates (Bong et al., 2018). Food wastes can be categorised as household FW i.e. organic fraction of municipal solid wastes (OFMSW) and commercial wastes i.e. processed FW and fog, grease &oil. Table 3-7 shows the ACoD potential of food wastes with other wastes that has been studied in the literature.

3.3.3.1 Organic fraction of municipal solid wastes

Food is a significant waste that has been found in almost all household garbage bins. Nearly two thirds of the food that could have been eaten normally threw out by average Victorian households in a week (SustainabilityVictoria, 2018). The report from Sustainability Victoria (SV) shows that the avoidable food waste makes up about 2.2 kg of the average household garbage bin, which is nearly a quarter of the bin by weight (2014). It also reported that food makes up about 40% of what is thrown out by weight. A detailed list of food composition in the household garbage bins are given in the report(2014). In this study a synthetic food waste of 10kg have been prepared following composition from the SV report for the whole experiment.

Food waste from different sources as residential, commercial (restaurants), institutional (such as cafeterias in educational premises), industrial sources (lunchrooms), food processing companies are producing wastes at an increasing rate due to the growing population and rising living standard (Dai et al., 2013). Almost all these food wastes are thrown to landfill both causing environmental pollution. Food wastes contain high percentage of biodegradable materials and have high potential for increasing the biogas yield (Alvarez and Lidén, 2008; Dai et al., 2013). Food waste is available year around and account for a significant portion of municipal solid waste (MSW). However, due to high biodegradability and low C:N ratio, AD as single substrate may encounter various potential inhibitions including VFA accumulations (Brown and Li, 2013). Therefore, these OFMSW could be beneficial in anaerobic co-digestion for high energy recovery as well as MSW reduction with very low or zero cost associated with this feed stocks (Brown and Li, 2013; Chen et al., 2014b). Table 3-7 shows a list review of anaerobic co-digestion of different types of food wastes.

Table 3- 7: Literature review on the ACoD of food wastes

FW wastes	Co-substrates	Mixture ratio	AD system	OLR	HRT (d)	T (°C)	Biogas yield	Methane (%)	Ref
Fruit & vegetable	Meat residue	50:50	Batch	-	30	30	0.42 m^3 CH_4/ kg TS	53	(Garcia-Peña et al., 2011)
FW from university canteen	Cow manure		Semi continuous	10g FW VS/L.d		35	0.317 m^3 CH_4/ kg VS		(Zhang et al., 2013)
Kitchen wastes	Rice straw: pig manure	0.4:1:1.6	batch		45	37	0.674 m^3 biogas/kg VS		(Ye et al., 2013)
FW from university canteen	Green wastes	40:60	batch	5g VS	24.5	37	0.272m^3 biogas / kg VS	65.5-70	(Chen et al., 2014b)
FW from university canteen	Fruit & vegetable, waste activated sludge	2:1:1	Semi continuous CSTR		25	35	0.706m^3 biogas / kg VS	64	(Sun et al., 2014)
Kitchen waste	Fruit & vegetable	8:5	Two-phase	2g VS / L.d	10	35	0.725 m^3 CH_4/ kg VS	60	(Wang et al., 2014)
Hotel, restaurants	Dairy manure	1:1 VS	Semi continuous	0.67-3 VS/L.d	178	36	0.4-0.64 m^3 CH_4/ kg VS	60-80	(Agyeman and Tao, 2014)
FW from university canteen	straw	5:1	Batch	5g VS/L	8	35	0.392m^3 CH_4/ kg VS	67.6	(Yong et al., 2015)
FW from university canteen	Rice husk: rice huller		Pilot plant Single stage	6 kg VS/ M^3.d		37	0.446 m^3 biogas / kg VS		(Jabeen et al., 2015)
Kitchen wastes from university canteen	Cow manure	1:1	Batch	8% TS	45	35	0.859 m^3 biogas / kg VS		(Zhai et al., 2015)
FW	Pig manure	1: 0, 4:1, 3:2, 2:3, 4:1, 0:1	Batch	5.2 g VS		37	0.521 m^3 biogas / kg VS		(Dennehy et al., 2016)
FW	Grease trap		CSTR				0.60 m^3 biogas / kg VS		(Wu et al., 2016)

3.3.2 Whey

Whey is a green-yellowish liquid wastewater that is extracted from the card produced during yoghurt making process (Siso, 1996). It is produced during cheese or yoghurt production in a large quantity, have high COD organic loading & saline content (Prazeres et al., 2012). Because of its high COD loading, these industries required treatment before they could through them into the wastewater stream. The waste management focused on valorisation, biological treatment, physico-chemical treatment (Prazeres et al., 2012). Through AD, COD removal of around 81–99% has been reported (Carvalho et al., 2013). Despite the significant COD removal, the whey has low buffering capacity and VFAs accumulates in the reactors leading the failure of anaerobic digester (Carvalho et al., 2013; Prazeres et al., 2012). Some kind of supplement of alkalinity is required in the start-up period (Siso, 1996). Therefore, it can also be co-substrates to co-digestion which has high buffering capacity. Table 3-8 shows a list review of anaerobic digestion of whey.

Table 3- 8: Literature review on the AD of whey

Substrates	Reactor type	pH	T (°C)	HRT (d)	OLR (kg/ m^3.d)	Gas yield	CH_4 (%)	COD removal (%)	Ref
Diluted whey	Vertical/ horizontal	7	37	3		3.3 / 3 m^3/ m^3.d	73	78/ 77	(Patel and Madamwar, 1997)
Diluted whey	Up-flow AD Sludge Blanket	4-7	35	2.06-2.46	22.6-24.6	23.4 l CH_4/L	77	95-97	(Ergüder et al., 2001)
Cheese whey	Up-flow AD Sludge Blanket	6.5-7.5	36	2.5	0.5-0.9	0.2-18.5 L/d	78	98	(Blonskaja and Vaalu, 2006)
Cheese whey	Two stages	6.5	37	5	19.8	10	<70	98.5	(Saddoud et al., 2007)
Pre-treated whey	Up flow AD filter	7.2	35	2-5	3-4	1.3-3.2 L/d	-	95-98	(Gannoun et al., 2008)
Sorghum: whey: liquid cow manure (55:40:5)	Two stage (CSTRs)	5.5 -8	37	1st stage- 0.5 d 2nd stage- 16 d	115 and 3.87	1.52 L/L.d	58.58	83.36	(Dareioti and Kornaros, 2015)
Whey: liquid fraction of raw manure (75: 25 & 60:40)	One stage Up-flow Sludge Blanket		35	2.2-1.3	20.9-28.7	13.2-16.6	53-56		(Rico et al., 2015)

3.3.3 Agricultural wastes

Agriculture sector accounts for the largest potential feedstock and most current researches and applications in anaerobic digestion and co-digestion. This sector mainly includes agro-industrial wastes such as animal farm wastes and waste water, agricultural wastes and residue, and industrial wastes associated with agriculture and food production (Kothari et al., 2014). Table 3-9, represents some characterisation and operating conditions of different agro-industrial wastes research work. According to data of the European Biogas Association, more than 14,000 AD plants are running in Europe, of which 80% plants are operating in the agricultural sector and are mostly of farm based (Bolzonella et al., 2018). However, Australia is still laking behind on this sector and required focus for the utillisation of the use of the low cost abundant agro-industrial wastes.

Most of the agriculture wastes /crop residues are lignocellulosic in nature i.e rich in carbohydrates,which exist mostly as the polysaccharides of cellulose and hemicelluloses, and are not readily available for microbes (Kothari et al., 2014). The structural contents (Cellulose, hemicelluloses, and lignin) are covalently linked with each other that protect the available carbohydrates from degradation (Paul and Dutta, 2018; Timung et al., 2015; Zheng et al., 2014).Therefore, some kind of pretreatment is required for the utilization of carbohydrates in AD process and section 3.4 has an detail overview of pre-treatment of lignocellulosic wastes. An study on the design parameters and optimization of Dry AD, using six different types of fresh and dry feedstocks suggested to use the mixed biomass feedstock for stable biogas production with high conversion efficiency and yield (Chanakya et al., 1997). Dry AD of residue from agriculture sector also offers some attractive advantages (Kothari et al., 2014)but, little information was found in literaure, which needs further research and development.

3.3.4 Overview of chicken litter co-digestion

Chicken litter co-digestion has been recent focus of some of the literaurte studies. A list of literatuire that has been publised on CL co-digestion are shown in Table 3-10.

Table 3- 9: Literature review of the AD of agro-industrial waste (Kothari et al., 2014)

Substrate	TS (%)	VS (% of TS)	C:N ratio	Biogas yield (m^3/kg VS)	HRT (d)	CH_4 (%)	Unexpected content	Chemical inhibition	AD process problems
Pig slurry	3-8	70-80	3-10	0.25-0.5	20-40	70-80	Wood shaving, bristles, H_2O, sand, cords, straw	Antibiotics, disinfectants	Scam layes, sediments
Cow slurry	5-12	75-85	6-20	0.2-0.3	20-30	55-75	Bristles, soil, H_2O, NH_4^+, straw, wood	Antibiotics, disinfectants	Scam layes, poor biogas yield
Chicken slurry	10-30	70-80	3-10	0.35-0.6	>30	60-80	NH_4^+, girt, sand, feathers, straw, soil	Antibiotics, disinfectants	NH_4^+ inhibition, scam layes
Whey	1-5		80-95	0.8-0.95	3-10	60-80	Transporttation impurities		pH reduction
Ferment. slops	1-5	80-95	4-10	0.35-0.55	3-10	55-75	Undegradable fruit remains		VFA accumulation
Leaves	80	90	30-80	0.1-0.3	8-20		soil	Pesticides	
Wood shaving	80	95	511				Unwanted material		Mechanical problems
Straw	70	90	90	0.35-0.45	10-50		Sand, girt		Scam layes, poor digestion
Wood wastes	60-70	99.6	723				Unwanted material		Poor bio-degradation
Garden wastes	60-70	90	100-150	0.2-0.5	8-30		Soil, cellulosic components	Pesticides	Poor inoculum of cellulosic component
Grass	20-25	90	12-25	0.55	10		Grit	Pesticides	pH reduction
Grass silage	15-25	90	10-25	0.56	10		Grit		pH reduction
Fruit wastes	15-20	75	35	0.25-0.5	8-20		Undegradable fruit remains	Girt pesticides	pH reduction
Food remains	10	80		0.5-0.6	10-20	70-80	Bones, plastic materials	Disinfectants	Sediments, mechanical problems

Table 3- 10: Overview of the ACoD of chicken litter

Substrate	TN (CM)	Ammonia removal	Ratio	C/N ratio	Reactor type	Biogas yield	Ref
Chicken Manure: corn straw	3.6%	No	1:1.4 (VS based)	20	Batch/ CSTR	281 m^3 CH_4/kg VS (batch), 255-223 m^3 CH_4/kg VS (CSTR)	(Li et al., 2014b)
Chicken Manure: coconut/cassava / coffee waste	**TAN-6.63 g/L**	**stripping**	**7:3 (VS)**	**17.1-21**	**Batch**	**426-631 m^3 CH_4 /kg VS**	**(Abouelenien et al., 2014)**
Chicken Manure: wheat/ rice straw, corn stalks	**TAN- 5.4 g/L**	**No**	**100:0, 83.3:16.7,75:25,50:50,25: 75,16.7:83.3, 0:100 (TS)**	**Cm-11.2, WS-91.2, RS- 92.9, CS-88.1**	**Batch**	**345-383 m^3 CH_4/kg VS**	**(Zhang et al., 2014b)**
Chicken droppings: Cymbopogon	TKN- 72.2 g/L	No		42.2	Batch	21.6-33.3 m^3 CH_4/ kg	(Owamah et al., 2014)
Chicken Manure: maize silage	TKN- 28 g/L	No	9:1, 4:1, 3:1, 7:3 (VS)	12.8-15.6	CSTR	309 m^3 CH_4/kg VS	(Sun et al., 2016)
Chicken Manure: sugar beet residues	4.9%	No	1:0, 3:1, 1:1, 1:4 (w/w)	CM-12.2, SBR- 33.9	CSTR	346 m^3 CH_4/kg VS	(Borowski et al., 2016)
Chicken Manure: Thai rice noddle wastewater	TKN-0.7 g/L	No	1:20, 1:10, 3:20, 1:5, 1:4 (w/w)	CM: 12.4	Batch	562 m^3/kg COD	(Jijai and Siripatana, 2017)
Chicken Manure: maize silage/ corn stover	0.05 g/L	Water extraction	1.01	Cm- 7.5, MS- 45.3, CS- 52.5	Batch		(Böjti et al., 2017)
Chicken Manure: spent poppy straw	TKN- 13.0-13.8 g/L	No	3.6 (VS)		CSTR	140-360 m^3 CH_4/ kg VS	(Bayrakdar et al., 2017)
Chicken Manure: microalgae Chlorella sp.	Protein- 16.3%	No	0:10, 2:8, 4:6, 6:4, 8:2, 10:0 (VS)	Cm-12.3, MC sp.- 4.9	CSTR		(Li et al., 2017)
Chicken Manure: oxidative cleaved wheat straw	TAN-1.3 g/L	No		20, 25	CSTR	296 m^3 CH_4/ kg VS	(Hassan et al., 2017)
Chicken Manure: corn stover	3.6%	No	3:1, 1:1 (VS)	Cm-9.9, CS- 53.7	CSTR	296 m^3 CH_4/ kg VS	(Yan et al., 2018)

3.3.5 Overview of lignocellulosic wastes

Lignocelluloses are plant material which contains high lignin, cellulose and hemicelluloses composition. From this definition the term includes woods, grain stalks and many other plant materials. Lignocellulose are commonly known for rigidity and are the components of plants that give the rigidly and strength, i.e. branches or stems. The Lignocellulosic components of plants have evolved to be resistant to many natural forms of degradation, thus it requires high degradation times in nature to break down most lignocellulosic matter.

The composition of lignocellulose varies significantly between plants variety and species which greatly effects their applications. Lignin, Cellulose and hemicelluloses exist in a web like bound matrix with hemicelluloses binding to outside of the cellulose clusters (Potters et al., 2010). These hemicelluloses bind to the lignin and form the matrix that holds the cellulose micro fibrils in place as shown in Figure 4.

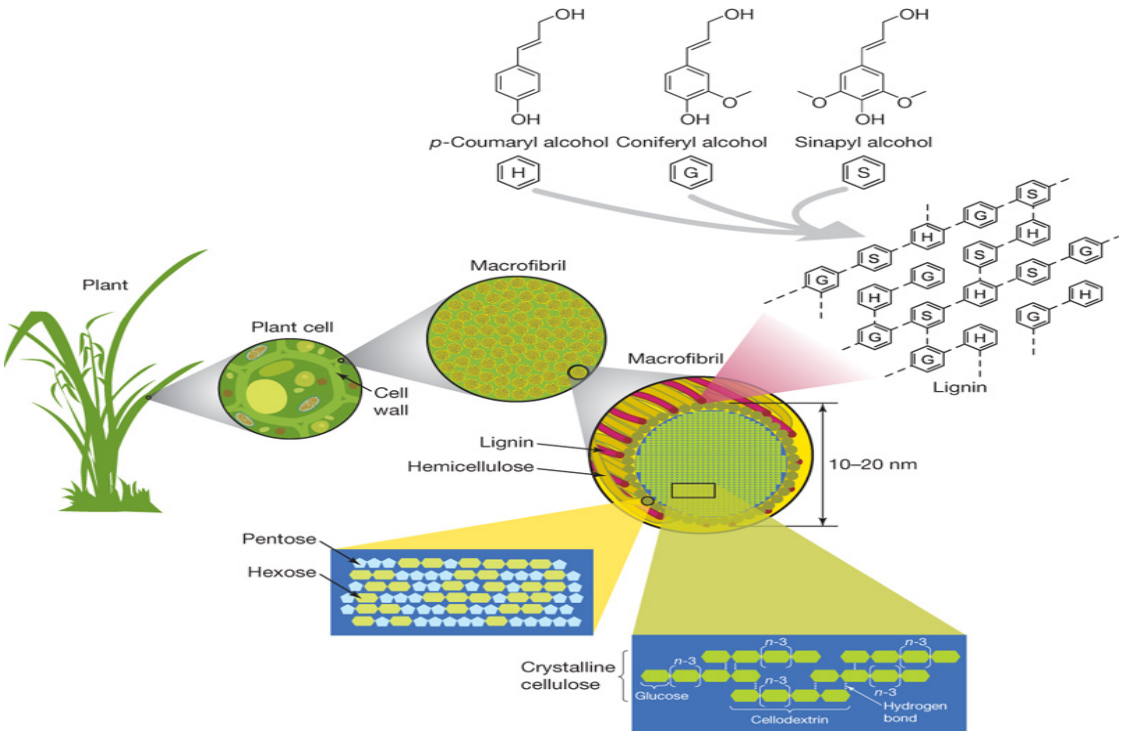

Figure 3- 4: Structure of lignocellulosic biomass (Potters et al., 2010)

3.3.5.1 Cellulose

Cellulose is the most abundant organic substance on earth and most prevalent hydrocarbon in the lignocellulosic material (Narayanaswamy et al., 2013). Cellulose exist as a chain of individual glucose molecules connected via β-1-4glycoside bondage forming chains of 100-10,000 glucose units (Chundawat et al., 2011). Cellulose chains bond onto the side of other cellulose chains to from large clusters known as microfibrils, these become tightly packed and from a crystalline structure. The cellulose is the major competent that will provide an energy-based product due to the high conversion of glucose to ethanol and CH_4 via biological means, while generally being the highest content of lignocellulosic material 30-60% depending on species and verity of Pre-treatment of lignocellulosic wastes.

Cellulose characteristics depend on:

- Degree of polymerization (DP), i.e. the number of glucose units that make up one polymer molecule.
- The nature of bond between the glucose molecules (β-1, 4 glucosidic) allows the polymer to be arranged in long straight chains. Later the hydrogen bonds in turn result in the formation of a compound that is comprised of several parallel chains attached to each other.
- Cellulose is found in both the crystalline and the non-crystalline structure. several polymer chains lead to the formation of micro fibrils obtain a crystalline structure

Common feature of cellulose:

- Cellulose is also insoluble in dilute acid solutions at low temperature.
- The solubility of the polymer is strongly related to the degree of hydrolysis achieved.
- At higher temperatures it becomes soluble, as the energy provided is enough to break the hydrogen bonds that hold the crystalline structure of the molecule.
- Cellulose is also soluble in concentrated acids, but severe degradation of the polymer by hydrolysis is caused.

- In alkaline solutions extensive swelling of cellulose takes place as well as dissolution of the low molecular weight fractions of the polymer (DP < 200).
- its decomposition starts at 180˚C

3.3.5.2 Hemicelluloses

Hemicelluloses are the semi long chains present though the cell area in between cellulose and represent a family of polysaccharides such as arabino-xylans, gluco-mannans, galactans, and others. Hemicelluloses fall into several classes xylans, mannas, mixed link β-glucans, xyloglucans (Chundawat et al., 2011; Potters et al., 2010). Hemicelluloses often contain links of 50-200 units and vary between differing plant/cell types and verity with hardwood containing mostly xylans and softwood mostly glucomannan (Mandal et al., 2010; Narayanaswamy et al., 2013). They often have a sturdy chian that acts as a backbone which binds along cellulose microfibrils surface with a branched component bonding with lignin as shown in Figure 2.

Hemicellulose characteristics

- Lack of crystalline structure, mainly due to the highly branched structure, and the presence of acetyl groups connected to the polymer chain
- Hemicellulose extracted from plants possesses a high degree of polydispersity, polydiversity and polymolecularity

Common feature:

- Hemicellulose is insoluble in water at low temperature. However, its hydrolysis starts at a temperature lower than that of cellulose, which renders it soluble at elevated temperatures.
- The presence of acid highly improves the solubility of hemicellulose in water

3.3.5.3 Lignin

Lignin is a highly complex phenyl-propanoid polymers (p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol) that are largely responsible for the difficulty in penetrating the cell wall (Mendu et al., 2011), as mentioned previously lignin bonds with the hemicelluloses to create the matrix which holds that cellulose. For most

biological degradation methods lignin must be degraded or removed to allow for effective and economy feasible methods of bio-energy generation. Lignin does not contain any saccharises in the structure and is hydrophobic these factors increase the resistant to degradability for the lignocellulosic materials greatly.

Lignin characteristics

- Lignin from softwood is made up of more than 90% of coniferyl alcohol with the remaining being mainly p-coumaryl alcohol units.
- Lignin contained in hardwood is made up of varying ratios of coniferyl and sinapyl alcohol type of units
- Lignin in wood behaves as an insoluble three-dimensional network.
- It acts as binder between cells creating a composite material that has a remarkable resistance to impact, compression and bending.

Common feature:

- Solvents that have been identified to significantly dissolve lignin include low molecular alcohols, di-oxane, acetone, pyridine, and dimethyl sulfoxide.
- It has been observed that at elevated temperatures, thermal softening of lignin takes place, which allows depolymerisation reactions of acidic or alkaline nature to accelerate

3.3.5.4 Challenges in lignocellulosic biomass degradation

There are numerous challenges to be overcome in the degradation of lignocellulosic materials. In thermos-chemical processing (gasification or combustion) many of these are avoided, but this comes at a cost of much higher activation energies requiring much higher operational temperatures (Chundawat et al., 2011; Narayanaswamy et al., 2013).

The Lignin-hemicelluloses matrix is the main cause for the lignocellulosic resistance to biological degradation; this matrix provides structure to the primary and secondary cell wall for plants and prevents access of enzymes that can degrade the cellulose. This matrix provides a hydrophobic interface that prevents many items entering or exiting the cell walls, this allows for the transportation of water within vascular cells.

The hydrophobic ability and high resistance to degradation within the lignin prevents enzymatic penetration of the cell and thus prevents the hydrolysis of the hemicellulose and cellulose(Chundawat et al., 2011).

As previously stated there are many factors that add to the resistance to degradation these include waxy coatings often found around plant tissue, arrangement and density of vascular bundles within the plant material, presence and volume of thick plant tissue, degree of lignification, the ratio of lignin to hemicellase and verities of these, arrangement between hemicelluloses and cellulose microfibrils, and crystallinity of the cellulose microfibrils (Himmel et al., 2007).

3.3.5.5 Composition of different lignocellulosic biomass

The composition of different lignocellulosic biomass reported in literature is summarised in Table 3-11. Among the different lignocellulosic biomass investigated, straws have comparatively less lignin than hardwood and softwood, whereas nut shells, newspaper and softwood have the high lignin content. Fibres, bagasse and woods have high cellulose content. Whereas woods, grasses and shells have high hemicellulose content. Therefore, woods, shells, grasses are required some sort of pre-treatment to breakdown the lignin and hemicellulose to obtain the cellulose.

Table 3- 11: Composition of lignocellulosic biomass from literature (Anwar et al., 2014)

Lignocellulosic biomass	Lignin (%)	Hemicellulose (%)	Cellulose (%)
Sugarcane bagasse	20	25	42
Sweet sorghum	21	27	45
Hardwood	18-25	24-40	40-55
Softwood	25-35	25-35	45-50
Corn cobs	15	35	45
Corn Stover	19	26	38
Rice straw	18	24	32.1
Nut shells	30-40	25-35	25-30
Newspaper	18-30	25-40	40-55
Grasses	10-30	25-50	25-40
wheat straw	16-21	26-32	29-35
Banana waste	14	14.8	13.2
Bagasse	23.33	16.52	54.87
sponge gourd fibres	15.46	17.44	66.59

3.3.5.6 Milling of lignocellulosic wastes

As some of the lignocellulosic biomass is large in size, it may require a grinding or mill which could significantly add to the running cost of any system (Potters et al., 2010). All process investigated in this report require milling of the lignocellulosic samples. The grinding can be one of the most energy intensive processes undertake during the energy generation, as desired particle matter for AD is as less as <1mm, this energy has been found to require ~ 2500kj/kg dry matter of wood material to reach this level of milling, this equates to ~12% of the biomass energy, thus realistically it may be of better benefit to operate a milling of ~4-5mm which equated to ~400-500kj/kg dry mater or ~2-3% of the potential bio-energy production (Miao et al., 2011). Modern Forestry and wood pulping industry for uses in energy reactions and chemical collection utilise Hammer milling as far more options in these verities.

3.4 Pre-treatment

The role played by the pre-treatment is to increase the ability for the enzymes to get inside the wood cells. As mentioned the lignin hemicellulose connections are largely responsible for this resistance that is why many of the pre-treatment techniques work to either destroy /dissolve the lignin or hemicellulose. The removal of either the lignin or hemicellulose greatly increases the ability for the wood to be converted into sugars and subsequently methane (Mendu et al., 2011; Miao et al., 2011; Narayanaswamy et al., 2013; Potters et al., 2010; Zheng et al., 2014). Therefore, the pre-treatment is necessary to break down the lignin structure, recover cellulose, decrease cellulose crystallinity, partially remove or break down bond between hemicellulose, increase surface area and porosity of biomass, increase accessibility of enzymes and microbes. The effect of pre-treatment on lignocellulosic biomass is shown in a simplified form in Figure 3-5.

There are many treatments available that are divided into mechanical, e.g. by milling or grinding, physical by steam explosion or radiation, chemical by acids, alkali or solvents, biological by enzymes and fungi, thermal, combined mechanical, thermal

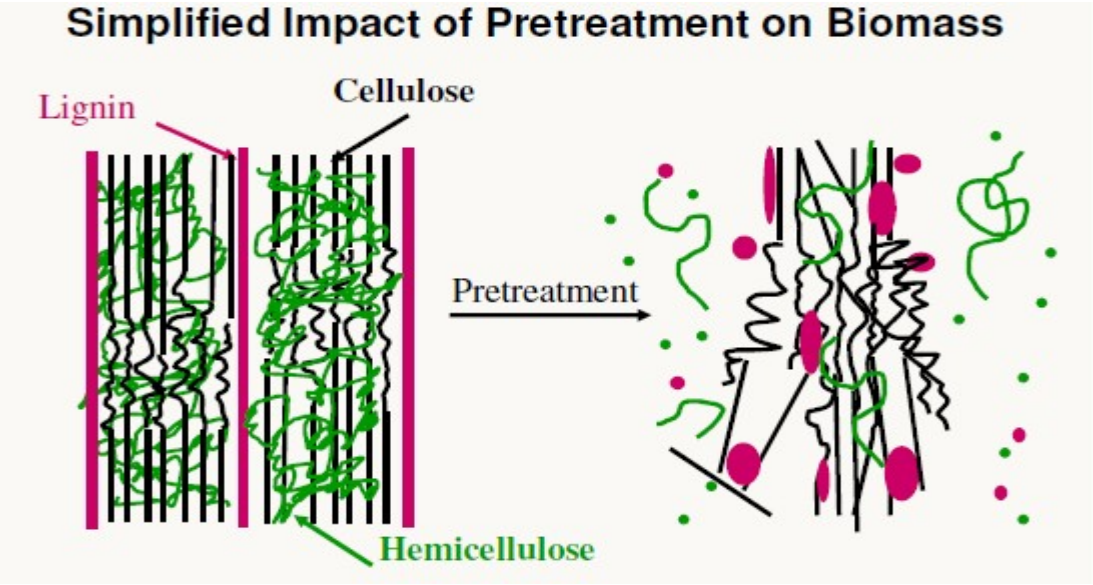

Figure 3- 5: Impact of pre-treatment on lignocellulosic biomass (Liu and Fei, 2013)

and chemical(Chandra et al., 2012; Chen et al., 2014a; Salehian and Karimi, 2013; Zhang et al., 2014a), Co-digestion with other organic wastes(Chen et al., 2014a)have been explored to improve the efficiency of degradation. Among the mechanical and physical pre-treatment, milling has a good potential to improve the hydrolysis by increasing the surface area of lignocellulosic materials (Miao et al., 2011; Potters et al., 2010) which makes this as a prerequisite before any other pre-treatment to apply.

Among the chemical treatments, the alkaline pre-treatment is one of the most important methods to break down the ester bonds between amorphous and cellulose contents by saponification and cleavage of lignin-carbohydrate linkage, decrease in polymerization and crystallinity, which increases porosity, internal surface area and structural swelling (Salehian and Karimi, 2013; Zheng et al., 2014). Sodium hydroxide (NaOH) has been extensively used in the studies to improve biogas from lignocellulosic wheat straw (Chandra et al., 2012), rice straw (He et al., 2009), corn stover (Wang et al., 2013a), hardwoods and softwoods (Salminen and Rintala, 2002; Zheng et al., 2014), paper and pulp sludge (Lin et al., 2009), oil palm empty fruit branches (Nieves et al., 2011). Studies showed that NaOH pre-treatment has increased methane yield from feedstocks like softwood, hardwood and pulp and paper. However, various treatment conditions, i.e. NaOH concentration, time, and temperature can show different results along with wood types. For example, NaOH

pre-treatment was more effective on hardwood (birch) than softwood (spruce) for methane yield in anaerobic digestion (Mohsenzadeh et al., 2012).

Cellulase and hemicellulose are the most commonly used enzymes for lignocellulosic biomass. However, the cost of enzymes and effectiveness in biogas production limited its applications in anaerobic digestions (Zheng et al., 2014).

Although several studies have been done on the effectiveness of pre-treatment, the promising results were found with the combination of two or more pre-treatment methods. Moreover, the effectiveness and selection of pre-treatment mostly depend on the types of lignocellulosic materials. In addition, the use of enzymes still needs to be investigated as it accelerates the cellulose and hemicellulose decomposition.

3.4.1 Effects of chemical pre-treatment

Chemical pre-treatment has been done by alkaline, acid, catalyst, wet oxidation and ionic liquid. A summary of literature review on chemical pre-treatment are given in Table 3-12. Therefore, depending upon the nature of the lignocellulosic biomass, chemical pre-treatment should be chosen carefully.

Table 3- 12: Summary of most effective chemical pre-treatment

Chemical pre-treatments summary (Serna et al., 2015)			
Pre-treatment	Reagents	Treatment effects	Reference
Alkaline	Sodium hydroxide, potassium hydroxide, calcium hydroxide, ammonium hydroxide, lime among others	Lignin removal	(Park and Kim, 2012)
Dilute acid	Sulphuric acid, hydrochloric acid, nitric acid, phosphoric acid among others	Hemicellulose fractionation	(Chandel et al., 2012)
Organosolv	Ethanol, acetic acid, formic acid, per acetic acid with organic and inorganic catalysts	Lignin removal and hemicellulose fractionation	(Amiri et al., 2014)
Ionic liquids	Anions from chloride, formate, acetate or alkyl phosphorate	Cellulose crystallinity reduction and partial hemicellulose and lignin removal	(Li et al., 2010)

3.4.2 Comparison of different pre-treatments

Different pre-treatment techniques have been discussed individually in the previous section. Now summary of all the pre-treatment has been provided in Table 3-13 showing the effect of pre-treatment on the compositional and structural alteration of lignocellulosic biomass.

The effect of pre-treatment has been divided in six categories which are accessible surface area, decrystallization of cellulose, solubilisation of hemicellulose, solubilisation of Lignin, alteration of lignin structure and formation of any inhibitory ingredient such as furfural. The effect was also categorised on the scale of 1-4 where 1 being the major effect and 4 would be no effect.

From Table 3-13, alkaline pre-treatment has major effect on lignin solubilisation and lignin structural alteration whereas acid pre-treatment has major effect on solubilisation of hemicellulose. Therefore, depending upon the lignocellulosic structure, the type of pre-treatment should be chosen. Sometimes, combination of two pre-treatment would be effective if the structural lignin and hemicellulose is required to be sequentially removed.

Table 3- 13: Effect of pre-treatment on the compositional and structural alteration of lignocellulosic biomass (Zheng et al., 2014)

Pre-treatment	Primary effect	Accessible Surface area	Decrystallization of cellulose	Solubilisation of hemicellulose	Solubilisation of lignin	Alteration of lignin structure	Formation of furfural/(HMF)
Particle size/ mechanical	-increase microbial access and activity	❶	❶	❹	❹	❹	❹
Ultrasonic	-disintegrates the particles -creates favourable conditions for biodegradation	❶	❸	❶/❷	❶/❷	❶/❷	❸
Irradiation		❶	❷	❷	❹	❹	❷
Steam explosion	-organic and inorganic compounds are partially solubilized	❶	❹	❶	❷	❶	❶
Liquid hot water	-partially solubilized	❶	❸	❶	❷	❷	❷
Catalysed steam-explosion	- solubilisation of organic and inorganic compounds	❶	❹	❶	❶/❷	❶/❷	❶
Acid	-higher solubilisation and biodegradation of cellulose, hemicelluloses and lignin	❶	❹	❶	❷	❶	❶
Alkaline		❶	❹	❷	❶	❶	❷
Oxidative		❶	❸	❹	❶	❶	❷
Ionic liquids		❶	❶	❷	❹	❹	❹
Thermal acid		❶	❸	❶	❹	❹	❶
Thermal alkaline		❶	❸	❷	❶/❷	❶	❷
Thermal		❶	❸	❷	❶/❷	❶	❷
Ammonia fibre explosion		❶	❶	❷	❶	❶	❷
Biological pre-treatment	- degrade lignin and hemicelluloses	❶	❸	❶	❶	❶	❹

❶= Major effect ❷= Minor effect ❸= Not determined ❹= no effect

3.5 Literature Research Findings

The main findings and researcg gap developed and identified through this literature review as described in this chapter, section 3.1 to 3.4, were summarised in five categories. The categories, findings and research gap are summarised in Table 3-14.

Table 3- 14: Summary of a list of literature review and findings

Area	Main findings	References
1.Chicken litter co-digestion	Recently few studies have been published on poultry wastes. However most of them are initial optimisation studies. More research needs for them to be implemented. -Bench scale reactors operated at semi-continuous conditions is more useful for full scale and industrial application (Li et al., 2014b). - Studies on continuous reactors for the ACoD of agro-industrial wastes are still limited so far. - The majority of studies on ACoD have been carried out using biomass mixes consisting of two or three materials, whereas only a few studies considered four substrates, or more (Poulsen and Adelard, 2016). **Remarks/gaps:** The agro-industrial wastes used this study has not studied as a combine co-substrate mixture in any studies which are the promising wastes available in Australian context.	(Hassan et al., 2016; Mei et al., 2016; Poulsen and Adelard, 2016; Shen and Zhu, 2016b; Sun et al., 2016)
2.Kinetics of digestion	Performance of AD/ the kinetics parameters i.e. such as biogas yield potential, maximum biogas production rate, hydrolysis rate constant and lag phase duration can be predicted with some established Mathematical models for example first-order regression model, the modified Gompertz model, transfer function model and Cone model. Models based on the composition of the waste have also been used for predicting the BMP without undertaking extensive and costly experiments. **Remarks/gaps:**	(Kafle and Chen, 2016; Li et al., 2015; Shen and Zhu, 2016a)

	-Kinetics has been limitedly studied and required more attention specially for AD of agro-industrial wastes
3.Pre-treatment	Solid wastes are mostly lignocellulosic in nature and have a recalcitrant and crystalline structure which resists bacterial attack and biodegradability. - Different pre-treatment methods, including thermal, mechanical by milling or grinding , physical by steam explosion or radiation , chemical by acids, alkali or solvents , biological by enzymes or fungi , combined mechanical, thermal and chemical (Chen et al., 2014a; Salehian and Karimi, 2013; Teghammar et al., 2014; Zhang et al., 2014a), and co-digestion with other organic wastes (Chen et al., 2014a) have been explored to improve the efficiency of degradation. - Removing selectively hemicellulose and lignin from biomass with dilute acid and alkali pretreatments eliminates the undesirable interaction between lignin and cellulose, as well as the physical barrier of hemicellulose leaving cellulose more accessible for enzymes (Alvira et al., 2010) - Dilute sulfuric acid pretreatments (DAP) efficiently hydrolyze hemicellulose without an excessive formation of inhibitory degradation by-products (Saha et al., 2005). Dilute sodium hydroxide (i.e., alkaline) pretreatments (AKP) are generally effective for lignin removal (Carrillo et al., 2005). They do not cause excessive sugar degradation and require much lower pressure and temperature compared to other thermochemical pretreatment methods (Mosier et al., 2005) - Selective fractionation of lignocellulose could increase yields. Therefore, sequential pretreatments may be a promising strategy to establish suitable industrial-scale processes with high rates of sugar recovery (Mussatto et al., 2010; Sanchez et al., 2015). **Remarks/gaps:** Work required to establish conditions of sequential diluted acid (DAP) and alkaline (AKP) pretreatments [i.e., DAP followed by AKP (DAP+AKP)] for maximizing sugar recovery from chicken litter and wheat straw. The pretreatment effectiveness could be evaluated by the

	indipendent factor and by the hydrocarbon yield achieved in fractional analysis. The comparison of pretreatment sequences could be done for structural breakdown and maximal sugar recovery that may be relevant for industrial-scale applications.	
4.Dry anaerobic digestion	Number of publication for D-AD increased 2010. Increasing efforts have been made to optimize the D-AD process for improving methane yield, stability, and benefit-to-cost ratio. Better understanding of mechanisms, such as mass transfer, biomass structure, and microbial distribution, during SS-AD could to be addressed using modeling, microbial community analysis, and microscope imaging and tracer techniques. Reactor development is the limiting factor for commercialization of SS-AD, and future studies needs to be focused on these aspects. **Remarks/gaps:** - Identification of possible feedstock and co-digestion -Evaluation of feedstock particle size, mixing, storage and pre-treatment -Development of reactor system for large/ commercial scale D-AD -Assessment and improvement of digestate post-treatment technology -Integration of D-AD with other process for value added products.	(André et al., 2016; Ge et al., 2016; Huang et al., 2016; Nkemka and Hao, 2016; Rajagopal and Massé, 2016; Riya et al., 2016; Saady and Massé, 2016; Xu et al., 2016)
5.RSM Modelling, statistical analysis	Use of Mathematical modelling for understanding D-AD mechanism, performance prediction and improvement of process control only trace back from 2011 and require further investigation for large scale establishment. **Remarks/gaps:** -Most of them are theoretical interpretation -limitation of theoretical and mathematical instrumentation -Most used with single or two standard substrates. Required experimental verification. -Requires models for scale up applications. -Combination of different approaches for D-AD modelling	(Batstone et al., 2015; Benbelkacem et al., 2015; Fernández-Rodríguez et al., 2013; Khan et al., 2016; Liotta et al., 2015; Pardo et al., 2017; Xu et al., 2015)

3.6 Analysis Techniques Used for Characterisation and Biogas Production

3.6.1 Sample preparation

Several batches of chicken litter and agricultural wastes were collected during field visit. The chicken litter stored in plastic airtight bucket at room temperature. Agricultural wastes i.e. wheat straw, hay grass, green wastes were grinded to make it below 5mm and stored in plastic screw cap container. Yoghurt whey was stored in refrigerator at 4˚C until used. The samples were characterised to check the variation of different batches as well as the shelf life of the samples.

A stock of synthetic food waste (SFW) of 10 kg has been prepared to use for a long period using compositional data collected by Sustainability Victoria, Australia (IWM020, 2014) and stored in small containers at -20˚C so that small portion of it can be used as required without losing the integrity of the whole sample. The characteristics of the samples were measured in regular interval to examine any changes in the stock sample.

3.6.2 Total solids, volatile solids, moisture and ash content

An optimised anaerobic digestion process can treat more waste in terms of dry weight. The initial substrate concentration influences the AD process at batch as well as continuous process at different temperature conditions.

The total solids were determined by the dry weight at 105 °C in a drying according to the standard APHA method 2540B (2012). For volatile solids and ash content the samples were placed in a muffle furnace at 550 °C for 20 min 575 °C with a temperature ramp following APHA methods 2540E (2012) and NREL method (Sluiter, 2008) respectively. The moisture content of the samples was determined by the moisture analyser.

The amount of moisture in litters varies from place to places depending on weather, humidity, manure management and collection type, sample storing duration etc. The total solids and moisture content varied with different samples of chicken litter collected from different areas as well as different times. However, the variation in volatile solids comparatively small and it is in the range of 40- 47%. The VS (%TS)

was around 50-52%. Furthermore, the chicken manure collected inside the sheds at 20 days shows totally different characteristics with 68% VS.

3.6.3 pH

pH changes at various stages of AD digestion process. During the hydrolysis process, the pH is around 6 or less and much of CO_2 is given off. In the later stages, the pH increases as the volatile acid is digested and CH_4 is produced (Jain et al., 2015). To maintain a stable process, it is necessary to maintain a stable pH range inside the digester.

When mixing different substrates and adding deionised water, the pH was in the range (between 6.5 and 7.5). In this range, the microorganisms are very active, and AD is very efficient. Above and lower these range are detrimental to the methanogenic organisms. Any sudden upset in the pH by the addition of any material would cause an imbalance in the bacterial population.

The measurement of pH was carried out using a calibrated pH meter (ThermoOrion, Model 550A). All the chicken litter were found with a pH higher than 8 which indicates high alkaline properties and subsequent buffering capacity of the samples. The lower pH of whey and SFW is an indication of high total volatile acids (TVA) of the food wastes and the lack of buffering capacity.

3.6.4 Total C, N, C: N ratio and total P

The major nutrients required for the bacterial growth in the digester are C, H_2, O_2, N_2, P and S (Jain et al., 2015). The carbon and nitrogen are the main food of anaerobic bacteria. Carbon is required for energy and nitrogen is used for building the cell structure. From literature, an operating C:N ratio range of 20:1–30:1 with an optimal ratio of 25:1 for anaerobic bacterial growth is recommended (Rahman et al., 2017). However, this ratio can vary for substrate to substrate as well as for co-digestion mixtures (Zahan et al., 2016b). Improper C/N ratios could result in high total ammonia nitrogen (TAN) released and high volatile fatty acids (VFA) accumulation in the digester (Jain et al., 2015). Both TAN and VFAs are important intermediates as well as potential inhibitors in the AD process. Having high concentrations of TAN and

VFAs in the digester would decrease the methanogen activity and cause possible failure of digester.

Leco (TruMac series) carbon nitrogen analyser was used to determine the C: N ratio for the substrates. The Total P (TP) has been determined by colorimetric techniques using a HACH (Model: DR/4000 U) spectrophotometer following the method 10127.

3.6.5 COD and TOC analysis

The COD test measures the organic matter concentration by measuring the oxidant consumption for the oxidation of the organic material. COD has been determined by colorimetric techniques using a HACH (Model: DR/4000 U) spectrophotometer following the method 8000 and TOC analysed by TOC analyser. The samples were centrifuged (Eppendorf 5702, Germany) at 4.4 rpm for 15 mins and then filtered through 0.45 µm filter paper (mixed cellulose esters membrane filter, Advantec, Japan), where the filtrate is used for soluble constituents.

3.6.6 Total nitrogen, total phosphorous and volatile acids

All living organisms require nitrogen to form their cell proteins. TN and TP are required to maintain proper balance inside the reactor, however, excess of these nutrient is detrimental. Short chain volatile fatty acids (VFA) are not toxic themselves but long chain VA can constitute inhibition to AD process (Kwietniewska and Tys, 2014). They are produced and used as nutrients in the AD digester. Access amount of VFA might lower the pH to undesirable level and methanogens would not be able to metabolise the acetate produced by the acetogenic organisms until the number of methanogenic organisms has increased sufficiently. This is especially true for rapidly hydrolysed feedstock (Kwietniewska and Tys, 2014).

Total phosphorus (TP), total nitrogen (TN), NH_3-N, volatile acids (VA) were measured by colorimetric techniques using a HACH (Model: DR/4000 U) spectrophotometer following the methods 10127, 10072, 10031 and 8196 respectively. The samples were centrifuged (Eppendorf 5702, Germany) at 4.4 rpm for 15 mins and then filtered through 0.45 µm filter paper (mixed cellulose esters

membrane filter, Advantec, Japan) where the filtrate is used for soluble constituents. Alkalinity was measured by APHA method 2320B.

3.6.7 Extractive, lignin, carbohydrate and ash analysis

Extractives were determined following NREL method (Sluiter et al., 2005c). The samples were subjected to exhaustive ethanol extraction only. The samples were done for three static cycles according to the parameter settings. The samples were then kept in container for air drying and stored for acid hydrolysis. The solvent ethanol was evaporated with evaporator (Centrivap DNA system, Labconca co., Australia) for determination of extractives.

Extractive free sawdust samples were hydrolysed with 72% H_2SO_4 for compositional analysis (Sluiter et al., 2011). The samples were autoclaved at 121˚C for 1hr. The autoclaved hydrolyse samples were vacuum filtered over filtering crucibles. The filtrate aliquots were preserved for acid soluble lignin and sugar analysis. The acid insoluble lignin was determined by gravimetric analysis of the solids over filtering crucibles and the acid soluble lignin of the filtrate liquid aliquots were read with UV-Visible spectrophotometer at a wave length of 240nm. The content of Glucose was determined from the filtrate liquid aliquots by the high-performance liquid chromatography (HPLC, Shimadzu UFLC).

Structural carbohydrates and sugars were analysed by high performance liquid chromatography (HPLC) connected with a Refractive Index (RI) detector (Thermo Fisher Scientific, Australia). The hydrolysed sugars were analysed on an ion-exchange column (Phenomonex Hi-Plex Pb^{2+} followed by Na^{+}, 7.7*300 mm, 8µm) at 85°C using 100% degassed deionised water as a mobile phase with a flow rate of 0.3 mL/min. Standard sugars of varying concentration (0.01-4 mg/mL) were pre-pared for making the calibration curve. The standard sugars used for making the standard curve are the high purity standards of D-cellobiose, D (+) glucose, D (+) xylose, D (+) galactose, L (+) arabinose, and D (+) mannose. Combined standard using all the sugars were also made and both (individual and combined) standard were run on HPLC for making the standard curve.

Percentage of ash was determined by gravimetric analysis of the sample in a muffle furnace by ramping the temperature program up to 575°C outlined NREL LAP 005 (Sluiter et al., 2005b).

3.6.8 FTIR analysis

The effects of pre-treatment on the structure of the lignocellulosic substrates were investigated by FTIR analysis. This analysis is mostly used to obtain information about the structure of the lignocellulosic biomass and chemical changes taking place in the material due to various treatments (Chen et al., 2010).The raw lignocellulosic samples were dried in oven at low temperature of 40˚C before doing the experiments to ensure no structural damage. Crystallinity of pure cellulose and lignin sample will also be analysed through FTIR.

Fourier transform infrared spectroscopy (FTIR) equipped with a universal ATR (attenuated total reflection) accessory was used for structural analysis and crystallinity of the treated and untreated samples. The spectra were obtained with an average of 64 scans from 4000 to 600 cm-1 with 4 cm-1 resolution. Crystallinity index (CI) and total crystallinity index (TCI), which are the absorbance ratio of A1430/A896 and A1375/A2900, respectively, are also calculated from the spectra.

Crystallinity index (CI), which is the absorbance ratio of A1430/A898, was calculated from the spectra. The 1438 and 898 cm-1 absorption bands allocated to the cellulose I and cellulose II, respectively.

3.6.9 Biogas and methane production

The biogas produced in each reactor was measured using a water displacement unit (Demirer and Othman, 2008). Biogas reactors were closed with rubber suba seals and one end of the water displacement unit has needle. When the needle is injected through the suba seal in the reactor, high pressure biogas comes out and displaces water of the water displacement unit. Thus, the displaced water amount can be read (in mL) with a measuring scale attached with the displacement unit. Reactor set up for batch, continuous and dry AD are shown in Figure 3-6.

(a) Batch Digestion reactor

(b) Semi- continuous digestion reactor

(c) Semisolid digestion reactor

Figure 3- 6: Anaerobic reactor set up

The composition of the biogas was analysed based on APHA method 2720C using a gas chromatography (Varian 450-GC, Varian Australia Pty Ltd., Netherlands) equipped with a packed column (GS Carbon plot 113-3132, 1.5 micron, 30m* 0.320mm, stainless steel, Agilent Technologies Inc., Australia) and a thermal conductivity detector (Varian). The carrier gas used was helium with a flow rate of 28ml/min. Temperature of the column, detector and injector were 70°C, 200°C, and 100°C respectively. The biogas was collected and manually injected using a 50mL FORTUNA® Optima glass syringe (Poulten & Graf, Germany). Calibration was done using three points and five levels of CH_4, CO_2 and nitrogen (BOC, Australia).

3.6.10 FOS-TAC and alkalinity

A biogas plant functions most efficiently when substrates are added in amounts that are tailored to the anaerobic process. For this purpose, the exact status of the degradation in the digester must be known and documented over a long period of time. This is achieved by regular, easily performed in-house laboratory analyses of the FOS/TAC ratio. FOS/TAC is defined as the ratio of volatile acids to alkaline buffer capacity is a measure of the risk of acidification of a biogas plant. It is measured with Nordmann method. The operator is provided with exact information of the biodegradation performance of the digester and therefore of the biogas production. Any interference with the process can be quickly identified and eliminated in a targeted manner to make the operation more efficient and cost-effective. In practice, a FOS/TAC ratio of 0.3 to 0.4 is normal, although each plant

has its own optimal ratio. This can only be determined by long-term observation and regular checks, as there is a strong dependence on the substrate. According to the FOC/TAC ratio the amount of biomass is low, and more biomass can be added in the reactors. The ratio of FOS/TAC and the required measure are provided in the table 3-15.

Alkalinity were measured according to APHA Methods 2320B. According to Metcalf and Eddy, 2003 in well stablished digester the alkalinity ranges between 2000-5000 mg/L, however this rang can be vary depending upon the substrates. For waste water operation, the optimum VA/ alkalinity ration is less than and equal to 0.1 and its expectable limit is 0.1 to 0.3. Over 0.34 inhibitions occurs.

3.6.11 Ammonia

Ammonia is produced during the digestion of protein-rich substrates, such as chicken, swine or cow manure. Ammonia can inhibit the digestion process and decrease its overall performance. Concentrations over 1,500 mg/L of ammonia-N have been reported to be inhibitory for the digestion process at high pH (i.e., > 7.4); however, acclimation to higher ammonia levels (>5,000 mg/L) has been also reported in manure systems. The limit for ammonia (TAN, total ammonia nitrogen) is 2500-3000 mg/L. Ammonium was determined by colorimetric techniques using a HACH (Model: DR/5000 U) spectrophotometer according to the method 10031 using centrifuged filtered samples.

Table 3- 15: Rules of thumb for the assessment of FOS/TAC ratios (empirical values provided by DEULA-Nienburg).

FOS/TAC ratio	Background	Measure
>0.6	Highly excessive biomass input	Stop adding biomass
0.5-0.6	Excessive biomass input	Add less biomass
0.4-0.5	Plant is heavily loaded	Monitor the plant more closely
0.3-0.4	Biogas production at a maximum	Keep biomass input constant
0.2-0.3	Biomass input is too low	Slowly increase the biomass input
<0.2	Biomass input is far too low	Rapidly increase the biomass input

3.6.12 Volatile acids (VA)

In a correctly designed and well-operated digester, the concentration of total VFA is typically below 500 mg/L as acetic acid. However, if the digester is undersized for the organic load this concentration can be higher. At VFA concentrations over 1,500 – 2,000 mg/L, biogas production might be limited by inhibition. However, rather than a specific concentration, it is a sudden and steady increase of VFAs in the effluent what can be a sign of a digester upset.

3.6.13 Phosphorus (TP)

High concentrations of soluble PO4-P (> 250 mg/l) were found to have a retarding effect on anaerobic digestion, reducing the rate of volatile solids digestion and methane production in comparison to control digesters. Phosphate can cause eutrophication (extraordinary growth of algae) when it is excessively discharged into closed natural water bodies. To control eutrophication, phosphate removal is often required if it is required to discharge to the receiving water bodies.

3.6.14 Effect of different parameters on AD of CL

Anaerobic digestion of poultry waste is the most viable option of manure disposal (Sakar et al., 2009) . Most of the studies on AD of poultry wastes were conducted on different types of reactors for varying ranges of operating condition and parameters such as HRT, pH, ORL and temperature. The influence of these parameters is very important, but the excessive levels of ammonia, toxic substances, sulphides, heavy metals, unstable pH or temperature can heavily inhibit the activity of micro-organism specially methanogens. The effect and necessity of controlling different parameter is given in table 3-16.

Table 3- 16: Parameters based on literature review

Parameter	Effect and necessity of controlling different parameter
Litter analysis	-Dry matter with ammonia inhibition - control parameters are moisture content, ammonia nitrogen, TN, TKN, TP, potassium, pH
C/N ratio	-Optimal ratio should be provided to promote the growth of methanogens populations, depending on the type of manure used as feed substrate. -Low values may become toxic for methanogenic bacteria resulting in a low gas production.
pH and alkalinity	-Optimal pH should be provided between 6.2 and 8.5 to achieve a rapid sludge granulation and to stimulate the reactive activity of methanogens. -Excessive increases or decreases in pH are detrimental on the reactor performance; this may be due to potential inhibition of methanogens. -Sufficient alkalinity should be supplied to buffer dramatic changes in pH.
Temperature	-Appropriate temperature ranges (35–37°C or 55°C) are necessary to maintain the stability on the bacterial activity and biogas production.
Volatile fatty acids (VFA)	-Sufficient buffering capacity should be provided for controlling of undissociated VFA accumulation. -Change in VFA concentrations is the most sensitive parameter affecting the predomination of methanogens or acetogens in the bioreactor.
Ammonia toxicity	-Toxicity threshold level should be attentively examined for varying operational conditions, particularly changes in the temperature and pH above 7. -$NH4^+$ and NH_3 may interchange rapidly depending on the pH. Toxicity threshold level should maintain below 1500–3000 mg L–1 (pH > 7.5).

Sulphides and heavy metals	-Increase in sulphide concentration above 200 ppm may strongly inhibit the metabolic activity of methanogenic bacteria, leading to the failure of the process. -Heavy metals are toxic to both major anaerobic populations even at very low concentrations.
Methane content	-Decreases in gas yield and methane content is an indicator of an unstable process conditions in anaerobic processes. -A well operated reactor may yield a biogas production having methane content above 65%. -The stability of the system should be attentively examined for the methane content below 65%.
HRT and ORL	-Feed substrate should be allowed to stay in the reactor long enough, depending on the manure characteristics. -Start-up with low initial OLRs may encourage granule/floc growth. -Start-up with long retention times may reduce solids loss due to low liquid up-flow velocities and promotes higher methanogen populations.
Microbial inoculum	-Seeding with actively digesting sludge from ongoing mesophilic or thermophilic digesters is strongly recommended to increase the efficiency of the digestion process. -More importantly, seeding with mature granular biomass may require less time for start-up, and biodegrade waste at a higher rate. -Seed acclimatised with litter for ammonia is good for starting for AD
Co-digestion wastes	-Co-digestion of animal manures with each other or other additional substances may yield an increase in the total methane production.

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Chapter 4 Anaerobic Digestion/Co-digestion Kinetic Potentials of Different Agro-industrial Wastes: A Comparative Batch Study for C/N Optimisation

In the previous chapter, an overview of Austrialian chicken litter, agricultural wastes, food wastes, anaerobic digestion system, pretreatment technologies, experimental methods are discussed. For different types of wastes available in the proximity of broiler farms, it is important to understand their characteristics, the biogas production potential, the hydrolysis and kinetic performance parameters such as lag phase, biogas production rate. The results can be applied to anaerobic co-digestion system for better performances optimization. This chapter presents a through characterization of wastes with their AD kinetic potential as well as co-digestion potential with optimization conditions.

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Abstract

Anaerobic digestion (AD) of different agro-industrial wastes and their co-digestion potential has been exhaustively studied in this research. It explores variation of feedstock characteristics such as biodegradability and methane potential during AD and anaerobic co-digestion (ACoD) of chicken litter (CL) with yoghurt whey (YW), organic fraction of municipal solid waste (OFMSW), hay grass (HG) and wheat straw (WS) under mesophilic conditions. Comparative performance was made at different loading concentrations (2, 3 and 4% VS) with 1:2 g/g VS of substrate to inoculum and carrying C/N ratio. Among different kinetic models, the AD of single substrates showed better fit to the modified Gompertz model (R2: 0.93-0.997) indicating variation in lag phase and methane production rate depending upon the substrate characteristics. During ACoD, the methane yield was improved by 9-85% by the addition of two, three or four substrates due to the synergistic effect as a result of increased biodegradability and optimum conditions (such as C/N ratio). A surface (optimisation) model indicated that maximum methane production was achieved by blending chicken litter (30-35%) and a (65-70%) mixture of yoghurt whey, hay and wheat straw with a C/N ratio of (26-27.5).

4.1. Introductions

Anaerobic digestion (AD) has received growing attention, as an eco-friendly technology for the management of various solid organic wastes such as manure, lignocellulosic wastes, industrial, and municipal wastes (Liotta et al., 2015; Naik et al., 2014; Poulsen and Adelard, 2016). In Victoria, Australia, agro-industries, such as the poultry industry operate at considerable scale, and produce around 0.4 million tonnes per year of chicken litter (CL) as well as additional solid wastes from the other food manufacturing industries such as dairy (Zahan et al., 2016a). Typically CL is a mixture of manure and bedding materials i.e. wood shaving and rice hulls(Ogunwande et al., 2008). Although this waste has a high biochemical methane potential (BMP), it is known that AD of CL is susceptible to inhibition due to the high level of proteins and amino acids which led to ammonia toxicity during digestion (Abouelenien et al., 2010; Chen and Jiang, 2014; Nie et al., 2015). This process is accelerated with organic overloading due to high solid loading and inadequate carbon: nitrogen (C/N ratio) (Abouelenien et al., 2009). Although many research studies have focused on this issue (Abendroth et al., 2015; Nie et al., 2015), the successful utilisation of CL as a single substrate for AD has not been reported to date and no large scale plants exist.

Ammonia, an important indicator of AD inhibition is produced by the biological degradation of proteins and urea (Niu et al., 2013; Uludag-Demirer et al., 2008) and accumulates in the AD process of CL(Liu et al., 2012). Unionised ammonia (NH_3) or free ammonia (FA) can diffuse across the cell membrane (Salminen and Rintala, 2002), therefore, FA are considered as an actual toxic agent (Borja et al., 1996; Liu et al., 2012) to anaerobic microorganisms, particularly methanogens (Salminen and Rintala, 2002). The increase in FA shifts the FA to ionised ammonia (NH4+) which causes an increase in pH with increased toxicity (Chen et al., 2008; Liu et al., 2012). The most effective mechanism to overcome ammonia inhibition is to eliminate or reduce the precursors for ammonia formation in the digester (Karthikeyan and Visvanathan, 2012a). This can be achieved by adjusting the C/N of the feedstock using carbon rich substrates (Karthikeyan and Visvanathan, 2012a; Wang et al., 2012), Numerous studies have shown that AD of CL can be improved by co-digestion with other organic waste streams such as agro-industrial wastes (Abouelenien et al., 2014; Poulsen and Adelard, 2016). Most studies have focused

on substrate mixture ratios and organic loading when determining the optimum process conditions, and there have been few studies on C/N optimization (Abouelenien et al., 2014; Hassan et al., 2016; Poulsen and Adelard, 2016). In addition, the selection of co-substrates is influenced by accessibility and availability as well as the transportation of the wastes (Zahan et al., 2016b).

Among the different other wastes from the neighbouring area, food wastes make up approximately 40% by weight of average household waste (IWM020, 2014). Moreover, yoghurt whey (YW), wheat straw (WS) and hay grass (HG) are also produced in considerable amounts. These solid wastes have high methane potential; but varying characteristics and composition, e.g. C/N ratio, pH, alkalinity, ammonia, structural re-calcitrance. In addition to the composition of the substrates, concentration is another significant factor for the stability and methane yield (Li et al., 2015), as excessive substrate concentration may inhibit the bio-methanation process.

Due to the role of microbes in the AD processes, kinetic models were applied to simulate the biodegradation during AD (Kafle and Chen, 2016). Understanding the phase of bacterial growth, biogas production rate showed a rising curve, the kinetics of methane production from substrates is important for designing and evaluating treatment plant (Gontupil, 2013). The first order kinetic model, modified Gompertz model, transfer function model and cone model for describing the AD processes of different wastes have been applied successfully (Gontupil, 2013; Li et al., 2015). To the best of our knowledge, no literature has reported the kinetics of methane production from the different agro-industrial wastes under the same test conditions using these four kinetic models.

The aim of this study is to investigate the anaerobic co-digestion (ACoD) of CL with varying agro-industrial wastes using the influent C/N ratio as a parameter for optimizing the ratios of different substrates in the influent. The rationale is to balance substrates addition such that it can control inhibition. This study focuses on chicken farms that have access to additional agro-industrial wastes from the neighbouring areas. It aims to assess the feasibility of AD facility in a designated area in Victoria, Australia, and seeks to convey sufficient technical detail to make results meaningful to the regions.

4.2. Materials and Methods

4.2.1 Characteristics of substrates and inoculum

The agro-industrial wastes used as potential substrates for AD were (i) CL, (ii) YW, (iii) organic fraction of municipal solid waste (OFMSW), (iv) WS, and (v) HG and collected from regional Victoria, Australia. The CL was collected from different broiler industries and kept in the plastic airtight bucket at room temperature. The CL used in this study is a complex substrate containing a high fraction of bedding materials which mostly contains lignocellulosic sawdust, wood shaving, rice hull or straw. After collection, WS and HG were ground and sieved to below 5 mm and stored in plastic screw cap container at room temperature. YW was stored in a refrigerator at 4˚C until used (Rico et al., 2015). The substrates were characterised at regular intervals for variation in characteristics in terms of TS, VS, COD and were used within a month after collection- variation was less than 5%.

In this study, OFMSW was also used. Due to the highly biodegradable nature of the OFMSW, a batch of synthetic OFMSW referred to here as synthetic food waste (FW) was prepared using compositional data collected by Sustainability Victoria, Australia (IWM020, 2014) from the Victorian garbage bin recipe and stored in small containers at -20˚C so that small portions could be used as required without losing the integrity of the whole sample. The characteristics of the samples were measured at regular intervals to examine variations in the stock samples.

The inoculum used in this experiment was collected from the anaerobic digester of Melton wastewater treatment plant, Melbourne operated at 37ºC. The characteristics of the substrates and the inoculum are shown in Table 4-1. The characteristics of lignocellulosic substrates (WS, HG and CL as it was mixed with lignocellulosic bedding materials) were also analysed for structural variations (such as carbohydrates, lignin and crystallinity) as shown in Table 4-2.

Table 4- 1: Characteristics of substrates and inoculum.

Parameters	Unit	#CL	#FW	#YW	#WS	#HG	Inoculum
##TS	%	77.2±3.3	27.9±0.3	5.20±01	82.9±5.1	84.6±1.9	3.5±0.0
##VS	%	39.1±2.5	26.3±0.4	4.42±0.06	79.78±2.85	81.1±0.5	2.62±0.04
##tCOD	mg/L	182.5±33.8*	25205±381	68550±106	ND	ND	31450±714
##sCOD	mg/L	21.15±1.15*	15900±565	42240±293	ND	ND	3410±71
##TN	mg/L	4.31±0.06*	860±13	196.5±24.7	ND	ND	199±4
Ammonium	mg/L	2.96±0.06*	111±4	129±7	ND	ND	829±24
##TP	mg/L	0.60±0.01*	2357±110	3306±174	ND	ND	350±18
VA	mgAcetic acid/L	3.56±1.12	5352±173	2572±96	ND	ND	501.5±34.6
Alkalinity	mgCaCO3/L	207±2.8	-	-	ND	ND	1260±18
pH	-	8.15±0.01	5.02±0.01	4.46±0.01	ND	ND	7.25±0.01
##C/N ratio	-	13.02±1.34	18.1±1.2	70.35±6.25	81.5±1.9	42.17±2.65	6.82±0.06

*the results are expressed in g/Kg

#CL- chicken litter; YW- yoghurt whey; WS- wheat straw; HW- hay grass; FW- synthetic food wastes

##TS- total solids: VS- volatile solids: tCOD- total chemical oxygen demand; sCOD- soluble chemical oxygen demand; TN- total nitrogen; TP- total phosphorous; VA- volatile acids; C/N ratio- carbon/nitrogen ratio

Table 4- 2: Structural carbohydrates and lignin

Substrates	Glucose (%)	Xylose (%)	Arabinose (%)	Galactose (%)	[a]AIL (%)	[b]ASL (%)	Lignin $_{ext\text{-}free}$ (%)	Extractives (%)	Ash (%)	*TCI
CL	12.55	5.09	0.94	0.91	28.05	7.59	34.4	3.44	40.75	1.898
WS	35.09	15.46	1.73	ND	20.55	7.02	24.0	12.81	4.85	1.572
HG	38.52	10.3	1.53	ND	11.72	6.45	17.7	2.81	4.22	1.371

[a]acid insoluble lignin

[b]acid soluble lignin

*Total crystallinity Index (calculated as A1375/A2900, where A1375 and A 2900 are FTIR absorbance spectra)

4.2.2 Experimental methodology

Batch tests were performed to determine the BMP of the individual substrates and varying combinations of wastes considering CL as the main substrate, mixed together to achieve the designated C/N ratios. All the BMP tests were performed in 500 mL glass bottles under mesophilic conditions according to the guideline of (Angelidaki et al., 2009). The headspace of the bottles was flushed with nitrogen gas for 2 minutes and the bottles were closed with a rubber suba seal. All batch tests were performed in duplicates. The bottles were kept at 37±1°C in an incubator shaker at a constant rotational speed of 100 rpm. Biogas measurement was carried out until the cumulative biogas production stabilised. The reported biogas volumes exclude the biogas produced from the inoculum.

4.2.2.1 Single substrates digestion

The BMP of single substrates was assessed in a batch assay conducted under the same anaerobic digestion conditions (same inoculum, mesophilic temperature, and time). The C/N ratio was maintained constant and a substrate: Inoculum (S:I) ratio of 1:2 (g/g VS) was applied. The BMP tests were undertaken with 2%, 3%, and 4% VS in the reactors (Table 3). Biogas and methane composition were analysed regularly, whereas the digestate was characterised at the end of the experiment.

4.2.2.2 Co-digestion

Co-digestion of the different wastes was carried out for two substrates (CL:YW, CL:HG, CL:WS), three substrates (CL:YW:HG, CL:YW:WS), and four substrates (CL:YW:HG:WS) combined at different ratios such that different C/N ratios were achieved for each group of wastes. The BMP of these wastes was carried out under

Table 4- 3: Composition of the feedstocks used in the BMP tests

Experiment type	Number of substrates	Substrates	Composition (g/g VS)	%VS	C/N ratio (Substrate)	Nomenclature
Single	one	CL	100	2	13.02	2% CL
				3		3% CL
				4		4% CL
	one	YW	100	2	70.35	2% YW
				3		3% YW
				4		4% YW
	one	WS	100	2	81.5	2% WS
				3		3% WS
				4		4% WS
	one	HG	100	2	42.17	2% HG
				3		3% HG
				4		4% HG
	one	FW	100	2	18.1	2% FW
				3		3% FW
				4		4% FW
Co-digestion	Two	CL:YW	90:10	3.73	19.45	YW_{10}
			70:30	3.55	25.01	YW_{30}
			50:50	3.38	27.65	YW_{50}
		CL:HG	90:10	3.83	14.34	HG_{10}
			70:30	3.84	16.66	HG_{30}
			50:50	3.84	19.99	HG_{50}
		CL:WS	90:10	3.83	14.48	WS_{10}
			70:30	3.84	17.40	WS_{30}
			50:50	3.85	22.34	WS_{50}
	Three	CL:YW:WS	90: 5: 5	3.77	17.31	YW_5WS_5
			70: 15:15	3.66	22.58	$YW_{15}WS_{15}$
			50: 25: 25	3.54	25.97	$YW_{25}WS_{25}$
		CL:YW:HG	90: 5: 5	3.77	17.40	YW_5HG_5
			70: 15:15	3.66	22.78	$YW_{15}HG_{15}$
			50: 25: 25	3.54	26.26	$YW_{25}HG_{25}$
	Four	CL:YW:WS:HG	85: 5: 5: 5	3.77	17.99	$YW_5WS_5HG_5$
			70:10:10:10	3.72	21.73	$YW_{10}WS_{10}HG_{10}$
			40:20:20:20	3.61	27.45	$YW_{20}WS_{20}HG_{20}$
			25:25:25:25	3.55	29.70	$YW_{25}WS_{25}HG_{25}$

* CL- Chicken litter; YW- Yoghurt whey; WS- Wheat straw; HW- Hay grass; FW- Synthetic food wastes

mesophilic conditions in 500mL digesters with 1:2 g/g VS of substrate to inoculum for all the reactors. The C/N ratio and substrate loading varied depending upon the mixture ratio of substrates (Table 4-3).

4.2.3 Analytical methods

TS, VS and alkalinity were measured according to APHA Methods 2540B, 2540E and 2320B, respectively (Rice et al., 2012). The COD (total and soluble), total phosphorus (TP), total nitrogen (TN), ammonium and volatile acids (VA) were determined by colorimetric techniques using a HACH (Model: DR/4000 U)

spectrophotometer according to the methods 8000, 10127, 10072, 10031 and 8196, respectively. The samples were centrifuged (Eppendorf 5702, Germany) at 4.4 rpm for 15 mins and then filtered through 0.45 µm filter paper (mixed cellulose-ester membrane filter, Advantec, Japan), to measure the soluble constituents. The pH was measured using a calibrated pH meter (ThermoOrion, Model 550A). The C/N ratio was determined using LECO (TruMac) analyser.

The composition of the lignocellulosic wastes such as structural carbohydrates, lignin, extractives and ash was determined following the procedures of National Renewable Energy Laboratory (NREL LAP 010, LAP 005 TP-510-42618 (Sluiter et al., 2011), (Sluiter et al., 2005a), (Sluiter et al., 2005c). Structural carbohydrates and sugars were analysed by high performance liquid chromatography (HPLC) connected with a refractive index (RI) detector (Thermo Fisher Scientific, Australia). The hydrolysed sugars were analysed with ion-exchange columns (Phenomonex Hi-Plex $Pb2^{+}$ followed by Na+, 7.7*300 mm, 8µm) at 85°C using 100% degassed deionised water as a mobile phase with a flow rate of 0.3 mL/min. Standard sugars of varying concentration (0.1-4 mg/mL) were prepared for making the calibration curve.

The volume of biogas was carried out with a water displacement unit. The biogas was normalised to the standard conditions (0^{o}C and 1 bar) and expressed as norm-litre (L_N). The composition of the biogas was analysed according to the APHA method 272^{0}C using gas chromatography (Varian 450-GC, Varian Australia Pty Ltd., Netherlands) equipped with a packed column (GS Carbonplot 113-3132, 1.5 µm, 30 m* 0.320 mm, stainless steel, Agilent Technologies Inc., Australia). The carrier gas used was helium at a flow rate of 28 mL/min. The temperature settings for the column, detector and injector were 70°C, 200°C and 100°C, respectively. The biogas figure 1was collected and manually injected using a 50mL FORTUNAOptima glass syringe (Poulten & Graf, Germany). Calibration was done using three points and five levels of CH4, CO2, and nitrogen (BOC, Australia).

Fourier transform infrared (FTIR) spectroscopy (Spectrum 100, PerkinElmer, USA) equipped with a universal ATR (attenuated total reflection) accessory was used for structural crystallinity analysis. The raw lignocellulosic samples were dried in an oven at low temperature (40˚C) before doing the experiments to ensure no structural damage. The spectra were obtained with an average of 64 scans from 4000 to 600 cm^{-1} with 4 cm^{-1} resolution.

4.2.4 Kinetic and statistical analysis

In this study four models; the first order regression model (Eq. (1)), the modified Gompertz model (Eq. (2)), the transfer function model (Eq. (3)), and the cone model (Eq. (4)) were chosen to fit the methane production from agro-industrial wastes. The first-order kinetics was based on the assumption that substrate availability as the limiting factor, and assumes that hydrolysis governs the overall process (Li et al., 2015). This model, however, does not predict the conditions for maximum biological activity, lag phase and system failures (Kafle and Chen, 2016). Researchers have modeled batch BMP data using first-order hydrolysis models and obtained valuable interpretations about hydrolysis kinetics (Kafle and Chen, 2016). The modified Gompertz model is an empirical non-linear regression model that describes cell density during methanogenic bacteria growth periods in terms of exponential growth rates and lag phase duration (I Nyoman and Seno, 2010). Transfer function model allows predicting the maximum methane production based only on accumulated methane production over time (Li et al., 2012) and analyzes the anaerobic digestion process as a system receiving inputs and generating outputs (Li et al., 2015). Whereas some literature has reported Cone model performs the best in fitting methane production from codigestion (El-Mashad, 2013; Li et al., 2015). Therefore, all the four models have been used to analysis the hydrolysis kinetics, lag phase duration, maximum methane production. These models are given below:

First order regression model: $B = B_0.(1 - e^{-kt})$ (4-1)

Modified Gompertz model: $B = B_0.\exp\{-\exp\left[\frac{R_m.e}{B_0}(\lambda - 1) + 1\right]\}$ (4-2)

Transfer function model: $B = B_0.\{1 - \exp\left[\frac{-R_m}{B_0}.(t - \lambda)\right]\}$ (4-3)

Cone model: $B = \frac{B_o}{1+(kt)^{-n}}$ (4-4)

Here, B= Cumulative methane yield at digestion time t, (mL/g VS)

B0= Maximum methane yield of substrate (mL/g VS)

K= Rate constant (1/d)

t= Digestion time (d)

λ= Lag phase time (d)

Rm= maximum methane production rate (mL/g VS.d)

e= exp (1) = 2.7182

n= Shape factor

The parameters were estimated for each substrate using MATLAB R2013b to obtain best fit. To compare the accuracy of the studied models, the Root Mean Square Prediction Error (rMSPE) was calculated for each model (El-Mashad, 2013).
Predictions in optimum mixture ration for substrates mixture ratio from batch test were obtained using MATLAB R2013b with surface and contour plot.

4.3. Results and Discussions

4.3.1 Single substrate digestions

The single substrates AD were carried out to investigate the BMP of agro-industrial substrates at different concentrations (2, 3, 4% VS). The cumulative and the daily biogas yields during the anaerobic digestion are shown in Figures 4-1(a)– 4-1(e) and 4-1(f) – 4-1(j), respectively. The BMP tests continued for 50 days until little or no biogas production was observed. The results presented are the net biogas yield from the feedstock after subtracting the control yield.
As shown in Figure 4-1(a)– 4-1(e), the highest biogas production was 316.73 $mL_N/g\ VS_{added}$ from CL at 3% VS loading and further increase in concentration decreased the biogas production. Of the five substrates tested, FW produced the highest amount of biogas (669.5 $mL_N/g\ VS_{added}$) at 4% VS concentration which is 2.1 times higher than the CL. The high yield from AD of FW and YW at VS concentration of 4% VS loading suggests AD at even higher solid concentrations may be possible. Among the lignocellulosic wastes, HG produced nearly 500 mL_N/g VS from all three concentrations, although 4% VS showed initial lag in biogas production. Increasing substrate concentration more than 2% showed decrease in biogas production for WS.
The per day biogas generation of lignocellulosic wastes i.e. HG, WS as well as CL was longer than the organic wastes. The daily biogas production continued in a scattered manner till 45 days for lignocellulosic wastes (CL, HG and WS) where as 80% biogas produced within 30 days for YW and SFW.

For all the reactors, the pH increased with increasing VS loading with exception of FW and ranged from 6.89-7.77 (Figure 2). For CL, TS and VS removal increased up

to 3% VS loading and then decreased. However, the ammonia concentration was below the optimum range (4 g/L) for CL. The inhibition may be because of the high lignin and crystallinity of the bedding materials that was mixed with CL. For YW and FW, TS and VS removal increased with increasing substrate loading. These results are in agreement with their biogas production. The reactors at 4% VS loadings had the maximum alkalinity. In a well stabilised digester the alkalinity ranged between 2000-5000 mg/L (Metcalf and Eddy), however this range can be vary depending upon the substrates. For wastewater operation, the optimum VA/Alkalinity ration is less than and equal to 0.1 and its expected limit is between 0.1 and 0.3. Over 0.34 inhibitions occurs. For all loadings, the VA/Alkalinity ratio was in the recommended range.

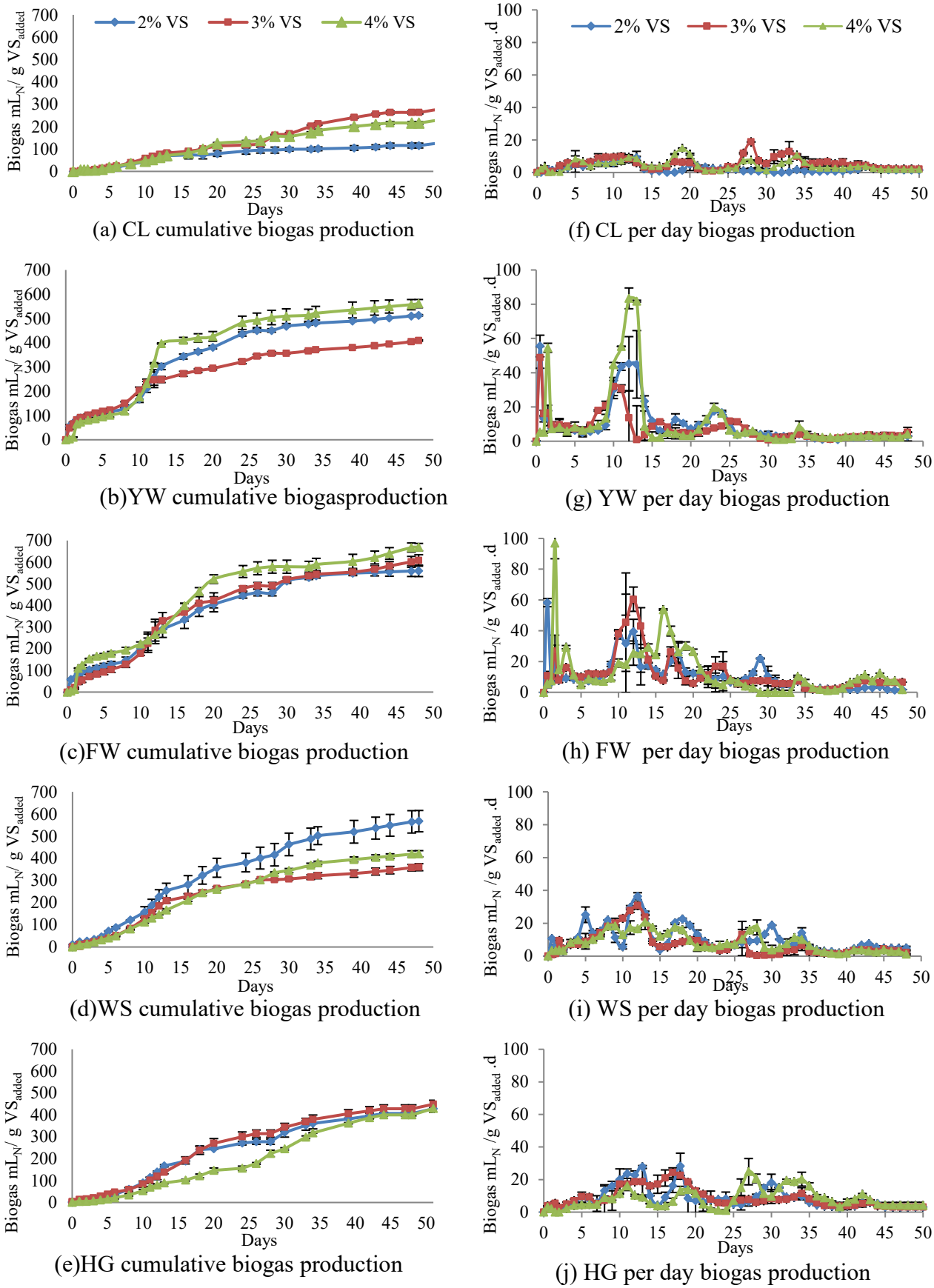


Figure 4- 1:Accumulative biogas production (a–e) and daily biogas yield (f–j) from batch experiments of single substrates.

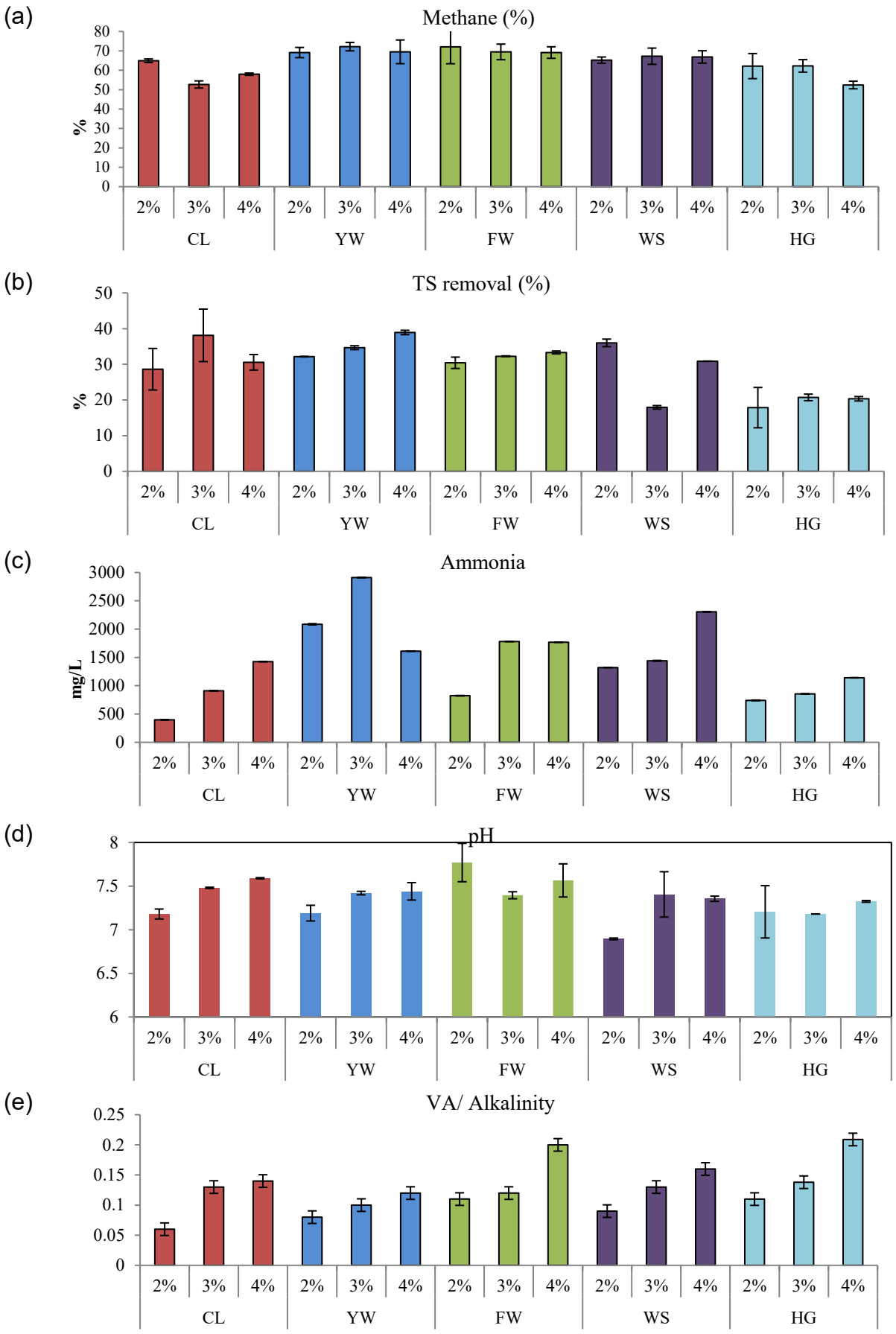


Figure 4- 2: Characteristics of final digestate of single substrates

4.3.2 Kinetic potentials of single substrates

In order to assess the performance of AD, the first order regression model, the modified Gompertz model, the transfer function model, and the cone model were studied. Parameters such as biogas yield potential, maximum biogas production rate, hydrolysis rate constant (first order) and lag phase duration were estimated for each case and summarized in table 4-4.

For all these models, the predicted final methane yield (B_0) decreased after 3% VS substrate concentration for CL. This is because the hydrolysis rate constant (first order kinetic model and cone model) was decreased from 0.03/d to 0.01/d when the substrate concentration was increased from 3% to 4% VS loading. Besides lower methane potential, longer lag phase was found with the increasing substrate concentration. The first order kinetic model only considers exponential stage in the production of biogas, therefore didn't fitted well. The modified Gompertz model was the best fit for the measured BMPs (R^2 = 0.93 to 0.99) and deviations between measured and the predicted BMPs were less than 10.0%. The low deviations obtained between the predicted and measured values (nearly equal to or lower than 10%) suggest that the proposed models predict the behavior of the reactors very accurately (Raposo et al., 2009). For HG, B_0 decreased over 3% VS concentration and for WS, B_0 decreased even after 2% substrate concentration. The hydrolysis rate constant was decreased for HG when more than 3% VS substrate concentration was added. For WS, at 2% VS substrate concentration, a high hydrolysis rate (0.06/d from first order kinetic model and 0.08/d from cone model) was observed which decreased with the increasing substrate concentration. Longer lag phase was found for both of the substrate with the increasing substrate concentration and HG had the highest lag phase among all the lignocellulosic substrate.

For high organic substrates (YW and FW), the predicted final methane yield (B_0) increased with the increasing substrate concentration. No lag phase, higher hydrolysis rate constant and maximum methane production rate was observed with the increase in substrate constant. The higher methane yield with better production rate from FW and YW is correlated with the presence of more nutrients (sCOD)

Table 4- 4: Summary of kinetic study using different models.

	Parameter		Units	CL			YW			FW			WS			HG		
				2%	3%	4%	2%	3%	4%	2%	3%	4%	2%	3%	4%	2%	3%	4%
First order kinetic model	rate constant (k)		1/d	0.03	0.03	0.01	0.07	0.05	0.07	0.05	0.04	0.05	0.06	0.02	0.03	0.03	0.03	0.02
	Methane yield	Predicted	$mL_N/g\ VS_{added}$	120.5	358.3	268.9	371.1	275.4	398.3	478.5	339.8	448.4	506.8	251.0	422.7	358.8	348.0	350.0
		measured	$mL_N/g\ VS_{added}$	108.5	166.9	136.7	354.8	255.6	390.2	416.8	292.4	413.1	370.5	242.2	281.3	307.1	312.2	258.8
		difference	%	11.11	114.7	96.69	4.60	7.73	2.08	14.81	16.23	8.55	36.78	3.65	50.29	16.83	11.47	35.26
	R-square			0.97	0.98	0.99	0.99	0.99	0.99	0.99	0.99	0.97	0.99	0.99	0.99	0.99	0.99	0.93
	rMSPE			5.47	8.10	4.81	8.93	6.24	10.17	10.66	3.76	21.35	11.75	6.00	11.66	7.56	4.41	23.94
Modified Gompertz Model	Lag phase (λ)		d	0.00	4.35	4.60	0.00	0.00	0.49	0.00	0.29	0.00	0.91	2.84	2.84	3.14	3.53	7.95
	Maximum methane production rate R_m		$mL_N/g\ VS_{added}.d$	2.29	3.85	3.00	13.86	10.00	18.96	15.28	9.54	15.72	12.77	11.82	10.22	8.48	9.02	6.41
	Methane yield	Predicted	$mL_N/g\ VS_{added}$	97.7	176.0	140.4	354.2	246.4	382.5	424.4	287.6	401.9	374.2	227.3	286.7	291.6	301.0	269.6
		measured	$mL_N/g\ VS_{added}$	108.5	166.9	136.7	354.8	255.6	390.2	416.8	292.4	413.1	370.5	242.2	281.3	307.1	312.2	258.8
		difference	%	9.93	5.46	2.69	0.16	3.62	1.97	1.83	1.63	2.71	0.99	6.13	1.94	5.05	3.59	4.19
	R-square			0.93	0.99	0.99	0.99	0.96	0.99	0.99	0.99	0.98	0.99	0.99	0.99	0.99	0.99	0.99
	rMSPE			7.82	4.69	2.34	7.16	14.55	12.98	8.65	8.28	19.31	9.86	7.32	6.39	10.08	8.27	4.78
Transfer function model	Lag phase (λ)		d	0.00	2.19	2.33	0.00	0.00	0.29	0.00	0.01	0.00	0.81	1.16	1.69	4.00	2.66	3.69
	Maximum methane production rate R_m		$mL_N/g\ VS_{added}.d$	3.03	4.12	3.35	22.90	10.83	29.64	21.96	13.17	22.54	15.86	15.87	12.20	12.43	12.76	5.84
	Methane yield	Predicted	$mL_N/g\ VS_{added}$	120.5	282.6	217.2	374.6	305.2	396.4	478.9	339.6	448.9	477.7	247.7	374.2	327.1	335.7	566.0
		measured	$mL_N/g\ VS_{added}$	108.5	166.9	136.7	354.8	255.6	390.2	416.8	292.4	413.1	370.5	242.2	281.3	307.1	312.2	258.8
		difference	%	11.11	69.34	58.87	5.59	19.38	1.60	14.91	16.16	8.67	28.93	2.29	33.05	6.51	7.53	118.7
	R-square			0.97	0.98	0.99	0.99	0.99	0.99	0.99	0.99	0.97	0.99	0.99	0.99	0.99	0.99	0.98
	rMSPE			5.47	7.15	3.89	10.16	7.01	12.57	11.01	4.21	21.18	10.44	6.02	9.41	7.84	5.04	14.37
Cone model	rate constant (k)		1/d	0.03	0.03	0.01	0.08	0.04	0.09	0.06	0.04	0.07	0.08	0.05	0.05	0.04	0.04	0.03
	Shape factor (n)			0.84	1.63	1.62	2.04	0.99	1.69	1.74	1.08	1.63	2.16	1.57	1.79	1.77	1.89	2.18
	Methane yield	Predicted	$mL_N/g\ VS_{added}$	263.0	211.9	185.0	373.9	383.4	418.9	479.5	452.4	462.9	472.7	245.6	341.0	339.7	345.1	304.7
		measured	$mL_N/g\ VS_{added}$	108.5	166.9	136.7	354.8	255.6	390.2	416.8	292.4	413.1	370.5	242.2	281.3	307.1	312.2	258.8
		difference	%	142.5	26.97	35.32	5.39	49.97	7.36	15.05	54.74	12.06	27.58	1.42	21.24	10.61	10.54	17.75
	R-square			0.97	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.98	0.99	0.99	0.99	0.99	0.99	0.99
	rMSPE			5.14	2.53	2.09	9.24	0.99	9.48	8.24	3.85	18.31	6.95	5.56	4.18	6.68	4.25	4.83

compared to the other reactors. As YW has highest sCOD, it showed highest methane production rate and highest hydrolysis rate constant among the organic substrates. The first order kinetic model showed better fit for organic substrates than the other agro-industrial substrates because of having no lag phase. However, the modified Gompertz model was the best fit for the measured BMPs (R^2 = 0.96 to 0.99) and deviations between measured and the predicted BMPs were less than 4.0%.

Overall, for all the substrates, the modified Gompertz model was the best fit for the measured BMPs (R^2 = 0.93 to 0.99) and deviations between measured and the predicted BMPs were less than 10.0%. CL, WS and HG, which are lignocellulosic in nature has higher lag phase compared to organic substrates (YW and FW), which didn't show any lag phase for substrate concentration up to 4% VS. The lignocellulosic substrates also have low hydrolysis rate except for WS at low concentration (2%). Therefore, addition of organic wastes in CL for ACoD should decrease the lag phase and accelerate the hydrolysis rate. The addition of WS at low concentration should also improve the hydrolysis rate however no improvement in lag phases.

4.3.3 Co-digestion

Considering CL as the main substrate, ACoD with other organic and lignocellulosic wastes, e.g. two, three and four substrates mixed at different ratios and at different C/N ratios were carried out in batch assays (Table 4-3). Both organic wastes FW and YW had similar biogas yield in the single substrate tests, however, from kinetic model analysis, as the YW had the highest hydrolysis and methane production rate, it was used as the organic substrate for co-substrate mixture ratio rather than FW. Lignocellulosic wastes were used to improve the C/N ratio. Overall, the cumulative biogas production increased as the percentage of agro-industrial wastes increased and the CL percentage in the feedstock decreased.

The biogas production from YW_{10} was 385.37 $mL_N/g\ VS_{added}$ which remained similar at YW_{30}. The same trend has been found for CL:HG up to 70:30 w/w VS where the C/N ratio is less than 20. However, when the C/N ratio is more than 20 i.e. the 50:50 substrates ratio for two substrates, a great improvement has been found in biogas production.

When three or more substrates had been mixed, a more balanced C/N ratio has been achieved and the biogas production improved a lot by adding 30% of the other wastes with CL. Similar amount of biogas production was observed when CL:other agro-industrial wastes has been mixed at a ratio of 50: 50 w/w VS which made a C/N ratio of 26-27.5. Adding more than 50% substrates, however, did not improve biogas much when the C/N ratio is 29 or more.

ACoD improved the biogas production rate with all of them having a single pick and the biogas production stabilised within 30 days of digestion (Figure 4-3, g-l). When CL was digested with lignocellulosic wastes i.e. with WS and HG, a small lag phase was found that was less than the lag phase for each substrate. However, When CL was digested with organic waste (YW) or combination of organic and lignocellulosic wastes no lag phase was found. The results are in conjunction with their kinetic model analysis i.e. inclusion of organic wastes decreases the lag phase.

From Figure 4 (a), increasing the amount of other agro-industrial wastes from 10% to 50% showed an increase in methane potentials ranging from 9% to 59% for two substrates and 9% to 60% for three substrates compared with the CL as a single substrate. The incorporation of four substrates showed the highest increase in methane yield of up to 85% increase in total yield.

The BMP assay of single substrates can be utilised to calculate the synergic effect of ACoD as additional methane yield over the weighted average of the individual substrates methane yield (Labatut et al., 2011). This is calculated according to Zahan et al (2016) (Zahan et al., 2016b) and shown in Figure 4-4(b). Synergistic effect was found in almost all the cases when agro-industrial wastes have been added to CL representing higher biodegradability. This is due to the adjustment in C/N ratios during the ACoD. From figure 4-4(b), when the C/N ratio is less than 20, the total improvement in biogas production is less than 10%. However, when C/N ratio is more than 20, the total improvement in biogas production is between 15-20%.

In this study, the concentration of ammonia was less than 1000mg/L for all the co-substrates mixtures. As the amount of co-substrates was increased compared to CL, i.e. at higher C/N ratio, the ammonium concentration in the co-digester decreased, showing no inhibition, lag phase and enhanced methane production. In case of ACoD of CL with the lignocellulosic substrates i.e. WS and HG, it was observed that the VS removal decreased with increasing the percentage of WS and HG. However,

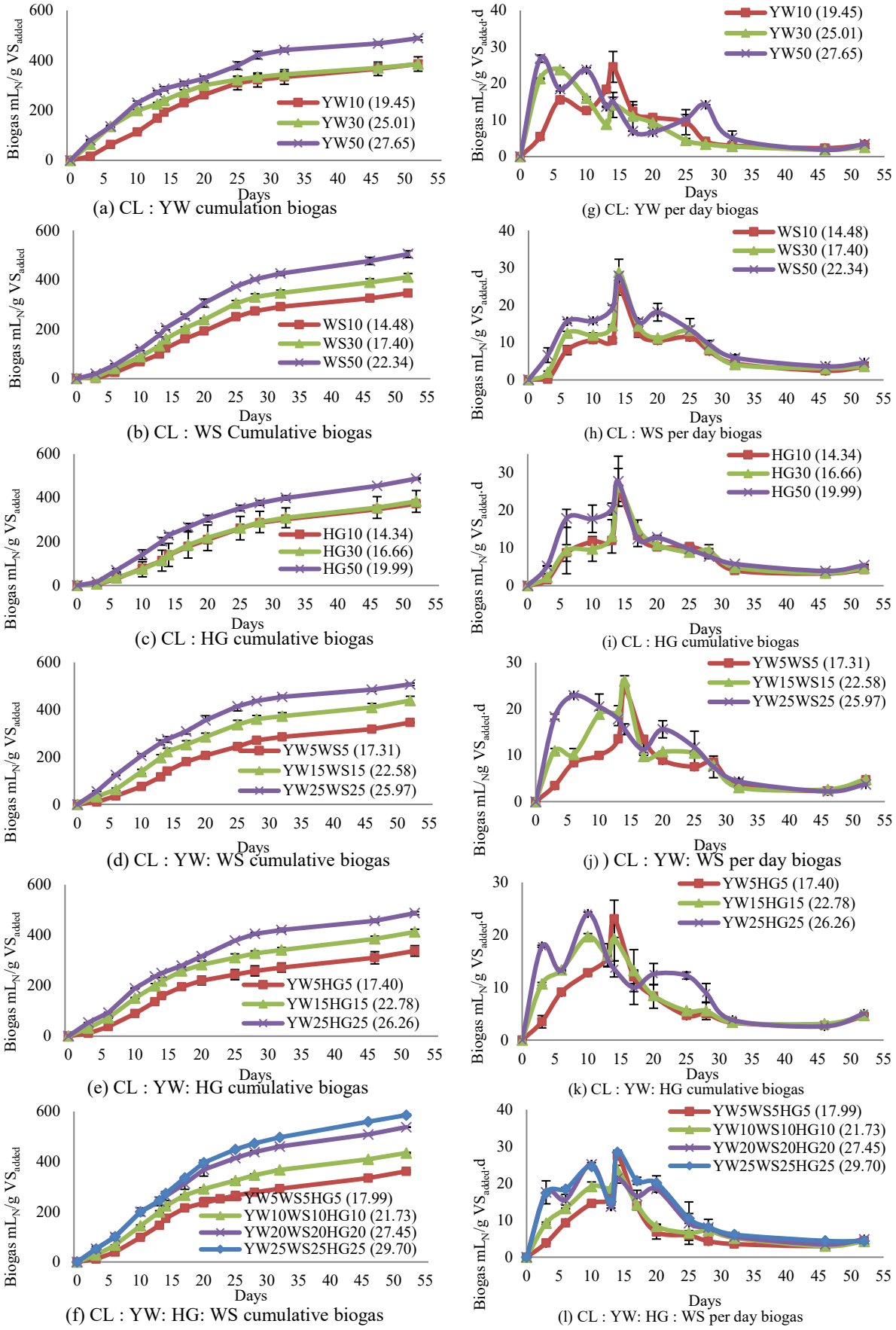


Figure 4- 3: Accumulative biogas production (a–f) and daily biogas yield (g–l) from batch co-digestion of agro-industrial wastes

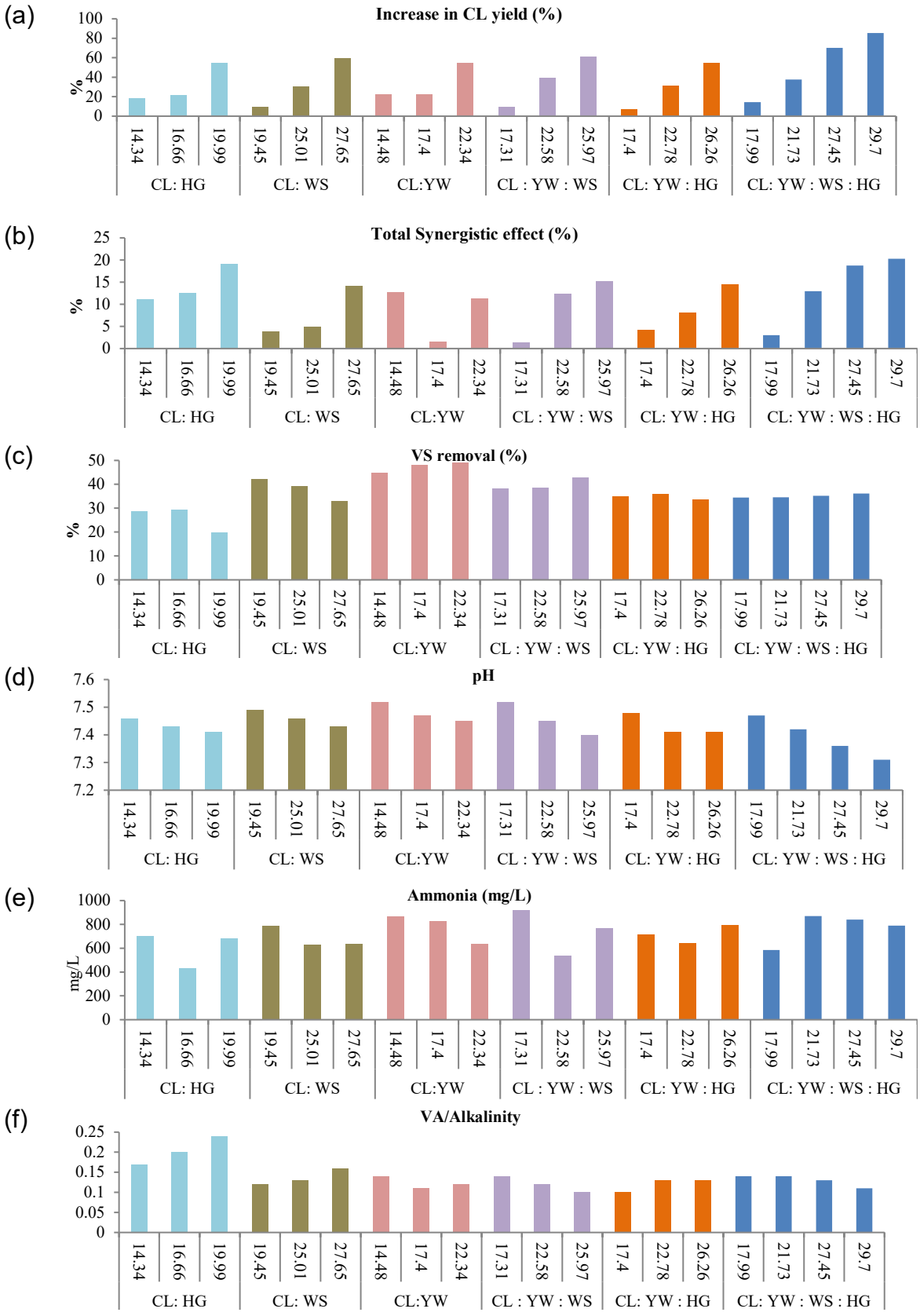


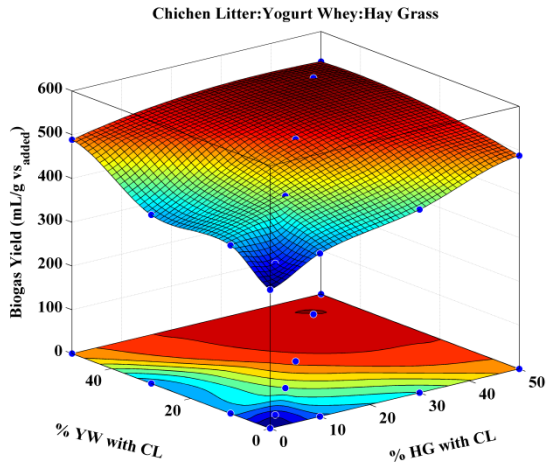
Figure 4- 4: Characteristics of final digestate of co-digestion of agro-industrial wastes

when YW, the substrate of high sCOD was added i.e. the ACoD of CL with three and four substrates, a more balanced C/N ratio and better process performances were obtained, e.g. increased VS removal. The pH was within the optimum range for all the reactors and was found to be decreased as the amount of co-substrates increased in the mixture. For all reactors, the VA/Alkalinity ratio was in the recommended range.

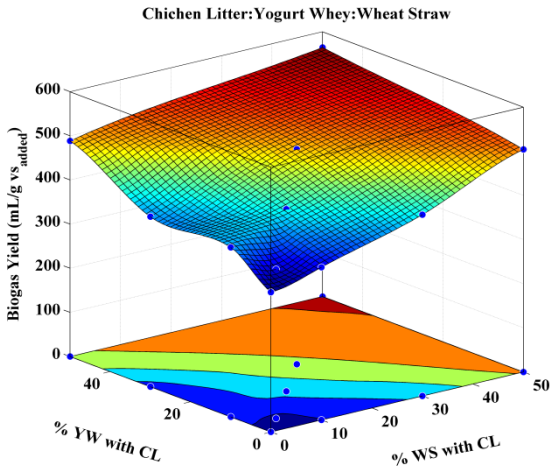
4.3.4 Optimum substrate mixture ration

Figure 4-5 shows the 3D model prediction of optimum combinations and ratios of agro- industrial wastes incorporated with CL, where the percentage of wastes in the substrate mixtures (i.e. WS, HG, YW) with CL are in the x and y axes and methane yield is the z axis. The red regions show the optimum conditions and the dark red area represents the maximum methane yield region. However, the figures show the reactors that had CL as the main substrate with up to 50% did not reach the optimum region. According to Figure 4-5, Only the CL:YW:HG (Figure 4-5 (a)) reached the maximum biogas yield region, however for all the mixtures combinations the optimum condition obtained were for CL (30-35%) mixed with (65-70%) mixture of YW, HG and WS to achieve a C/N ration of 26-27.5.

(a)



(b)



(c)

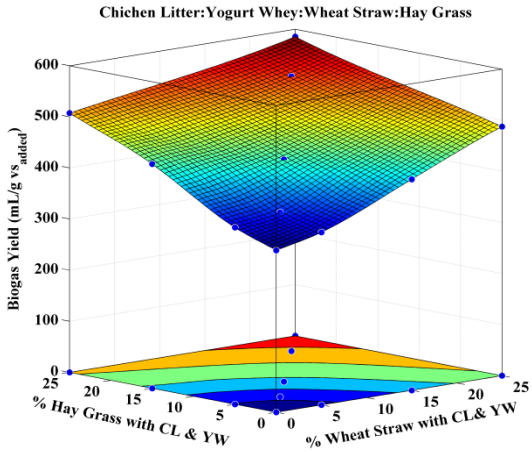


Figure 4- 5: 3D prediction of optimum substrate mixture ratio (a) CL: YW: HG (b) CL: YW: WS (c) CL: YW: WS: HG

4.4. Conclusions

CL, HG, FW, YW and WS have variations in characteristics and composition. The lignocellulosic wastes (CL, WS and, HG) showed longer lag phase and lower biogas production rates with the increase in their ratio into substrate mixture. This work also demonstrated by the kinetic model study. The AD of FW and YW did not show symptoms of inhibition to biogas production for up to 4% VS solid concentration. ACoD of agro-industrial wastes is beneficial not only enhancing the methane yield but also for improving the process performances as it balances the C/N ratio in the reactors. The optimum ACoD of the waste tested was observed condition was found at a C/N ratio of 26-27.5 with 35-40% CL and 65-70% other agro-industrial wastes.

This study addresses a significant gap in the knowledge concerning the

understanding of the ACoD of the multiple wastes and provides important data concerning the characteristics of different agro-industrial waste streams and their kinetic and co-digestion potential parameters in AD.

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Chapter 5 Semi-continuous Anaerobic Co-digestion of Chicken Litter with Agricultural and Food Wastes: A Case Study on the Effect of C/N Ratio, Substrate Mixing Ratio and Organic Loading

In this chapter, the semi-continuous anaerobic digestion potential of CL and agro-industrail wastes is investigated for process performance and stabilisation. The experimental design is done in such a way that the C/N ratio of the substrates mix become 20 or higher according to the biogas production potential and synergistic effects found during co-digestion batch assay, presented in the previous chapter. A new concept that C/N ratio and lignocellulosic structure degradation through fractionation are two important and interdependent factors for lignocellulosic agricultural substrates during AD, is reported to understand any variation in the process performance and biogas production. The effect of organic loading rate and feed concentration on biogas production during continuous AD are also reported here. The interection and correlation between different process performances parameters is shown to better interpret the results and process.

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Abstract

In this study, four agro-industrial substrates, chicken litter (CL), food waste (FW), wheat straw (WS) and hay grass (HG) were assessed as feedstock for anaerobic digestion (AD) under semi-continuous conditions at organic loading rates (OLRs) of 2.0-3.0 g TS/L.d and hydraulic retention time (HRT) of 20 days. Six different substrate mixtures were prepared such that the C/N ratio of each was 20 or more. Using principal component analysis 68.1% of data variability was explained. Biogas production from CL, as single substrate, was 181.3±9.8 mL_N biogas/g VS_{added} at OLR of 2.0 gTS/L.d. The optimum substrates mixture was CL: FW:WS 60:20:20, where 73.0%, 167.2% and 116.9% increase in total biogas production at OLR of 2.0, 2.5, 3.0 gTS/L.d, respectively, compared to that from CL, was obtained. Digestate sequential fractionation revealed carbohydrate degradation is an important factor that can explain the variation in performance and production of biogas for feedstocks of balanced C/N ratio.

5.1. Introduction

The sustainable management of the increased amounts of solid organic wastes such as manure, lignocellulosic, industrial and organic wastes have emerged as an area of major concern worldwide (Bong et al., 2018; Zahan et al., 2018c). Among the different waste management technologies, anaerobic digestion (AD) has received attention for offering biogas of high calorific value, less sludge production, small footprint, lower overall operating and maintenance costs and promoting renewable alternatives (Demirel and Yenigün, 2002; Mao et al., 2015).

In Victoria, Australia, around 0.45 million tonnes of chicken litter (CL) and considerably large amounts of other agro-industrial wastes are produced every year. Although these wastes have high bio-energy potential, varying characteristics and complex structure have limited their use as a resource for bio-energy production (Abouelenien et al., 2010; Hassan et al., 2016; Nie et al., 2015; Pardo et al., 2017; Riya et al., 2016). CL for example, comprises manure mixed with bedding materials such as wood shavings, rice hulls and straw that can amount to around 70% of the CL (Ogunwande et al., 2008). CL also has high level of proteins and amino acids which are precursors for ammonia toxicity and inhibition of volatile acids during digestion (Abouelenien et al., 2010; Chen and Jiang, 2014; Nie et al., 2015). This inhibition can be accelerated with organic overloading either by high solid loading or low carbon: nitrogen (C/N ratio) (Abouelenien et al., 2009; Zahan et al., 2018c).

Ammonia, an important indicator of AD inhibition is produced by the biological degradation of proteins and urea (Niu et al., 2013) where unionised ammonia (NH_3) or free ammonia accumulates as an actual toxic agent to anaerobic microorganisms, particularly methanogens (Borja et al., 1996; Liu et al., 2012). This also causes an increase in pH with increased toxicity (Chen et al., 2008; Liu et al., 2012). The most effective mechanism that can overcome inhibition is reducing the precursors for ammonia formation in the AD process (Karthikeyan and Visvanathan, 2012a). This can be achieved by adjusting the C/N ratio of the feedstock using carbon rich biomass substrates through co-digestion (Karthikeyan and Visvanathan, 2012a; Wang et al., 2012).

Numerous studies have shown that anaerobic co-digestion (ACoD) of CL with other organic waste streams such as agro-industrial wastes could achieve a nutrient-balance i.e. better C/N ratio and optimal pH, as well as increase specific methane yield (Shen and Zhu, 2016b; Siddiqui et al., 2011; Wang et al., 2012; Zahan et al., 2018c) . Most studies have focused on mixing substrates at different ratios to form feedstocks and on the OLRs. But there have been few studies on optimization of process performance based on feedstock's C/N ratio (Abouelenien et al., 2014; Hassan et al., 2016; Poulsen and Adelard, 2016). A review on the anaerobic co-digestion (ACoD) of animal manure and crop residues substrates revealed that ACoD at a proper percentage of C- and N-rich substrates can significantly increase the biomethane potential (BMP) (Esposito et al., 2012). It was also reported that, C/N ratio between 20 and 30 is considered optimal but there are indications that stabilised production can be achieved within a wider ranges of C/N ratios, e.g. 9-30, depending on the substrates. Therefore, special attention should be given to optimisation with the aim of AD process stability (Siddiqui et al., 2011; Vivekanand et al., 2018; Zahan et al., 2016b). There are however, only few studies published regarding the the effect of balancing the feedstock C/N ratio on the AD of agro-industrial wastes (Zahan et al., 2018c). In addition its effect on reactor performance especially under continuous flow conditions has not been reported.

The selection of substrates for ACoD was done based on their availability, quantities as well as transportation (Pokój et al., 2015; Zahan et al., 2016b). Among the different other wastes from the neighbouring areas, food wastes (FW) represents approximately 40% by weight of average household wastes and around 887,000 tonnes/yr were sent to landfill (SustainabilityVictoria, 2018). Moreover, wheat straw (WS) and hay grass (HG) are also produced in considerable amounts. These solid wastes have high methane potential; but varying characteristics and composition, e.g. C/N ratio, pH, alkalinity, ammonia, structural recalcitrance. In addition to the composition of the substrates, concentration is another significant factor for the stability and methane yield (Li et al., 2015), as excessive substrate concentration may inhibit the bio-methanation process.

The majority of studies on methane production during anaerobic co-digestion have been carried out using feedstock consisting of two or three different biomass

materials, whereas only a few studies considered feedstock to AD through mixing of four biomass materials or more (Poulsen and Adelard, 2016; Zahan et al., 2016a). Given that most large-scale co-digestion plants often digest materials from several different sources (Li et al., 2014a; Poulsen and Adelard, 2016), this study will focus on the ACoD of feedstock consisting of three or more biomass materials, specifically improving the C/N ratio, in order to better understand the potential for improving biogas production.

Most agro-industrial wastes i.e. WS, HG or even CL have bedding materials with a complex lignocellulosic structure which slowly degrades during anaerobic digestion (Li et al., 2014b; Mohseni Kabir et al., 2014; Mohsenzadeh et al., 2012; Zhang et al., 2014a). Therefore, it is important to understand the mechanism of how lignocellulosic wastes breakdown during continuous AD to improve of methane yield and stability of the process (Park and Kim, 2012; Paul and Dutta, 2018).

C/N ratio and lignocellulose structure degradation through fractionation are two important factors for agro-industrial substrate degradation. With these two parameters, it will be possible to understand any variation in the process stability and performance, however, very little research has focused on these two parameters (Zahan et al., 2017).

Most of the anaerobic co-digestion published research studies were carried out under batch conditions. But under batch conditions methane yield is usually higher compares continuous flow conditions (Pokój et al., 2015; Zahan et al., 2017) and it is also difficult to determine the cause of the process stability i.e. changes in volatile acids concentration or digestate composition (Zahan et al., 2016b). A few reports have correlated the effect of OLR on anaerobic co-digestion (Pokój et al., 2015; Shen et al., 2018). It has been consistently and widely believed that OLR can reflect the capacity of anaerobic digestion and this has become a key technical factor for controlling and efficient operation of anaerobic digestion of agricultural waste (Shen et al., 2018). To date no study has looked at the combined effect of OLR, C/N ratio and degradation of substrates through digestate fractionation. Considering that continuous flow AD is usually the mode of operation in full-scale digestion (Abouelenien et al., 2014) and that the knowledge of process stability and

performance of continuous AD for the ACoD of CL, agricultural and FW is still limited so far (Poulsen and Adelard, 2016), this study seeks to fill this knowledge gap.

The main objective of this study is to improve the continuous ACoD efficiency of agro-industrial wastes and investigate the relationship between the substrates carbohydrate composition and C/N ratio with biogas production at different OLRs. To achieve this, sequential fractionation of agro-industrial wastes was conducted during anaerobic digestion to understand how wastes behave. The rationale is to balance the addition of substrates such that it can control inhibition and ensure process stability.

5.2. Materials and Methods

5.2.1 Characteristics of substrates and inoculum/ sample preparation

In this study, four different agro-industrial wastes, CL, FW, WS, and HG were chosen as potential substrates for semi-continuous AD. The CL, WS and HG were collected from regional Victoria, Australia whereas the FW was prepared. The CL was stored in plastic airtight buckets as described by (Stephenson et al., 1990). The WS and HG were ground and sieved to smaller than 5 mm for better mixing and stored in airtight containers at room temperature (Zahan et al., 2018c). For this study, a batch of synthetic food waste referred to here as food waste (FW) was prepared using compositional data collected by Sustainability Victoria, Australia (IWM020, 2014) and stored in small containers at -20˚C (Zahan et al., 2018c). The characteristics of the samples were measured at regular intervals to observe any variations in the stock samples.

The inoculum used in this experiment was collected from the mesophilic anaerobic digester at Melton wastewater treatment plant, Melbourne and kept at 37˚C under anaerobic conditions for several days in order to minimise its background biogas production.
The characteristics of the substrates and the inoculum are summarised in Table 5-1. The lignocellulosic substrates were also analysed for structural carbohydrates, lignin and crystallinity (Table 5-1).

5.2.2 Semi-continuous anaerobic digestion

CL was mixed with the agro-industrial wastes WS, HG and the FW at the designated ratio of manure to waste (Table 5-2) and used as feedstocks. The experiments were carried out in 500 mL glass reactors, designed to allow feeding and nitrogen flushing simultaneously, at 37±1°C in an incubated shaker at a constant rotational speed of 100 rpm. Reactors were operated in triplicate for each condition. The reactors received the substrates at a concentration between 4% and 6% TS and OLR between 2.0 and 3.0 kg TS/m^3.d. The reactors were operated at a sludge retention time of 20 days (equivalent to hydraulic retention time, HRT, in this case) and were fed and wasted semi-continuously (intermittent mode, 24hr). The biogas was collected daily before feeding the reactors. Measurements of the biogas, feeding and wasting were done within a 15 min window out of the incubator. The reactors were monitored weekly for biogas quality, TS, VS, VA; and the digestate was analysed at the end of each cycle for total COD (tCOD), soluble COD (sCOD), FOS/TAC, and NH_4 as well as fractionation for water soluble content, lignin, cellulose and hemicellulose. The pH was measured every alternating day.

Table 5- 1: Characteristics of substrates and inoculum used in this study

Parameters	Unit	CL	FW	WS	HG	Inoculum
TS	%	77.2±3.3	27.9±0.3	82.9±5.1	84.6±1.	3.5±0.0
VS	%	39.1±2.5	26.3±0.4	79.78±2.	81.1±0.	2.62±0.1
tCOD	mg/L	182.5±33.8	25205±38	ND	ND	31450±7
sCOD	mg/L	21.2±1.2*	15900±56	ND	ND	3410±71
Ammonia	mg/L	2.96±0.1*	111±4	ND	ND	829±24
TP	mg/L	0.60±0.0*	2357±110	ND	ND	350±18
VA	mg Acetic	3.6±1.1	5352±173	ND	ND	501.5±34
Alkalinity	Mg $CaCO_3$	207±2.8	-	ND	ND	1260±18
pH	-	8.2±0.1	5.0±0.1	ND	ND	7.3±0.1
C/N ratio	-	13.0±1.3	18.1±1.2	81.5±1.9	42.2±2.	6.8±0.1
Cellulose	%	12.64	10.62	36.89	38.38	19.82
Hemi-cellulose	%	22.67	23.54	28.59	22.57	14.01
Lignin	%	49.82	1.67	9.92	13.85l	7.95
Water soluble	%	14.19	53.52	20.58	21.75l	25.29
Ash	%	40.75	ND	4.85	4.22	ND

* Unit are expressed as mg/g

Table 5- 2: Experimental design of intermittent anaerobic digestion

Substrate	Composition	Nomenclature	C/N ratio	HRT (days)	Day 8-63	Day 64-115	Day 116-165
CL	100	CL_{100}	13.15	20			
CL: FW	60:40	FW_{40}	15.35	20	ORL 2.5 g TS/ L.d at 5% feed	ORL 3.0 g TS/ L.d at 6% feed	ORL 2.0 g TS/ L.d at 4% feed
CL: WS	60:40	WS_{40}	23.75	20			
CL: HG	60:40	HG_{40}	22.09	20			
CL: FW: WS	60:20:20	$FW_{20}WS_{20}$	21.87	20			
CL: FW: HG	60:20:20	$FW_{20}HG_{20}$	22.68	20			
CL: FW: WS: HG	60:14:13:13	$FW_{14}WS_{13}HG_{13}$	22.43	20			

5.2.3 Analytical methods

TS and VS were measured by gravimetric analysis according to the Standard Method 2540B and 2540E respectively (Rice et al., 2012). The tCOD and sCOD were measured using HACH method 8000. The total phosphorus (TP), total nitrogen (TN), ammonium, volatile acids (VA) were measured by colorimetric techniques using a HACH (Model: DR/4000 U) spectrophotometer according to methods 10127, 10072, 10031 and 8196, respectively. The samples were centrifuged (Eppendorf 5702, Germany) at 4.4 rpm for 15 mins and then filtered through a 0.45 µm filter paper (mixed cellulose esters membrane filter, Advantec, Japan), to measure the soluble constituents. Measurement of pH was carried out using a calibrated pH meter (ThermoOrion, Model 550A). The C/N ratio was determined using a LECO (TruMac) analyser and alkalinity was measured according to APHA method 2320B (Rice et al., 2012).

The volume of biogas was normalised to standard conditions comprising dry gas, standard temperature and pressure (0ºC and 1 bar) according to method described by (Strömberg et al., 2014)) and results are presented as norm-liter (L_N). The headspace was corrected for methane (CH_4) and carbon dioxide (CO_2) to 100% according to VDI 4630 (2006) (VDI, 2006). The composition of the biogas was analysed according to APHA method 2720C using gas chromatography (Varian 450-GC, Varian Australia Pty Ltd., Netherlands) equipped with a packed column (GS Carbonplot 113-3132, 1.5 micron, 30m* 0.320 mm, stainless steel, Agilent Technologies Inc., Australia) and a thermal conductivity detector. The carrier gas

used was helium at a flow rate of 28 ml/min. Temperature of the column, detector and injector were 70°C, 200°C and 100°C, respectively. The biogas was collected and manually injected using a 50 mL FORTUNA® Optima glass syringe (Poulten & Graf, Germany). The sequential fractionation of substrates and digestate was done according to the method reported by (Opatokun et al., 2015)).

5.2.4 Statistical analysis

The AD runs were designed according to the general full factorial design with two factors- substrate mixture (seven levels) and OLR (3 levels) in three replicas using the statistical software Minitab 17.1.0. Main effects and interaction of AD process performances in relation to feedstock composition, i.e. substrate mixture, and OLR were analysed and the conditions at which an optimum performance can be achieved were determined.

Data were also analysed by multivariate statistics (principal component analysis, PCA and cluster analysis) and univariate statistics (analysis of variance, ANOVA). Multivariate statistics were applied to all the experiments' variables. PCA analysis was undertaken to establish the relationships between variables studied. The data used for the PCA were the co- digestion process performance parameters. The principal components (PCs) were selected to explain why more than 70% of data variance occurred (Cestonaro et al., 2015). Cluster analysis (CA) of the experiments' variables was performed based on the matrix of Euclidean distances by average linkage with hierarchical aggregation clustering. Both multivariate techniques were applied to all the variables observed. ANOVA was used to compare the biogas production from the different substrate mixtures.

5.3. Results and Discussion

5.3.1 Effect of substrate composition and C/N ratio on semi-continuous ACoD of CL

The designated substrate mixtures shown in Table 5-2 were used as feedstocks for the AD reactors. CL (single substrate) was utilised for continuous AD as well as with agro-industrial substrates for two, three and four substrate mixtures in different reactors to improve the C/N ratio.

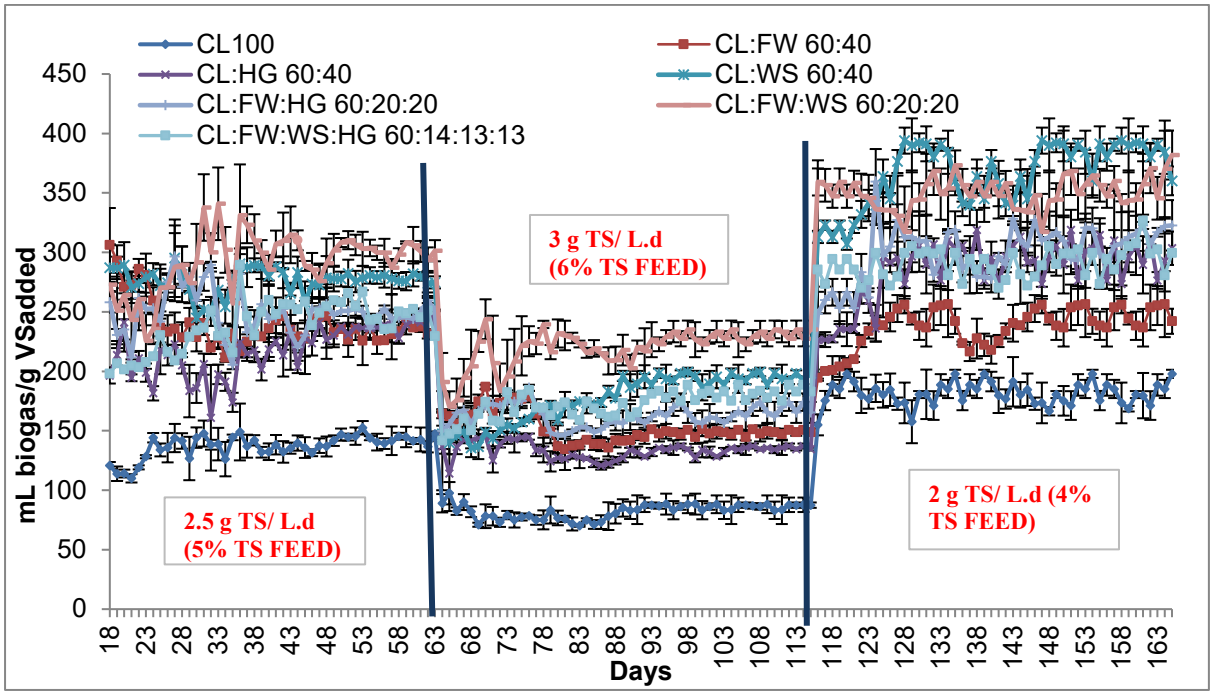

Figure 5- 1: Daily biogas production during the co-digestion of agro-industrial wastes at different substrates mix ratio

From Table 5-2, in all the mixtures, where CL was considered to be the main substrate, 60% CL (VS-based) was utilised. CL was mixed with agro-industrial substrates and FW as different substrate mixtures, in order to balance the C/N ratio more than 20 according to (Zahan et al., 2018c)). All the substrate mixtures had a C/N ratio higher than 20, except for $FW_{40,}$ where both the substrates had a low C/N ratio. The average amounts of daily biogas produced using different feedstocks tested under semi-continuous flow AD is shown in Figure 5-1. The biogas yield from CL as a single substrate (CL_{100}) was 181.3±9.8 mL_N biogas/g VS_{added} (at 4% TS feed). It can be seen from Figure 5-1 and Table 5-2 that for $FW_{40,}$ where the feedstock had a C/N ratio of 15.35 i.e. slightly improved C/N ratio compared to CL_{100} (C/N ratio of 13.15), there was a 31.02% increase in biogas production than $CL_{100.}$ This means that C/N exerted a major influence on biogas yield. This also constituted the poorest improvement in biogas production compared to all the other feedstocks. The reactors that received feedstocks of C/N ratio higher than 20 performed well in terms of biogas yield (almost 102% improvements in total). This result is consistent with the BMP batch results (Zahan et al., 2018c), where higher biogas production was observed at a C/N ratio over 20.

Also, the results (Figure 5-1) show that for feedstock containing HG, less biogas produced (285.4±13.3 mL_N biogas/g VS_{added}) compared to the feedstock that had WS (350.5±10.1 mL_N biogas/g VS_{added}). This is despite both of them having a similar C/N ratio. Furthermore, the optimum biogas production was obtained for feedstock $FW_{20}WS_{20}$ (C/N ratio of 21.87), where the C/N ratio was slightly less compared to other substrates mix of balanced C/N ratio.

Therefore, mixing CL with different agro-industrial substrates and FW, improved not only the C, N balance (i.e. C/N ratio) but also the daily biogas production. The same trend was observed when poultry droppings were co-digested with lignocellulosic co-substrates, namely wheat straw and meadow grass (Rahman et al., 2017). The effect of balanced C/N ratio on biogas improvement was also described by (Esposito et al., 2012)) and (Zahan et al., 2018c)), but they carried out their experimental work in batch AD reactors. However, at a similar C/N ratio, substrate composition had an effect on biogas production. This scenario is explained in more depth in section 3.5 of this paper.

5.3.2 Effect of OLR on semi-continuous ACoD of CL

The average daily biogas (reported as mL_N/gVS_{added} fed to the reactor) results were measured for OLRs ranging from 2.0 to 3.0 g TS/L.d up to 165 days. From Figure 5-1, the biogas yield from CL as a single substrate (CL_{100}) produced biogas of 129.0±6.9 mL_N biogas/g VS_{added} for OLR of 2.5 gTS/L.d. The biogas production fell to 82.4±3.3 mL_N biogas/g VS_{added} when the OLR rose from 2.5 to 3.0 gTS/L.d. Biogas production yield improved again to 181.3±9.8 mL_N biogas/g VS_{added} when the OLR decreased to 2.0 gTS/L.d. The same trend was observed for the other substrate mixtures. However, semi-continuous ACoD improved the biogas production better than the CL_{100} as a single substrate at the same OLR.

For the OLR of 2.5 gTS/L.d (left-hand side of Figure 5-1), the increase in biogas for semi-continuous ACoD ranged between 57.0 to 117.0% compared to CL_{100} (129.0±6.9 mL_N biogas/g VS_{added}) in the order of HG_{40} (57.0%) < $FW_{14}WS_{13}HG_{13}$ (72.7%) < $FW_{20}HG_{20}$ (80.4%) < FW_{40} (86.6%) < WS_{40} (105.7%) < $FW_{20}WS_{20}$ (117%). These increments in biogas production can be divided in three categories. In the first category, where more than a 2-fold increment in biogas was observed for the mixing ratios $FW_{20}WS_{20}$ (C/N ratio of 21.87) and WS_{40} (C/N ratio of 23.73) with the

associated biogas production of $_{279}$.9± 9.8 mL_N biogas/ VS_{added} and 265.5±15.3 mL_N biogas/ $VS_{added,}$ respectively, compared to CL_{100} (129.0±6.9 mL_N biogas/g VS_{added}). In the second category, around 1.7- 1.85-fold increments in biogas were observed for $FW_{14}WS_{13}HG_{13}$ (C/N ratio of 22.43), $FW_{20}HG_{20}$ (C/N ratio of 22.68) and FW_{40} (C/N ratio of 15.35) with the amounts of biogas produced being 222.9±10.7, 232.8±6.5 and 240.8±11.9 mL_N biogas/ VS_{added} respectively. In the third category, a smaller than 1.6-fold increment in biogas production was observed for HG_{40} (C/N ratio of 22.09) with a value of 202.6±5.7 mL biogas/ $VS_{added.}$ This is almost 38% lower than the first category. It is worth noting that although FW_{40} had a lower C/N ratio than HG_{40}, it produced more biogas at OLR 2.5 gTS/L.d i.e. 5% TS feedstock. The FW produced higher amount of biogas than HG at the very start of the experiment, mainly due to the high soluble COD content (Table 5-1). But with the continuous operation of the reactors, the FW soluble organics and low C/N lead to non-stable conditions, e.g. accumulation of VAs. These conditions resulted in lower biogas yield compared to HG which has a higher C/N ratio.

When the OLR was increased to 3 gTS/L.d (middle portion of Figure 5-1), almost 20-36% decline in biogas production was observed compared to OLR 2.5 gTS/L.d. However, 9.3-167% increments in biogas production were reported for semi-continuous ACoD compared to $CL_{100\ at}$ the same OLR 3 gTS/L.d in the order of FW_{40} (9.3%) < HG_{40} (61.8%) < $FW_{20}HG_{20}$ (97.7%) < $FW_{14}WS_{13}HG_{13}$ (109.4%) < WS_{40} (115.4%) < $FW_{20}WS_{20}$ (167.2%). This increment in biogas production clearly had four ranges. In the first range, almost 2.7-fold increase in biogas was observed for $FW_{20}WS_{20}$ (220.1±11.4 mL_N biogas/VS_{added}). In the second range, a doubled increase was observed for WS_{40} (177.4±7.9 mL_N biogas/ VS_{added}), $FW_{14}WS_{13}HG_{13}$ (172.5±20.1 mL_N biogas/ VS_{added}) and $FW_{20}HG_{20}$ (162.8±4.6 mL_N biogas/ VS_{added}). In the third range, a 1.6-fold increase in biogas production was noted for HG_{40} (133.3±8.4 mL_N biogas/VS_{added}) which was 39.4% lower than the best combination. Finally, with reference to the fourth category, only a 1.1-fold increase in biogas was observed for FW_{40} (90.02±2.1 mL_N biogas/ VS_{added}). As opposed to OLR 2.5 gTS/L.d, FW_{40} produced the smallest amount of biogas among all the mixture combinations because it had the lowest C/N ratio. It can be also seen that as the OLR increased to 3 gTS/L.d, combining three and more substrate mixtures were less

affected by inhibition. In other words, it led to more biogas being produced whereas the two substrate mixtures did not.

As OLR 3 gTS/L.d demonstrated an inhibitory effect (less biogas production), the OLR decreased to 2 gTS/L.d (right-hand side of Figure 5-1), which improved the overall biogas production. The increasing biogas for semi-continuous ACoD was valued between 31.0% and 101.9% compared to CL_{100} in the order of FW_{40} (31.0%) < HG_{40} (57.4%) < $FW_{14}WS_{13}HG_{13}$ (60.4) < $FW_{20}HG_{20}$ (65.6%) < $FW_{20}WS_{20}$ (93.4%) < WS_{40} (102.0%). These increments in biogas production were again visible in three categories. In the first category, a greater than 2-fold increment in biogas was observed for the substrates-mixes WS_{40} (366.0±12.7 mL_N biogas/g VS_{added}) and $FW_{20}WS_{20}$ (350.5±10.1 mL_N biogas/g VS_{added}) compared to CL_{100} (181.3±9.8 mL_N biogas/g VS_{added}). However, at this low OLR, WS_{40} suppressed $FW_{20}WS_{20}$ AD and did not make maximum biogas production possible. The second category was observed for $FW_{20}HG_{20}$ (300.2±13.1 mL_N biogas/ VS_{added}), $FW_{14}WS_{13}HG_{13}$ (290.7±9.3 mL_N biogas/ VS_{added}) and HG_{40} (285.4±13.3 mL_N biogas/ VS_{added}) with 1.6-1.65-fold increases in biogas production. In the third category, FW_{40} (237.5±15.6 mL_N biogas/ VS_{added}) was observed where a 1.3-fold increment in biogas production occurred. As the OLR increased the production of biogas from FW_{40} also rode but did not exceed substrate mixture HG_{40}. FW_{40} had the lowest C/N ratio of all the mixtures, although when the experiment started it revealed a large amount of biogas being produced, compared to some other mixtures. However, eventually it could not cope with the abrasive OLR changes and reported the lowest increment in biogas production among all the mixtures.

It can therefore be stated that among the OLRs, 2 gTS/L.d performed the best performed the best for all six substrate mixture ratios. It had a feeding concentration of 4% and $FW_{20}WS_{20}$ was observed to be the best condition. That is also evident that at low OLR, the combination of two substrate mixtures worked far better than a combination of three or more such substrate mixtures. Nonetheless, because of better mixing and balance of C/N, as the OLR increased the combination of three and more substrates mixtures worked batter than two substrates mixtures. Overall, as the organic loading increased the amount of biogas being produced decreased.

5.3.3 Processes' performance

In addition to the biogas yield, different parameters were monitored at the end of each cycle to assess the quality of the supernatant and digestate. The process performance parameters measured are summarised in Table 5-3. From Table 5-3, for CL100, the TS removal was 23.7%, 16.9% and 24.5%, respectively, and the corresponding VS removal was 27.8%, 24.4% and 31.4%, respectively, for OLRs of 2.5, 3 and 2 gTS/L.d i.e. 5%, 6% and 4% TS feed. Similarly, (Salminen and Rintala, 2002) reported VS removal of 31% at 3.1% TS feed in semi-continuous AD of poultry slaughterhouse wastes. It was observed that the VS removal under semi-continuous AD of 31.4%, is similar to the VS removal of 32.7%, obtained using BMP assays at 4% TS feed (Zahan et al., 2018c). This indicates that BMP test can be used as indication to assess performance under-continuous conditions, if well designed.

At the start, the pH was 7.6 for CL_{100} at OLR 2.5 gTS/L.d which increased to 7.9 at OLR 3 gTS/L.d. As the OLR increased, because of substrate overloading, not all the substrates were able to degrade, and this subsequently increased the pH level in the reactor. In this way, inhibition in the form of decreased TS, VS removal was evident. The pH again decreased to 7.6 at OLR 2gTS/L.d which led to an improvement in biogas production and VS removal. The same trend was observed for all the other substrate mixtures. It was also observed that the pH value remained relatively stable at around 7.1 to 7.3 for the high performing reactors which is an ideal situation for stable methanogenesis.

When a new feed was prepared at the start of a new OLR, a lag phase was noted in biogas production (Figure 5-1). Low pH and low organic content removal were also observed. This lag phase could be due to biomass adaptation and the acclimatisation of the inoculum to the new feed. However, after the lag phase the reactors indicated stable biogas production.

Co-digestion with food waste and agricultural waste improved the overall processes' performance. The removal of TS, VS and COD was around 40, 45% and 60%, for the best performing reactors $FW_{20}WS_{20}$ and WS_{40}, respectively. These results were comparable with AD performance of wastewater i.e. sewage sludge co-digestion (Davidsson et al., 2008; Silvestre et al., 2011; Zahan et al., 2016b). From Table 5-3,

the incorporation of FW improved the TS, VS and COD removal greatly; this was likely due to the biodegradability of FW being probably close to 100% (Zahan et al., 2016b). However, production of biogas did not improve significantly because the reactor's C/N ratio did not improve much (15.35). From the batch test results, the optimum C/N ratio for ACoD of agro-industrial wastes was 26.5-27.6. However, a huge improvement in biogas production can be obtained when a C/N ratio of 20 or more is used (Zahan et al., 2018c).

No accumulation of VAs was evident and the highest VA observed was HG_{40} (0.96 g/L) which is well below the inhibition threshold (4 g/L) (Siegert and Banks, 2005). The VA accumulation threshold can vary from substrate to substrate (Luostarinen et al., 2009).The accumulation of VA is what causes instability of the process and inhibits of acetotrophic methanogenesis (Girault et al., 2012). The VAs in the reactors at OLR 3 gTS/L.d increased hugely, demonstrating an inhibitory effect and much less biogas production. The ammonia-N content in all the reactors was between 0.6 and 0.71 g/L which was below the inhibition range, this being 1.5–2.0 g/L (Woon and Othman, 2012).

From the results it can be summarised that when the OLR increased, the TS removal, VS removal, COD removal and methane (%) also decreased. This was very consistent with their biogas production results. On the other hand, the pH, VA, ammonia, TN and TP tended to increase when the OLR also increased. The Specific biogas production and methane yields depended on the origin of the substrates, composition, and operational conditions (SRT, temperature). Therefore, the results reported here should be applied to a small pilot scale continuously fed anaerobic digester before they are used in a large-scale scenario.

Table 5- 3: Reactor performances during continuous digestion

Substrate	OLR (g TS/L.d.)	Methane (%)	% TS Removal	% VS Removal	% COD Removal	pH	VA mg Acetic acid/L	Ammonia (mg/L)	Total Nitrogen (mg/L)	Total Phosphorus (mg/L)
CL_{100}	2.5	64.5±5.7	23.7±3.6	27.8±1.6	44.8±6.7	7.6±0.1	564.0±32.0	507.5±15.3	1050±50.0	72.5±1.5
	3.0	60.7±3.3	16.9±2.1	24.4±8.8	38.1±5.5	7.9±0.3	838.0±45.6	647.5±30.1	1400±20.5	210.0±0.5
	2.0	65.3±2.9	24.5±4.6	31.4±1.7	47.3±3.9	7.6±0.2	456.0±25.7	485.6±10.5	875±10.5	68.5±0.5
FW_{40}	2.5	69.5±8.6	39.9±8.8	45. 8±1.4	58.2±4.4	7.4±0.3	426.0±18.7	445.0±8.7	500±20.0	75.0±1.0
	3.0	68.7±4.2	30.1±3.3	35.1±2.7	54.0±6.2	7.6±0.1	755.7±30.5	540.0±20.5	825±5.5	95.0±2.5
	2.0	70.2±2.9	40.6±6.2	45.7±4.7	63.7±3.6	7.4±0.1	575.0±23.2	450.6±9.6	425±10.0	62.3±0.5
WS_{40}	2.5	65.5±2.6	41.0±3.6	45.0±3.1	53.7±5.8	7.1±0.2	637.0±15.7	330.0±13.3	400±10.0	290.0±5.0
	3.0	66.4±2.2	32.7±2.8	36.8±1.2	48.3±4.3	7.3±0.1	754.0±12.0	350.0±10.8	475±15.5	247.5±2.5
	2.0	67.3±6.1	33.0±4.1	37.8±0.8	61.5±3.8	7.1±0.0	215.3±8.7	298.0±7.5	365±5.5	222.3±3.3
HG_{40}	2.5	61.4±6.4	33.4±3.3	38.3±1.1	46.0±2.4	7.3±0.1	909.0±32.7	300.0±10.1	450±5.5	162.5±2.5
	3.0	58.6±3.2	25.8±2.4	29.9±2.1	41.5±2.6	7.4±0.2	960.0±40.0	310.0±5.8	524±1.5	235.0±5.0
	2.0	62.3±1.9	35.6±1.9	40.5±0.5	46.3±3.2	7.3±0.1	528.7±60.0	270.0±3.2	392±2.5	132.5±1.5
$FW_{20}WS_{20}$	2.5	68.6±2.6	32.8±6.8	36.4±9.4	54.1±2.9	7.2±0.0	495.0±25.0	517.5±10.8	575±5.0	80.0±0.5
	3.0	68.2±3.2	30.1±5.1	35.3±6.7	47.9±4.8	7.3±0.0	941.0±15.0	595.0±15.7	725±5.5	162.5±2.5
	2.0	69.7±5.1	36.0±3.5	41.7±2.8	58.4±5.2	7.1±0.1	254.0±5.7	480.5±7.6	495±15.5	78.3±3.5
$FW_{20}HG_{20}$	2.5	64.1±3.5	30.3±6.9	34.6±5.7	47.0±8.8	7.3±0.2	618.0±27.3	405.0±3.8	450±10.0	222.5±5.5
	3.0	63.8±5.8	21.6±4.4	25.7±3.6	40.4±4.9	7.3±0.5	824.0±64.3	450.0±22.5	575±15.0	227.5±10.5
	2.0	65.3±6.5	35.1±2.6	41.3±3.3	50.1±3.8	7.2±0.5	520.0±20.7	355.0±9.5	380±10.0	180.5±5.0
$FW_{14}WS_{13}HG_{13}$	2.5	66.5±2.6	32.5±4.3	37.6±5.6	49.7±3.3	7.2±0.3	677.0±31.3	475.0±25.3	400±10.0	75.0±0.5
	3.0	65.1±3.6	25.6±3.7	29.4±5.5	46.4±4.6	7.3±0.2	885.0±29.3	460.0±3.5	525±5.0	202.5±1.5
	2.0	66.2±4.7	35.7±6.1	40.8±2.7	51.0±2.7	7.1±0.2	429.3±20.0	425.0±10.0	350±10.0	95.3±2.3

5.3.4 Effect of fractionation and C/N ratio

As the substrates were lignocellulosic in nature, the feedstock and digestate at the end of each cycle, during the AD tests, were analysed for cellulose, hemicellulose and lignin. For this purpose, sequential fractionation was employed, and the results are depicted in Figure 5-2.

From Figure 5-2 (a), as the OLR increased, the fraction of cellulose in the rectors increased for all the substrates mixtures. For CL_{100}, FW_{40}, HG_{40}, $FW_{20}HG_{20}$, cellulose (%) was higher, at the end of the AD cycle, for all OLRs tested, than in the feedstock. This is mainly because cellulose had degradability under the conditions tested which led to its accumulation in the reactor. Whereas, for mixtures containing WS, the cellulose (%) at the end of OLR cycles were lower than feed cellulose which correlated with the higher volumes of biogas production from these substrates mixtures i.e. WS_{40} and $FW_{20}WS_{20}$. The highest amount of degradation of cellulose was found for WS_{40} which produced the maximum biogas. As shown in Figure 5-2(b), the hemicellulose (%) in the digestate showed no significant variation for all OLRs tested almost for all feedstock, i.e. little degradation occurred under the conditions tested. These results indicated hemicelluloses were not much accessible to the anaerobic microorganism under these conditions (Zhang et al., 2014a). (Ghosh et al., 1985) reported that, cellulose was utilised in preference of hemicellulose during mesophilic and thermophilic AD in nitrogen-rich environment and were converted at a higher efficiency, compared to lower nitrogen environments, because the metabolism of cellulose breakdown requires least investment of enzymes and energy.

From Figure 5-2(c), the water soluble content in the digestate increased as the OLR decreased and was higher than the water soluble contents in the reactor at the start of the cycle. The reactors that received a feedstock containing WS, had the highest amount of water soluble content at the end of the AD cycle. This indicated high rate of hydrolysis of WS, compared to other substrates, under the conditions tested. From Figure 5-2(d), for all the reactors, lignin (%) in the digestate indicated 15-20% of lignin degraded under the AD conditions tested which indicates the presence of some cellulolytic microbes in AD (Zhu et al., 2010), however there were still 30-40% lignin remaining inside the reactors. The complex bonding of lignin with

hemicellulose limited their degradation, therefore, pre-treatment to breakdown the bondage is suggested for enhancement in their removal (Zhang et al., 2014a).

Overall, from Figure 5-2, with the variation in OLR, an inverse relationship was observed between the water soluble content and cellulose contents. This means that when increasing the OLR, more lignocellulosic substrates were added to the reactor. Therefore, more undigested cellulose was found at high OLR which was not been able to convert to soluble content and reduced the amount of biogas being made. The smallest fraction of cellulose obtained was for the $FW_{20}WS_{20}$ and WS_{40} mixtures which correlated with biogas production i.e. maximum biogas production was observed for those mixtures. As well, the highest amounts of water soluble content were found for those mixtures. The feedstock's that resulted in lower biogas production, FW_{40}, HG_{40} and $FW_{20}HG_{20}$, all experienced low cellulose degradation, thus clearly indicating the correlation of biogas production with cellulose degradation. Almost all the reactors showed some lignin degradation compared to the feed. This is because some cellulolytic activity and specific growth rate of cellulolytic microbes happens in AD. Therefore the hydrolysis of native lignocellulosic biomass usually occurs and degrades the hydrocarbon, although any growth that occurs is very small and its rate is much slower (Zhu et al., 2010).

Different agro-industrial wastes were mixed to improve the C/N ratio during ACoD. It is clear from the results that increasing C/N from 15.15 to 23.75 increased biogas production. However, the complexity of structure or cellulose degradation of the wastes could have an effect on biogas production. For example, a C/N ratio of 21.87, i.e. $FW_{20}WS_{20}$ was found to generate the most biogas which entailed degrading the largest amount of cellulose during the ACoD. This was despite the other reactors having a similar or higher C/N ratio. The reactors fed with HG mixtures have high cellulose content and low water soluble content after digestion compared to the reactors fed with WS mixtures. This may explain why the reactors fed with HG mixtures produced smaller amounts of biogas. The results showed a correlation between biogas production and carbohydrate content with varied OLR. We can conclude here that although biogas production depends on balanced C/N ratio, for lignocellulosic substrates with similar C/N ratios, biogas production is further reliant on their lignocellulosic properties.

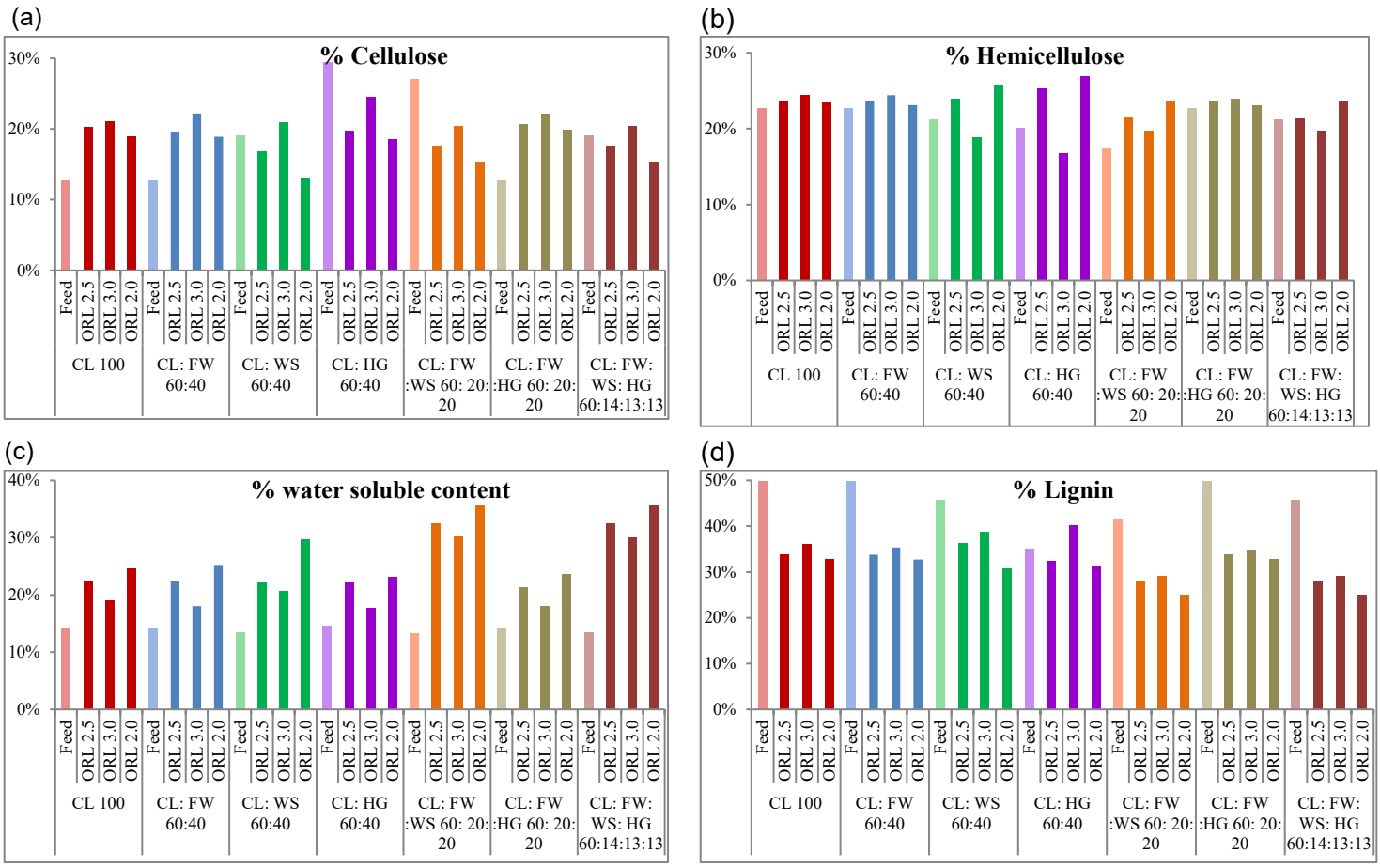

Figure 5- 2: Sequential fractionation analysis of feedstock and digestate

5.3.5 Principal component analysis

Table 4 shows the correlation coefficients between the variables used for principal component analysis (Figure 5-3). Numerous correlations of moderate to high magnitude between the variables, highlighting the inter-dependence between them and the importance of using a statistical analysis tool such as PCA. This tool can address such a complex pattern of variable inter-dependence. Two principal components (PCs) selected by PCA were able to explain 68.1% of data variability. Figure 5-3 illustrates the associations between these PCs and the variables and experimental conditions. In PC1, the most relevant variables, which explained 48.4% of data variability, were as follows: lignin (%),cellulose (%), pH, VA (mL Acetic Acid/g) (negatively correlated); water soluble content (%), COD removal (%), VS removal (%), TS removal (%), biogas (mL/g $_{VSadded}$) (positive correlation) (Figure 5-3). PC1 was characterised by a relationship between organic fraction consumption (represented by the variables having a positive correlation) and the phenomena

triggered by this consumption (represented by variables with negative correlation), these being biogas production, nutrient concentration, and sample stability changes. Thus, PC1 can be referred to as 'organic fraction consumption/productivity', because higher rates of organic fraction consumption led to higher digester productivity.

All variables related to digester productivity revealed a strong correlation with PC1 (Figure 3). Moreover, COD (%), water soluble content (%) and cellulose (%) were the organic fraction consumption variables strongly correlated with PC1. Consequently, amongst the organic fraction consumption variables, it was evident that COD (%), and cellulose (%) and water soluble content (%) levels explained most of the productivity of digesters. Meanwhile VA (mL Acetic Acid/g), lignin (%) and hemicellulose (%) levels, which displayed moderate correlation to some (but not all) productivity variables, explained only part of the biogas productivity in our experiments.

The experimental replicates of FW_{40}, WS_{40} and $FW_{20}WS_{20}$ demonstrated the highest weights among productivity variables, while experimental replicates displayed higher weights for organic fraction variables. Therefore, productivity and organic fraction

Table 5- 4: Linear correlation values (the bold value means the correlations of moderate to high magnitude between the variables)

	A	B	C	D	E	F	G	H	I	J
A	1									
B	**-0.908**	1								
C	-0.137	-0.017	1							
D	**-0.722**	0.449	-0.486	1						
E	-0.351	0.311	0.064	0.286	1					
F	-0.137	0.075	-0.263	0.389	0.397	1				
G	0.043	0.003	0.344	-0.360	-0.404	-0.547	1			
H	0.278	-0.215	0.105	-0.573	-0.591	-0.521	**0.727**	1		
I	0.273	-0.205	0.100	-0.572	-0.595	**-0.710**	**0.725**	**0.997**	1	
J	**0.891**	-0.240	0.378	**-0.781**	-0.584	-0.606	**0.767**	**0.714**	**0.715**	1

Where A- Water soluble component (%), B- Lignin (%), C- Hemicellulose (%), D- Cellulose (%), E-pH, F- VA (ml Acetic Acid/g), G- COD Removal (%), H- VS Removal (%), I- TS Removal (%), J- Biogas (mL/g VS)

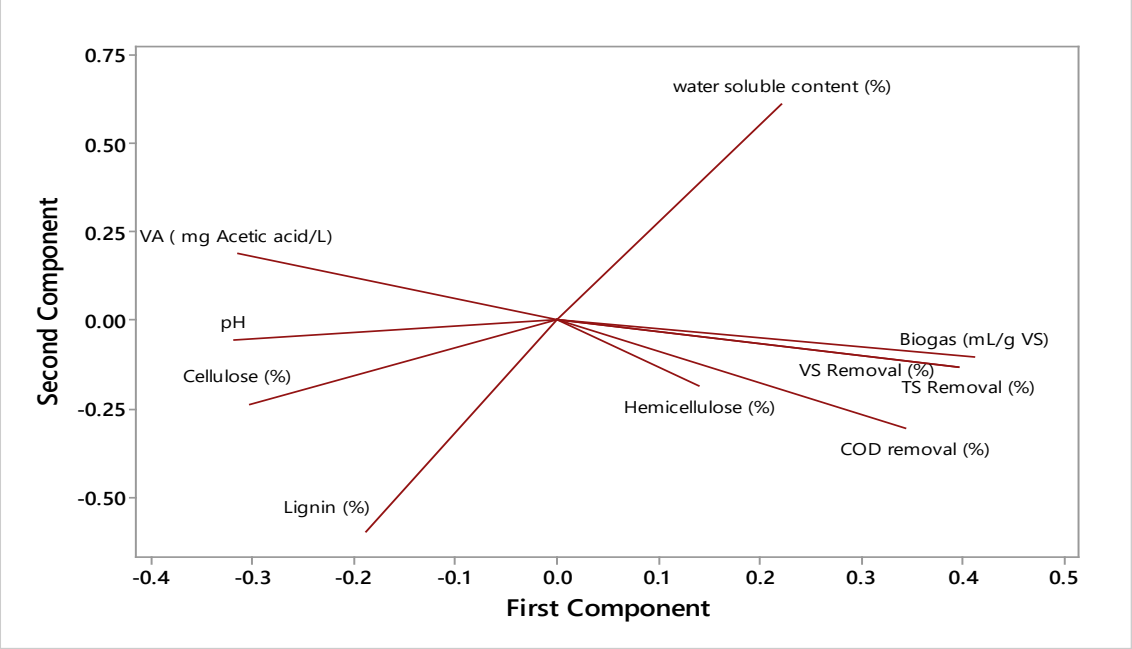

Figure 5- 3: PCA analysis relative to parameter variations during anaerobic co-digestion of agro-industrial wastes

variables were mostly influenced by manure mixtures with higher $FW_{20}WS_{20}$ or lower CL_{100} ratios, respectively. In particular, manure mixtures with $FW_{20}WS_{20}$ resulted in increased organic fraction consumption, biogas production, nutrient concentration, and biofertilizer stability when compared to mixtures with CL_{100}.

The most relevant variables for PC2, which explained 19.7% of data variability, were: firstly, lignin (%) and cellulose (%) (negatively correlated); and secondly, biogas production potential per unit of VS_{added}, water soluble content (%), VS removal (%), TS removal (%), COD removal (%) (positively correlated). In this component, cellulose levels were negatively correlated with variables describing biogas production potential, suggesting that cellulose consumption had a greater influence on biogas production when compared to that of hemicellulose (Cestonaro et al., 2015). (Yue et al., 2013) described that differences in the consumption of cellulose and hemicellulose fractions during AD varied mainly in terms of the composition of the original substrates. Also, rupture of the lignin matrix by animal digestion facilitated enzymatic access to cellulose in animal manure (Triolo et al., 2011). Furthermore if the lignin layer is no longer present, cellulases rapidly degrade the easily accessible cellulose (Cestonaro et al., 2015).

5.3.6 Main effect and interaction

Figure 4 shows the main effects and interaction plots concerning the processes' performance parameters with varied OLR at different substrate mixture ratios during AD. From Figure 5-4, the OLR had a sharply declining relationship with biogas production and COD removal (%); and a sharply increasing relationship was observed with VA. Referring to TS and VS removal (%), when the OLR rose from 2 to 2.5 gTS/L.d, a slight reduction in TS and VS removal (%) was observed. However, a sharp decrease occurred with any further increase in OLR with TS and VS removed. In case of pH, similar trend was observed with an exact inverse relationship. In all the scenarios $FW_{20}WS_{20}$ performed the best where the highest biogas production was found.

Figure 5-5 is a dendrogram produced by cluster analysis of the experimental conditions tested. Cluster analysis indicated two separate groups, the first formed by biogas (mL/g VS_{added}), TS removal (%), VS removal (%), COD removal (%) and water soluble content (%) (where TS removal and VS removal were most similar to each other) and the second represented by VA (mg Acetic acid/L), pH, cellulose (%) and lignin (%). Overall, these results confirm two things: firstly, that the ACoD of agro-industrial wastes is more efficient and secondly, there is a stronger correlation with water soluble content, cellulose and lignin degradation as well as VA (mg Acetic acid/L) and pH.

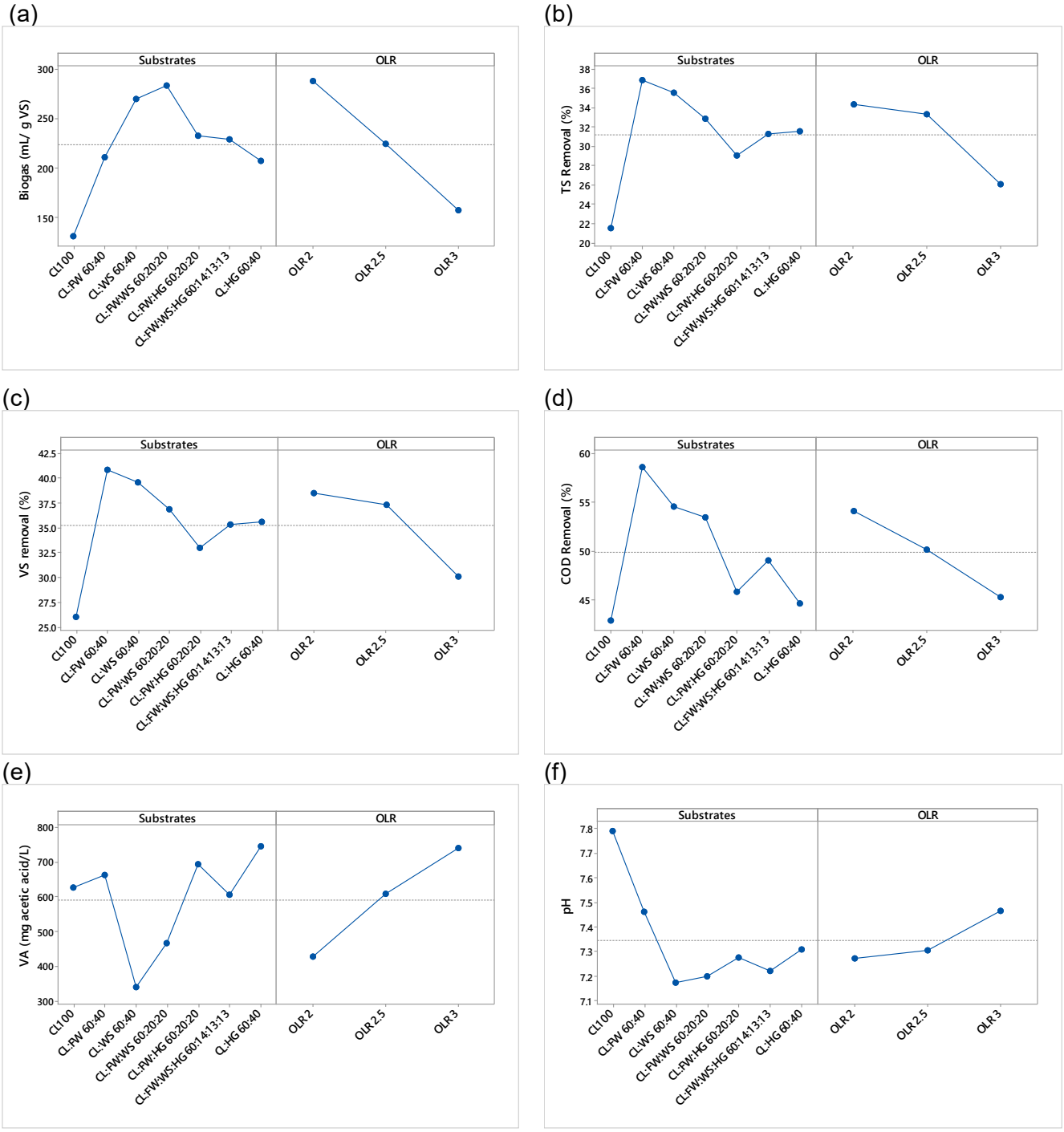

Figure 5- 4: Main effects and interaction of different substrate mixtures at different OLRs for parameters- (a) Biogas (mL/g VS_{added}), (b) TS Removal (%), (c) VS Removal (%), (d) COD Removal (%), (e) VA (mg acetic acid/L) and (f) pH

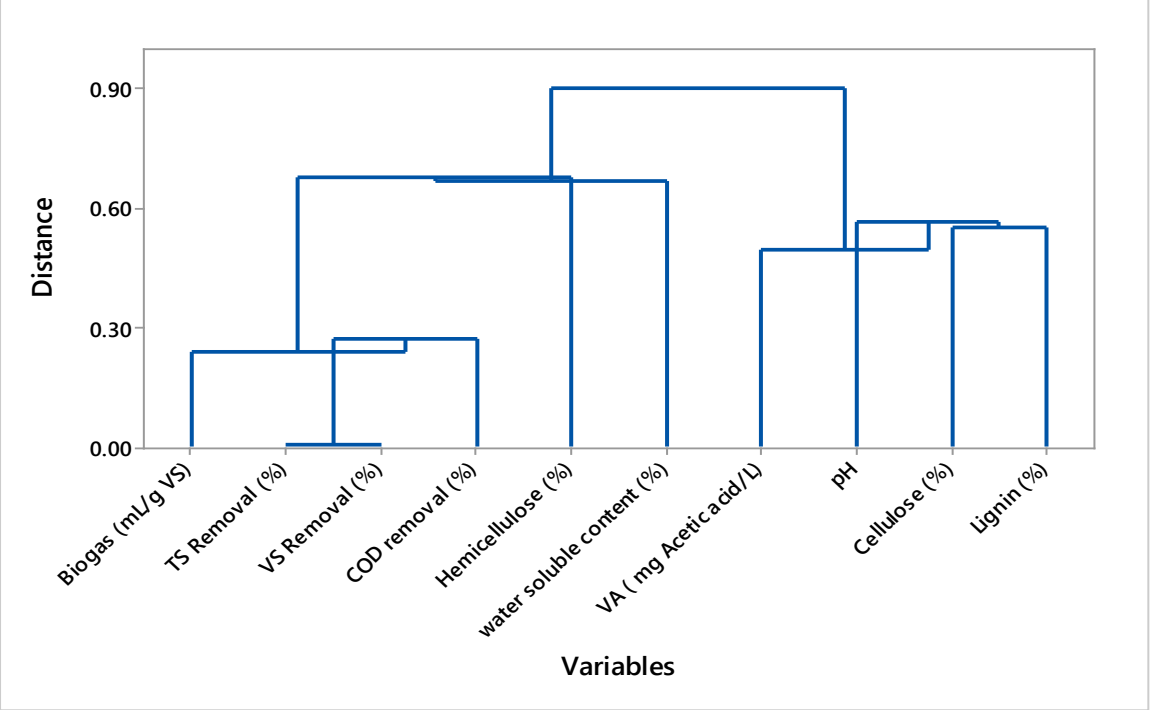

Figure 5- 5: Dendrogram showing the results of cluster analysis of the different experimental conditions, considering all biogas production variables and process performance

5.4. Conclusions

In this study, anaerobic co-digestion of agro-industrial wastes for four different substrate mixtures were studied under semi-continuous conditions, for different C/N ratio, substrate types (as per fractionation analysis) and three different OLRs. The best operational scenario was the feedstock comprised of CL:FW: WS 60:20:20 operated at OLR of 2.0 gTS/L.d and fed with a feedstock of 4% TS. Although biogas production depends on the C/N ratio, the sequential fractionation analysis revealed that for lignocellulosic substrate mixture, biogas production is further dependent on the structural breakdown of lignocellulose, i.e. cellulose degradation and water soluble content. During AD, the removal of TS, VS and COD also decreased as the ORL increased.

This study focused on chicken farm wastes and agricultural residues and FW that are available from neighbouring areas of a designated area, Victoria, Australia. Therefore, the outcomes of the study is important to assess the potential of an ACoD facility in the region and seeks to convey sufficient technical details to inform the design and operation of such.

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Chapter 6 Degradation of Structural Carbohydrates of Agro-industrial Wastes Through Sequential Pre-treatment Strategies

In the previous chapter, the semi- continuous anaerobic co-digestion of chicken litter with agricultural and food waste was presented where it was shown that the ACoD of these substrates can be operated successfully at low total solids (or organic) loading. It was also shown that agricultural substrates, being lignocellulosic in nature have an effect on the digestion process. Therefore, in this chapter, the breakdown of the lignocellulosic structure through pre-treatment has been investigated. In chapter 3, the effect of different pre-treatments was discussed. Therefore, after careful consideration alkaline (NaOH) and acid (H_2SO_4) pre-treatment were selected to be investigated for their potential to enhance lignin and hemicellulose degradation and consequently enhance the anaerobic digestion of these substrates. The pre-treatment processes were assessed at different conditions according the experiment design method and response surface optimisation was utilised to determine optimum conditions in terms solids recovery, cellulose content and lignin degradation. The results are presented in this chapter and will also be submitted for consideration for a journal publication (e.g. Biomass and Bioenergy).

6.1 Introduction

In Victoria, Australia, agro-industries, such as the poultry industry, operate at considerable scale, and produce around 0.45 million tonnes per year of chicken litter (CL) (Scott et al., 2009). The farmers either gave it freely or sometimes had to pay for the collection and transport of this waste to a composting facility. Typically, CL is a mixture of manure and bedding materials, e.g. wood shaving, rice hulls, wheat or rice straw (nearly around 70% is bedding materials) (Ogunwande et al., 2008). Wheat straw is another abundant by product from wheat production in farming in the neighboring areas to poultry farming. Both wastes can be used as a valuable feedstock to produce biofuels and biogas. However, these abundant low-cost biomasses are lignocellulosic in nature and has a recalcitrant and crystalline structure which resists bacterial attack and biodegradability (Mohseni Kabir et al., 2014; Mohsenzadeh et al., 2012; Zhang et al., 2014a). Moreover, the crystalline

cellulose in these wastes is tightly bound with amorphous components such as lignin and hemicellulose which is difficult to break down and required some sort of pre-treatment (Chen et al., 2014a; Xu et al., 2012).
As the bedding materials in the CL is comprised of different types of lignocellulosic wastes, it has varied types of fibers and structural constituent properties (Salehian and Karimi, 2013; Salehian et al., 2013). For examples, bioconversion of wood shaving is more difficult than straw for its long fiber and highly matrix structure (Salehian et al., 2013). Wood shavings from different types of trees or even distinctive parts of the same tree, although all parts are from either hardwoods or softwoods, have various compositions that may result in variations in bio-energy production (Salehian and Karimi, 2013).

Different pre-treatment methods, including thermal, mechanical by milling or grinding (Janzon et al., 2014), physical by steam explosion or radiation (Feng et al., 2018; Ramos, 2003), chemical by acids, alkali or solvents (Kapoor et al., 2018; Terán Hilares et al., 2018), biological by enzymes or fungi (Brémond et al., 2018; Zhao et al., 2018), combined mechanical, thermal and chemical (Chen et al., 2014a; Salehian and Karimi, 2013; Teghammar et al., 2014; Zhang et al., 2014a), and co-digestion with other organic wastes (Chen et al., 2014a) have been explored to improve the degradation of lignocellulosic materials.

Among the chemical treatments, the alkaline pre-treatment is widely used to break down ester bonds between amorphous and cellulose contents by saponification and cleavage of lignin-carbohydrate linkages, and by the reduction in polymerization, cross links and crystallinity, which increases the porosity, internal surface area, and structural swelling (Salehian et al., 2013; Zheng et al., 2014). Sodium hydroxide (NaOH) has been investigated to improve biogas from wheat straw (Chandra et al., 2012), rice straw (He et al., 2009; He et al., 2008), corn stover (Wang et al., 2013a; Zhu et al., 2010), hardwoods and softwoods (Chen et al., 2014a; Mirahmadi et al., 2010; Mohsenzadeh et al., 2012; Salehian and Karimi, 2013; Salehian et al., 2013), paper and pulp sludge (Lin et al., 2009), and oil palm empty fruit branches (Nieves et al., 2011). However, various treatment conditions, such as NaOH concentration, time, and temperature showed variations in results along with the type and origin of the lignocellulosic materials (Mohseni Kabir et al., 2014). Mohsenzadeh et al. (2012)

reported that NaOH pre-treatment was more effective on hardwood (birch) than softwood (spruce) for methane yield in anaerobic digestion (Mohsenzadeh et al., 2012). Although several studies investigated on the effectiveness of pre-treatment, the promising results were obtained using combination of two or more pre-treatment methods.

Dilute sodium hydroxide e. g. alkaline pretreatments (AKP) are generally effective for lignin removal (Carrillo et al., 2005) and dilute acid pretreatments (DAP) efficiently hydrolyze hemicellulose without an excessive formation of inhibitory degradation by-products (Saha et al., 2005). AKP detaches and partially hydrolyzes lignin contained in lignocellulosic biomass by breaking the ester bonds that cross-link lignin and xylan (Bjerre et al., 1996; Sun and Cheng, 2002).

Therefore, sequential pretreatments may be a promising strategy to establish suitable industrial-scale processes with high rates lignocellulosic structure degradation (Mussatto et al., 2010; Sanchez et al., 2015). The aim of this study was to investigate the effectiveness of AKP and DAP for enhancinh CL and WS anaerobic degradation and ultimately biogas production.

6.2 Materials and Methods

6.2.1 Characteristics of substrates

The agro-industrial wastes CL and WS, collected from regional Victoria, Australia. The CL was collected from broiler chicken farm and kept in an airtight plastic bucket at room temperature (Zahan et al., 2018c). The CL used in this study is a complex substrate containing a high fraction of bedding materials which mostly contains lignocellulosic bedding material (sawdust or straw). After collection, WS was ground and sieved to below 5 mm and stored in plastic air tight container at room temperature. The characteristics of the CL and WS are shown in Table 6-1. These lignocellulosic substrates were also analysed for carbohydrates, lignin and crystallinity and the results are also shown in Table 6-1.

Table 6- 1: Characteristics of substrates

Parameters	Unit	CL	WS
TS	%	77.2±3.3	82.9±5.1
VS	%	39.1±2.5	79.78±2.8
tCOD	mg/g	182.5±33.8	ND*
sCOD	mg/g	21.2±1.2	ND
TN	mg/g	4.31±0.06	ND
Ammonia	mg/g	2.96±0.1	ND
TP	mg/g	0.60±0.0	ND
VA	mg Acetic acid/g	3.6±1.1	ND
Alkalinity	Mg CaCO3/g	207±2.8	ND
pH	-	8.2±0.1	ND
C/N ratio	-	13.0±1.3	81.5±1.9
Cellulose	%	12.97	31.76
Hemi-cellulose	%	17.24	15.22
Lignin	%	46.30	24.70
Water soluble content	%	22.84	18.96
Glucose	%	12.55	35.09
Xylose	%	5.09	15.46
Arabinose	%	0.94	1.73
Galactose	%	0.91	-
[a]AIL	%	28.05	20.55
[b]ASL	%	7.59	7.02
Lignin $_{ext.free}$	%	34.4	24.0
Extractives	%	3.44	12.81
Ash	%	40.75	4.85
[c]TCI (A1375/A290)		1.898	1.572

*Not determined
[a]AIL- Acid insoluble lignin
[b]ASL- Acid soluble lignin
[c]TCI – Total crystallinity index

6.2.2 Pre-treatment

6.2.2.1 Alkaline pre-treatment (AKP)

Alkaline pre-treatment has been found to be very effective particularly for reducing the lignin content of lignocellulosic biomass without significant loss in celluloses, thus increases the bio-digestibility. NaOH pre-treatment was applied for both CL and WS substrates. Every 1g of substrate was immersed in 10mL alkaline solutions for different periods of time under different operational conditions. The effectiveness of AKP conditions, NaOH concentration (1-5% w/v), reaction time (30-90 min), and temperature (60-120°C) was investigate according to the response surface

methodology (RSM) with a three-level factorial experimental design, design 1 (Table 6-2). This pre-treatment design was used for both CL and WS.

After pre-treatment, the samples were thoroughly washed to achieve a pH of around 7and stored at 4°C for further use. The liquid fractions from Alkali pre-treatment were recovered and samples were stored at -20°C for further analysis.

6.2.2.1 Sequential alkaline-acid pre-treatment

Sequential pre-treatment was carried out according to the methods proposed by Sanchez et al. (2015) (Sanchez et al., 2015) and at the identified for the AKP. WS and CL were immersed in 1%, 2%, and 3% (w/v) H_2SO_4 solutions as per the RSM design 2 (Table 6-2). The DAP pre-treatment of the substrates was carried out under three different temperatures, 60, 90 and 120°C for 30, 60 and 90 mins.

After pre-treatment, the samples were washed to achieve a pH of around 7. After that, the samples were vacuum filtered and stored at 4°C for further use. The liquid fractions after pre-treatment were recovered stored for further analysis for mass balance.

6.2.3 Compositional analysis

The composition of the WS was determined according to the methods by the National Renewable Energy Laboratory (NREL). The total solids contents were determined by heating the samples in a drying oven at 105°C until a constant weight was obtained (NREL LAP 001) (Sluiter et al., 2008). Volatile solids (VS) were calculated from igniting the sample at 550°C for 20 mins after total solids were determined. Percentage of ash was determined by gravimetric analysis of the sample in a muffle furnace by ramping the temperature program up to 575°C as outlined in NREL LAP 005(Sluiter et al., 2005a).

Extractives were determined according to the NREL method, NREL LAP 010 (Sluiter et al., 2005c). The samples were then taken out of the cell and dried in the oven at 37°C and stored for acid hydrolysis. The solvent ethanol was evaporated with Centrivap DNA Concentrator (Labconco corp., Kansas, USA) at a temperature of 40°C.

The extractive free WS was analysed for structural carbohydrates and lignin compositions according to the NREL method, NREL/TP-510-42618 (Sluiter et al., 2011). All the analyses were performed in duplicate and the average results are presented in Table 6-1.

AS CL has lots of impurities, the composition of CL was done according to (Opatokun et al., 2015) for lignocellulosic characteristics determination.

6.2.4 Analytical methods

TS and VS were measured by gravimetric analysis according to the Standard Method 2540B and 2540E respectively (Rice et al., 2012). The tCOD and sCOD were measured using HACH method 8000. The total phosphorus (TP), total nitrogen (TN), ammonium, volatile acids (VA) were measured by colorimetric techniques using HACH (Model: DR/4000 U) spectrophotometer according to the methods 10127, 10072, 10031 and 8196, respectively. The samples were centrifuged (Eppendorf 5702, Germany) at 4.4 rpm for 15 mins and then filtered through 0.45 µm filter paper (mixed cellulose esters membrane filter, Advantec, Japan), to measure the soluble constituents. The measurement of pH was carried out using a calibrated pH meter (ThermoOrion, Model 550A). The C/N ratio was determined using LECO (TruMac) analyser and Alkalinity was measured by APHA method 2320B.

Structural carbohydrates and sugars were analysed by high performance liquid chromatography (HPLC) connected with a refractive index (RI) detector (Thermo Fisher Scientific, Australia). The hydrolysed sugars were analysed with ion-exchange columns (Phenomonex Hi-Plex Pb2+ followed by Na+, 7.7*300 mm, 8µm) at 85°C using 100% degassed deionised water as a mobile phase with a flow rate of 0.3 mL/min. Standard sugars of varying concentration (0.1-4 mg/mL) were prepared for making the calibration curve. The standard sugar used for making the standard curve is the high purity standard sugars.

Fourier transform infrared (FTIR) spectroscopy (Spectrum 100, PerkinElmer, USA) equipped with a universal ATR (attenuated total reflection) accessory was used for structural analysis and crystallinity of the treated and untreated samples. The samples were dried in oven at low temperature of 40°C before doing the experiments

Table 6- 2: Design matrices of pre-treatments for CL and WS

Design 1 (AKP)				Design 2 (DAP after AKP)			
Std Order	NAOH (%)	Time (Min)	Temperature(°C)	Std Order	H_2SO_4 (%)	Time (min)	Temperature(°C)
14	3	60	90	9	2	30	60
11	3	30	120	11	2	30	120
6	5	60	60	3	1	90	90
7	1	60	120	8	3	60	120
3	1	90	90	6	3	60	60
10	3	90	60	14	2	60	90
4	5	90	90	5	1	60	60
1	1	30	90	10	2	90	60
5	1	60	60	4	3	90	90
15	3	60	90	12	2	90	120
2	5	30	90	2	3	30	90
8	5	60	120	13	2	60	90
9	3	30	60	7	1	60	120
13	3	60	90	15	2	60	90
12	3	90	120	1	1	30	90

to ensure no structural damage. The spectra were obtained with an average of 64 scans from 4000 to 600 cm-1 with 4 cm-1 resolution monitored by Spectrum software. Crystallinity index (CI) and total crystallinity index, which are the absorbance ratio of A1430/A896 and A1375/A2900, respectively, were also calculated from the spectra.

6.2.5 Response surface methodology

The classical approach does not depict the combined effect of all the process parameters and time consuming as well as requires number of experiments to determine the optimum levels, which may be unreliable thereby elevating the overall process cost. The limitations of the classical method could be eliminated by statistical experimental design such as response surface methodology (RSM). The main objective of the RSM was to estimate the optimum operational conditions of the pre-treatment process with respect to temperature, percentage of alkali/acid and time. Therefore, the experiments were designed according to the Box-Behnken Design (BBD) using MiniTab. The combined effects of the various parameters on the structural carbohydrate yields by sequential AKP and DAP, by MINITAB16. The

statistical importance of each variable was computed and any non-significant at $p > .10$ was removed. Furthermore, cellulose yield optimization was carried out using RSM models.

6.3 Results and Discussions

6.3.1 Characterisation of biomass

The details of basic physical properties and chemical compositions of the biomass i.e. CL and WS are shown in Table 6-1. As CL is a mixture of litter and bedding lignocellulosic bedding material, it has lots of inert and inorganics, making it a substrate of high ash content. The C/N ratio of both substrates were also very different, where WS had a C/N ratio 6.27 times higher than CL C/N ratio. The low C/N ratio indicate less C-content which implies that the anaerobic digestion of the substrates would result in the accumulation of ammonia in the digester which may inhibit the process. It was also reported that, C/N ratio between 20 and 30 is considered optimal but some studies reported stabilised biogas production within a wider range of C/N ratios, e.g. 9-30, depending on the substrates (Zahan and Othman, 2019).

The carbohydrates content of CL and WS were analysed using fractional analysis as well as NREL method and both are reported to show the variation in the two processes. Both methods showed similar content of cellulose (glucose). However, the lignin content was different, especially for CL with fractional analysis (46.8%) compared to NREL method. The fractionation analysis is a gravitational analysis method of total fractions; therefore, the lignin content also included ash content, which explains the high value of lignin content (Zahan and Othman, 2019). The substrates cellulose compound in WS (34.8%) was almost 2.7 times higher compared to CL (12.97%). The total crystallinity index (TCI) for WS and CL was found to be 1.572 and 1.898, respectively. CL had high TCI because of the presence of more crystalline hardwood i.e. wood shavings compared to WS (Salehian et al., 2013; Zheng et al., 2014).

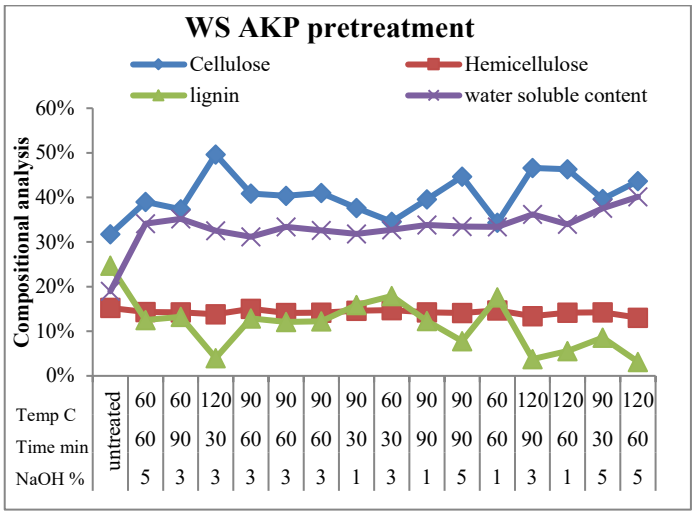
6.3.2 Compositional changes after AKP

Pre-treatment of CL was different from the treatment of other lignocellulosic biomass due to its higher lignin and ash content. AD of CL was mostly unutilised as a feedstock because of its high N-content (Hassan et al., 2017). Although, published research has focused on utilisation of CL as a co-substrate especially in anaerobic digestion, however, there is no literature on the pre-treatment of CL. On the other hand, WS pre-treatment using different chemicals has been reported (Bjerre et al., 1996; Carrillo et al., 2005; Chandra et al., 2012; Saha et al., 2005; Sanchez et al., 2015). WS has 36.9% cellulose, 9.82% lignin, 28.67% hemicellulose and 20.19% water soluble content. Minor variation in composition of WS from previously reported studies exist, likely because of the natural variation in growing conditions, location and age of sample.

AKP i.e. NaOH pre-treatment were done under the conditions shown in Table 6-2. After AKP, CL and WS were recovered and subjected to compositional analysis. CL and WS showed significant compositional changes through AKP (Figure 6-1) with varying levels of solubilisation of the lignin, hemicellulose, cellulose components and water soluble contents (Figure 6-1). It was found that AKP pre-treatment had very little effect on hemicellulose solubilisation for both CL (1-12.5%) and WS (1-14%), compared to lignin solubilisation. The maximum hemicellulose was obtained at 5% NaOH at 120°C for 30 min pre-treatment for both WS and CL. It was also observed that at higher temperature maximum hemicellulose was solubilised for AKP pre-treatment.

Under all pre-treatment conditions, lignin was significantly hydrolysed for both CL (1-40%) and WS (27.5-87%). Because of the presence of the hardwood i.e. wood shavings, CL had comparatively less lignin degradation compared to WS. From Figure 6-1, lignin solubilisation in AKP pre-treated CL and WS, was significantly impacted by both the AKP concentration and temperature and was less impacted by treatment duration. Increasing AKP concentrations led to greater solubilisation of the lignin for all conditions tested. Pre-treatment CL and WS containing the lowest lignin content were those produced using from 5% AKP at 120 °C for 60min which contained only 3.15% and 28.15 w/w lignin, respectively for WS and CL.

(a)



(b)

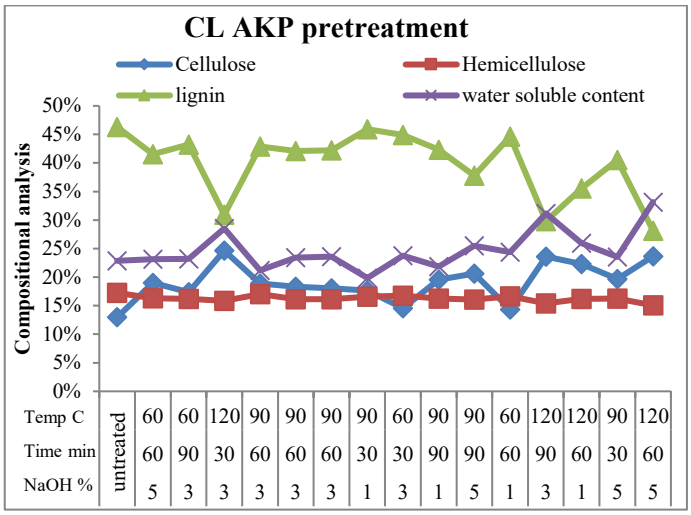


Figure 6- 1: Compositional changes of (a) WS and (b) CL after AKP pre-treatment

An apparent increase in the cellulose and water soluble content of CL and WS was observed with decrease in lignin content. It was also reported that, NaOH pre-treatment increased glucose content and was associated with decrease in lignin content (Sanchez et al., 2015). The increase in water soluble content was 40-53% for WS and 0-31% for CL; and the cellulose content increase was found to be 7.5-32% for WS and 9.5-45% for CL. A relatively higher amount of water soluble content was found for WS compared to CL, as CL had high inorganic ash content which could not be converted. The highest amount of cellulose (49.65%) was observed at 3% NaOH at 120°C for 30 min for WS and 5% NaOH at 120°C for 30 min for CL (23.64%), whereas the highest water soluble content was observed at 5% NaOH at 120°C for 30 min for both WS and CL. From Figure 6-1, there is an opposite relationship between lignin solubilisation and cellulose content i.e. if the amount of lignin solubilisation increased cellulose content decreased or vice versa. Whereas, no such relationship was found with water soluble content with cellulose or hemicellulose solubilisation.

6.3.2.1 RSM optimisation of AKP

While several researchers have undertaken studies into AKP pre-treatment of WS, none have extensively investigated the impact of solvent concentration, treatment duration and temperature and/or the interaction effects between these variables for

AKP pre-treatment of CL. The cellulose and other carbohydrates content in the CL and WS discussed in Section 6.3.1 and 6.3.2 was used to calculate coefficients for the RSM equation. These coefficients were then used to generate the second order polynomial equation which was used to predict the conditions for optimum cellulose content for AKP pre-treatment CL and WS.

The statistical analysis of the RSM indicated that among the three individual pre-treatment variables (X_1, X_2 and X_3), X_1 and X_3 were of significance. The coefficients were fitted to Eq. (6-1) and (6-2) to generate the model for the CL and WS cellulose. The fit of data into the model demonstrates a correlation with R^2 value of 82.3% and 93.09%, respectively for WS and CL. The model for pre-treatment parameters and cellulose content for CL and WS were described by the following equations:

$$Y_{CL\_NaOH} = 0.069867 + 0.014894X_1 + 0.000473\,X_2 + 0.000198\,X_3 + 0.000927\,X_1^2 + 0.000007\,X_2^2 + 0.000011\,X_3^2 - 0.000038\,X_1X_2 - 0.000139\,X_1X_3 - 0.000011\,X_2X_3 \quad (6\text{-}1)$$

$$Y_{WS\_NaOH} = 0.150283 + 0.037394X_1 + 0.000779\,X_2 + 0.001865\,X_3 - 000199\,X_1^2 + 0.000005\,X_2^2 + 0.000010\,X_3^2 + 0.000128\,X_1X_2 - 0.00031\,X_1X_3 + 0.000016\,X_2X_3 \quad (6\text{-}2)$$

where Y_{CL_NaOH} and Y_{WS_NaOH} is the cellulose content of the CL and WS following treatment, X_1, X_2 and X_3 are NaOH concentration (%), pre-treatment time (min) and treatment temperature (°C), respectively. These values are in un-coded units. Predictions from the second order polynomial to describe the effect of pre-treatment variables on the CL and WS cellulose content are described in the following section.

According to the RSM predictions, the optimum conditions for cellulose enrichment are 1% NaOH, at 120 °C for 30min for WS (48.47% cellulose) and 5% NaOH, at 120 °C for 90min for CL (23.74% cellulose), respectively. Validating the model was undertaken to confirm the accuracy within the investigated range, the optimised conditions of CL and WS. The predicted and measured values for the cellulose content of the AKP pre-treated CL were 22.96% and 21.77%, respectively and 47.72% and 47.05% respectively, for WS. The predicted values generated by the model are comparable with the measured results and are within the 5% error associated with the model. These results confirm the model can be used to determine the cellulose content of the CL and WS following AKP pre-treatment at conditions within the range investigated.

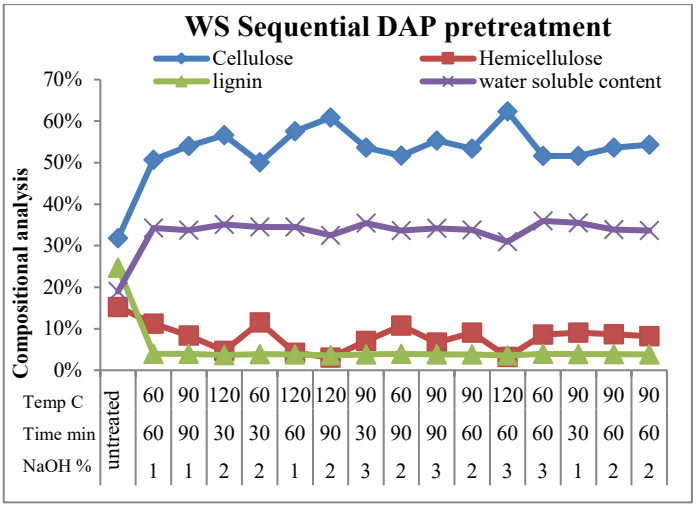
6.3.3 Compositional changes for sequential DAP after AKP

AKP showed huge effect on lignin degradation compared to hemicellulose. Therefore, further pre-treatment with dilute acid was investigated with an aim to enhance hemicellulose solubilisation. On the other hand, for WS, DAP pre-treatment has been reported by (Timung et al., 2015), however, has variation in chemical concentrations, time and temperature very less, making this study more robust.

After AKP, sequential DAP i.e. H_2SO_4 pre-treatment was applied according to the conditions shown in table 6-2. After DAP, CL and WS were recovered and subjected to compositional analysis. CL and WS showed significant compositional changes with varying levels of solubilisation of the lignin, hemicellulose and cellulose components, as well as water soluble contents (Figure 6-2). It was found that unlike AKP, DAP pre-treatment had very little effect on lignin degradation for both CL and WS, compared to hemicellulose degradation (27-80%). The lignin content showed no changes for all concentrations, time and temperatures and all the pre-treatment had the similar amount of lignin content.

Under all pre-treatment conditions, hemicellulose was significantly hydrolysed for both CL (35-82%) and WS (26.5-80%). For both CL and WS, DAP was found to be very effective for hemicellulose degradation. From Figure 6-2, the hemicellulose solubilisation of DAP pre-treated CL and WS, was significantly impacted by both the

a)



(b)

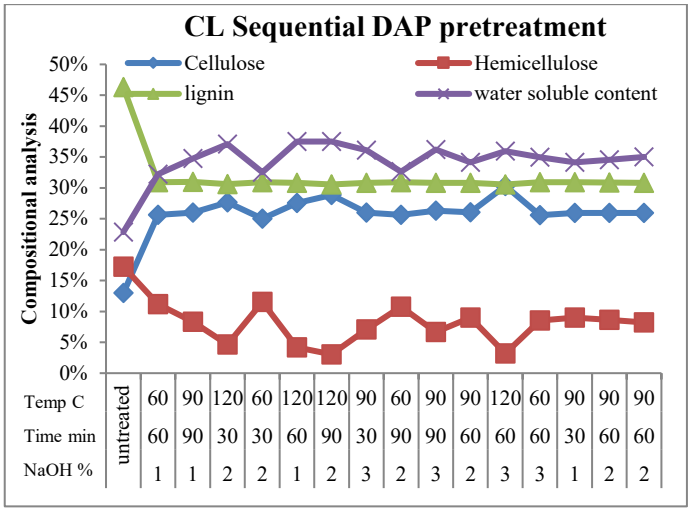


Figure 6- 2: Compositional changes of (a) WS and (b) CL after sequential DAP pre-treatment

DAP concentration and treatment temperature. For WS, hemicellulose was less impacted by treatment duration compared to CL. Increasing DAP concentrations led to greater solubilisation of the hemicellulose for all conditions tested. The CL and WS containing the lowest hemicellulose content were those produced from 3% H_2SO_4 at 120 °C for 60min which contained only 3.55% and 3.18 w/w hemicellulose, respectively for WS and CL. An apparent increase in the cellulose and water soluble content of CL and WS was observed with decrease in hemicellulose content. Previously, it was also reported that, H_2SO_4 pre-treatment increase the glucose content with the decrease in hemicellulose content (Timung et al., 2015). The increase in water soluble content was 38-46.5% for WS and 29-40% for CL; and the cellulose content increase was found to be 37-49% for WS and 49-55% for CL was found compared to raw substrates without pre-treatment. A relatively higher amount of water soluble content was found for WS compared to CL for DAP as well, as CL had high inorganic ash content which could not be converted. The highest amount of cellulose was observed at 3% H_2SO_4 at 120°C for 60 min for WS (63.32%) and CL (30.34%), whereas the highest water soluble content was observed at 3% H_2SO_4 at 60°C for 60 min for WS (35.96%) and at 2% H_2SO_4 at 120°C for 90 min for CL (37.5%). From Figure 6-2, there is a relationship between hemicellulose solubilisation and cellulose or water soluble content was observed i.e. if the amount of hemicellulose solubilisation increased cellulose and water soluble content increased. Whereas, no such relationship was found with lignin with hemicellulose solubilisation.

6.3.3.1 RSM optimisation of sequential DAP after AKP

While several researchers have undertaken studies into the pre-treatment of WS using AKP, only few research published investigated sequential treatment of DAP after AKP for WS and none have extensively investigated the combined effect of the main process factors, solvent concentration, treatment duration and temperature and/or the interaction for CL.

The cellulose and other carbohydrates content in the CL and WS discussed in Section 6.3.1 and 6.3.3 was used to calculate coefficients for the RSM equation. These coefficients were then used to generate the second order polynomial equation

which was used to predict the conditions for optimum cellulose content for sequential DAP after AKP of CL or WS.

The statistical analysis of the RSM indicated that all the three individual pre-treatment variables X_1, X_2 and X_3 were of significance ($p < .10$). The coefficients were fitted to Eq. (6-3) and (6-4) to generate the model for the CL and WS cellulose. The fit of data into the model demonstrates a correlation with R^2 value of 86.03% and 90.23%, respectively for WS and CL. The model for pre-treatment parameters and cellulose content for CL and WS after sequential DAP after AKP were described by the following equations:

$$Y_{CL\_H_2SO_4} = 0.3489 - 0.0298\ X_1 + 0.000165\ X_2 - 0.002097\ X_3 + 0.00271\ X_1^2\ \% - 0.000002\ X_2^2 + 0.000011\ X_3^2 + 0.000023\ X_1X_2 + 0.000238\ X_1X_3 + 0.000002\ X_2X_3 \quad (6\text{-}3)$$

$$Y_{WS\_H_2SO_4} = 0.5797 - 0.0258\ X_1 + 0.000450\ X_2 - 0.002580\ X_3 + 0.00293\ X_1^2 - 0.000005\ X_2^2 + 0.000016\ X_3^2 - 0.000057\ X_1X_2 + 0.000321\ X_1X_3 + 0.000007\ X_2X_3 \quad (6\text{-}4)$$

where $Y_{CL\_H_2SO_4}$ and $Y_{WS\_H_2SO_4}$ is the cellulose content of the CL and WS following treatment, X_1, X_2 and X_3 are H_2SO_4 concentration (%), pre-treatment time/duration (min) and treatment temperature (°C), respectively. These values are in un-coded units. Predictions from the second order polynomial to describe the effect of pre-treatment variables on the CL and WS cellulose content for sequential DAP after AKP are described in the following section. According to the RSM predictions, the optimum conditions for cellulose enrichment are 3% H_2SO_4, at 120 °C for 30min for WS (59.66%) and 3% H_2SO_4, at 120 °C for 90min for CL (30.23%). Validating the model was undertaken to confirm the accuracy within the investigated range, the optimised conditions of CL and WS. The predicted and measured values for the cellulose content of the sequential DAP after AKP pre-treated CL were 30.16% and 29.27% and WS sequential DAP following AKP were 59.41% and 58.52% respectively. The predicted values generated by the model are comparable with the measured results and are within the 5% error associated with the model. These results confirm the model can be used to determine the cellulose content of the CL and WS for sequential DAP following AKP pre-treatment at conditions in the range investigated within the model.

6.4 Conclusions

Sequential pre-treatments of AKP followed by DAP showed a positive effect on CL and WS degradation, improving cellulose yield and solibilisation of hemicellulose and lignin compared with those obtained with single AKP pre-treatment. The total cellulose yield achieved with DAP after AKP indicated that this sequential pre-treatment was very effective in reducing lignin and hemicellulose, which was associated with an increase in cellulose content. AKP could unlock approximately 40-87% of the residual lignin from the lignin–cellulose complex, and DAP unlocked 35-80% of the residual hemicellulose leaving cellulose more accessible for utilisation, thus resulting in higher cellulose yields as well as water soluble content. Interestingly, time was not as important as temperature in the range of experimental conditions for AKP and sequential DAP. Therefore, feasibility of AKP and DAP pre-treatment versus the enhanchment in biogas yield worth assessing before scale-up of pre-treatment processes and an AD facility.

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Chapter 7 Effect of Pre-treatment on Sequential Anaerobic Co-digestion of Chicken Litter with Agricultural and Food Wastes under Semi-Solid Conditions and Comparison with Wet Anaerobic Digestion

In this chapter, the effect of pre-treatment on the sequential anaerobic co-digestion of chicken litter with agro-industrial wastes under High-solids (15% TS) condition is presented. As most of the agro-industrial wastes are solid substrates, performing high solid anaerobic digestion is of interest to the broiler industries. In previous chapters i.e., Chapter 6 enlightened with pre-treatment of agro-industrial wastes and chapter 4 presented the optimum substrate mixture ratio for anaerobic co-digestion with optimum C/N ratio. This chapter utilises those results and conditions to run anaerobic digestion at a high solid condition and analyses their performances with showing the effect of pre-treatment. Additionally, comparison with their wet anaerobic digestion performances obtained in chapter 4 and 5 are also presented which confirms the successfulness of high solid anaerobic digestion for pre-treated agro-industrial wastes.

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Abstract

Sequential co-digestion batch assays were conducted using feedstocks of chicken litter (CL), food waste (FW) and wheat straw (WS) mixed to a C/N ratio of 26.5 and 15% TS. Untreated mixture produced biogas of 321.6±13.4 mL_N /g VS_{added} which improved up to 50% when either CL or WS pre-treated substrates were fed. However, when both pre-treated CL and WS were fed, 80% and 88% increase in total biogas were found with associated VS removal of 49% and 55%, respectively, for alkali and sequential acid pre-treatment. Also, reactors received pre-treated substrates showed reduction in ammonia and digestate cellulose fraction with an increase in water soluble contents. Biogas production using sequential AD at 15% was almost 38% less than BMP biogas at 4%, however this was negated with the pre-treatment indicating that co-digestion at high TS of 15% is achievable. Further testing is required to confirm these results under semi-continuous conditions.

7.1 Introduction

There has been a growing attention on the use of livestock manure especially chicken litter as an alternative source for bioenergy production (Abouelenien et al., 2016; Zahan et al., 2018b).This is mainly due to the continuous land, environmental and economic concerns and obligations facing by the farmers and governments (Ragauskas et al., 2006). In Victoria, Australia, the agro-industries, such as the poultry industry produces around 0.45 million tonnes per year of chicken litter (CL) (Scott et al., 2009; Zahan et al., 2018a). This study focuses on chicken farms and aims to investigate the potential of CL and other agro-industrial wastes, from the neighbouring area available for a semi-solid anaerobic digestion (AD), using a designated area in Victoria, Australia as a case study. To complete the investigation the organic fraction of municipal solid wastes (FW) and agricultural residue such as wheat straw (WS) were also included; being abundant and available for a potential AD facility. This will reduce the huge cost for managing the wastes as well as can become energy source for those farms and broilers. In a broader context, advance resource recovery technologies will be researched which will be directly applicable to Australian agro-industrial wastes management and resource recovery.

AD is considered to be one of the advantageous processes for organic waste management, which produces biogas of high calorific value and soil conditioner while reducing the environmental hazard of these wastes (Bong et al., 2018; Poulsen and Adelard, 2016). AD is usually classified, based on the anaerobic digester's total solids (TS) content, as wet, semi-solid and solid state (dry), referring to TS percentages of <10%, 10-15% and >15%, respectively (Abouelenien et al., 2016; Karthikeyan and Visvanathan, 2013; Kothari et al., 2014). Some literature also classified semi solid as 10-20 % and solid AD at >20% TS (Karthikeyan and Visvanathan, 2013). Solid state AD has several benefits over conventional wet AD, including smaller reactor volume, less heating and energy requirement, less wastewater and digestate (Huang et al., 2018; Rodriguez-Verde et al., 2018). However, while wet AD of manure has been widely established and studied, very few studies have been conducted on the semi-solid and solid state AD, especially of poultry or chicken litter (Abouelenien et al., 2016; Rodriguez-Verde et al., 2018; Zahan and Othman, 2018).

CL, that is generated from broiler farms, is a mixture of manure and bedding materials (wood shaving, rice hull and straw) and has high biochemical methane potential (BMP) which is comparable to sludge BMP potentials (Zahan et al., 2016b). However, AD of CL is prone to inhibition due to the high level of protein and amino acid. This proteins and urea, during AD, degrade to ammonia (Niu et al., 2013), where unionised or free ammonia (NH3) generally accumulates and reaches threshold concentration that is toxic to the anaerobic microorganisms, particularly methanogens (Borja et al., 1996; Liu et al., 2012), which are associated with an increase in pH (Liu et al., 2012). Although many published papers focused on inhibition by ammonia and also volatile acids (Abendroth et al., 2015; Akindele and Sartaj, 2018; Nie et al., 2015), successful AD of CL as a single substrate has not been reported nor there are large scale dry AD plants. The most effective mechanism to overcome ammonia inhibition is to reduce the precursors for ammonia formation in the AD process (Karthikeyan and Visvanathan, 2012a). The organic substrates of high C-content, i.e. high C/N ratio can mix with high N-content substrate (CL) with the aim to dilute the N-content. Wheat straw and FW both has comparatively high C/N ratio than CL. Therefore, the anaerobic co-digestion (ACoD) of CL with other agro-industrial wastes with an aim for balancing the influent C/N ratio could be utilised to control inhibition (Nie et al., 2015).

Both CL and wheat straw is lignocellulosic in nature and has a recalcitrant and crystalline structure which resists bacterial attack and biodegradability (Mohsenzadeh et al., 2012; Zhang et al., 2014a). Therefore, pre-treatment prior to AD is required to increase the biodegradability of lignocellulose by decreasing its crystallinity and eliminating its network structure with amorphous components (Salehian et al., 2013). Among different pre-treatment method, the chemical methods of pre-treating lignocellulosic materials are widely employed in many pilot and demonstration plants since they maximise lignin removal with minimum sugar loss i.e. not more than 5% (Taherzadeh and Karimi, 2007). Some of the chemical pre-treatment are even cost effective at low concentrations with minimum heat and power requirements (Nitsos et al., 2017). The alkaline pre-treatment is widely used to break down ester bonds between amorphous and cellulose contents by saponification and cleavage of lignin-carbohydrate linkages, and by the reduction in polymerization, cross links and crystallinity, which increases the porosity, internal

surface area, and structural swelling (Zheng et al., 2014). Sodium hydroxide (NaOH) pre-treatment was investigated with an aim to improve biogas from lignocellulosic wheat straw (Chandra et al., 2012), rice straw (He et al., 2009; He et al., 2008), corn stover (Wang et al., 2013a; Zhu et al., 2010), hardwoods and softwoods (Chen et al., 2014a; Mirahmadi et al., 2010; Mohsenzadeh et al., 2012; Salehian and Karimi, 2013), paper and pulp sludge (Lin et al., 2009), and oil palm empty fruit branches (Nieves et al., 2011). However, various treatment conditions, such as NaOH concentration, time, and temperature can show variations in results along with lignocellulosic material types (Mohseni Kabir et al., 2014).

Removing selectively hemicellulose and lignin from biomass with dilute acid and alkali pre-treatments eliminates the undesirable interaction between lignin and cellulose, as well as the physical barrier of hemicellulose leaving cellulose more accessible for enzymes (Alvira et al., 2010). Dilute sodium hydroxide (i.e., alkaline) pre-treatments (AKP) are generally effective for lignin removal (Carrillo et al., 2005) and dilute sulfuric acid pre-treatments (DAP) efficiently hydrolyse hemicellulose without an excessive formation of inhibitory degradation by-products (Saha et al., 2005). Both alkaline and acid pre-treatment, do not cause excessive sugar degradation and require much lower pressure and temperature compared to other thermochemical pre-treatment methods (Mosier et al., 2005). Therefore, sequential pre-treatments may be a promising strategy to establish suitable industrial-scale processes with high rates of sugar recovery (Mussatto et al., 2010; Sanchez et al., 2015).

There is limited information in the effect of chemical pre-treatment on CL during the ACoD with agricultural wastes and food wastes for enhancing biogas yield, VS reduction and digestate quality. The authors of this paper in their recent publication reported the BMP for the co-digestion of these substrates under wet AD of 2-4% TS (Zahan et al., 2018c) and their semi-continuous assays at 4-6% TS (Zahan et al., 2018b) and the co-substrates mixture that produced the highest yield and showed process stability were used to inform the experiment design in this study. The main goals of this study were (i) to investigate the potential of semi-solid ACoD of CL at optimum C/N ratios and (ii) to assess the effect of pre-treatment of lignocellulosic based substrates on enhancing biogas yield from the substrates. Methane production, ammonia accumulation, volatile acid production, pH as well as fractional

analysis and degradation of cellulose were used as evaluation parameters.

7.2 Materials and Methodology

7.2.1 Characteristics of substrates and inoculum

In this study, three different agro-industrial wastes: CL, FW and WS were chosen as potential substrates for AD. The substrates (CL and WS) were collected from regional Victoria, Australia (Zahan et al., 2017). The CL was stored in plastic airtight buckets. WS samples were ground, sieved to below 5 mm and stored in plastic container (Zahan et al., 2018c).

The FW substrate was prepared to simulate the organic fraction of municipal solid waste (OFMSW). A batch of synthetic OFMSW referred to here as food waste (FW) was prepared using compositional data collected by Sustainability Victoria, Australia (IWM020, 2014). The FW was then stored in small containers at -20˚C (Zahan et al., 2018b). The characteristics of the samples were measured at regular intervals to determine the variation in compositions and the shelf life of the FW (Table 7-I).

Table 7- 1: Characterisation of substrates

Parameters	Unit	CL	FW	WS	Inoculum	Acclimatised inoculum
TS	%	77.2±3.3	27.9±0.3	82.9±5.1	3.50±0.10	11.92±0.57
VS	%	39.1±2.5	26.3±0.4	79.78±2.8	2.62±0.04	7.51±1.56
tCOD	mg/L	182.5±33.8*	25205±381	ND	31450±714	ND
NH_4	mg/L	2.96±0.06*	111±4	ND	829±24	1505±26
TP	mg/L	0.60±0.01*	2357±110	ND	350±18	ND
VA	mg/L	3.56±1.12*	5352±173	ND	501.5±34.6	523±5
pH	-	8.15±0.01	5.02±0.01	ND	7.25±0.01	7.40±0.2
C/N ratio	-	13.02±1.3	18.1±1.2	81.5±1.9	6.82±0.06	ND
Glucans	%	12.6	ND	32.1	ND	ND
Xylans	%	5.1	ND	15.5	ND	ND
Lignin	%	34.4	ND	24.0	ND	ND
Ash	%	40.75	ND	4.85	ND	ND
*TCI (A1375/A290)	-	1.898	ND	1.572	ND	ND

* Unit are expressed as mg/g

** ND- not determined

#For standard deviation (±), at least three replicates were taken

Here, TS- total solids, VS- volatile solids, tCOD-total COD, NH_4- ammonia, TP-total phosphorous, VA-volatile acid, C/N ratio- carbon/nitrogen ratio, TCI- total crystallinity index

The inoculum used in this experiment was collected from the mesophilic anaerobic digester at Melton wastewater treatment plant, Melbourne and was kept at 37˚C under anaerobic conditions for several days to minimise its background biogas production (Zahan et al., 2018b). The inoculum was then acclimatised with the feedstock's (a mix of different combinations of substrates) over a long period of time (six months) to get high solid inoculum before use in the reactors (Zahan and Othman, 2018).The characteristics of the substrates and the inoculum are shown in Table 7-1. The lignocellulosic substrates were also characterised for structural carbohydrates and lignin (Zahan and Othman, 2018).

7.2.2 Pre-treatment

7.2.2.1 Alkaline pre-treatment

Alkaline pre-treatment (AKP) has shown to be very effective in reducing the lignin content of lignocellulosic biomass without significant loss in carbohydrates, thus increases the bio-digestibility. NaOH pre-treatment was applied for both CL and WS substrates. Every 1 g substrate was immersed in 10mL alkaline solution for different periods of time under different operational conditions. Independent factors including NaOH concentration (1-5% w/v), reaction time (30-90 min), and temperature (60-120°C) were explored according to experimental design developed using response surface methodology (RSM) with a three-level factorial experimental design, design 1 (Table 7-2). This pre-treatment design was used for both CL and WS.

7.2.2.2 Sequential alkaline-acid pre-treatment

Sequential pre-treatment was carried out according to the methods proposed by Sanchez et al. (Sanchez et al., 2015) and at the best conditions determined for the alkali pre-treatment (as described in section 2.2.1). WS and CL were immersed in 1%, 2%, and 3% (w/v) H2SO4 solutions as per the RSM design 2 (Table 7-2). The substrates were treated under three different temperature, 60°C, 90°C and 120°C for 30, 60 and 90 mins. After pre-treatment, the samples were washed with de-ionized water to achieve a pH of around 7 and stored at 4°C for further use.

Table 7- 2: Experimental range and levels of independent process variables

(a) For alkaline pre-treatment (AKP)			(b) For sequential acid pre-treatment (DAP) after AKP (at optimum condition)		
NaOH (1-5%)	Time (30-90 Min)	Temperature (60-120 °C)	H_2SO_4 (1-3%)	Time (30-90 min)	Temperature (60-120°C)
3	60	90	2	30	60
3	30	120	2	30	120
5	60	60	1	90	90
1	60	120	3	60	120
1	90	90	3	60	60
3	90	60	2	60	90
5	90	90	1	60	60
1	30	90	2	90	60
1	60	60	3	90	90
3	60	90	2	90	120
5	30	90	3	30	90
5	60	120	2	60	90
3	30	60	1	60	120
3	60	90	2	60	90
3	90	120	1	30	90

7.2.2.3 Optimum pre-treatment condition

The optimum pre-treatment conditions were determined by analysing the response surface methodology (RSM) models were-

1. Alkaline pre-treatment
   (a) CL - 5% NaOH 90min 120˚C
   (b) WS - 1% NaOH 30min 120˚C
2. Sequential alkaline and acid pre-treatment
   (a) CL - 5% NaOH 90min 120˚C+ 3%H2SO4 90min 120˚C
   (b) WS- 1% NaOH 30min 120˚C + 3% H2SO4 30min 120˚C

7.2.3 Experimental methodology

A sequential batch assay was conducted to determine biogas production under same AD conditions (inoculum, mesophilic temperature and time) applied for the

biochemical methane potential (BMP) assays. The sequential tests were carried out at 15% TS over 215 days in six cycles, where the substrates were mixed such that CL:FW:WS is 35:32.5:32.5 (g/g VS) to achieve a C/N ratio of 26.5 which was the optimum C/N ratio obtained through BMP assays under wet AD conditions (Zahan et al., 2018c).

All the sequential tests were performed in 1L reactors at 37±1ºC. Inoculum to substrates (S:I ratio) of 2:1 were applied and a total of 12 reactors in triplicates were used to assess performance at four different conditions. The headspace in the bottles was flushed with nitrogen gas for 2 minutes and the bottles were closed with a rubber suba seal (Zahan et al., 2018c). The bottles were kept in an incubator shaker at a constant rotational speed of 100 rpm. The volume of biogas produced was measured using a water displacement unit and the gas composition was determined using gas chromatography.

For the first 2 cycles, all the reactors received untreated samples, and then pre-treated samples were fed, i.e. during cycles, 3-6 cycles (Table 7-3). Reactor R1 always received untreated substrate as a control and also served as an indicator of reproducibility. Reactors R2, R3, and R4 received pre-treated WS, pre-treated CL and both pre-treated CL and WS, respectively. The reactors during cycle 3 and 4 were operated at the same conditions, where the lignocellulosic CL and WS were pre-treated using NaOH at the optimum condition described in Table 7-3. During cycle 5 and 6 the reactors received sequential alkaline and acid pre-treated CL and WS.

Biogas and methane composition were analysed regularly, whereas digestate characterisation was carried out at the end of experiments. The digestates were characterised for pH, NH4, FOS/TAC, VA and fractional analysis for cellulose, hemicellulose and lignin. The results obtained were also compared with the performances under wet AD conditions.

Table 7- 3: Experimental design for sequential AD

Reactor	Samples included	Ratio (VS based)	Pre-treatment	Notation
R_1	Chicken litter: Food waste: Wheat straw	35: 32.5: 32.5	All 7th Cycle: no pre-treatment	All 7th Cycle: CL_{35}:$FW_{32.5}$:$WS_{32.5}$
R_2	Chicken litter: Food waste: Wheat straw	35: 32.5: 32.5	1st & 2nd Cycle no pre-treatment	1st & 2nd Cycle CL_{35}:$FW_{32.5}$:$WS_{32.5}$
			3rd & 4th cycle Wheat straw alkali pre-treated (**A1**- 1% NaOH 30min 120^{0}C)	3rd & 4th cycle CL_{35}:$FW_{32.5}$:$WS_{32.5}$ **(A1)**
			5th & 6th cycle Wheat straw sequential alkali and acid pre-treated (**AA1**- 1% NaOH 30min 120^{0}C + 3% H_2SO_4 30min 120^{0}C)	5th & 6th cycle CL_{35}:$FW_{32.5}$:$WS_{32.5}$ **(AA1)**
R_3	Chicken litter: Food waste: Wheat straw	35: 32.5: 32.5	1st & 2nd Cycle no pre-treatment	1st & 2nd Cycle CL_{35}:$FW_{32.5}$:$WS_{32.5}$
			3rd & 4th cycle Chicken litter alkali pre-treated (**A2**- 5% NaOH 90min 120^{0}C)	3rd & 4th cycle $CL_{35(A2)}$: $FW_{32.5}$: $WS_{32.5}$
			5th & 6th cycle Chicken litter sequential alkali and acid pre-treated (**AA2**- 5% NaOH 90min 120^{0}C+ 3%H_2SO_4 90min 120^{0}C)	5th & 6th cycle $CL_{35(AA2)}$: $FW_{32.5}$:$WS_{32.5}$
R_4	Chicken litter: Food waste: Wheat straw	35: 32.5: 32.5	1st & 2nd Cycle no pre-treatment	1st & 2nd Cycle CL_{35}:$FW_{32.5}$:$WS_{32.5}$
			3rd & 4th cycle **A1 & A2**	3rd & 4th cycle CL_{35} **(A2)**: $FW_{32.5}$:$WS_{32.5}$ **(A1)**
			5th & 6th cycle **AA1 & AA2**	5th & 6th cycle CL_{35} **(AA2)**: $FW_{32.5}$:$WS_{32.5}$ **(AA1)**

7.2.4 Analytical methods

TS, volatile solids (VS) and alkalinity were measured according to APHA Methods 2540B, 2540E and 2320B, respectively (Rice et al., 2012). The COD (total and soluble), total phosphorus (TP), total nitrogen (TN), ammonium and volatile acids (VA) were determined by colorimetric techniques using a HACH (Model: DR/5000 U) spectrophotometer according to the methods 8000, 10127, 10072, 10031 and 8196, respectively (Zahan et al., 2018c). The samples were centrifuged (Eppendorf 5702, Germany) at 4.4 rpm for 15 mins and then filtered through 0.45 µm filter paper (mixed cellulose-ester membrane filter, Advantec, Japan), to measure the soluble constituents. The pH was measured using a calibrated pH meter (ThermoOrion, Model 550A). The C/N ratio was determined using LECO (TruMac) analyser.

The composition of the biogas was analysed according to the APHA method 2720C using gas chromatography (Varian 450-GC, Varian Australia Pty Ltd., Netherlands) equipped with a packed column (GS Carbonplot 113-3132, 1.5 µm, 30 m* 0.320 mm, stainless steel, Agilent Technologies Inc., Australia). The carrier gas used was helium at a flow rate of 28 mL/min. The temperature settings for the column, detector and injector were 70°C, 200°C and 100°C, respectively. The biogas was collected and manually injected using a 50 mL FORTUNA® Optima glass syringe (Poulten and Graf, Germany). The volume of biogas was normalised to standard conditions i.e. standard temperature and pressure (0ºC and 1 bar) according to method described by (Strömberg et al., 2014)) and results are expressed as norm-litre (LN). The headspace was also corrected for methane (CH4) and carbon dioxide (CO2) to 100% according to VDI 4630 (2006) (VDI, 2006).The sequential fractionation of untreated and pre-treated substrates as well as the digestate were done according to the method reported by (Opatokun et al., 2015; Zahan et al., 2018b).

Fourier transform infrared (FTIR) spectroscopy (Spectrum 100, PerkinElmer, USA) equipped with a universal ATR (attenuated total reflection) accessory was used for structural analysis and crystallinity of the treated and untreated samples. The samples were dried in oven at low temperature of 40°C before doing the experiments to ensure no structural damage. The spectra were obtained with an average of 64 scans from 4000 to 600 cm-1 with 4 cm-1 resolution monitored by

Spectrum software. Crystallinity index (CI) and total crystallinity index, which are the absorbance ratio of A1430/A896 and A1375/A2900, respectively, were also calculated from the spectra.

7.2.5 Statistical analysis

The RSM design for each pre-treatment comprised fifteen experiments which were carried out in duplicate according to the scheme mentioned in Table 7-2 and then the results were analysed using the statistical software MINITAB Version 17.1.0. Optimum values of the selected variables were obtained by solving the regression equation and by analyzing the response surface contour plots.

7.3 Results and Discussion

7.3.1 Characterisation and pre-treatment of the lignocellulosic CL and WS

The details of basic physical properties and chemical compositions of the substrates i.e. CL and WS are shown in Table 7-1. The weight percentage of ash and moisture content of the substrates varied and lied in the ranges of 40.75%-4.85% and 22.8%-17.1% for CL and WS, respectively. The C/N ratio of the WS was 6.27 times higher than of the CL. Among the studied biomass, CL showed the lowest C/N ratio, whereas, for FW it was slightly higher at 18.1. The lower C/N ratio in the biomass implies less C-content during the digestion which paves the accumulation of ammonia during AD. It was also reported that, C/N ratio between 20 and 30 is considered optimal but some studies reported stabilised biogas production within a wider ranges of C/N ratios, e.g. 9-30, depending on the substrates (Zahan et al., 2016b).The composition of the lignocellulosic substrates used, CL and WS, i.e. cellulose, hemicellulose, lignin and water soluble content (untreated and pre-treated) were determined by fractional analysis (Figure 7-1). Significant difference was observed for CL and WS in terms of structural carbohydrates. Hot water extraction process was performed to remove the water soluble contents. It was observed that CL and WS contained similar amounts of water soluble contents (21-23%) and hemicelluloses (17.3-18.5%), respectively.

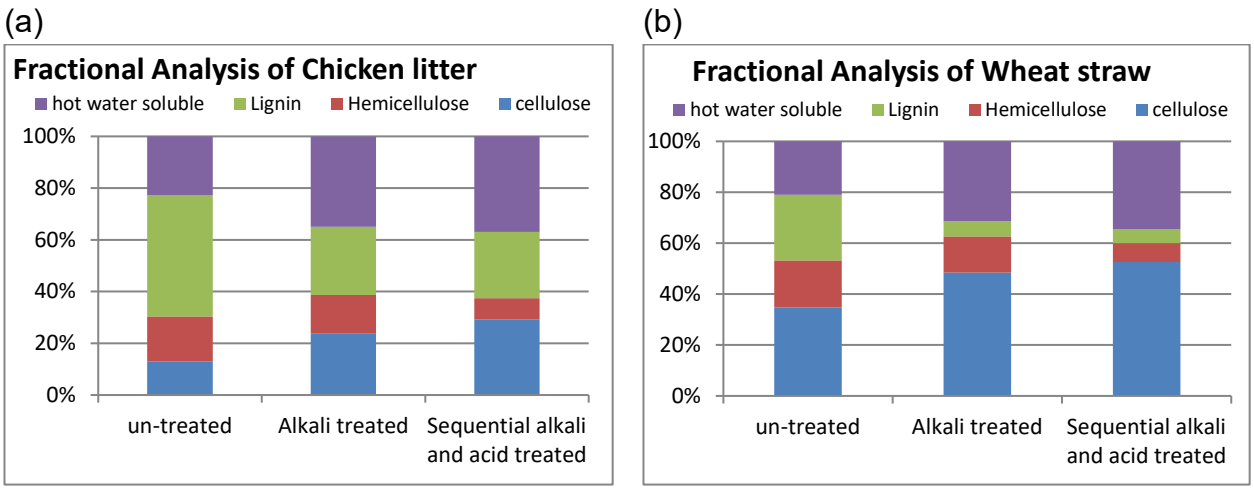

Figure 7- 1: Fractionation analysis of lignocellulosic substrate before and after pre-treatment for the optimum conditions for (a) CL and (b) WS

The substrate cellulose compound in WS (34.8%) was almost 2.7 times higher compared to CL (12.97%). The total crystallinity index for WS and CL were found to be 1.572 and 1.898 respectively. This is due to the presence of more crystalline hardwood i.e. wood shavings in CL compared to WS (Salehian et al., 2013; Zheng et al., 2014). The lignin content in CL (46.8%) was very high. The fractionation analysis is a gravitational analysis method of total fractions; therefore, the lignin content presented here also included a high fraction of ash content in it.

After NaOH pre-treatment, the degradation of lignin was found to be 2-40% and 30-70% removal for CL and WS, respectively for the different conditions tested. The amount of cellulose and water soluble content increased drastically (1.6-2 times higher), however the degradation of hemicellulose was much lower. Therefore, alkali pre-treated CL and WS (at the optimum condition), were sequentially acid pre-treated. After the sequential pre-treatment, though the lignin content was not degraded much, a huge degradation of hemicellulose content was observed i.e. almost 50% of the hemicellulose content degraded. The cellulose, hemicellulose, lignin and water soluble content of the untreated and for alkali and sequential alkali-acid pretreatment were shown in Figure 7-1.

7.3.2 Sequential anaerobic digestion

The average daily biogas production (reported as mL_N/gVS_{added} fed to the reactor) were measured at 15% TS feedstock for 215 days, for the substrates CL:FW:WS at a ratio of 35:32.5:32.5 to obtain C/N ratio of 26.5, the optimum substrate mix ratio found using batch ACoD assays (Zahan et al., 2018c). From Figure 7-2, consistent biogas production of 321.8±13.4 mL_N biogas/g VSadded was observed over first 2 cycles, for all the four reactors, where untreated substrates were fed. The results presented here are the average of triplicate values for each reactor. After the 1st and 2nd cycle, pre-treatments were applied to CL and WS fed into reactors R_2, R_3 and R_4. It can be observed from Figure 7-2, that ACoD of the pre-treated substrates showed improved biogas production compared to the untreated substrates, at the same conditions.

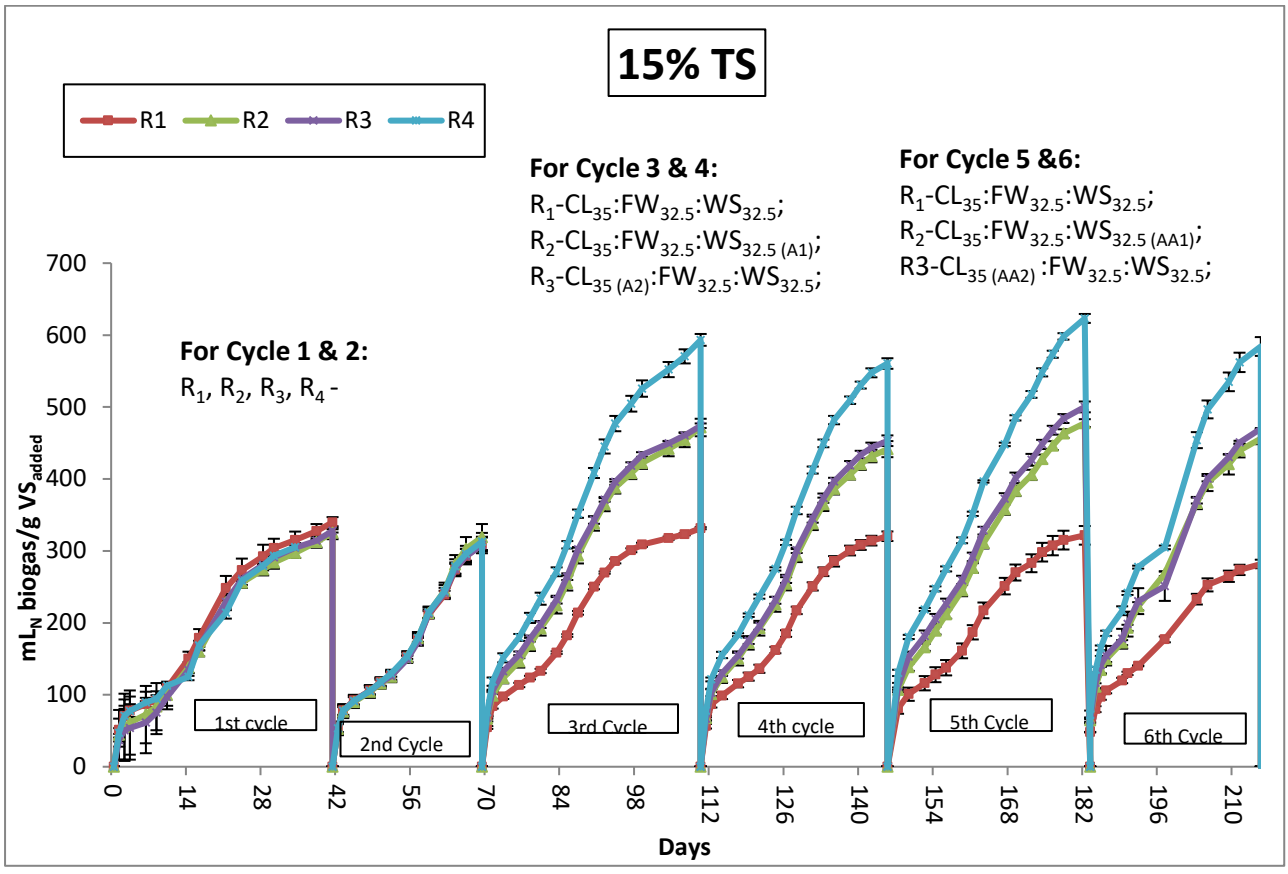

Figure 7- 2: Biogas production during sequential AD

A1- 1% NaOH 30min 120°C pre-treated WS;
AA1- 1% NaOH 30min 120°C + 3% H2SO4 30min 120°C pre-treated WS;
A2- 5% NaOH 90min 120°C pre-treated CL;
AA2- 5% NaOH 90min 120°C+ 3%H2SO4 90min 120°C pre-treated CL

During cycle 3 and 4, alkali pre-treatments were applied to CL and WS. From Figure 7-2, an improvement of around 42% and 45% in total biogas was observed compared to untreated substrates for reactors R_2 (receiving alkali treated WS) and R_3 (Receiving alkali treated CL) respectively. However, when both CL and WS were alkali pre-treated and fed into reactor R_4, up to 80% increase in total biogas was observed compared to untreated substrates (reactor R_1) and almost 25% increase compared to the single pre-treated WS (R_2) or CL (R_3).

During cycles 5 and 6, sequential alkali and acid pre-treatment were applied to CL and WS. R_2 received sequential alkali-acid pre-treated WS feedstock, R_3 received sequential alkali-acid pre-treated CL mixture and R_4 received both pre-treated substrates (CL and WS). Biogas production improved by 44% and 51% compared to untreated substrates for reactors R_2 and R_3 respectively, when either of WS or CL was pre-treated. However, when both CL and WS were sequentially alkali-acid pre-treated and fed into the AD reactor (i.e. Reactor R_4), up to 88% increase in total biogas was observed compared to untreated substrates (reactor R_1). With an extra pre-treatment added (acid pre-treatment), the improvement in total biogas was only around 5% compared to single alkali pre-treatment, as seen during cycles 3 and 4.

From Figure 7-3, during 1st and 2nd cycles, all the reactors showed a high initial peak between 51-60 mL_N biogas/g VS_{added}.d at day 1 and at day 3 a lag phase is observed. The high amount of biogas produced on dat 1 are most likely because the reactors recieved acclimatised inoculum to the feedstock hence the anaerobic microorganisms were available and active to degrade the readily available soluble substrates. During 3rd and 4th cycle, an initial peak of 66, 68 and 75 mL_N biogas/g VS_{added}.d at day 1 was observed for reactors R_2, R_3 and R_4, respectively. During the 5th and 6th cycle, the biogas production in day 1 was the highest (% higher than cycle 3 and 4), and it was almost 52%, 53.3% and 87% higher for reactors R_2, R_3 and R_4, respectively than the initial peak from the untreated substrate mixture (reactor R_1).

After the initial peak, a lag phase of 7 days starting from day 3 was observed. However, when pre-treated CL and WS were fed into reactors R_2, R_3 and R_4 for cycles 3 to 6, the amount of lag phase at day 3 was improved by 5-12 mL_N biogas/g VS_{added}.d (Figure 7-3) and the highest improvement was observed for reactor R_4

when both the pre-treated samples were used. A second peak was noticed on day 17-20 of 13-18 mL_N biogas/g VS_{added}.d during both the 1st and 2nd cycle. At 3rd and 4th cycle, the 2nd peak increased by 2-7 mL_N biogas/g VS_{added}.d for reactors R_2, R_3 and R_4 with alkali pre-treatment, however, it didn't improve further when sequential acid pre-treatment were applied. Rather they had some scattered peaks after high initial 1st peak.

The biogas' methane composition was between 60-67% for the untreated sample, (for cycle 1 and 2) and for the alkali pre-treated samples, the methane content was between 66-69% (cycle 3 and 4). When the AD reactors received sequential alkali-acid pre-treated substrates, the methane composition was found between 67-71%. Therefore, pre-treatment not only improved the total biogas production, it also improved the quality of the biogas, i.e. the percentage of methane.

For reactor R_1, which received untreated substrates for all six cycles, the biogas production slightly decreased i.e. around 10% compared to the initial cycle with the continual operation of the reactor. This indicates slight inhibition occurred in the sequential batch AD over the duration of the experiment (215 days) probably

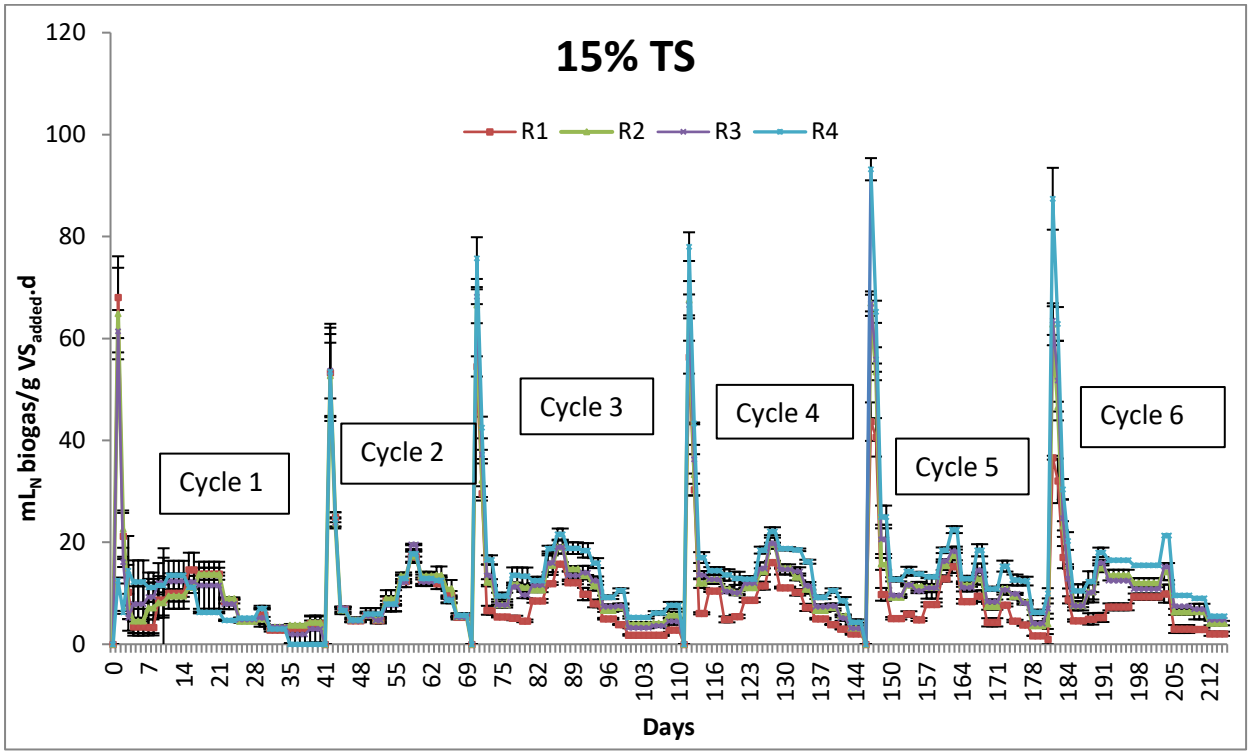

Figure 7- 3: Daily biogas production during sequential AD

because of ammonia or lignocellulosic material accumulation. This phenomenon was explained in detail in section 3.2.

Overall, it can therefore be stated that biogas quality improved with the pre-treatment of the lignocellulosic CL and WS. The daily biogas production was also improved by 50-80%. With alkai pre-treatment 40-80% improvement in biogas production was achievable when the AD reactor received feedstock where either of or both the CL and WS were pre-treated. However, sequential alkali/acid pretreatment only increased total biogas by 5% compared to alkali pre-treatment, though the initial daily biogas yields were higher.

7.3.2 Reactor performances

In addition to the biogas yield, different parameters were monitored at the end of each cycle to assess the quality of the digestate and supernatant. The process performance parameters measured are summarised in Table 7-4 and Figure 7-4.

Table 7- 4: VS removal during sequential batch AD assays

Reactors	VS Removal %					
	1st Cycle	2nd cycle	3rd cycle	4th cycle	5th cycle	6th cycle
R1	40.7±0.2	35.98±0.2	33.8±0.1	34.63±0.6	32.36±0.98	30.65±1.50
R2	36.25±0.2	36.32±0.3	42.60±0.1	41.78±0.2	45.62±1.25	44.96±1.44
R3	36.11±0.1	36.25±0.6	39.53±0.8	40.27±0.3	44.32±1.71	43.95±1.77
R4	33.99±0.4	35.68±0.1	49.58±0.9	50.12±0.5	55.20±1.27	55.69±1.18

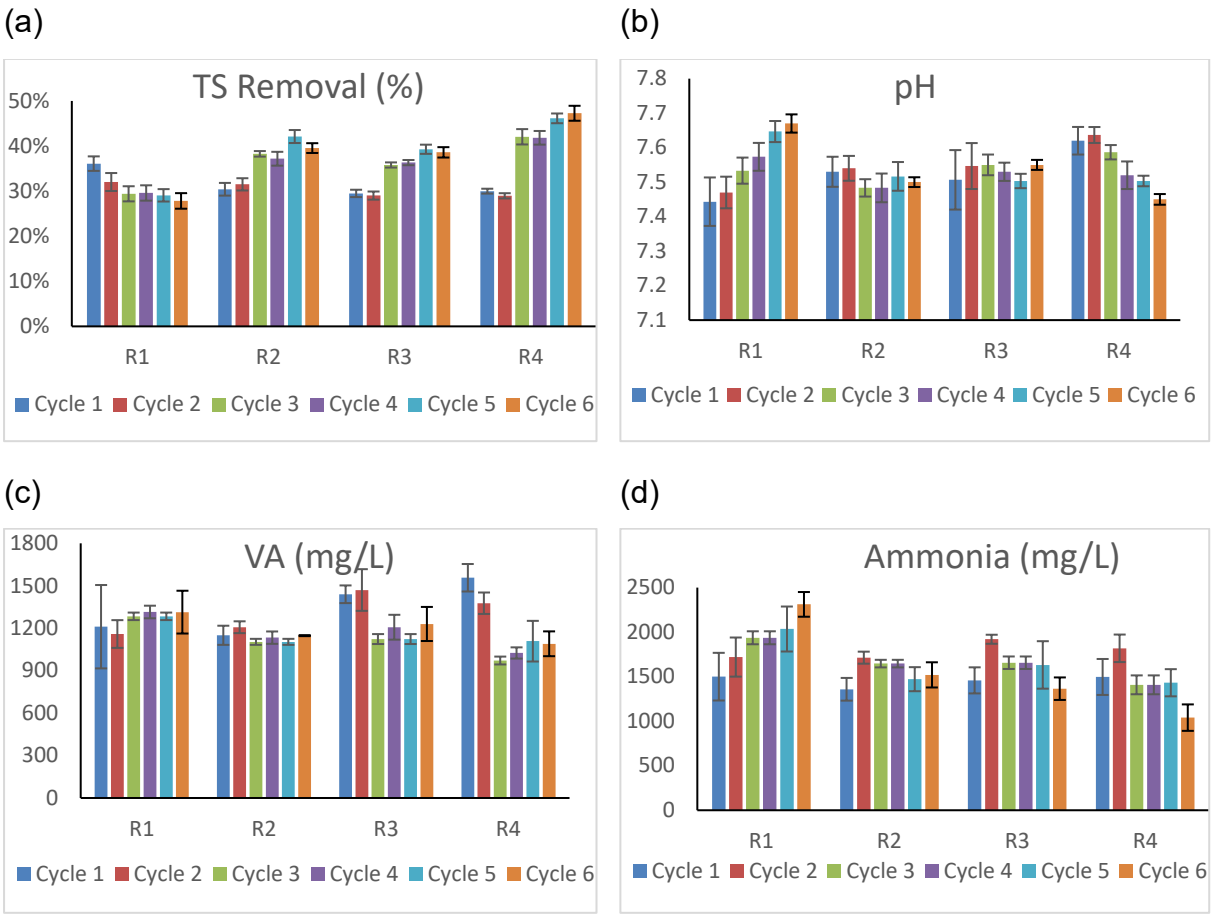

Figure 7- 4: Reactor performance during sequential AD- (a) TS removal (%), (b) pH, (c) VA (mg/L) and (d) ammonia

The VS removal at the end of each cycle is shown in Table 7-4. In all the reactors, the VS removal for the first 2 cycles (untreated substrates) was around 36.7±1.9%. It was observed that the VS removal under semi-continuous AD was 41.7% (Zahan et al., 2018b) and the VS removal of 42.8%, obtained using BMP assays at 4% TS feed (Zahan et al., 2018c) for CL ACoD with agricultural wastes and FW. As the reactors used acclimatised inoculum as anaerobic microbial source, therefore, the inoculum had already adapted to the different substrates-mix (feedstock), which could explain the TS and VS removal at these high TS of 15% being comparable to the AD at 4% TS, though slightly less.

For reactor R1, the VS removal decreased to around 34.8%-30.65% with the continual operation of the reactors, which synchronised with the trend in biogas production. As shown in Figure 7-4, the inhibitory parameters (NH4 and VA)

increased in the reactors with times, causing less activity of the methanogenic bacteria, which led to 15.4% decrease in VS removal.

When either CL or WS was alkali pre-treated, VS removal of around 42.6% and 39.5% respectively were observed for reactors R2 and R3. The VS removal from AD reactors that received pre-treated WS mixture was around 2% higher compared to the treated CL mixtures, as WS has more cellulose and less lignin content than CL. VS removal of around 50% was obtained when both the alkali pre-treated samples were fed into the reactor R4 in cycle 3 and 4. During the 5th and 6th cycle, when sequential alkali and acid pre-treatment were applied, the VS removal ranged between 44% to 55%, for reactors R2-R4, which is almost 20-50% higher than the initial removal. From Figure 7-4 (a), the TS and VS removal correlated with the biogas production trends. For reactor R1, the TS removal efficiency decreased with continual operation. The highest TS removal was around 47% for reactor R4 when feedstock that contains sequential alkaline and acid pre-treated CL and WS mixture was applied.

Pre-treatment also improved the AD process performance. Characteristics at the end of each cycle are shown in Figure 7-4. At the start, the pH was between 7.3-7.6 for cycle 1 and 2 (Figure 7-4 (b)), which increased to 7.7 with subsequent repeats of feeding for reactor R1. With each repeat, the reactors were overloaded with CL as indicated by the increase in pH, associated with the accumulation of ammonia from the uric acid and proteins, and this subsequently increased the pH level (7.7) in the reactor. In this way, inhibition in the form of decreased TS, VS removal was evident for reactor R1 (Table 7-4 and Figure 7-4). The pH level decreased (7.4-7.45) with pre-treated feed which led to an improvement in biogas production and VS removal. With sequential pre-treatment, the pH level decreased further within the optimal range for the high performing reactors which are an ideal situation for stable methanogenesis (Zahan et al., 2018b).

No accumulation of VAs was evident and the highest VA observed with untreated feed (1.55 g/L) which is well below the inhibition threshold (4 g/L) (Siegert and Banks, 2005). The VA accumulation threshold can vary from substrate to substrate depending upon several factors and tolerances of microbial source .The accumulation of VA can cause instability of the process by inhibiting acetotrophic

methanogenesis (Zahan et al., 2018b). From Figure 7-4 (c), the VAs in the reactors decreased further with pre-treated substrates, indicating no inhibitory effect for VA and was associated with increased biogas production.

From Figure 7-4(d), for reactor R1, the NH4 increased with the continual cycle repeats feeding cycles. For wastewater, if the NH4 level is between 1.5–2.0 g/L, inhibition occurs (Zahan et al., 2016b). The ammonia level in reactor R1 was in the inhibition range and with each repeat the ammonia level increased. As acclimatised inoculum was used, it improved the tolerance for NH4 level and the AD reactor was continued to produce biogas. Eventually, however, inhibition occurred in the reactor, if no measure to stop inhibition was taken. For reactor R2, a decrease in NH4 was observed for cycles 3 and 4. Similar trend was observed for R3 which received feedstock including CL pre-treated samples. The pre-treatment improved the process performance, most likely through the increased water soluble organics as well as soluble organics which served to provide balance environment in terms of C/N and degradation products utilisation. From Figure 7-4(d), the NH4 level in reactor R3 is slightly lower than reactor R2. This is probably due to the pre-treatment of CL which removed some of the free ammonia in CL samples whereas in reactor R3, untreated CL was used. For reactor R4, where both CL and WS pre-treated samples were fed, best performance was observed, and the ammonia was below 1500 mg/L.

From the results it can be summarised that with each sequential repeat feed the removal of TS and VS decreased with increase in pH, and ammonia. This was very consistent with their biogas production trends. On the other hand, the pH, VA, ammonia level decreased with pre-treated feed with increased TS and VS removal. The inhibition in the reactors occurred more of an occurrence of ammonia inhibition rather than VA accumulation. The specific biogas production and CH4 yield depended mostly on the origin of the substrates, composition, and operational conditions i.e. SRT, temperature for large reactors (Zahan et al., 2018b). Therefore, the results reported here should be applied to a continuous process and a small pilot scale continuously fed anaerobic digester before they are used in a large-scale scenario.

7.3.3 Sequential fractionation

As the substrates (CL and WS) were lignocellulosic in nature, the digestate collected from each reactor at the end of each cycle, during the AD tests, were analysed to observe the changes to the composition, e.g., cellulose, hemicellulose and lignin with the continual operation of the AD reactors. For this purpose, sequential fractionation of the digestate was employed and the results are depicted in Figure 7-5.

From Figure 7-5 (a), the fraction of cellulose in R1 digestate was increased (24.29- 29.13%) with sequential repeats of feeding in to reactor R1 which was fed with untreated substrate mix. But less changes in hot water soluble content was observed. The reactor R1 received untreated CL and WS, hence the non-degradable lignin and cellulose, remained almost non-degraded and accumulated in the reactor with the repeated feeding, showing decrease in biogas production and lower VS removal (Figure 7-2 and Table 7-4). As shown in Figure 7-5(c), the hemicellulose (%) in the digestate showed no significant variation for R1, i.e. little degradation occurred under the conditions tested. These results indicated hemicelluloses were not much accessible to the anaerobic microorganism under these conditions (Zhang et al., 2014a). It has been reported that, cellulose was utilised in preference of hemicellulose during mesophilic and thermophilic AD in nitrogen-rich environment and were converted at a higher efficiency, because the metabolism of cellulose breakdown requires least investment of enzymes and energy (Zahan et al., 2018b). From Figure 7-5(d), for 1st and 2nd cycle, lignin (%) in the digestate with untreated substrates was similar to feed lignin content. As the fractionation analysis is a gravitational analysis method of total fractions; therefore, the lignin content included the high amount of ash content of the substrates indicating no degradation of lignin. Lignin degradation of 15-20% under the AD was reported in literature because of the presence of some cellulolytic microbes in AD (Zahan et al., 2018b), however there will be still higher amount of lignin remaining inside the reactors. Therefore, pre-treatment to breakdown the bondage of lignin and hemicellulose are always suggested for enhancement in their removal (Zhang et al., 2014a).

From Figure 7-5, pre-treatment improved the degradation of the lignocellulosic components, with the fraction of cellulose being decreasing and the increase in hot

water soluble contents in the reactors during the digestion. This indicated more soluble content were available for micro-organisms to produce biogas. For reactor R2 and R3, during 3rd and 4th cycle, where alkali treated CL and WS were fed, the decrease in cellulose was 26% to 23% with an increase in hot water soluble content from 20% to 26% compared to R1. For R4, where both the pre-treated substrates were fed, the cellulose degradation was around 20% which is almost 40% less than the cellulose feedstock content. The hot water soluble contents increased to 30 % compared to 14.3% in the initial feed. For 5th and 6th cycle, nearly around 22% degradation of cellulose and 90% increase in water soluble content were found for reactor R2 and R3 with the sequential alkali-acid pre-treated CL and WS feed. The highest amount of degradation of cellulose found was for R4 which produced the maximum biogas. Almost 1.4 times increase in water soluble content and 0.43 times decrease in cellulose content were also observed compared to feedstock content, indicating sequential alkali-acid pre-treatment was effective in enhancing the biogas production. Overall, from Figure 7-5 (a) and (b), an inverse relationship was

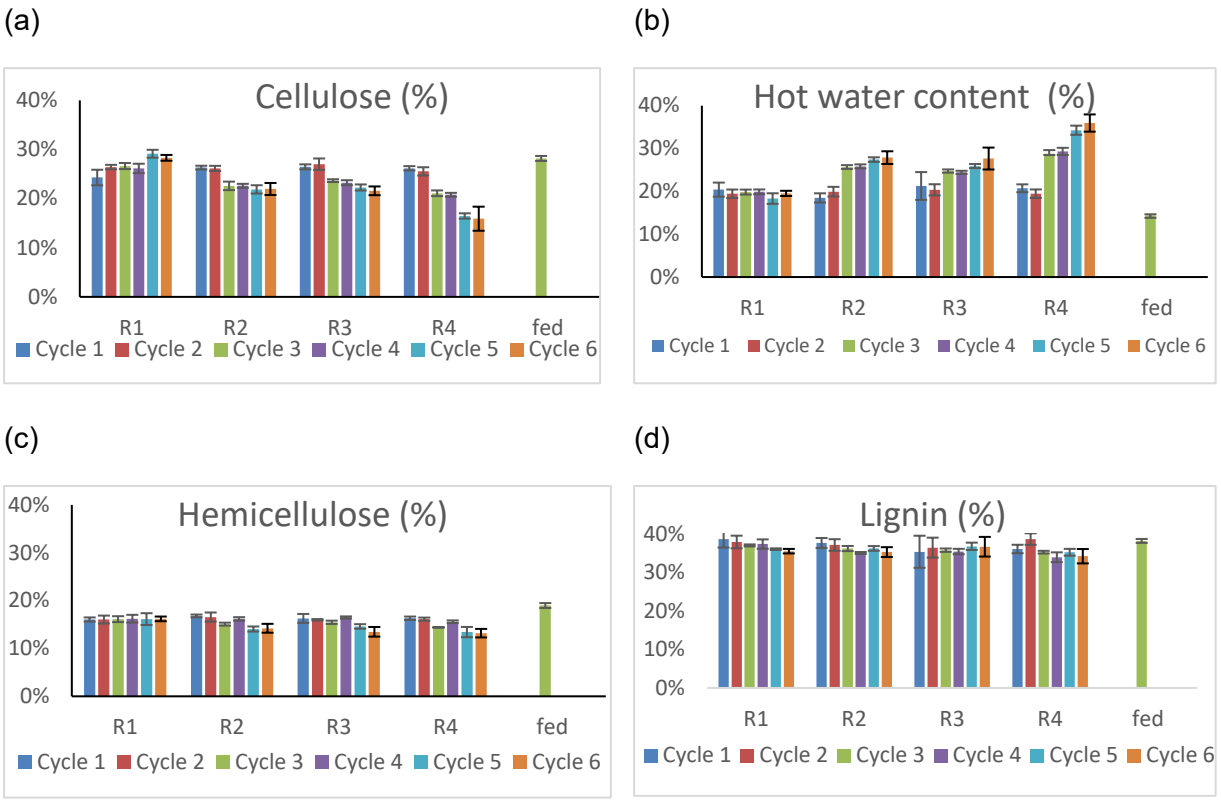

Figure 7- 5: Sequential fractionation analysis of feedstock and digestate for (a) Cellulose (%), (b) Water soluble content (%), (c) Hemicellulose (%) and (d) Lignin (%)

observed between the water soluble content and cellulose contents. This means that water soluble contents had strong correlation with the biogas production.

From Figure 7-5(c), during 3rd and 4th cycle, hemicellulose content didn't degrade much where alkali-treated substrate mixture was feed. This is because the breakdown of hemicellulose bondage was less during alkali pre-treatment (Figure 7-1). During 5th and 6th cycle, degradation of hemicellulose was observed which is synchronised with their pre-treatment and biogas productions results (Figure 7-1 and 7-2). From Figure 7-5 (d), lignin contents in the digestate collected from the reactor R4 decreased, indicating slight degradation, where both the pre-treated CL and WS were fed. However, in most of the cases, the lignin content after digestion was found to be unchanged. This was probably due to the nature of the fractionation analysis method where the lignin content included the high amount of ash content as well as the inorganic content of the substrates at the end. This can be a limiting factor of this method for determining the lignin in the digestate mixture. However, it is still an indicative method for cellulose content for assessing the degradation process.

Therefore, the results showed a correlation between biogas production and carbohydrate content. The more the degradation of cellulose and water soluble content found in the reactors, the higher the biogas production. It can be concluded that, pre-treatment unlocks the shell structure of lignocellulose and make the biodegradable materials available to the microbes enabling almost double the biogas production.

7.3.4 Comparison of semi-solid and wet anaerobic digestion

The performance results of semi-solid AD (15% TS) were compared with their wet AD performances (4% TS) under batch and continuous assays at mesophilic conditions (Zahan et al., 2018b; Zahan et al., 2018c) and were shown in Table 7-5. The biogas production was 517.2± 12.6 mL_N biogas/g VS_{added} with the associated VS removal of 42.8% at 4% TS loading under batch assays. From continuous anaerobic digestion at 4% TS feed (HRT 20days), it was found to be have 366 mL_N biogas/g VS_{added} biogas production with a VS removal rate of 41.7%. Whereas, at 15% TS the

Table 7- 5: Comparison of AD at different TS percentage with chicken litter, agro-industrial wastes and food wastes

Biogas ($mL_N/g\ VS_{added}$)				
Batch at 4% TS (Zahan et al., 2018c)	Continuous at 4% TS (Zahan et al., 2018b)	Batch at 15% TS (This study)		
Untreated	Untreated (20 days)	Untreated	Alkali pre-treated	Sequential alkali and acid pre-treated
517.2	366	321.6	440-590	470-625
VS removal (%)				
Batch at 4% TS (Zahan et al., 2018c)	Continuous at 4% TS (Zahan et al., 2018b)	Batch at 15% TS (This study)		
Untreated	Untreated (20 days)	Untreated	Alkali pre-treated	Sequential alkali and acid pre-treated
42.8	41.7	36.7	40-45	49-55

biogas production was 321.8±13.4 mL_N biogas/g VS_{added} which is almost 38% less biogas production compared to the batch assay at 4% TS as well as lower than biogas production from the continuous AD. The VS removal of around 36.7% is also lower compared to wet digestion which is synchronised with the biogas production. This lower biogas production and VS removal is probably due to the substrate overloading at high TS conditions which led to ammonia accumulation and lower degradation of lignocellulosic substrates. Although the biogas production is 38% less in semi-solid AD (15% TS), the reactor size will be almost 3.75 times lower compared with wet AD. This will allow more waste feeding with less waste and digestate handling; less heating and energy.

With pre-treatment, however, the biogas production can be improved and even possible to get higher biogas production than that at wet AD with untreated samples. From Table 7-5, biogas production of around 440-625 mL_N biogas/g VS_{added} can be achievable at high solid conditions (15% TS) with VS removal up to 55%. Therefore, these results indicate the possibility AD at semi-solid condition is possible, though required to take measures to mitigate the inhibitory parameters with untreated substrates. However, with pre-treated substrates, a continuous production of biogas is possible without any inhibition with possible reduction in total volume of digester

plant by four times.

7.5 Conclusions

CL can be successfully co-digested with agro-industrial wastes and food wastes under semi-solid conditions of 15% TS for feedstock of C/N ratio of 26.5 at mesophilic AD conditions. Biogas production using the sequential batch AD was 38% less than that at wet AD of 4% TS for untreated substrates. But sequential alkali-acid pre-treatment of the lignocellulosic substrates improved the biogas production by 88% with an associated VS reduction of around 55%. Therefore, this study provides strong evidence that ACoD at high TS can be achieved and offers promising opportunities compared to wet AD for agro-industrial wastes.

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Chapter 8 Conclusions and Recommendations

8.1 Conclusions

This chapter summarises the main conclusions that can be drawn from the investigation of anaerobic digestion (AD) and codigestion (ACoD) potential of chicken litter (CL) wirh agro-industrial and food wastes (FW) in Australian context. In this reaserch work, single substrates digestion potential, co-digestion potential, optimum co-substrate mixture and condition, continuous ACoD, improvement of biodegradibility through pretreatment, and finally high solids (HS) AD pontial were conducted. Conclutions and major findings from each of these experiments are provided below with recommendations for future works which offer pathways to develop the findings of the work.

8.1.1 Single substrates digestion in batch assays

Single substrate digestion work was conducted with chicken litter (CL), youghert whey (YW), organic fraction of municipal food wastes (FW), wheat straw (WS) and hay grass (HG). The highest biogas yield of 316.73 mLN/g VS_{added} was from CL at 3% VS loading. Further increase in VS concentration decreased biogas production for CL. The ammonia concentration was below the inhibition threshold of 1.5-2.0 g/L for CL; however, the inhibition at higher VS concentration occured because of the high lignin of the bedding materials of CL. Among the different co-substrates tested, FW produced the highest amount of biogas (669.5 mLN/g VS_{added}) at 4% VS concentration which is 2.1 times higher than the CL's yield. Lignocellulosic wastes i.e. HG, WS as well as CL had low per day biogas generation and the biogas generation had longer duration than the organic wastes to stabilise. The digestate characterisation results were also in agreement with their biogas production.

Among the four model, i.e. the first order regression model, the modified Gompertz model, the transfer function model, and the cone model, the modified Gompertz model was the best fit for the measured BMPs (R2 = 0.93 to 0.99) and deviations between measured and the predicted BMPs were less than 10.0%. Therefore, this model could accurately simulate and predict the duration of lag phase, meathane production rate which would help in selection substrates for co-digestion. For organic

wastes (YW and FW), no lag phase, higher hydrolysis rate constant and maximum methane production rate were observed with the increase in substrates VS concentrations. YW and wheat straw (WS) showed high hydrolysis rate among all the wastes. Therefore, the addition of organic wastes in AcoD of CL should decrease the lag phase and addition of WS at low concentration should improve the hydrolysis rate.

8.1.2 ACoD potential in batch assays

The ACoD of CL with YW, FW, WS and HG showed that ACoD had synergistic effect and when C/N ratio was more than 20, 15-20% increase in biogas production was achieved. The biogas yield increased as the percentage of agro-industrial wastes increased and CL percentage decreased in the feedstock. An improvement of 85% in biogas production, compared with the monodigestion of CL, was achieved by adding 10-50% of other substrates. ACoD also improved the lag phase compared to single substrates digestions as well as the biogas production stabilised within 30 days of digestion. The biogas production rate with all the different substrates mix showed a single peak, which is in synchronisation with the kinetic model prediction of single substrate digestion. The methane yield improved as the C/N ratio improved with adding more of other subatrates with CL. In case of ACoD of CL with the lignocellulosic substrates i.e. WS and hay grass (HG), the VS removal decreased with the increase in the percentage of WS and HG. However, when organic wastes was added i.e. the ACoD of CL with FW or FW, WS and HG, a more balanced C/N ratio and better process performances were obtained. Maximum biogas yield was achieved for feedstock of C/N ratio of 26-27.5 comprised of chicken litter (30-35%) and mixture of YW, HG and WS (65-70%), determined using surface optimisation model.

8.1.3 Continuous ACoD

Mixing CL with different agro-industrial substrates and FW, improved not only the C, N balance (i.e. C/N ratio) but also the daily biogas production under semi-continuous AD. AD reactors performed the best at organic loading rate (OLR) of 2 gTS/L.d and CL:FW:WS at 60:20:20 ratio showed the best performance. As the OLR increased more that 2.5 to 3 gTS/L.d, the biogas production decreased by 20-36%. At the low

OLR of 2-2.5 gTS/L.d, AD reactors that received a mixture of two substrates showed better bio-methane yield than reactors that received combination of three or more substrates. That was because, at low OLR i.e. at less VS feeding, the reactors had less effect of C/N rato imbalance. However, as the OLR increased, combining three and more substrates showed better performances than feedstocks of binary substrates, mostly because of better balanced C/N ratio. Increasing the OLR to 2-3 gTS/L.d, the removal of TS, VS, and COD decreased and the pH, VA, ammonia, TN and TP increased. A correlation was found between biogas production and carbohydrate content with varied OLR. Although biogas production depends on balanced C/N ratio, for lignocellulosic substrates with similar C/N ratios, biogas production is further reliant on their lignocellulosic properties.

Principal component analysis (PCA) were able to explain 68.1% of data variability with PC1 (first componet) explained 48.4% and PC2 (second component) explained 19.7% data variability. There was a strong correlation between biogas production and the water soluble content, cellulose and lignin degradation as well as VA (mg Acetic acid/L) and pH.

8.1.4 Pre-treatment

Alkaline pretreatment (AKP) with 1-5% NaOH and sequencial dilute acid pretreatment (DAP) with 1-3% H_2SO_4 were used to pre-treat lignocellulosic CL and WS using four response surface optimisation (RSM) models. AKP increased lignin removal and DAP was effective for hemicellulose removal. After AKP, the degradation of lignin was 2-40% and 30-70% for CL and WS, respectively for the different conditions tested. The cellulose and water soluble content increased drastically (1.6-2 times higher), however the hemicellulose did not degrade much. After DAP following AKP, little removal of lignin and almost half of the hemicellulose removal were observed. Therefore, with AKP+DAP pre-treatment, more cellulose and water soluble content could be achievable which could be utilised by the microorganism during AD process for maximising energy production. With sequential AKP+DAP, an increament of biogas yield of around 25% were observed compared with single pre-treatment. Through Minitab analysis, Chemical concentration (i.e.

percentage of NaOH and H_2SO_4) and temperature had more effect over time duration on the pre-treatment and charbohydrate degradation.

8.1.5 HS-AD in sequential batch assays

High solids anaerobic digestions (HS-AD) were performed at 15% TS with untreated and pre-treated substrates (optimum pre-treated subatrates found in the previous section) with the optimum conditions found for ACoD with surface optimisation model. Biogas production and quality improved when pre-treated CL and WS were used compared to untrated substrates at HS-AD. The daily biogas production was also improved by 50-88%. With AKP substrates, 40-80% improvement in biogas production was achievable by using only pre-treated CL or WS, or conbined pre-treated CL and WS, however, further sequential acid pretreatment (DAP) only added 5% more total biogas. With each sequential repeat feed the removal of TS and VS was decreased with increase in pH, and ammonia. The pH, VA, ammonia level again decreased with pre-treated feed with increased TS and VS removal. The inhibition in the reactors occurred more of an occurrence of NH4 inhibition rather than VA accumulation. The more the degradation of cellulose and water soluble content found in the reactors, the higher was the biogas production. Pre-treatment unlocked the shell structure of lignocellulose enabling almost double the biogas production.

Biogas production at HS-AD was almost 38% less compared to the batch assays at 4% TS, as well as lower than yield obtained for the ACoD of the substrates under semi-continuous feeding conditions. Pre-treatment enhanced biogas production and a higher biogas yield than W-AD with untreated samples were observed with pre-treated substrates. Therefore, with pre-treated substrates, at HS-AD, a reduction in the reactor size almost 3.75 times with high biogas yield could be achievable.

8.1.6 Key research findings

- From kinetic model analysis, organic wastes had the highest methane production rate and low lag phase, whereas WS had a high hydrolysis rate. These properties are very useful in designing ACoD for maximasing biogas production.

- Co-digestion had synergistic effect, where the methane yield improved as the C/N ratio improved by adding high C-rich substrates with CL. The optimum biogas yield was found with chicken litter (30-35%) and a mixture of organic and lignocellulosic wastes (65-70%) to reach a C/N ration of 26-27.5.

- The maximum biogas yield under semi-continuous AD were found for a mixture of CL:FW:WS at 60:20:20 ratio of C/N ratio higher than 20 at OLR of 2.0 gTS/L.d for a feedstock of 4% TS.

- Although at low OLR i.e. at less VS feeding, the semi-continuous reactors didn't show any effect of C/N ratio imbalance, biogas production had dependancy on the C/N ratio at high OLR and mixing three or more substrates balnced the C/N ratio with more biogas production and less inhibition.

- At balanced C/N ratio, biogas production from lignocellulosic substrates were further dependent on their structural charbohydrate breakdown i.e. lignin, hemicellulose, cellulose, water soluble content.

- AKP pre-treatment increased the lignin degradation and DAP sollubilised hemicellulose content. Therefore, by combining both of the pre-treatement, more cellulose and water soluble content were available for anaerobic microorganism during AD. Chemical concentration (i.e. percentage of NaOH and H_2SO_4) and temperature had more effect over the duration of pre-treatment on the charbohydrate degradation.

- The maximum biogas yield were obtained with sequential AKP+DAP with an increment of around 25% compared with single pre-treatment.

- With AKP+DAP pretreated feed, up to 88% increase in total biogas was observed with a VS removal of around 55% during HS- AD (15% TS).

- HS-AD (15% TS) has 38% less biogas production than W-AD however reduced the reactor volume by 3.75 times. Pre-treated feed can mitigate the low biogas production at HS-AD.

8.2 Recommendations for Future Works

This research demonstrated the potential of biogas production from CL and the impact of ACoD of CL with agro-industrial and food wastes. Biomethane potential (BMP) tests were carried out at different AD conditions to find out the optimum substrate mixtures, balanced C/N ratio, solids removal and other conditions i.e pH, ammonia, VA. This work conducted in this research could be continued and the following recommendation are suggested for the future work.

- The HS-AD were run in sequential batch assays. The best conditions found are suggested to be examined under semi-continuous conditions, i.e. daily feeding for process stabilisation and inhibition.
- After the pretreatement (AKP and DAP), the liquor found could be recycled and reused. Further investigation is required on recovery of chemicals and reuse after pre-treatment.
- The digestate could be investigated for digestate quality for fertiliser ammendment purposes.
- The research could be expanded on ash analysis of the CL and other lignocellulosic substrates. To examine the effect of dirt, inorganic and ash on digestate quality and the maximum removal of solids could be of interest.
- The experimental work was continued till 15% TS. Because of time limitation work beyond that had not been possible. Further investigation on D-AD (20-40% TS) might be possible with the optimum conditions found in HS-AD.
- It is essential to evalute the economic feasibility of the total process for large-scale commercialisation purposes. One of the cost enhancements was due to the cost for pre-treatment that required to be justified with the overall improvements to be applied in the commercial scale.

Appendix

Anaerobic Codigestion of Municipal Wastewater Treatment Plant Sludge with Food Waste: A Case Study

In this chapter, the effect of the codigestion of food manufacturing and processing wastes with sewage sludge is presented. Batch biochemical methane potential tests as single substrates as well as bench scale semi-continuous anaerobic digestion results are studied and reported, exhaustively. This is a separate case study performed with two different wastes that has not been explored in the previous chapters. Commercial food wastes are investigated here, whereas earlier chapters considered household food wastes. Municipal waste water treatment plant sludge is produced in a huge quantity and required particular attention. Although this chapter has only reported anaerobic digestion of these wastes; it has been included in the research to provide an idea of anaerobic using these wastes with chicken litter and agro-industrial wastes to build a decentralised AD facility.

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Abstract

The aim of this study was to assess the effects of the co-digestion of wastes from food manufacturing and processing (FW) with sewage sludge (SS) i.e. municipal wastewater treatment plant's primary sludge (PS) and waste activated sludge (WAS). Bench scale mesophilic anaerobic reactors, were fed intermittently with varying ratios of SS and FW; operated at a hydraulic retention time of 20 days and organic loading of 2.0 kg TS /m^3.d. The specific biogas production (SBP) increased by 25% to 50% with the addition of 1-5% FW to SS which is significantly higher-than the SBP from SS (284±9.7 mL_N/g VS_{added}). Although, the TS, VS and tCOD removal slightly increased, the biogas yield and methane content improved significantly, and no inhibitory effects were observed as indicated by the stable pH throughout the experiment. Metal screening of the digestate suggested bio-solids meet guidelines for use as a soil conditioner. Batch biochemical methane potential tests at different FW: SS were used to determine the optimum FW to SS ratio using surface model

analysis. The results showed that up to 47-48% FW can be codigested with SS. Overall these results confirmed that co-digestion has great potential in improving the methane yield of SS.

1. Introduction

Sludge production from municipal wastewater treatment plants (MWTPs) is expected to continue to increase with the increasing number of wastewater treatment plants being constructed or upgraded due to the growing population connected to the sewage networks of Australia. The disposal of sludge generated at the MWTPs is a problem of increasing importance, representing up to 50% of the current operating costs of a wastewater treatment plant (Appels et al., 2008). In Australia, MWTPs produces approximately 360,000 dry tones of stabilised sewage sludge to dispose off which costs about $100M per year (Gale, 2007; Pritchard et al., 2010). Hence, Water Authorities operating these plants in Australia have been actively investigating alternative sustainable and economic sludge management pathways (Woon and Othman, 2012). Although different disposal routes are possible, anaerobic digestion (AD) seems to be the most promising sludge management alternative for its ability to generate bioenergy by the reduction of the sludge volumes to be disposed off (Cavinato et al., 2013; Dai et al., 2013; Shizas and Bagley, 2004; Wang et al., 2013b).

Sewage sludge (SS) contains high percentage of organic matter (60-70% of the dry matter) and nutrients; and typically comprises of primary sludge (PS) and waste activated sludge (WAS) (Bouallagui et al., 2010; Silvestre et al., 2011). However, WAS has low biodegradability, hence AD of WAS has low efficiency from both processing and economic standpoint (Park et al., 2012). Among the different strategies to enhance the performance of AD is the co-digestion of sludge with other organic wastes as it increases biodegradable organic matter and provide for a feedstock with an optimum C/N ratio (Appels et al., 2008; Bouallagui et al., 2010; Cavinato et al., 2013; Dai et al., 2013; Kalogo et al., 2008; Park et al., 2012; Shizas and Bagley, 2004; Silvestre et al., 2011; Wang et al., 2013b; Woon and Othman, 2012). Among the factors that limit the co-digestion are the selection and type of new organic wastes and the transportation cost of co-substrates to the MWTPs

(Bouallagui et al., 2010; Girault et al., 2012; Kalogo et al., 2008; Park et al., 2012; Silvestre et al., 2011).

Food waste (FW) from different sources e.g. residential and commercial are producing wastes at an increasing rate due to the growing population and rising living standard (Dai et al., 2013). FW is available all year around and account for a significant portion of municipal solid waste. In Victoria (Australia), FWs contribute 35.6% of total municipal solid wastes when source separated form waste garbage bin (2014). Almost all these FWs are disposed off to land fill. FW contains high percentage of biodegradable materials and have high potential for increasing the biogas yield. However, due to high biodegradability and volatile acids, AD as a single substrate may encounter various potential inhibitions including volatile fatty acids (VFA) accumulations (Brown and Li, 2013). Therefore, these FWs could be beneficial in anaerobic co-digestion for high energy recovery as well as solid waste reduction.

The application of anaerobic co-digestion in treating SS has been steadily growing attention for improving the biogas yield, solid destruction and digestate quality to use as a fertiliser (Koch et al., 2015). Full scale applications of anaerobic co-digestion of SS with FW can become an environmentally sound renewable energy source by creating opportunity to recover the energy potential from these very low or zero cost FW and getting benefit from high organic matter to increase methane yield. This will result in significantly less bio-solids disposal and reduction in municipal solid wastes as well as operating cost of the plant.

Many authors reported increased biogas yield during the co-digestion of SS with different types of food and/or food processing wastes. For example, co-digestion of sludge mix with FOG from a meat processing plant (46% VS added to the feed) increased the methane yield by 60% (Luostarinen et al., 2009). Similarly, methane yield was 2.6 times higher when SS was co-digested with oil and grease (48% of total VS load) from restaurant (Kabouris et al., 2009). Under mesophilic conditions, the highest methane production rate was observed when FW was mixed in the range of 30-40% VS with SS (Kim et al., 2003; Koch et al., 2015).

A MWTP in Melbourne, Australia produces about 3600 kg solids/day of which 627 kg is WAS and remaining is PS. This plant is in the progress of upgrading the existing old anaerobic digestion reactor, hence they were interested in assessing the feasibility of co-digestion of sludge with two streams of wastes, namely, grease trap

collected from food businesses in the area, referred to in this study as fat, oil and grease (FOG) and waste from a food products manufacturing factory at small ratio. The MWTP interest is to maximise methane yield, enhance solids removal and maintain or improve biosolids quality.

The aim of this study, therefore, was to access the effect of sludge: waste ration on the biogas yield, digestate and supernatant nutrient quality under semi-continuous conditions, subsequently, to monitor process performance and stability during co-digestion experiments.

2. Materials and Methods

2.1. Characteristics of substrates and inoculum

The sludge feed-stock used in this study were thickened PS and WAS collected from Melton Recycled Water Treatment Plant, Victoria, Australia. The PS and WAS were mixed at a ratio in-line with their flow rates such that the final mixed SS's TS is 4%. The raw PS and WAS were collected several times while running the experiment and each time they were characterised and mixed as described. The characterisations of different SS samples are reported in Table 1. The SS was stored in a sealed plastic container at 4°C.

The FWs used were, (i) thickened grease trap, referred to in this paper as GT, obtained through a commercial business that collects FOG from restaurants and food businesses in the Western area and (ii) wastes from a processed food products manufacturing factory denoted as PF. These FWs mostly composed of cooking oil, butter, cheese, meat, bread, meat fat and bones, mayonnaise, salad dressing etc. The food wastes were collected regularly, homogenised using a high-speed homogeniser, then characterised for the parameters shown on Table 1. The TS of the substrates (SS, PF and GT) was adjusted such that the AD reactors receive a feedstock of consistent TS and chemical oxygen demand (COD) concentration throughout the duration of the experiments. The inoculum used in this experiment was collected from the mesophilic anaerobic digester at Melton wastewater treatment plant. The characteristics of the feed-stocks (SS, PF and GT) and the inoculum are shown in Table 1.

2.2. Batch experiments

Batch tests were performed to determine the biochemical methane potential (BMP) of the individual substrates (SS, PF, GT) and mixtures of the SS and FW (mixture of PF and GT at 50:50 w/w) at different ratios. The experimental design is shown in Table 2. All the BMP tests were performed in 500mL glass bottles at 37˚C according to the guideline of (Angelidaki et al., 2009). Each reactor contained 4000 mg VS with $VS_{substrate}$: $VS_{inoculum}$ ratio of 0.25. In addition, two reactors received only inoculum as a control to determine the BMP of the inoculum. Headspace of the bottles was flushed with nitrogen gas for 2 minutes and the bottles were closed with a rubber suba seal. All batch tests were performed in duplicates. The bottles were kept at 37±1°C in an incubated shaker at a constant rotational speed of 100 rpm. The volume of biogas produced was measured with a water displacement and the gas composition was monitored using a gas chromatography. The volume of biogas (or methane) from the control was subtracted from the volume of biogas (or methane) produced in each reactor to obtain net production of biogas or BMP of the feed substrates into the reactor.

2.3. Semi-continuous experiments

SS was mixed with the wastes from the processed food products manufacturing and/or FOG, at the designated ratio of sludge to waste (SS:PF, SS:GT). The experiments were performed in 500 mL glass reactors, designed to allow feeding

Table 1: Characteristics of substrates and inoculum

Parameters	Unit	SS	PF	GT		Inoculum
				1st	2nd sample	
TS	%	3.7±0.1	18.77±0.8	7±0.2	26.1±0.2	1.85±0.2
VS	%	3.13±0.1	18.06±0.7	6.8±0.16	25.55±0.2	1.32±0.12
tCOD	g /L	53.73±8.	239.1±0.9	405.3±50	475.5±10	12.9±2.8
sCOD	g/L	3.95±0.6	3.42±0.04	2.98±0.9	3.8±0.7	1.4±0.7
Total N	g/L	2.6±0.1	3.55±0.15	3.5±0.2	3.54±0.2	1.86±0.003
Ammonium	g/L	0.11±0.0	0.11±0.00	0.14±0.0	0.26±0.00	0.48±0.007
Total PO_4^{3-}	g/L	1.5±0.05	1.1±0.04	2.56±0.0	2.58±0.1	0.9±0.3
Total VA	g Acetic Acid/L	0.6±0.01	1.98±0.15	1.9±0.2	2.03±0.2	0.17±0.013
Alkalinity	$gCaCO_3$/L	2.7±0.00	1.42±0.00	1.3±0.00	2.1±0.01	4.1±0.002
pH		6.36±0.0	5.54±0.01	5.0±0.6	6±0.3	7.55±0.13

and nitrogen flushing simultaneously, at 37±1°C in an incubated shaker at a constant rotational speed of 100. The reactors received the substrates at a concentration of 4% TS and organic loading rate of 2.0 kg TS/m^3.d. The reactors were operated at a sludge retention time of 20 days (equivalent to hydraulic retention time, HRT, in this case) and were fed and wasted once a day. Duplicate reactors for each condition were operated. The biogas was collected daily before feeding the reactors. The biogas measurement, feeding and wasting were done within 15 min window out of the incubator. The reactors were monitored weekly for biogas quality; and the wastage was analysed every ten days for pH, TS, VS, total COD (tCOD) and soluble COD (sCOD). The feedstock to the reactors was prepared from different substrates at the ratios shown on Table 2.

Table 2: Composition of the feed-stocks used in the BMP and semi-continuous tests

Experiment type	Substrates in feedstock	Substrates	Composition (w/w)	Nomenclature
Batch	Single	SS	100	100% SS
		PF	100	100% PF
		GT	100	100% GT
	Two	SS:PF	99:01	1% PF
		SS:PF	98:02	2% PF
		SS:PF	90:10	10% PF
		SS:PF	75:25	25% PF
		SS:PF	50:50	50% PF
		SS:GT	99:01	1% GT
		SS:GT	98:02	2% GT
		SS:GT	90:10	10% GT
		SS:GT	75:25	25% GT
		SS:GT	50:50	50% GT
	Three	SS:PF:GT	95:2.5:2.5	5% FW
		SS:PF:GT	80:10:10	20% FW
		SS:PF:GT	50:25:25	50% FW
		SS:PF:GT	33.3:33.3:33.3	66.67% FW
Semi-continuous	Single	SS	100	100% SS
	Two	SS:PF	99:1	1% PF
		SS:PF	98:2	2% PF
		SS:GT	99:1	1% GT
		SS:GT	98:2	2% GT
	Three	SS:PF:GT	95:2.5:2.5	5% FW

[#] Run in duplicate

[#]FW= Mixture of PF and GT at ratio 50:50 (w/w)

2.4. Analytical methods

TS and VS were measured by gravimetric analysis according to the Standard Method 2540B and 2540E respectively (Rice et al., 2012). The tCOD and sCOD was measured using HACH method 8000. The total phosphorus (TP), total nitrogen (TN), ammonium, volatile acids (VA) were measured by colorimetric techniques using a HACH (Model: DR/4000 U) spectrophotometer according to the methods 10127, 10072, 10031 and 8196, respectively. The samples were centrifuged (Eppendorf 5702, Germany) at 4.4 rpm for 15 mins and then filtered through 0.45 µm filter paper (mixed cellulose esters membrane filter, Advantec, Japan), to measure the soluble constituents. The measurement of pH was carried out using a calibrated pH meter (ThermoOrion, Model 550A) and Alkalinity was measured by APHA method 2320B.

The volume of biogas was normalised to standard conditions compromising dry gas, standard temperature and pressure (0ºC and 1 bar) according to method described by Strömberg *et. al.* (Strömberg et al., 2014) and results are presented as norm-liter (L_N). The headspace was corrected for methane (CH_4) and carbon dioxide (CO_2) to 100% according to VDI 4630 (2006) (VDI, 2006). The composition of the biogas was analysed according to APHA method 2720C using gas chromatography (Varian 450-GC, Varian Australia Pty Ltd., Netherlands) equipped with a packed column (GS Carbonplot 113-3132, 1.5 micron, 30m* 0.320 mm, stainless steel, Agilent Technologies Inc., Australia) and a thermal conductivity detector. The carrier gas used was helium at a flow rate of 28 ml/min. Temperature of the column, detector and injector were 70°C, 200°C and 100°C, respectively. The biogas was collected and manually injected using a 50 mL FORTUNA® Optima glass syringe (Poulten & Graf, Germany). Calibration was done using three point and five levels of CH_4, CO_2 and nitrogen (BOC, Australia). Screening of the metals in the digestate samples were tested for Sodium (Na) to Cerium (Ce) from a commercial lab (ALS Environmental Division: Water research group).

2.5 Statistical analysis

Predictions in optimum mixture ration for two and three substrates from batch test were obtained using MATLAB R2013b. Furthermore, a predictive model for optimum

FW incorporation was prepared with surface and contour plot. To determine the significance of difference in cumulative methane yields over the digestion period, each set of co-digestion feed stock was statistically analysed with 100% SS using one-way analysis of variance (ANOVA) at α= 0.05 in MATLAB R2013b.

3. Results and Discussion

3.1 Batch experiments

Batch experiments were carried out to investigate the optimum ratio of FW to incorporate in SS. The effect of two substrates and three substrates were also investigated at different mixture ratios. The accumulative methane yields and the daily biogas yields during the anaerobic co-digestion are shown in Figure 1: (a-d) and (e-h), respectively. The BMP tests continued for 46 days until little or no biogas production was observed. The results presented are the net biogas and methane yield from the feedstock after subtracting the control yield.

From Figure 1(a), the BMP of 100% SS was 192±12.3 mL_N CH_4/g VS_{added}, whereas the processed food wastes, 100% PF and 100% GT had a BMP of 466.2±0.73 and 408.7±6.6 mL_N CH_4/g VS_{added} respectively, which is 1.42 and 1.12 times higher than 100% SS alone. For 100% SS, the biogas production started after 2 days and reached the first peak at day 8 with a rate of 21.5 mL_N biogas/g $VS_{added.\ d}$ [Figure 2(e)]. The second peak occurred at day 17 with a peak value of 46.1 mL_N biogas/g $VS_{added.\ d}$ and after 21 days slowly decreased. Both the food wastes started biogas production after day one and obtained the first peak at day 17 with daily biogas yields of 54.3, 45.4 mL_N biogas/g $VS_{added.\ d}$, respectively for PF and GT. The second peak values were 56.3, 25.3 mL_N biogas/g $VS_{added.\ d}$ from PF and GT respectively at day 28 and 36. The technical digestion time i.e. $T_{80\text{-}90}$ (the time for 80-90% of the maximum biogas production) was calculated to be in between 20-27, 31-35 and 37-40 days for SS, PF and GT, respectively. The technical digestion time can be used as a HRT for continuous anaerobic digestion for these substrates (Kafle and Kim, 2013).

The co-digestion of SS with PF enhanced the BMP from 199.6±20.6 to 616.8±30.2 mL_N CH_4/g VS_{added} for PF fractions of 1% to 50% i.e. 4% to 287% increase in methane yield than 100% SS alone [Figure 1(b)]. Although, with 1% PF to 10% PF

incorporation, a lag phase of 2 days was observed, 25-50% PF mixture with SS immediately started biogas production. For 1% PF to 25% PF, a single peak in daily biogas yield was observed at day 17 with peak values of 36.4±0, 43.9±7.1, 67.2±4, 86.7±2.7 mL_N biogas/g $VS_{added.\ d}$, respectively, for 1% PF, 2% PF, 10% PF, and 25% PF [Figure 1(f)]. A trend of rise was observed in peak value with increasing PF ratio. The production of biogas was decreased after 20 days and almost ceased after 36 days. However, for 50% PF, an inhibition in biogas production was obtained with two peaks. At day 15, first peak of 42.4±0 mL_N biogas/g $VS_{added.\ d}$ with easily degradable organic materials and at day 28, a small second of 29.6±8.7 mL_N biogas/g $VS_{added.\ d}$ with slow degradation was observed. $T_{80\text{-}90}$ was calculated between 20-26, 21-27, 21-27, 21-27 and 27-33 days, respectively for 1%-50% PF incorporation.

SS mixed with GT enhanced the BMP of SS from 200± 2.6 to 561.3±16.9 mL_N CH_4/g $VS_{added,}$ i.e. 5% to 260% increase in methane production by adding up to 50% GT during co-digestion [Figure 1(c)]. It was observed that increasing the GT fraction in the feedstock from 1% to 50% caused increase in BMP up to 17 days and started decreasing until completely ceased at around 36 days [Figure 1(g)].The peak values were 47.3±2.4, 47.7±3.1, 42.3±1.8, 75.4±13.8 and 89.8±22.8 mL_N biogas/g VS_{added} .d, respectively, for 1% GT, 2% GT, 10% GT, 25% GT and 50% GT. No inhibition was observed with $T_{80\text{-}90}$ between 20-26, 20.5-26, 21-28, 21-28 and 25-32 days, respectively for 1%-50% GT incorporation.

For three substrates, biogas production improved up to 50% FW addition (632.8±10.1 mL_N CH_4/g VS_{added}) and decreased at mixture ratio of 66.7% FW (603.3±6.7 mL_N CH_4/g VS_{added}) [Figure 1 (d)]. An early peak at day 8 was observed for 5% FW with a peak value of 40.7±7.9 mL_N biogas/g $VS_{added.}$d. It was, however, observed at day 17 for 20-66.7% FW with peak values of 63.6±0.5, 74.8±6.8 and 73.3±0 mL_N biogas/g $VS_{added.\ d}$, respectively [Figure 1 (h)]. $T_{80\text{-}90}$ was calculated between 24-35, 25-32, 24-30 and 26-37 days, respectively for 5%-66.7% FW incorporation.

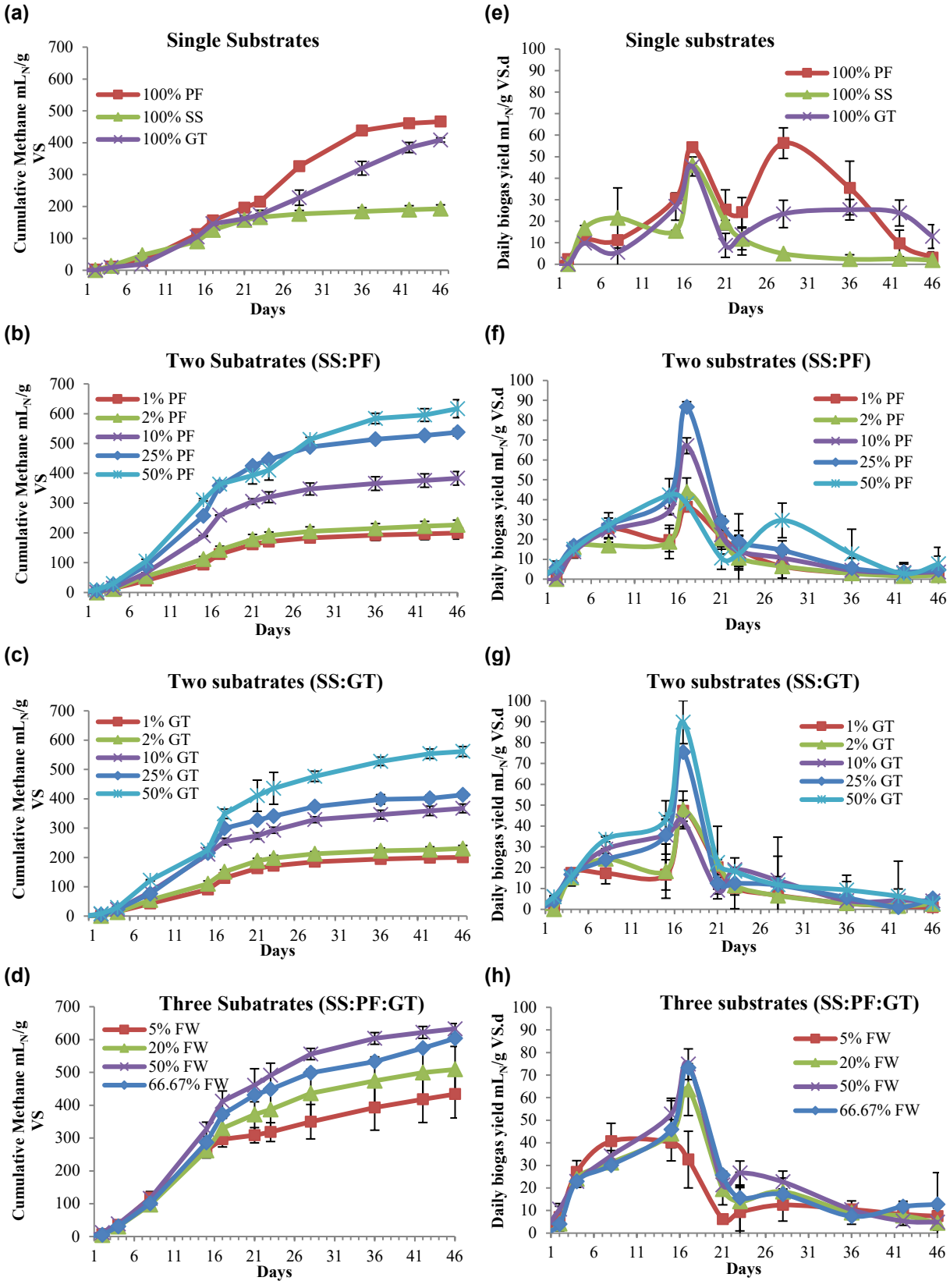


Figure 1: Accumulative Methane production (a-d) and daily biogas yield (e-f) from batch experiments of single, two and three substrates

Therefore, the addition of FW with SS decreased the technical digestion time with single peak. The VAs that is usually associated with the GT seems to be below inhibition up to 50%. However, the inhibition effect at 50% PF indicates there is NH_4 that reached a threshold (2.1±0.1 g/L). Ammonia which is an important indicator of AD produces by the hydrolysis of proteins and urea (Niu et al., 2013; Uludag-Demirer et al., 2008) and accumulates in the AD process(Liu et al., 2012). FW which is reach in proteins showed an inhibition when more than 50% FW incorporated with SS.

The BMP assay can be utilised to calculate the synergic effect of co-digestion as additional methane yield over the weighted average of the individual feedstock's methane yield (Labatut et al., 2011). The weighted experimental methane was calculated from single substrates following the formulas:

$$\text{Weighted } EMY_{FW} = EMY_{100\%SS} * P_{100\%SS} + EMY_{100\%PF} * P_{100\%PF} + EMY_{100\%GT} * P_{100\%GT} \quad (1)$$

$$\text{Weighted } EMY_{PF} = EMY_{100\%SS} * P_{100\%SS} + EMY_{100\%PF} * P_{100\%PF} \quad (2)$$

$$\text{Weighted } EMY_{GT} = EMY_{100\%SS} * P_{100\%SS} + EMY_{100\%GT} * P_{100\%GT} \quad (3)$$

Where, weighted EMY_{FW}, EMY_{PF} and EMY_{GT} represent the weighted average of experimental methane yield of the substrates FW, PF, and GT, respectively. $P_{100\%SS}$, $P_{100\%PF,}$ and $P_{100\%GT}$ refer to the percentage composition and $EMY_{100\%SS}$, $EMY_{100\%PF,}$ and $EMY_{100\%GT}$ are the experimental methane yield for substrates SS, PF, and GT, respectively in the co-substrate mixture. According to Y. Li. *et al* if the difference (EMY- weighted EMY) was higher than the standard deviation of EMY, synergic effect could be observed (Li et al., 2013a). The EMYs of the co-digestion substrates during the digestion period were analysed statistically with respect to EMYs of 100% SS. From Table 3, 1-2% PF and GT did not have very significant synergistic effects, however, increasing the amount of food wastes resulted in a very significant ($p<0.05$) increase in methane yield compared to the digestion of SS alone. Synergic effect was found in almost all of the cases when food wastes were added with SS representing higher biodegradability. This might be due to the adjustment in C/N ratios during co-digestion (Li et al., 2013a) than the single substrates. The C/N ratio is a good indicator of the efficiency of AD that can be limited by inadequate amount and diversity of waste from a single resource. For example, high carbon content of a sample can cause rapid acidification and methanogenesis will be

inhibited by the low pH. The optimum C/N ratio is waste specific over a range from 9 to 30 (Siddiqui et al., 2011). The C/N ratio of SS used in this study was 8.16 which is lower than the C/N ratio of PF and GT (17.64 and 15.5, respectively). Incorporating 50% food wastes in SS increased the C/N ratio of the reactors up to 12-13. Antagonism (probably due to inhibition) was observed for 50% PF. In case of three substrates, 5% FW showed the highest increase in methane yield than further incorporation. Luostarinen *et. al.* also reported inhibition with grease trap sludge to SS of more than 50% (Luostarinen et al., 2009). However, these inhibitory effects were only deduced from pattern of methane production and synergistic effects and will require further investigations.

To investigate the optimum mixture ratio of FW and SS with respect to methane yield, a trend was predicted using MATLAB [Figure 2 (a-c)]. The R^2 co-relation values were 0.999, 0.993 and 0.885 for %PF, %GT and % FW incorporation with SS, respectively, thus indicating a good fit exist between experimental and predicted values. The results showed that methane yield obtained a maximum value of 614.6, 562, 651.1 mL_N CH_4/g VS_{added} when 47% PF, 61.4% GT and 48% FW were incorporated with SS improving the C/N ratio of 12.5. Figure 3 shows the 3D model of optimum FW incorporation with SS where %PF and % GT with SS in x and y axis with methane yield in z axis. The dark red area represents the maximum methane yield region. FW incorporation up to 48% with the mixture of GT and PF according to the dark red region will produce the maximum biogas. Considering, SS as the main substrate, mixtures with more than50% of SS was suggested from batch experiments. However, inhibition of continuous operation of a plant also depends upon factors such as organic loading rate (OLR), HRT and reactor configurations. Therefore, a small pilot scale continuous reactor should be operated before incorporating the mixture ratio.

Table 3: Synergistic effect evaluation of co-digestion of SS with PF, GT and FW (mixture of PF:GT)

Substrates ratio[a]	EMY	SD	Weighted EMY	Difference	Increase in EMY (%)	p-Value	Synergistic effect
1% PF	199.6	20.6	195.7	3.9	2.0	0.9310	Not clear
2% PF	226.6	16.3	198.4	28.2	14.2	0.6106	Not significant
10% PF	383.1	22.9	220.3	162.8	73.9	0.0462	Synergistic
25% PF	537.5	12.3	261.3	276.2	105.7	0.0084	Synergistic
50% PF	616.8	30.2	329.6	287.2	87.2	0.0066	Synergistic
1% GT	200.8	2.6	195.1	5.16	2.7	0.9067	Not clear
2% GT	230.6	10.3	197.3	33.32	16.9	0.5423	Not significant
10% GT	317.3	14.8	214.5	102.8	47.9	0.0467	Synergistic
25% GT	413.2	10.1	246.7	166.3	67.4	0.0259	Synergistic
50% GT	561.3	16.9	300.8	260.5	86.6	0.0081	Synergistic
5% FW	433.7	72.7	205.2	228.5	111.4	0.0176	Synergistic
20% FW	508.9	70.1	241.8	267.1	110.4	0.0110	Synergistic
50% FW	632.8	16.1	315.2	317.6	100.8	0.0038	Synergistic
66.67% FW	603.3	6.7	352.4	250.9	71.2	0.0066	Synergistic

EMY: experimental methane yield (mL/g VS_{added}); SD: standard deviation; Weighted EMY: weighted average of experimental methane yield for co-substrates

[a]Percentage of food wastes (PF, GT, FW) mixed with SS

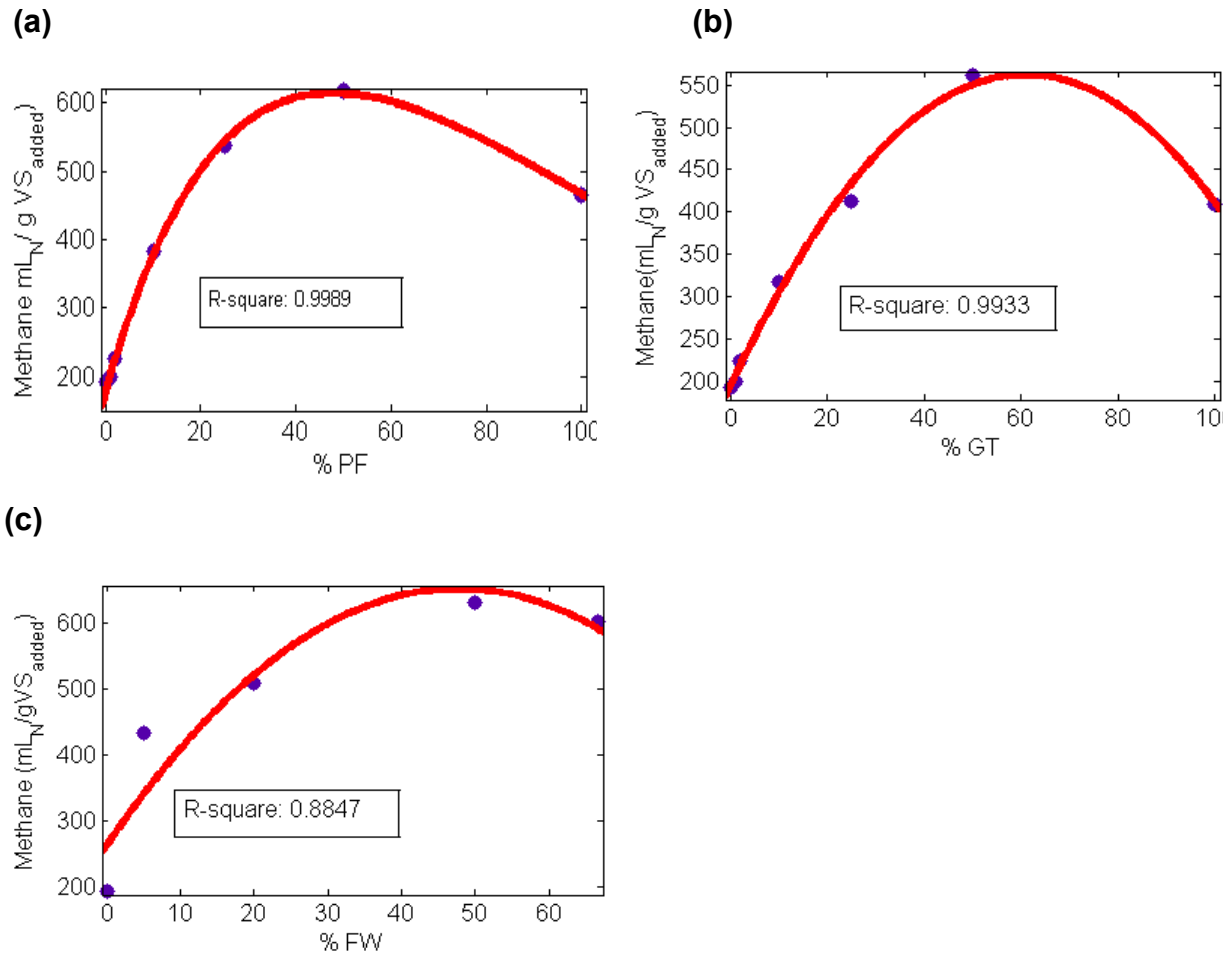
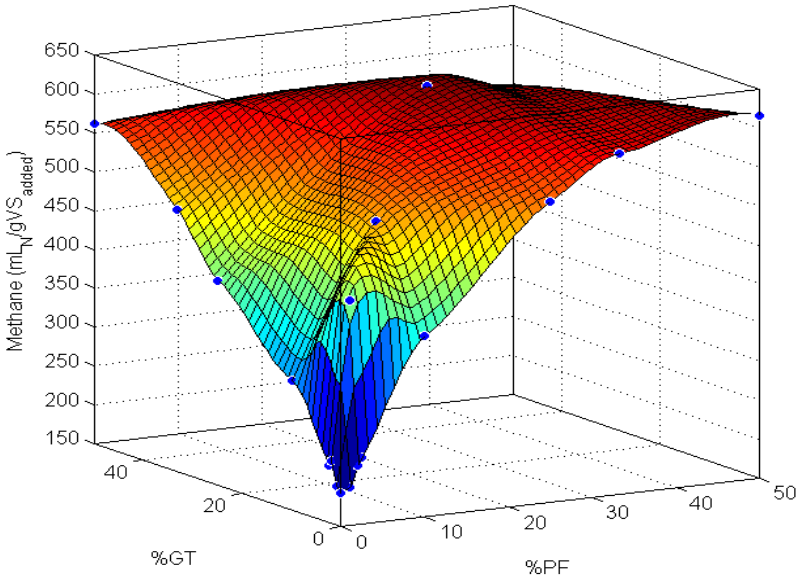


Figure 2: Prediction in optimum SS and FW mix ratio according to the methane yield: (a) %PF, (b) % GT and (c) % FW

(a)



(b)

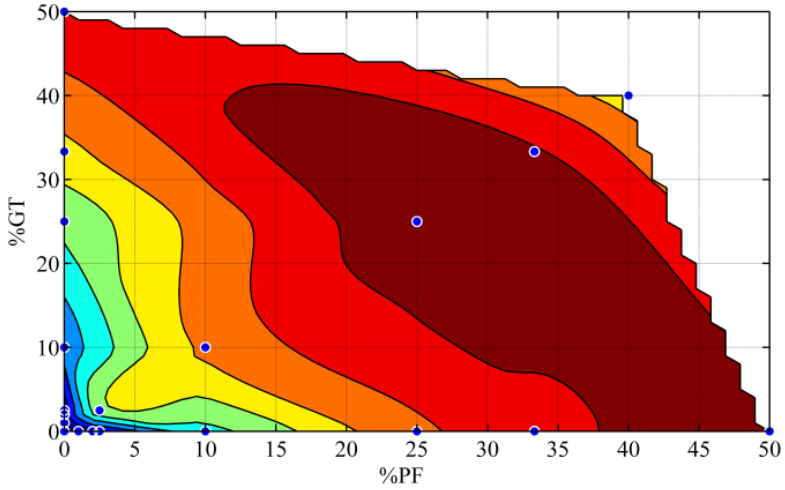


Figure 3: 3D prediction of optimum FW incorporation (a) Surface plot and (b) Contour plot

3.2 semi-continuous experiments

According to the requirement of the plant only 5% or less food waste incorporation was tested for process performances under semi-continuous conditions for six HRT cycles of 20 days each. Figure 4 represents specific biogas and methane production from the four cycles (20-100 days) reported as $mL_N/g\ VS_{added}$ fed to the reactor. The average daily methane yield from SS (100% SS) and different mixture ratios of SS with PF and GT (1% to 2%) varied between 212 to 415 mL/g VS added. For small amounts of FW incorporation, biogas production was proportional to the percentage of FW and the biogas yield for 5% FW was the highest throughout the experiment duration which is coherent with BMP assays (Park et al., 2012).

For 100% SS, the average SBP was 284 ±9.7 mL/g VS with methane content in the range 64% and 66%. The average TS, VS and tCOD removal for 100% SS was 41%, 50% and 58% respectively, which was in agreement with COD and VS removal of 35% and 36% respectively, reported by Silvestre *et. al.* for continuous AD of sludge mix of 70% PS and 30% WAS at an OLR of 1.5 to 1.7 kg $VS/m^3.d$ and HRT of 20 days (Silvestre et al., 2011). A low SBP of 236 ± 6.6 mL_N/g VS was observed during the third HRT cycle (40- 60days) comparing to HRT cycle two (20-40 days) when a new batch of feed was prepared with newly collected sample. Low TS, VS and tCOD removal were also found during the period. This lag phase might be because of the biomass adaptation with the new feed (Silvestre et al., 2011). The pH varied between 6.9 and 7.1 during the whole experiment.

The average SBP of 1% PF and 2% PF were 359 ±9 and 367± 11 $mL_N/g\ VS_{added}$ which is 25% and 32% higher than the SBP from 100%SS alone. Similarly, 23% and 47% increase in SBP was observed for 1% GT and 2% GT with an average SBP of 355± 9 and 367 ± 3 $mL_N/g\ VS_{added}$. As FOG has high biodegradability and BMP value (when added below 20% of the influent COD) (Girault et al., 2012), co-digestion with small proportion of GT produced more biogas than the other food waste of same amount. The co-digestion of three wastes SS: PF: GT at 95:2.5:2.5 (5% FW) produced an average SBP of 424 ± 10 mL_N/g VS (methane yield 327 mL_N/g VS) which is 50% higher than 100% SS (single substrate). These results are in agreement with the results reported by Luostarinen *et. al.* (Luostarinen et al., 2009) and Davidsson *et. al.* (Davidsson et al., 2008). They worked with SS and grease trap sludge (95:5 w/w) and reported methane yield of 374 and 295-308 mL/g VS

corresponding to the organic loading of 1.67-2.23 and 2.5 kg VS/m^3.d of HRT 16 and 13 days respectively. The addition of food wastes also increased the methane content and the average methane content was 69-72% in this experiment.

The TS removal for 1-2% food wastes (GT, PF) were between 42% and 49% and the corresponding VS removal were found to be between 50% and 56% (Table 4). This is similar to the VS removal from previous studies (Davidsson et al., 2008; Luostarinen et al., 2009). At the start of the second HRT cycle (20-40 days), the pH was at between 6.8 and 6.9 for all the reactors which might be because of high VA production at the beginning. The pH started increasing after that indicating the consumption of produced VA due to acidification and inoculum acclimatisation (Kawai et al., 2014). However, when a new feed was prepared in the fourth HRT cycle (60-80 days), a lag phase was observed with a low organic content removals, pH as well as low biogas production. However, after the lag phase the reactors produced stable biogas production in last two HRT cycles of the co-digestion.

Methane production was increased significantly from 2% GT after the lag phase which might be because the methanogens were acclimated to inoculum (Woon and Othman, 2012). However, GT which is mainly lipid rich material (Long et al., 2012) has found to have wide variation in characteristics (from Table 1, where characteristics results from two different sample collection are shown).

The daily biogas production was observed to be fluctuating, although the feedstocks were prepared by homogenising to constant TS loading throughout the experiments.

As the FWs had high variations in the characteristics, feeding a very small proportion in the reactors every day (from a batch of prepared feedstock) resulted in variations. Therefore, the average biogas production over each HRT cycle was shown in Table 4 and a trend of rise was seen because of the acclimatisation of the inoculum to the feedstock.

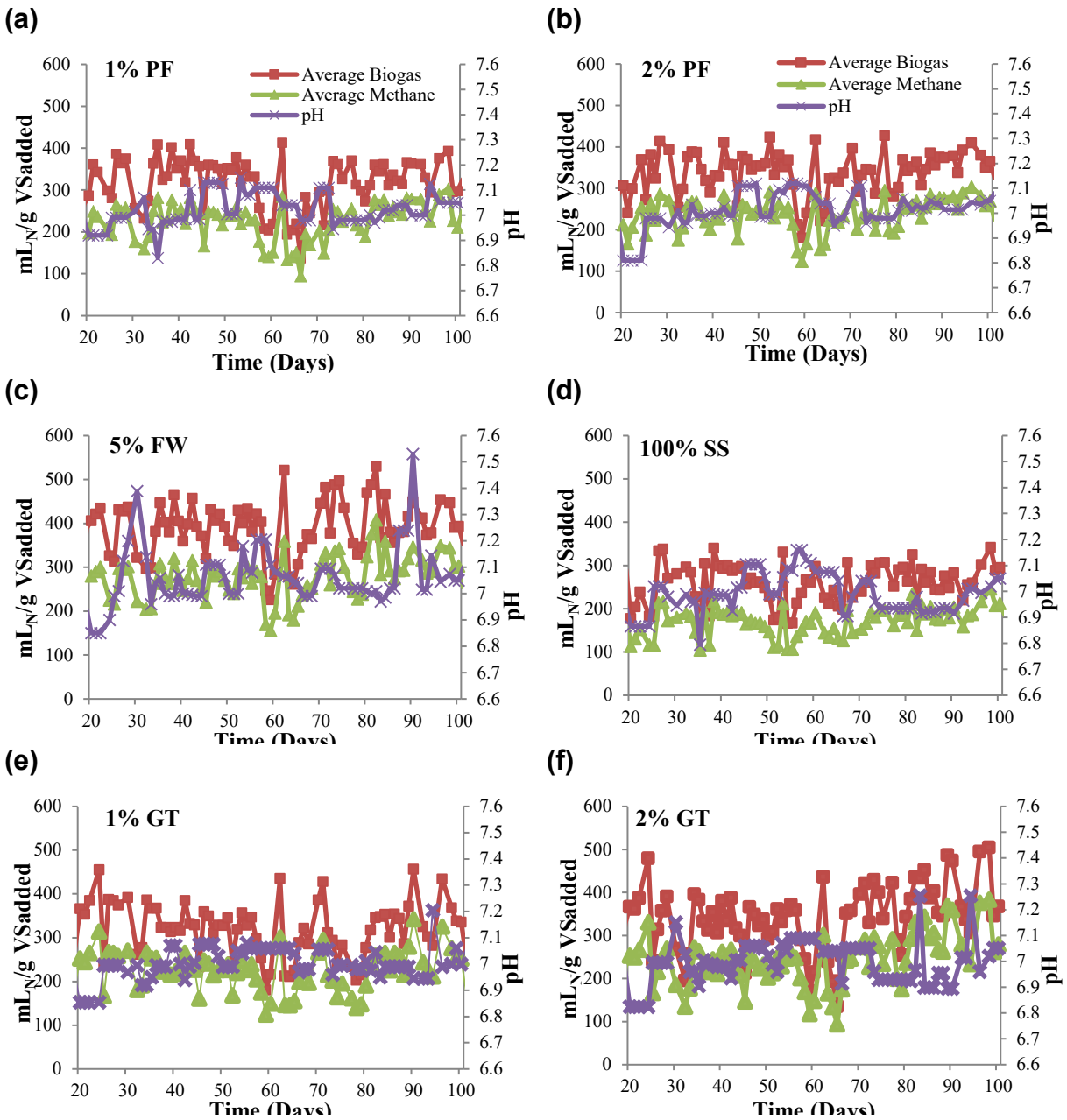


Figure 4: Daily biogas production, methane yield and variation of pH during the co-digestion of MWTP sludge with food wastes at different mix ratios: (a) 1% PF, (b) 2% PF, (c) 5%FW, (d) 100% MS, (e) 1% GT and (f) 2% GT

Table 4: Biogas production and process performance in terms of TS, VS and COD removal

Feedstocks	Parameters	Period I (0-20 d)	Period II (20-40	Period III (40-	Period IV (60-80 d)	Period V (80-100 d)	Period VI (100-120 d)
1% PF	Avg Biogas	256±16	337±14	320±1.5	284±8	339±7	355±9
	CH4%		69±3.2	65±7.81	69±2.8	77±2.8	71±6.7
	TS removal	43±0.01	43±0.03	40±0.04	40±0.4	46±1	41±4.5
	VS removal	49±0	51±0.02	49±0.03	45±0.1	57±3.3	50±2.1
	COD	61±0.06	58±0.01	53±0.02	59±0.03	59±0.03	55±2.03
	pH	7.4±0.4	7.0±0.0	7.1±0.0	7.01±0.06	7.02±0.05	7.07±0.03
2% PF	Avg Biogas	252±14	335±9	334±2	322±1	364±2	367±3
	CH4%		69±2.4	66±3.4	69±2.4	74±1.4	69±5.4
	TS removal	43±0.02	45±0.01	41±0.05	45±2.5	46±0.06	45±2.2
	VS removal	51±0.01	52±0.01	50±0.04	52±0.05	55±0.03	53±1.6
	COD	58±0.1	54±0.01	55±0.06	59±0.03	57±0.03	57±2.05
	pH	7.3±0.4	7±0.04	7.1±0.0	7.01±0.01	7.03±0.02	7.05±0.04
5%FW	Avg Biogas	281±1	376±2	386±3	393±2	415±19	424±10
	CH4%		69±5.5	68±7.5	69±7.8	77±4.9	72±5.1
	TS removal	49±0.01	52±0.02	52±0.08	44±0.06	50±1.0	52±0.07
	VS removal	56±0.01	60±0.02	60±0.02	55±0.08	54±1.01	59±4.05
	COD	58±0.1	54±0.01	55±0.02	60±0.04	54±0.08	58±1.01
	pH	7.3±0.5	7.09±0.	7.08±0.	7.05±0.07	7.1±0.3	7.05±0.04
1% GT	Avg Biogas	253±36	285±15	308±7	348±5	345±12	361±1
	CH4%		69±2.51	67±2.3	69±2.12	75±2.8	69±7.1
	TS removal	44±0.01	45±0.01	45±0.06	43±0.01	44±0.06	45±3.05
	VS removal	52±0.01	52±0.02	46±0.06	50±1.02	48±0.04	48±5.09
	COD	63±0.04	59±0.01	63±0.04	65±1.06	56±2.3	59±1.06
	pH	7.3±0.5	7±0.06	7.02±0.	7±0.05	7±0.12	7.08±0.13
2% GT	Avg Biogas	284±9	336±5	312±3	329±7	405±4	395±8
	CH4%		68±2	66±5.1	69±4.2	76±4.9	72±4.0
	TS removal	46±0.02	45±0.01	46±0.04	45±0.01	44±0.02	46±2.04
	VS removal	54±0.01	54±0.02	53±0.03	53±0.01	57±0.08	53±0.03
	COD	65±0.06	60±0.01	60±0.05	60±0.04	56±0.01	59±0.04
	pH	7.25±0.	7±0.09	7.03±0.	7±0.06	7.01±0.2	7.06±0.07
100% SS	Avg Biogas	212±1.7	271±5.8	236±6.6	264±3.16	269±3.5	284±9.7
	CH4%		64±4.5	62±1.5	64±7.8	66±2.8	66±9.6
	TS removal	43±0.02	41±0.03	40±0.06	40±0.01	39±0.03	40±6.08
	VS removal	50±0.01	54±0.02	46±0.06	50±1.3	51±2.6	53±1.7
	COD	60±0.03	58±0.03	55±0.06	56±0. 6	55±0. 8	55±1.01
	pH	7±0.12	6.99±0.	7.04±0.	6.93±0.02	7.03±0.05	7.05±0.04

Biogas and methane production potential of the food wastes was very high because of its high fat and protein content. Therefore, incorporation of FWs at a very small ratio (1-5%) with SS in co-digestion had significantly improved the biogas production from the SS alone. Although the biogas production improved a lot, the VS and COD removal was not improved significantly (Table 4). This was likely due to the huge amount of more slowly degradable and/ or inert material in the SS (60% degradable) (Luostarinen et al., 2009). The biodegradability of Food wastes on the other hand was probably close to 100% due to the dilution with SS which caused the high biogas production.

Although, SBP and methane yield depends on substrates origin, composition and operational conditions (SRT, temperature), the results reported by Silvestre *et. al.* (Silvestre et al., 2011) and Davidson *et. al.* (Davidsson et al., 2008) showed a methane yield lower than this study when a small percentage of wastes from dissolved air flotation unit of a wastewater treatment plant and kitchen grease wastes were added with SS. In addition to the biogas yield, different parameters were monitored at the end of each cycle to assess the quality of supernatant and digestate (Table 5). It is observed that the pH value remained relatively stable at around 7 throughout the operation of the reactors. The alkalinity in all the reactors was around 2.5 to 2.75 g/L which also indicates no accumulation of VAs and the highest VA was observed from 2% GT (0.315 g/L) which is well below the threshold of inhibitory (4 g/L) (Siegert and Banks, 2005). The VA accumulation might cause the instability of the process and an inhibition of acetotrophic methanogenesis (Girault et al., 2012). However, the VAs in the reactors indicates stable process conditions. Luostarinen *et. al.* observed total VA accumulation of not more than 0.43 g/L with a high ratio of grease trap to sludge in the feedstock (71% of the feed VS) while working with mixture of PS, WAS and grease trap sludge (Luostarinen et al., 2009).The ammonia-N content in all the reactors was between 0.6 to 0.71 g/L which is below the inhibition range (1.5-2.0 g/L)(Woon and Othman, 2012).

The last aspect to consider in the anaerobic co-digestion refers to the possibility to produce high quality compost (or a fertiliser). In this case, the dewatered digestate characteristics for heavy metal contents need to be considered when assessing the effect of co-digestion (Cavinato et al., 2013).

In Australia, concentration of contaminants presents in the biosolids and the microbial quality are two important parameters for biosolids classifications. Contaminant grade (C1 and C2) and treatment grade (T1, T2 and T3) are the classification of Biosolids based on the factors described where C1/T1 are high quality product and can be used without restriction. According to the EPA guideline, biosolids from wastewater treatment plant are categorised as C2/T3 (EPAVictoria, 2004). Integration among the AD and composting can be possible where composting can play the role of curing step to overcome the phyto-toxicity limit for VA and ammonia (Di Stefano et al., 2008). In the AD reactors, no inhibition of VA and ammonia N were observed. The digestate characteristics were adequate for the

production of good quality compost by integrating a simple aerobic post stabilisation and dewatering step for biological stability.

In Australia, regulation of heavy metals in fertilizers of organic origin is given by Fertilizer working group, department of agriculture, AU government (www.daff.gov.au). The concerning heavy metals are zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), lead (Pb), chromium (Cr) and mercury (Hg) and their allowable limits are shown in Table 6.

Table 5: Bench scale AD reactors' performance at the end of the experiment

Parameter	Unit	1% PF	2% PF	5% FW	1% GT	2% GT	100% SS
TS	g/L	21.15±2.43	20.62±2.57	21.13±5.1	20.02±3.46	20.45±0.26	20.34±0.97
VS	g/L	15.67±2.1	17.72±1.61	17.16±1.97	15.33±0.28	14.61±0.26	14.90±2.92
tCOD	g/L	28.025±0.2	26.65±0.07	28.9±0.21	26.075±7.8	28.55±0.21	29.025±5.6
sCOD	g/L	2.05±0.10	1.765±0.06	3.24±0.10	1.755±0.02	2.285±0.04	1.925±0.11
TS removal	%	45±2	47±3	52±5	48±3	48±1	46±1
VS removal	%	52±2.1	53±1.6	55±2	53±0.3	57±0.3	53±3
COD removal	%	57±2.5	59±0.7	59±2.1	60±7.8	59±2.1	55±5.7
TP	g/L	0.36±0.03	0.38±0.04	0.44±0.04	0.4±0.02	0.42±0.01	0.41±0.001
TN	g/L	0.93±0.035	0.895±0.04	0.98±0.035	0.877±0.02	0.965±0.01	0.945±0.03
TKN*	g/L	1.9±0.3	2±0.42	2.4±0.3	2.2±0.2	2.3±0.15	2.2±0.3
NH4-N	g/L	0.62±0.014	0.575±0.03	0.685±0.05	0.7±0.014	0.71±0.014	0.67±0.014
VA	g/L	0.147±0.00	0.148±0.01	0.266±0.07	0.253±0.05	0.315±0.01	0.208±0.01
pH		7±0.00	7.08±0.00	7.13±0.02	7.05±0.02	7.09±0.05	7.04±0.02
Alkalinity	g/L	2.498±0.29	2.7±0.06	2.756±0.08	2.711±0.05	2.678±0.03	2.671±0.05

*analyses were carried out at a commercial lab (ALS, Australia)

Table 6: Digestate heavy metals concentration* in mL/g at the end of the experiment (after six HRT cycle of 20 days)

Parameter	1%PF	2%PF	5%FW	1% GT	2%GT	100% SS	Limit**(mg/kg)
Ca	430±4	455±5	480±6	450±5	440±4	450±7	
Mg	82±1	85±0.5	89±0.7	89±0.4	85±0.3	87±0.5	
Ca Hardness	1100±2	1100±0.5	1200±1	1100±0	1100±0.5	1100±0	
Mg Hardness	340±0	350±0	370±1	360±2	350±5	360±5	
Al	170±0	170±0	180±0	170±0	170±0	200±0	
As	<1	<1	<1	<1	<1	<1	20
Cd	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1
Cr	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	400
Cu	11±1	11±1	12±0	12±1	12±0	14±0	100
Fe	160±10	160±5	170±10	170±10	170±5	170±5	
Pb	<0.5	<0.5	<0.5	<0.5	0.5±0.1	0.6±0.1	300
Hg	<1	<1	<1	<1	<1	<1	1
Ni	0.4±0.1	0.4±0.1	0.5±0.1	0.4±0.1	0.4±0.1	0.4±0.1	60
Zn	19±0.5	20±0.5	21±1	20±0.5	21±1	22±1.5	200
Si	170±5	180±5	170±5	170±5	180±5	210±10	
Si-SiO2	350±10	380±5	370±5	370±5	380±5	440±10	
S	160±5	170±0	180±5	170±5	170±5	200±5	
S-SO4	480±10	520±5	540±5	520±5	520±5	600±10	

* Heavy metal screening of the digestate samples were carried out by a commercial lab (ALS Environmental Division: Water research group).

**Contaminant upper limits for biosolids as grade C1(EPAVictoria, 2004)

These heavy metals may be present in concentrations above legal limits can potentially harm environment, and affect crop quality, crop yield, and soil fertility. Heavy metal concentration may increase during AD due to the microbial mineralization and loss of volatile solids (Ciavatta et al., 1993). Most national regulations prohibit the use of organic fertilizers, e.g. digestate, if the concentrations of one or more heavy metals are higher than the threshold concentrations. There are also evidences suggesting that AD increases the complexation of heavy metals with organic ligands and hence lower the mobility of heavy metals in the digestate (Lavado et al., 2005; Marcato et al., 2009). However, the metal contents found in these experiments were less than the allowable limit used in Australia for high quality

amendments. Table 6 shows the concentration of heavy metal in the digestate collected reactors after six HRT cycle (at the end of the experiment).

4. Conclusions

Food processing wastes are a suitable co-substrate for the anaerobic co-digestion of MWTPs sludge. From batch experiments, food wastes can be added up to 47%-48% (v/v) without inhibition. The addition of 5% FW to the SS increased the SBP up to 50% during semi-continuous experiments. Although, the TS, VS and tCOD removal slightly increased, the quality of the methane also improved by co-digestion. The reactors showed stable pH and performance with no inhibitory effect. Overall these results reveal the possibility of MWTPs sludge co-digestion with food waste for methane yield and quality.

5. Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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