AMERICAN UNIVERSITY OF BEIRUT

STARTUP OF THERMOPHILIC ANAEROBIC DIGESTION OF ORGANIC WASTE: ONE STAGE VS TWO STAGE

by

DANA NAZIH AL-SANIOURA

A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Science in Environmental Sciences
to the Interfaculty Graduate Environmental Science Program
(Environmental Technology)
of the Faculty of Engineering and Architecture
at the American University of Beirut

Beirut, Lebanon

January 2015

AMERICAN UNIVERSITY OF BEIRUT

STARTUP OF THERMOPHILIC ANAEROBIC DIGESTION OF ORGANIC WASTE: ONE STAGE VS TWO STAGE

by

DANA NAZIH AL-SANIOURA

APPROVED BY:

Dr. Mutasem El-Fadel, Associate Professor
Civil and Environmental Engineering,
Advisor

Dr. Sophia Ghanimeh, Assistant Professor
Civil and Environmental Engineering
NDU, Lebanon
Member of Committee

Dr. Pascal Saikaly, Assistant Professor
Environmental Science and Engineering,
KAUST, KSA
Member of Committee

Date of thesis defense: 14-January-2015

AMERICAN UNIVERSITY OF BEIRUT

THESIS, DISSERTATION, PROJECT RELEASE FORM

Student Name: _____
Last First Middle

☒ Master's Thesis Master's Project ⬭ Doctoral Dissertation

I authorize the American University of Beirut to: (a) reproduce hard or electronic copies of my thesis, dissertation, or project; (b) include such copies in the archives and digital repositories of the University; and (c) make freely available such copies to third parties for research or educational purposes.

I authorize the American University of Beirut, **three years after the date of submitting my thesis, dissertation, or project,** to: (a) reproduce hard or electronic copies of it; (b) include such copies in the archives and digital repositories of the University; and (c) make freely available such copies to third parties for research or educational purposes.

_____

Signature Date

ACKNOWLEGEMENTS

Above all I am thankful to God for giving me the patience and the perseverance that has driven me during my research.

I would like to express my appreciation and thanks to my advisor professor Dr. Mutassem El-Fadel, I am thankful for his help, encouragement and the opportunities that he has given me. I would also like to express my gratitude to professor Dr. Sophia Ghanimeh for introducing me to the topic and guiding me all the way to my defence, without her help and persistence this dissertation would not have been possible. I would also like to thank my committee member professor Dr. Pascal Saikaly for his insightful input and suggestions.

In addition, I like to extend my appreciation to professor Dr. Lucy Semerjian, for her support, advice and making the EERC laboratory feel like a second family to me. As well as my sincere thanks to Mr. Joseph Daoud, Mr. Joseph Nassif, Mr. George Jurdi and Mr. Ramzi Safi for their technical support, input in my work and for being my delightful neighbors. In addition I am much obliged to Mrs. Zakia Deeb and her help.

Special thanks to my lab mates at the EERC: Lisa Dahdah, Jihan Hashisho, Sara Bin Ahmed, Khaled Salam, Rania Maroun, Lea Korban, Hamdan Hamdan, Carla Aoun, Abeer Hamzé, Mirna Alameddine, Christiane Zoghbi, Grace Rashid and Joline Chakhtoura, for the stimulating conversations, words of encouragement and for all the coffee one might need for the long nights we were working together. I would also like to thank Hisham Hassan for his assistance with the laboratory testing.

Additionally, I want to thank the National Council for Scientific Research for their financial support, this work would not have been possible without their funding.

Last but not least, a special thanks to my beloved family. Thank you for understanding the times I had to go through to finish my thesis. Words cannot express how grateful I am to my mother, my father, Firas, Mohamed and Lamis Jaleledine for all the sacrifices that you have made for me. I will be eternally grateful for your support, encouragement and patience.

AN ABSTRACT OF THE THESIS OF

Dana Nazih Al Sanioura for Master of Science in Environmental Sciences

Major: Environmental Technology

Title: Startup of thermophilic anaerobic digestion of organic waste: One stage vs two stage

The anaerobic digestion of food waste is an attractive option for waste disposal. In the following study, a one stage digester was compared to a two stage digester for the treatment of food waste under thermophilic temperature. In addition, an innovative strategy for accelerating the startup of a one stage digester while using a fast incremental loading was explored.

In this regard, two one-stage bench-top digesters and a two-stage digester were operated to treat food waste under thermophilic temperature in separate studies. In the first study (Appendix A and C), the one stage digester produced 30% higher specific methane rate and exhibited better stability in terms of lower intermediate-to-partial alkalinity (IA/PA) ratio. However, the two stage digester exhibited better organic matter destruction exhibited by 52 % lower average TCOD, 64% lower SCOD and 5% higher VS% reduction than in the one stage system. In the second study (Appendix B), a biomass acclimation strategy that involved gradual and incremental increase of inoculum and loading rate over 20 weeks was contrasted with a faster 12-week startup. Both reactors showed similar operational stability, there was a 30% reduction in startup time with the fast startup reactor. Despite a 15% reduction in specific methane generation, it remained within the reported values for similar waste. In addition, the reactor achieved high alkalinity, low overall IA/PA ratio and VS% and TCOD removal values within the recommended value.

CONTENTS

ILLUSTRATIONS

TABLES

ABBREVIATIONS

AD	:	Anaerobic Digestion
C:N	:	Carbon Nitrogen Ratio
CH_4	:	Methane
CO_2	:	Carbon Dioxide
COD	:	Chemical Oxygen Demand
FW	:	Food Waste
HCl	:	Hydrochloric Acid
HRT	:	Hydraulic Retention Time
IA/PA	:	Intermediate Alkalinity to Partial Alkalinity Ratio
OLR	:	Organic Loading Rate
SCOD	:	Soluble Chemical Oxygen Demand
TCOD	:	Total Chemical Oxygen Demand
TDS	:	Total Dissolved Solids
TS	:	Total Solids
VDS	:	Volatile Dissolved Solids
VFA	:	Volatile Fatty Acids
VS	:	Volatile Solids

CHAPTER 1

INTRODUCTION

1. Background

Interest in anaerobic digestion technologies has been rising with the increased concern of finding alternative energies, such technologies offer a wide range of advantages that make them appealing for organic waste disposal. Popular disposal methods such as landfilling, composting and incineration are usually limited by stringent regulations and disregard to the combined benefits of anaerobic digestion such as generation of biogas, small area requirements, recovery of organic matter and nutrients, and utilization of the effluent as soil conditioner (Nair et al. 2005; Pavan et al. 2000; Yenigün and Demirel 2013; Zhu et al. 2011). Despite these benefits, the process has been plagued by operational difficulties, issues such as inhibition due to accumulation of certain chemicals (Ammonia, VFA, H_2) and slow startups that may take up to one year (Maroun and El Fadel 2007; Yenigün and Demirel 2013), have rendered the process economically less attractive.

AD is governed by complex biochemical pathways carried out by different groups of interdependent bacterial communities that are mainly classified as hydrolytic-fermentative, acidogenic, proton- reducing acetogens and methanogenic bacteria (Demirel and Scherer 2008; Fdez-Güelfo et al. 2010; Griffin et al. 1998; Velmurugan and Ramanujam 2011) (**Error! Reference source not found.**). The process starts with hydrolysis whereby the hydrolytic bacteria liquefy naturally occurring fibers of the organic substrate by degrading long chain polymers into smaller monomers that can be utilized by subsequent groups of bacteria (Bouallagui et al. 2004; Mata-Álvarez 2003). In fact, this ensuing product can be consumed in three different ways: (a) via fermentative bacteria that will transform it into acetate, hydrogen, carbon

dioxide, pyruvate, volatile fatty acids and alcohols, (b) by obligate hydrogen-producing acetogens through oxidation of fatty acids into hydrogen, carbon dioxide and acetate, and (c) by acidogenic bacteria into organic acids (VFAs), alcohols, hydrogen and carbon dioxide (Bouallagui et al. 2004; Demirel and Scherer 2008; Mata-Álvarez 2003). Next, acetogenic bacteria convert the organic acids and alcohols, produced by the acidogens, as well as previously produced soluble hydrogen and carbon dioxide into acetic acid. However this pathway is reversible depending on the concentration of soluble hydrogen, meaning that at high concentrations of hydrogen, acetogenesis is favored and acetate is produced, whereas at low hydrogen concentrations acetate oxidation is prevalent and acetate is oxidized to hydrogen and carbon dioxide (Demirel and Scherer 2008). Later on hydrogenotrophic methanogens will consume the hydrogen and the carbon dioxide, and the acetotrophic methanogens will consume the acetic acid (the dominant pathway). Both pathways will eventually produce methane, carbon dioxide and water, though the abundance and prevalence of which pathway is usually dictated by environmental factors (configuration of the digester (Nair et al. 2005), temperature, concentration of free ammonia and pH (Demirel and Scherer 2008)). Hydrogenotrophic methanogens are actually less susceptible to high ammonia concentrations than their acetotrophic methanogens, in fact high free ammonia (FA) concentrations coupled with a pH higher than 7.4 is inhibitory to the latter (Demirel and Scherer 2008; Karakashev et al. 2005).In addition this reaction is also dependent on temperature and acetate concentration: in thermophilic reactors acetate oxidation is prevalent at low acetate concentrations while aceticlastic methanogens are predominant at high acetate concentrations (Karakashev et al. 2006). The importance of which pathway is used lies in the mechanism that hydrogenotrophic methanogens use to produce methane and thus

reduce the amount of acetate, free hydrogen and VFA present in the reactor and thus preventing their inhibitory effect on acetogenesis and methanogenesis (Bouallagui et al. 2004; Zuo et al. 2013): hydrogenotrophic methanogens are in a syntrophic association with acetate oxidizers, this process thermodynamically endergonic reaction (Amani et al. 2010) has a higher acetate consuming ability and thus higher eventual yield of methane (Karakashev et al. 2006).

Advances in the field has led to the creation of the two stage system, where the complex biochemical process is optimized by separating two groups of bacteria according to their environmental needs (Nasr et al. 2012; Shen et al. 2013). This process has proven to be a success in lab-scale experiments, in terms of generation of biogas and reduction of organic matter, and prevention of process inhibition, however implementing this configuration in large-scale productions has been challenging. In addition, a number of studies have recently pointed out the lack of blatant advantages that this configuration has over the one stage system (Nasr et al. 2012; Park et al. 2008; Pavan et al. 2000; Schievano et al. 2012).

Additionally, the startup period is usually the critical step of the process, it determines the quality of the microorganism that are present in the digester and thus the quality of the procedure (Amani et al. 2010). During this sensitive period, intermediate metabolites can be susceptible to accumulation due to overwhelmed and unaccustomed bacteria. Moreover, the increase kinetics when the process is treated under thermophilic temperatures (50-55°C), produces higher amounts of biogas and methane, however it amplifies the risk for process inhibition during period of organic shock, such as startup (Amani et al. 2010; Yenigün and Demirel 2013). Therefore it is important to find means of shortening this period but still maintaining a stable digester.

2. Objectives and Scope of Work

The scope of this study was a comparative evaluation of two possibilities of using one stage digesters or two stage digesters for the anaerobic digestion of food waste. While the two stage design was used in lab-scale experiment, it was less adopted in commercial applications. Despite the general favorability of the two stage design, there is an apparent controversy in the literature. On one hand the separation of the two stages provides a multitude of advantages (better COD removal, better resistance to loading fluctuation…etc.), on the other hand the separation of the stages is supposed to hinder the transfer of hydrogen between syntrophs which could result in a failed startup or diminished performance (Reith et al. 2003; Schievano et al. 2012). In addition, various strategies can be found that propose means of shortening the startup period down to mere three weeks (Griffin et al. 1998), however these options are deemed aggressive and risky. In contrast slower strategies have also been proposed such as accommodating loading to the "activated biomass" that is actually present in the reactor. Accordingly, the following research addresses these approaches to assess the importance of two stage designs and come up with a method that is both fast and microbially safe for the one stage process.

3. Thesis structure

Besides this introductory chapter, the thesis consists of two appendices which include the detailed results, discussions and conclusions of the main investigation in appendix A and a follow up research in appendix B.

- *Appendix A* is a journal paper article. (In review for Waste Management): It presents a comprehensive analysis of the comparison of the one stage and two stage digesters as well as a detailed literature review of the anaerobic digestion process.

- *Appendix B* is a conference paper. It presents the analytical results and findings from the shortening of the startup experiment.

4. References

Angelidaki I, Chen X, Cui J, Kaparaju P, L. E. 2006. Thermophilic anaerobic digestion of source-sorted organic fraction of household municipal solid waste: Start-up procedure for continuously stirred tank reactor. Water Research 40:2621-2628.

Bouallagui H, Torrijos M, Godon JJ, Moletta R, Ben Cheikh R, Touhami Y, et al. 2004. Two-phases anaerobic digestion of fruit and vegetable wastes: Bioreactors performance. Biochemical engineering journal 21:193-197.

Demirel B, Scherer P. 2008. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: A review. Reviews in Environmental Science and Bio/Technology 7:173-190.

Griffin ME, McMahon KD, Mackie RI, Raskin L. 1998. Methanogenic population dynamics during start-up of anaerobic digesters treating municipal solid waste and biosolids. Biotechnology and bioengineering 57:342-355.

Karakashev D, Batstone DJ, Angelidaki I. 2005. Influence of environmental conditions on methanogenic compositions in anaerobic biogas reactors. Applied and Environmental Microbiology 71:331-338.

Nair S, Kuang Y, Pullammanappallil P. 2005. Enhanced degradation of waste grass clippings in one and two stage anaerobic systems. Environmental technology 26:1003-1012.

Nasr N, Elbeshbishy E, Hafez H, Nakhla G, Hesham El Naggar M. 2012. Comparative assessment of single-stage and two-stage anaerobic digestion for the treatment of thin stillage. Bioresource technology 111:122-126.

Pavan P, Battistoni P, Cecchi F, Mata-Alvarez J. 2000. Two-phase anaerobic digestion of source sorted ofmsw (organic fraction of municipal solid waste): Performance and kinetic study. Water science and Technology 41:111-118.

Reith JH, Wijffels RH, Barten H. 2003. Bio-methane and bio-hydrogen: Status and perspectives of biological methane and hydrogen production:Dutch Biological Hydrogen Foundation.

Schievano A, Tenca A, Scaglia B, Merlino G, Rizzi A, Daffonchio D, et al. 2012. Two-stage vs single-stage thermophilic anaerobic digestion: Comparison of energy production and biodegradation efficiencies. Environmental science & technology 46:8502-8510.

Zhu H, Parker W, Conidi D, Basnar R, Seto P. 2011. Eliminating methanogenic activity in hydrogen reactor to improve biogas production in a two-stage anaerobic digestion process co-digesting municipal food waste and sewage sludge. Bioresource technology 102:7086-7092.

Zuo Z, Wu S, Zhang W, Dong R. 2013. Effects of organic loading rate and effluent recirculation on the performance of two-stage anaerobic digestion of vegetable waste. Bioresource technology 146:556-561.

APPENDIX A

Startup of Thermophilic Anaerobic Digestion Systems with High Solids Food Waste Influent

D. Al-Sanioura*, S. Ghanimeh**, P. Saikaly***, M. El-Fadel*†

** Department of Civil & Environmental Engineering, American University of Beirut, Lebanon*
*** Department of Civil & Environmental Engineering, Notre Dame University, Lebanon*
**** Water Desalination and Reuse Center, Division of Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology, Environmental Science and Engineering, Thuwal, Kingdom of Saudi Arabia*

† Corresponding Author: mfadel@aub.edu.lb

ABSTRACT

The accumulation of volatile fatty acids (VFA) during the anaerobic digestion (AD) of highly biodegradable substrate such as food waste (FW) is associated with an inhibitory effect on methane producing biochemical pathways. While inconclusive at times, the usage of two-stage digesters has been argued as a potential solution for this issue. In this study one-stage vs. two-stage thermophilic digesters, fed with FW, were examined during the startup phase by increasing the loading rate from 0.5 to 2 gVS/l/d. While showing a 30% higher ammonia concentration, the one-stage system exhibited better overall stability expressed by a faster decrease of intermediate-to-partial alkalinity ratios (< 0.3) and resulting in about 30% higher methane generation rates. However, the two-stage digester exhibited better organic matter removal with 52 and 64 % lower average TCOD and SCOD, respectively.

Keywords: Thermophilic anaerobic digestion, one-stage vs. two-stage, startup, food waste

A.1. INTRODUCTION

The growing pressure towards effective disposal of food waste coupled with the rising interest in carbon neutral energy, has encouraged research on clean energy extraction from biomass and waste (Demirel and Scherer 2008). Food waste (FW), with its high moisture content and biodegradability, is a good candidate for anaerobic digestion (AD) (Bouallagui et al. 2004; Zuo et al. 2013) offering the advantage of producing methane and soil conditioners. However with its low carbon-to-nitrogen (C/N) ratio, AD of FW is associated with a low stability due to the accumulation of intermediate byproducts such as volatile fatty acids (VFA) (Jia Lin et al. 2011). This problem is more pronounced under thermophilic temperatures (50-55°C) due to faster kinetics compared to mesophilic systems (30-40°C) leading to an even higher accumulation of VFA, especially with highly biodegradable wastes such as FW (Mata-Álvarez 2003).

The separation of the AD process (Figure 1) has been promoted in some studies to alleviate the accumulation of metabolites by buffering the loading rate and organic matter in the first stage allowing a healthier methanogenesis in the second stage (Alvarez et al. 2008; Ganesh et al. 2014). As such the AD process is separated into two stages with the first stage encompassing the fermentative hydrolytic, the acidogenic and the acetogenic bacteria, while the methanogenic bacteria can be separated in a second stage due to their lower resilience, tolerance to upsets, and growth rate (Mata-Álvarez 2003). The two stage digesters can reportedly achieve higher methane generation and COD removal (Nasr et al. 2012), lower VFA accumulation (Ali et al. 2011), improved functional stability for waste with poor cellulose such as food waste (Vandevivere et al. 2003), better resistance to loading fluctuation and more tolerance of higher loading rates (Ganesh et al. 2014; Park et al.

2008; Vandevivere et al. 2003). In contrast, other studies showed that a well-designed and adequately operated one stage CSTR can perform similar or better than two stage systems (Bolzonella et al. 2003; Forster-Carneiro et al. 2008). Furthermore, the separation of the hydrolysis/acidogenesis and methanogenesis phases (or increasing the distance between acidogens and methanogens) is argued to hamper the syntrophic associations and prevent the transfer of hydrogen between both group of species negatively affecting the relations between the "producing" and "consuming" microorganisms" and increasing the accumulation of propionate and butyrate, both being methanogenic inhibitors (Amani et al. 2010; Blonskaja et al. 2003; Reith et al. 2003; Schievano et al. 2012).

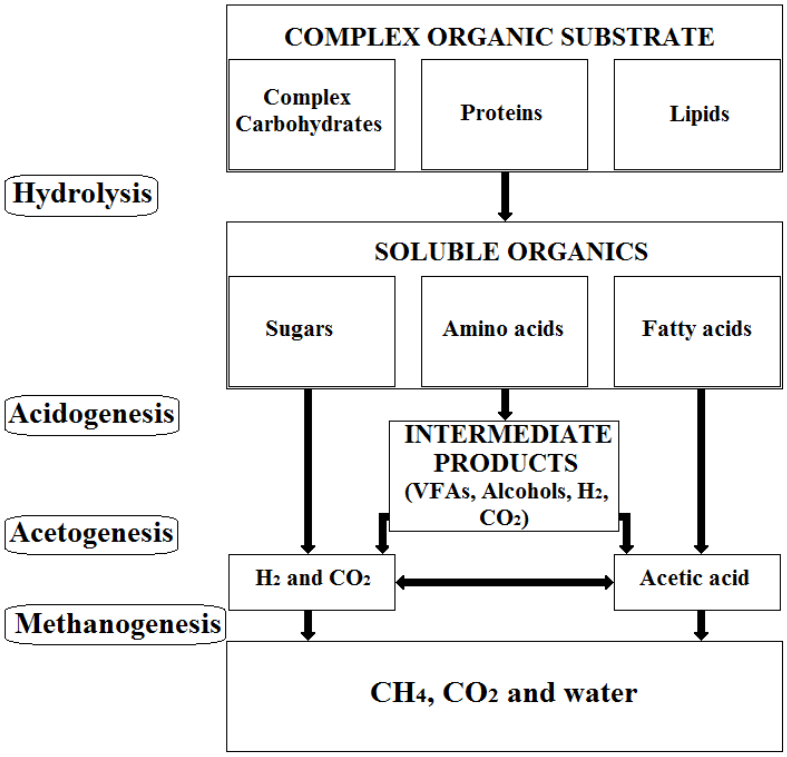


Figure A. 1 Anaerobic digestion biochemical pathways

It is worth noting that most reported two stage AD systems were operated under mesophilic temperatures using various types of digesters (CSTR, UASB and up flow sludge bed) (Ali et al. 2011; Alvarez et al. 2008; Ergüder et al. 2001; Zuo et al. 2013), and treating waste such as cheese whey (Ergüder et al. 2001; Ghaly 1989), or fruit and vegetable waste (Bouallagui et al. 2004; Bouallagui et al. 2009; Ganesh et al. 2014; Zuo et al. 2013). Similarly, studies comparing one- with two-stage digesters have been reported under mesophilic temperatures (Ganesh et al. 2014; Shen et al. 2013) or temperature phased stages (Nielsen et al. 2004), using synthetic substrate (Azbar and Speece 2001), high cellulosic substrate (Nair et al. 2005) or a combination of swine manure and market biowaste (Schievano et al. 2012). Comparisons of the startup of one- and two-stage digesters treating the same waste under thermophilic temperature are limited to non-existent (Ganesh et al. 2014). Given the importance of the startup phase and the inconsistency regarding the effectiveness of the two stage design, this work targets a comparative analysis of the one stage versus the two stage design treating food waste under thermophilic conditions during the startup of continuously stirred tank reactors (CSTR). The assessment contrasts the performance (in terms of biogas and methane generation), treatment efficiency (organic solid reduction) and stability (alkalinity and VFA) of both systems.

A.2. MATERIALS AND METHODS

A.2.1. Feed preparation and characteristics

Food waste was collected over two weeks from households and local fruit and vegetable markets to ensure a varied and representative sample. The feed was ground, mixed, homogenized and stored in 150 ml cups at -20°C. The purpose of early collection and storage of FW samples is to reduce fluctuations in substrate composition. Prior to use, the food samples were thawed and diluted with distilled

water to reach the required volume. The feed's characteristics (Table 1) are comparable to reported values (COD = 292000 mg/l (Park et al. 2008); TS= 22.61 % and C/N= 11.5 (Shen et al. 2013)).

Table A. 1 Physico-Chemical Characteristics of the food waste

Parameter		**Value**
TS%		20.93%
VS%		19.90%
VS(%TS)		95.06%
COD	Total	389172 mg/l
	Soluble	13540 mg/l
Ammonia-N		30 mg/l
Alkalinity	(5.75)	0 mg of $CaCO_3$ /l
	(4.30)	369 mg of $CaCO_3$/l
C:N Ratio		11

A.2.2. Experimental procedure

The one stage digestion was carried out in a bench-scale digester (9 l working volume, Bioflo 110, New Brunswick Scientific Co.) and the two-stage digestion was carried in two digesters in series: B1 and B2 (9 l in total, 4.5 l working volume each, Anaerobic Digester W8, Armfield Ltd.) (**Error! Reference source not found.**). Both systems were inoculated with digestate collected from a stable one-stage digester operating at similar conditions (fed with food waste at 55^{o}C) and running at an organic loading rate (OLR) = $2gVS.L^{-1}.d^{-1}$ for over a year. All three digesters were inoculated with 50% of their working volume (4.5 l in digester A and 2.25 l in digesters B1 and B2, respectively). After seeding, distilled water was added to all digesters till final volume was reached (digester A = 9 l; digester B1 = 4.5 l; digester B2 = 4.5 l). The pH of digester B1 was adjusted on day 1 to be between 5 and 6 with HCl (5M), whereas digester A and B2 had the desired pH 7 (Kastner and Schnitzhofer, 2011). The digesters were not fed until the third day after seeding and

physico-chemical parameters and gas monitoring started on day 16 after inoculation. A portion of the digestate removed from B1 was discarded considering that its hydraulic retention time (HRT) was double that of the B2 digester (**Error! Reference source not found.**). The one stage digester (A) was started at an OLR of 0.5 $gVS.l^{-1}.d^{-1}$ for the first 32 days. The OLR was then raised starting day 33 till day 89 from 0.5 to 2.1 $gVS.l^{-1}.d^{-1}$. The OLR remained steady at 2.0 $gVS.l^{-1}.d^{-1}$ till the end of the experiment. The two stage system (digesters B1 and B2) was started at an OLR of 0.3 $gVS.l^{-1}.d^{-1}$ for the first 30 days. The OLR was then increased from 0.3 to 2.1 $gVS.l^{-1}.d^{-1}$ between days 31 and 88 and maintained constant afterwards till the end of the experiment. The startup period was divided into three consecutive durations according to the loading pattern and rate: Run 1 (Steady low rate), Run 2 (Incremental rate), and Run 3 (Steady high rate) (Figure 2).

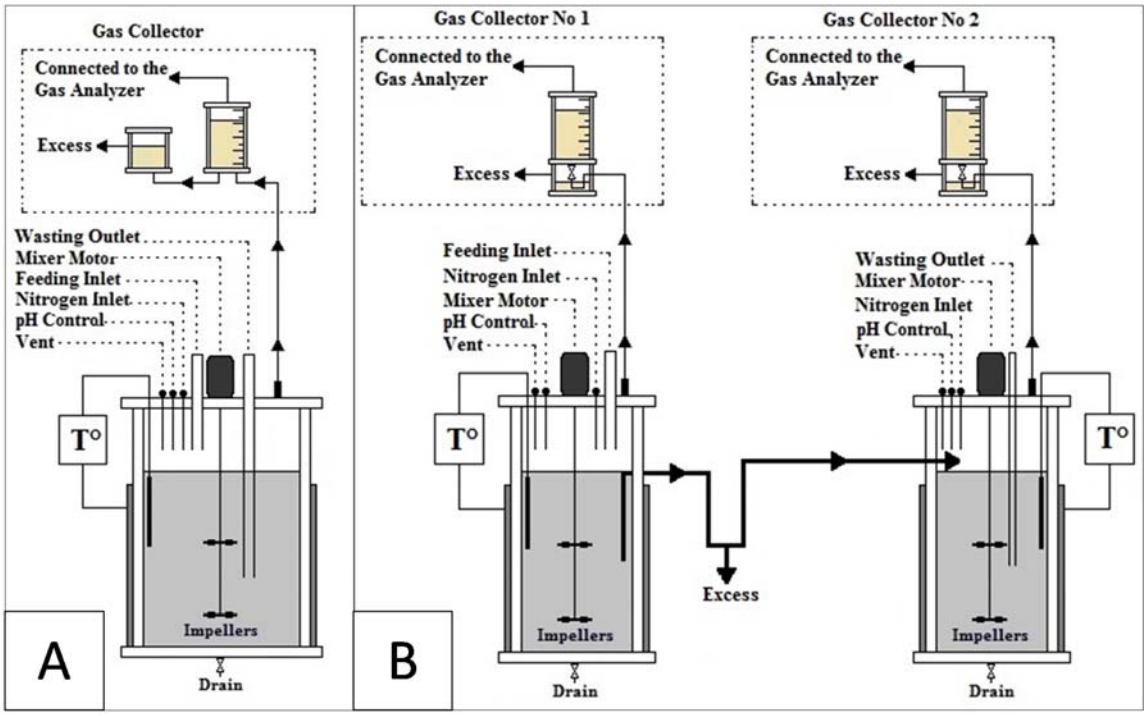


Figure A. 2 Layout of both digesters, the one stage digester, digester A (A), and the two stage digester, acidogenic reactor (B1) is on the left and methanogenic reactor (B2) is on the right

Table A. 2 Summary of the experimental conditions

	A	B1	B2
OLR (g VS.l^{-1})	0.5 to 2.1	0.5 to 4.2	0.3 to 2.4
HRT (Days)	30	10	20
Digestion period	112	105	105

A.2.3. Monitoring methods

The temperature of the digesters was maintained at 55±1°C and monitored using thermostatically controlled electric heating jackets connected to built-in temperature probes. The pH was monitored with an immersed probe and adjusted through a manually operated peristaltic pump connected to a NaOH (5N) solution or an HCl (5N) solution depending on the reactor. The biogas volume and composition (methane and carbon dioxide) were monitored once or twice per day by the water displacement method and a dual wavelength infrared cell with reference channels (GEM-2000 monitor, Keison Products, UK). Physico-chemical parameters were monitored on a weekly basis with samples collected from discarded digestate prior to feeding. A portion of the sample was centrifuged at 13000 rpm for 20-40 minutes depending on the quality of the sample using a Thermo Scientific Sorvall ST16 Centrifuge and then filtered using Whatman microfiber filter 47mm (pore size: 1.5 μm), the filtered portion constituted the soluble fraction. Soluble and total COD was carried out using the modification of Standard Methods 5220D procedure (Conklin et al. 2006) through photometric measurements using COD Digestion Vials, High Range Plus, 0 to 15,000 mg/L. Total, dissolved and volatile solids (TS/VS, and TDS/VDS) were determined using Standard Methods (Way 2012) for the analysis of water and wastewater, TDS/VDS was calculated by subtracting the two previous parameters; while ammonia content was determined by spectrophotometry using High Range Ammonia Nitrogen

by the AmVer™ Salicylate Test 'N Tube™ Method. Both COD and ammonia testing were conducted using a HACH DR/2010 Spectrophotometer. Total and partial alkalinity were determined by titration with HCl (0.2 N) to pH 4.3 and 5.75 respectively using a Thermo Scientific Orion 3 STAR benchtop pH, whereas the intermediate alkalinity was calculated as the difference between the two parameters.

A.3. RESULTS AND DISCUSSION

A.3.1. Biogas production

In the one-stage digester (A), methane yield was relatively stable at an average of 0.23-0.29 $l.gVS^{-1}.d^{-1}$,despite reaching a high of 0.32 $l.gVS^{-1}.d^{-1}$ during the last week of run 3 (Table 3 and Figure 4), typical of food waste fed systems (Chen et al. (2010), Ghanimeh et al. (2012),Gómez et al. (2010). Overall, methane content was constant at 33±1% during the first two runs, despite decreasing from 32% to 19% during the second week of run2 (**Error! Reference source not found.**) which was paralleled by an increase in the CO_2/CH_4 ratio indicating methanogenic distress (Ghanimeh et al. 2012) (**Error! Reference source not found.**).This could have been caused by the OLR increase during the start of run 2 which could have caused an initial shock to the bacterial community. The CO_2/CH_4 ratio kept increasing throughout the rest of the experiment to reach an average of 0.6 during run 3, indicating general reactor distress. Even though the overall biogas yield decreased by 18 % (from 0.79 to 0.65 $l.gVS^{-1}.d^{-1}$) between run 1 and run 3, there was a general improvement in biogas composition methane yield to reach an average of 45% and 0.29 $l.gVS^{-1}.d^{-1}$, respectively, in the last run .

Table A. 3 Methane and biogas yields and content for reactor A, B1, B2 and overall average of digester B

		RUN 1	RUN 2	RUN 3
Average Biogas Yield ($l.gVS^{-1}.d^{-1}$)	A	0.79	0.69	0.65
	B1	0.56	0.31	0.05
	B2	0.59	0.45	0.35
Average Methane Composition (%)	A	32	34	45
	B1	0	0	0
	B2	4	33	59
Average Methane Yield ($l.\ gVS^{-1}.d^{-1}$)	A	0.26	0.23	0.29
	B1	0	0	0
	B2	0.02	0.15	0.22

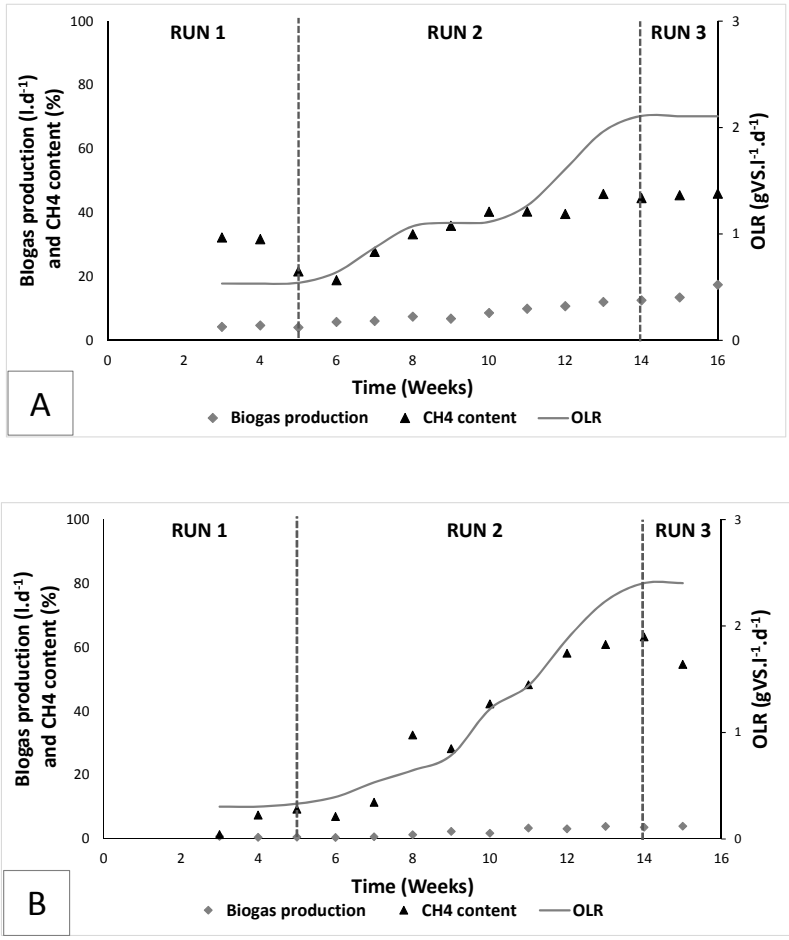


Figure A. 3 Biogas production and methane content in Digester A and B2

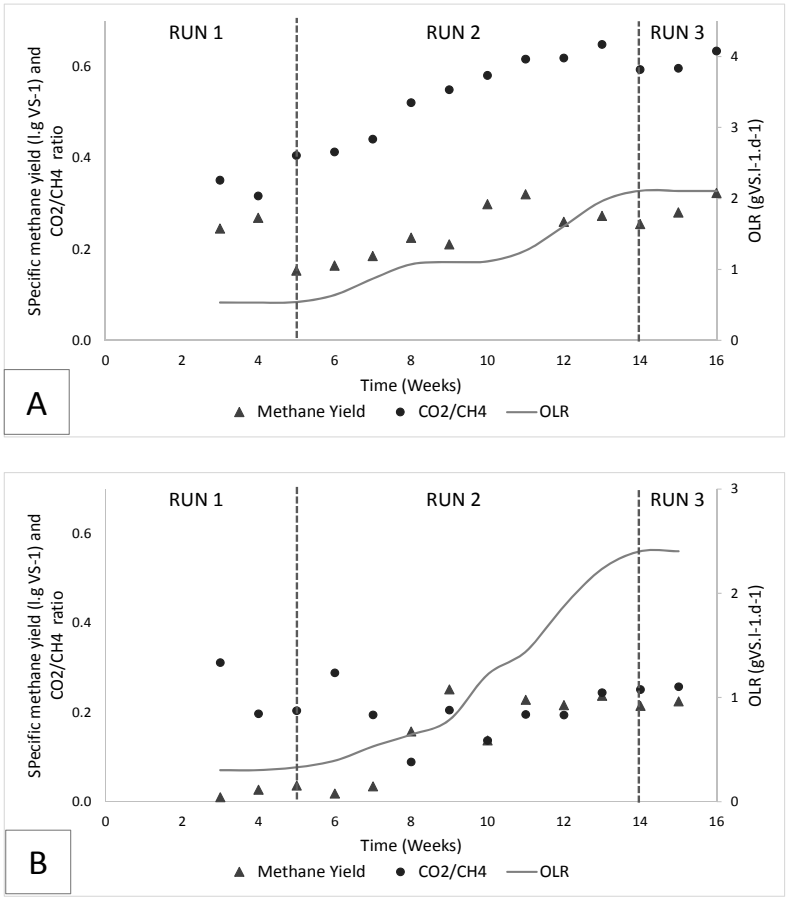


Figure A. 4 Weekly specific methane yield and CO2/CH4 ratio for digester A (A) and digester B2 (B)

In comparison, the methane yield and biogas production in B2 (the methanogenic phase of the two-stage system) started at low levels of <0.1 $l.gVS^{-1}$ and <1 $l.d^{-1}$, respectively. As the OLR reached 0.6 g $VS.l^{-1}.d^{-1}$, on week 8, the CH_4 yield increased to 0.16 $l.gVS^{-1}$ (Figures 3 and 4). This was concomitant with a rise in the overall buffering capacity of the system to over 3500 mg $CaCO_3.l^{-1}$and a decrease in the IA/PA ratio to about 0.22. Starting week 9, the methane yield stabilized at 0.12±0.04 $l.gVS^{-1}$ and the biogas production at 3 $l.d^{-1}$; but remained below the production level of digester A. However, the methanogenic activity in reactor (B2) was healthy by the end of the experiment, evident by the acceptable CO_2/CH_4 ratio of 0.25 during run 3 and the methane content ranging between 54% and 62% (and reaching up to 70% on

some days).

A.3.2. Alkalinity and VFAs

As a process indicator, pH is dependent on the concentration of free VFA and the overall alkalinity of the reactor (Björnsson et al. 2001). The IA/PA ratio is important as a representation of VFA alkalinity (Banks et al. 2011), therefore its increase is an indication of a surge in free VFA and thus a distress in the biochemical pathway. In both digesters, A and B2, the pH range remained high throughout the experiment, varying within the acceptable range of 7.4 and 7.9 (Park et al. 2008) and eliminating the need for pH control. Similarly, average IA/PA ratio in A and B2 was over 0.3, the recommended threshold for such digesters when total alkalinity is between 4000 and 8000 mg $CaCO_3.l^{-1}$ (Martín-González et al. 2013)..

Despite starting off at a high 0.6, the IA/PA ratio in reactor A gradually decreased to below 0.2 by the start of run 2 and remained so during this run. Digester B2 produced an initial IA/PA ratio that was even higher than digester A, and persisted until week 5 (**Error! Reference source not found.**). This could have inhibited the methanogenic community leading to the low methane generation observed in B2. In week 5, the IA/PA ratio in B2 decreased to below 0.4 (two weeks after digester A), accompanied by a decrease in total alkalinity to slightly below 4000 $mg.l^{-1}$ possibly indicating that a decrease in free VFA occurred due to alkalinity buffering. Interestingly, the pH in both digesters, A and B2, was not affected by the initial high concentration of VFA in both systems due to temporary compensation by the high buffering capacity presented by the total alkalinity (TA). The total buffering capacity expressed as TA, was stable and remained above 4000 and 3500 mg $CaCO_3.l^{-1}$ for digester A and B2, respectively, all throughout the experimental program, an acceptable limit according to Ganesh et

al. (2014). However, the TA did decrease by 41% (from 6800 to 4000 mg $CaCO_3.l^{-1}$) in digester A and 32% (from 5300 to 3600 mg $CaCO_3.l^{-1}$) in digester B2, between weeks 11 and 12, when the OLR was increased by 26%. This was followed by a doubling in the IA/PA ratio (from 0.15 to 0.37 in A and from 0.05 to 0.12 in B2), indicating system upset due to increased substrate loading. Nevertheless this shock was immediately subsided and the reactors recovered with an average IA/PA ratio of 0.28±0.05 and 0.10±0.02 and an average TA of 5320±273mg and 5809±563 mg of $CaCO_3.l^{-1}$in digesters A and B2, respectively. Regardless of these oscillations, pH was stable remaining on average above 7.5 and requiring no intervention.

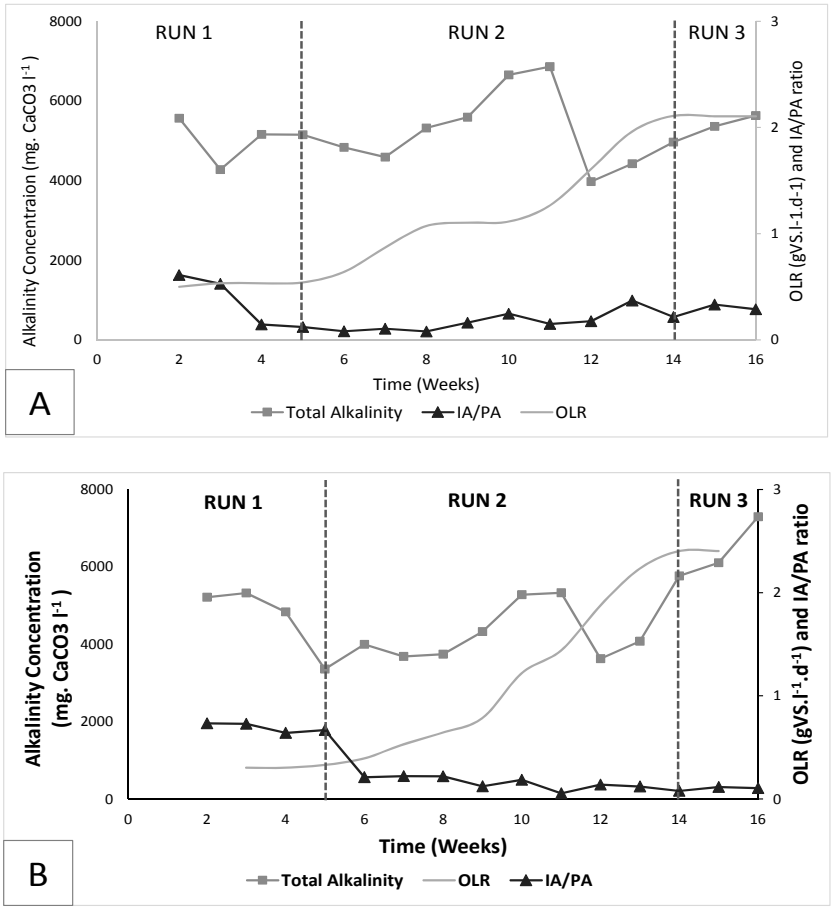
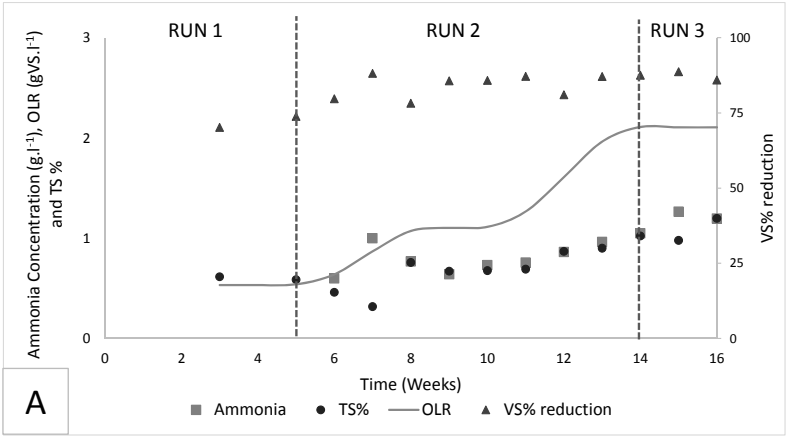


Figure A. 5 Alkalinity concentrations and IA/PA ratio for reactor A and reactor B2

A.3.3. Digestate characteristics

Upon increasing the OLR at the start of run 2, ammonia concentrations in digester A spiked from 0.6 to 1 $g.l^{-1}$ on week 7. In then decreased in week 8, only to slowly increase again till reaching an average of 1.2 $g.l^{-1}$ during run 3 (**Error! Reference source not found.**) – slightly higher than the inhibition threshold of 1 $g.l^{-1}$ for a similar waste type and process design (Yenigün and Demirel 2013). In comparison, ammonia levels in digester B2 remained above the 1 $g.l^{-1}$ threshold until week 9 of run 2, when they dropped to a reasonable level of 0.2 $g.l^{-1}$. This decrease lead to an enhanced degradation of intermediate metabolites resulting in an increase in total alkalinity (form 3740 to 5282 mg $CaCO_3.l^{-1}$) (Vandevivere et al. 2003). The drop in ammonia was followed by a subsequent gradual increase which paralleled the increase in OLR, to a final value of 0.7$g.l^{-1}$ during the last week.



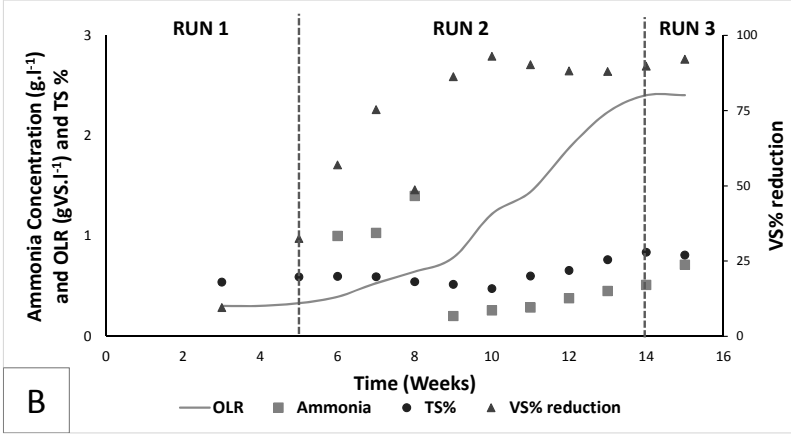


Figure A. 6 Ammonia concentrations, total solids content (TS%) of the liquor and volatile solids (VS%) removal in digesters A and B2

Total and soluble COD concentrations in reactor A remained below 20 $g.l^{-1}$and 5 $g.l^{-1}$, respectively, which is consistent with reported values (Ganesh et al. 2014) and indicates proper use of the hydrolytic products by the methanogenic bacteria. In comparison, the results of digester B2, during run 3, were even lower with average total and soluble COD of 5.1 $g.l^{-1}$and 1.2 $g.l^{-1}$, respectively, indicating a better degradation of organic components. Similarly, total and suspended solids were lower in B2, with TS = 1.02 % in A vs, 0.81 % in B2; and TSS = 0.52 % in A vs, 0.14 % in B2 (Figure 7). The lower removal rates in digester A during run 3 can be explained by the rise in ammonia levels during this run coupled with the high alkalinity of the system which could hinder bacterial functions at the end of the startup (Yenigün and Demirel 2013). Similarly, both systems underwent good degradation of organic matter and resulted in a high removal of volatile solids. Also, VS destruction was higher in the two-stage system with an average of 90±2% in B2, compared to an average of 85±3% in A, both comparable to reported results (88% from Ward et al. (2008), 83% from Verrier et al. (1987).

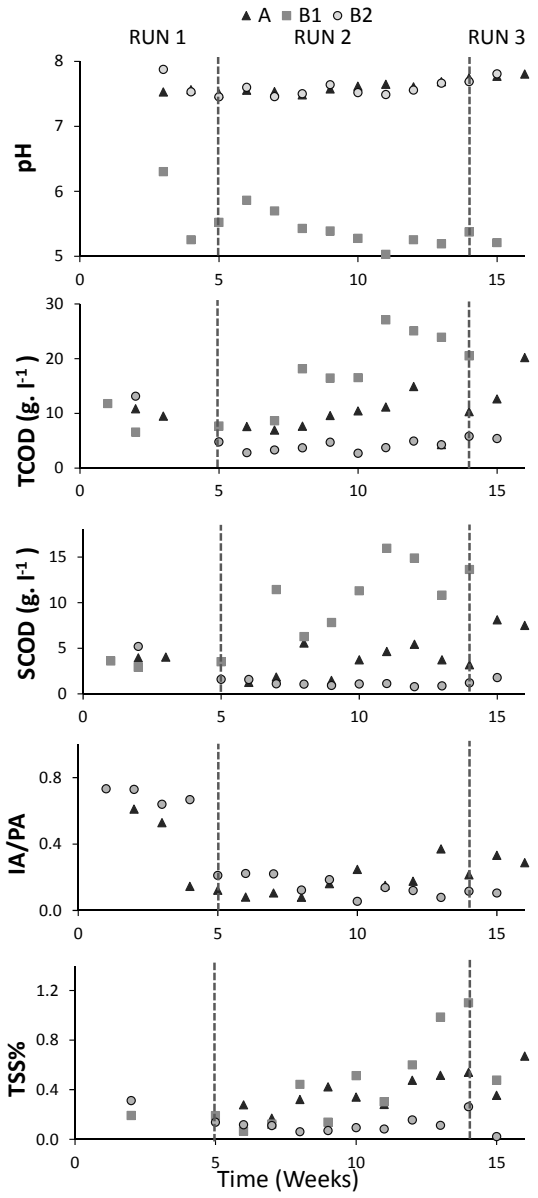


Figure A. 7 Effluent characteristics for reactors A, B1 (except for IA/PA ratio) and B2

The concentration of the organic substrate in B1 liquor was the highest of all three digesters (TS% = 1.09%, VS% = 0.7% and TSS = 0.43%) with SCOD more than two folds of the one-stage system (11 $g.l^{-1}$ compared to 4.2 $g.l^{-1}$ in A). This can be attributed to the high activity of fermenters and the absence of methanogenic activity to utilize hydrolyzed products. Whereas the organic substrate in reactor B2 was the lowest among all three digesters (TCOD = 5 $g.l^{-1}$, SCOD = 1.5 $g.l^{-1}$, TSS = 0.13%, TS = 0.64%)

arguably because most of the hydrolysis and degradation of larger polymers is achieved in the first stage, and the SCOD is consumed by methanogens (Ganesh et al. 2014) (**Error! Reference source not found.** and 7). It is worth noting that prior to the decrease in ammonia concentration during week 9, the VS reduction in the B2 effluent was low, averaging at 43%, and improved significantly to an average of 90% afterwards, exceeding the results of digester A. This is expected in two stage digesters, where organic matter destruction is superior than in one stage digesters, due to fermenters thriving in optimal acidic environments (Nasr et al. 2012).

A.3.4. Impact of stage separation

Despite similar seeding and operating conditions (temperature, mixing, overall HRT and OLR) in the one- and two-stage systems, differences in the microbial component are expected, leading to disparities in various aspects (CH_4 generation, ammonia levels, solids removal, and stability). Considering environmental parameters (pH and specific HRT to each reactor) imposed in the two-stage compartments, a difference in the abundance and activity of some microbial communities is inevitable (Zhang and Noike 1991). This is likely to lead to a superior hydrolysis and better destruction of organic matter due ideal acidic conditions provided to the hydrolytic and acidogenic communities (Nasr et al. 2012). In fact, the final percentage of total and suspended solids was lower than the one-stage system. Similarly, reduction in total and soluble COD was 52% and 64%, respectively, higher than the one-stage system. These findings are consistent with those reported by Liu et al. (2006), Massanet-Nicolau et al. (2013), and Nasr et al. (2012) but inconsistent with the findings of Nair et al. (2005) where the lower organic matter degradation in two stage digesters was attributed to a decrease in the protozoan population. Also, the results do not concur

with those of Ergüder et al. (2001) and Park et al. (2008) who did not find significant difference in COD removal between both systems.

On the other hand, separating the digestion phases in the two-stage system increases the distance between syntrophic bacteria and hinders the transfer of hydrogen, needed for hydrogenotrophic methanogenesis (Amani et al. 2010; Blonskaja et al. 2003; Reith et al. 2003; Schievano et al. 2012). Furthermore, the abundance of hydrolyzed substrates and easily fermentable organic matter, mainly VFAs, transferred from the acidogenic reactor, may prove to cause shocking to the sensitive methane producing community in the methanogenic reactor (Schievano et al. 2012) leading to reduced activity of methanogens and lower methane generation. This is further confirmed by the results of this study whereby the one-stage digester generated during the last run, 32% more methane per gram of VS fed to the system (0.29 in A vs. 0.22 $l.gVS^{-1}.d^{-1}$ in B2) which contradicts the general perception that two-stage systems have a superior methane generation compared to one-stage systems. While Massanet-Nicolau et al. (2013) and Shen et al. (2013) reported similar observations when treating wheat feed pellets and food waste, respectively, under mesophilic conditions, Schievano et al. (2012) reported little difference in energy generation between the two systems.

A.4. CONCLUSION

One and two-stage anaerobic digestion of food waste were compared during thermophilic start-up at OLR $\leq$ 2 $gVS.l^{-1}.d^{-1}$. The digesters were monitored for biogas generation and methane content, COD removal, intermediate-to partial alkalinity ratio and ammonia concentration. The adequacy of the one stage system was demonstrated and exhibited by a higher methane production and a more stable performance at such loading rates, whereas the two stage digester exhibited better removal of COD and total and soluble solids.

Acknowledgments

This research was supported by a grant from the National Council for Scientific Research, Lebanon. Special thanks are extended to the United States Agency for International Development for its support in acquiring the equipment used in the experimental program.

References

Ali A, Hashmi N, Ehsanullah, Khan KA, Iqbal J, Nosheen G. 2011. Performance evaluation of the two-stage uasb reactor using methanolic wastes. Electronic Journal of Environmental, Agricultural and Food Chemistry 10:2735-2743.

Alvarez JA, Armstrong E, Gomez M, Soto M. 2008. Anaerobic treatment of low-strength municipal wastewater by a two-stage pilot plant under psychrophilic conditions. Bioresource technology 99:7051-7062.

Amani T, Nosrati M, Sreekrishnan TR. 2010. Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects-a review. Environmental Reviews 18:255-278.

Angelidaki I, Chen X, Cui J, Kaparaju P, L. E. 2006. Thermophilic anaerobic digestion of source-sorted organic fraction of household municipal solid waste: Start-up procedure for continuously stirred tank reactor. Water Research 40:2621-2628.

Azbar N, Speece RE. 2001. Two-phase, two-stage, and single-stage anaerobic process comparison. Journal of Environmental Engineering 127:240-248.

Banks CJ, Chesshire M, Heaven S, Arnold R. 2011. Anaerobic digestion of source-segregated domestic food waste: Performance assessment by mass and energy balance. Bioresource technology 102:612-620.

Björnsson L, Murto M, Jantsch TG, Mattiasson B. 2001. Evaluation of new methods for the monitoring of alkalinity, dissolved hydrogen and the microbial community in anaerobic digestion. Water research 35:2833-2840.

Blonskaja V, Menert A, Vilu R. 2003. Use of two-stage anaerobic treatment for distillery waste. Advances in Environmental Research 7:671-678.

Bolzonella D, Innocenti L, Pavan P, Traverso P, Cecchi F. 2003. Semi-dry thermophilic anaerobic digestion of the organic fraction of municipal solid waste: Focusing on the start-up phase. Bioresource technology 86:123-129.

Bolzonella D, Cavinato C, Fatone F, Pavan P, Cecchi F. 2012. High rate mesophilic, thermophilic, and temperature phased anaerobic digestion of waste activated sludge: A pilot scale study. Waste Management 32:1196-1201.

Bouallagui H, Torrijos M, Godon JJ, Moletta R, Ben Cheikh R, Touhami Y, et al. 2004. Two-phases anaerobic digestion of fruit and vegetable wastes: Bioreactors performance. Biochemical engineering journal 21:193-197.

Bouallagui H, Lahdheb H, Ben Romdan E, Rachdi B, Hamdi M. 2009. Improvement of fruit and vegetable waste anaerobic digestion performance and stability with co-substrates addition. Journal of environmental management 90:1844-1849.

Chen X, Romano RT, Zhang R. 2010. Anaerobic digestion of food wastes for biogas production. International Journal of Agricultural & Biological Engineering 3.

Demirel B, Scherer P. 2008. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: A review. Reviews in Environmental Science and Bio/Technology 7:173-190.

Ergüder TH, Tezel U, Güven E, Demirer GN. 2001. Anaerobic biotransformation and methane generation potential of cheese whey in batch and uasb reactors. Waste Management 21:643-650.

Fdez-Güelfo LA, Alvarez-Gallego C, Sales Márquez D, Romero García LI. 2010. Start-up of thermophilic–dry anaerobic digestion of ofmsw using adapted modified sebac inoculum. Bioresource technology 101:9031-9039.

Forster-Carneiro T, Perez M, Romero LI. 2008. Anaerobic digestion of municipal solid wastes: Dry thermophilic performance. Bioresource technology 99:8180-8184.

Ganesh R, Torrijos M, Sousbie P, Lugardon A, Steyer JP, Delgenes JP. 2014. Single-phase and two-phase anaerobic digestion of fruit and vegetable waste: Comparison of start-up, reactor stability and process performance. Waste Management 34:875-885.

Ghaly AE. 1989. Biogas production from acid cheese whey using a two-stage digester. Energy Sources 11:237-250.

Ghanimeh S, El Fadel M, Saikaly P. 2012. Mixing effect on thermophilic anaerobic digestion of source-sorted organic fraction of municipal solid waste. Bioresource technology 117:63-71.

Gómez X, Cuetos MJ, Tartakovsky B, Martinez-Nunez MF, Moran A. 2010. A comparison of analytical techniques for evaluating food waste degradation by anaerobic digestion. Bioprocess and biosystems engineering 33:427-438.

Jia Lin, Jiane Zuo, Lili Gan, Peng Li, Fenglin Liu, Kaijun Wang, et al. 2011. Effects of mixture ratio on anaerobic co-digestion with fruit and vegetable waste and food waste of china. Journal of Environmental Sciences 23:1403-1408.

Kastner V, Schnitzhofer W. Anaerobic fermentation of food waste: Comparison of single stage and two-stage fermentation. In: Proceedings of the International IWA-Symposium on Anaerobic Digestion of Solid Waste and Energy Crops, 2011 2011, Vol. Aug 28 - Sep 1.

Liu D, Liu D, Zeng RJ, Angelidaki I. 2006. Hydrogen and methane production from household solid waste in the two-stage fermentation process. Water research 40:2230-2236.

Martín-González L, Font X, Vicent T. 2013. Alkalinity ratios to identify process imbalances in anaerobic digesters treating source-sorted organic fraction of municipal wastes. Biochemical engineering journal 76:1-5.

Massanet-Nicolau J, Dinsdale R, Guwy A, Shipley G. 2013. Use of real time gas production data for more accurate comparison of continuous single-stage and two-stage fermentation. Bioresource technology 129:561-567.

Mata-Álvarez J. 2003. Biomethanization of the organic fraction of municipal solid wastes:IWA publishing.

Nair S, Kuang Y, Pullammanappallil P. 2005. Enhanced degradation of waste grass clippings in one and two stage anaerobic systems. Environmental technology 26:1003-1012.

Nasr N, Elbeshbishy E, Hafez H, Nakhla G, Hesham El Naggar M. 2012. Comparative assessment of single-stage and two-stage anaerobic digestion for the treatment of thin stillage. Bioresource technology 111:122-126.

Nielsen HB, Mladenovska Z, Westermann P, Ahring BK. 2004. Comparison of two‐stage thermophilic (68° c/55° c) anaerobic digestion with one‐stage thermophilic (55° c) digestion of cattle manure. Biotechnology and bioengineering 86:291-300.

Palatsi J, Laureni M, Andrés MV, Flotats X, Nielsen HB, Angelidaki I. 2009. Strategies for recovering inhibition caused by long chain fatty acids on anaerobic thermophilic biogas reactors. Bioresource technology 100:4588-4596.

Park Y, Hong F, Cheon J, Hidaka T, Tsuno H. 2008. Comparison of thermophilic anaerobic digestion characteristics between single-phase and two-phase systems for kitchen garbage treatment. Journal of bioscience and bioengineering 105:48-54.

Reith JH, Wijffels RH, Barten H. 2003. Bio-methane and bio-hydrogen: Status and perspectives of biological methane and hydrogen production:Dutch Biological Hydrogen Foundation.

Schievano A, Tenca A, Scaglia B, Merlino G, Rizzi A, Daffonchio D, et al. 2012. Two-stage vs single-stage thermophilic anaerobic digestion: Comparison of energy production and biodegradation efficiencies. Environmental science & technology 46:8502-8510.

Shen F, Yuan H, Pang Y, Chen S, Zhu B, Zou D, et al. 2013. Performances of anaerobic co-digestion of fruit & vegetable waste (fvw) and food waste (fw): Single-phase vs. Two-phase. Bioresource technology 144:80-85.

Vandevivere P, De Baere L, Verstraete W. 2003. Types of anaerobic digester for solid wastes. Biomethanization of the organic fraction of municipal solid wastes:111-140.

Verrier D, Roy F, Albagnac G. 1987. Two-phase methanization of solid vegetable wastes. Biological wastes 22:163-177.

Ward AJ, Hobbs PJ, Holliman PJ, Jones DL. 2008. Optimisation of the anaerobic digestion of agricultural resources. Bioresource technology 99:7928-7940.

Yenigün O, Demirel B. 2013. Ammonia inhibition in anaerobic digestion: A review. Process Biochemistry 48:901-911.

Zhang TC, Noike T. 1991. Comparison of one-phase and two-phase anaerobic digestion processes in characteristics of substrate degradation and bacterial population levels. Water Science & Technology 23:1157-1166.

Zuo Z, Wu S, Zhang W, Dong R. 2013. Effects of organic loading rate and effluent recirculation on the performance of two-stage anaerobic digestion of vegetable waste. Bioresource technology 146:556-561.

APPENDIX B

Shortening the Startup of Thermophilic AD of Food Waste

D. Al-Sanioura[*], S. Ghanimeh[**], P. Saikaly[***], M. El-Fadel[*†]

[*]*Department of Civil and Environmental Engineering, American University of Beirut, Lebanon*
[**]*Notre Dame University - Louaize, Lebanon*
[***]*King Abdullah University of Science and Technology, Saudi Arabia*

[†]*Corresponding Author: mfadel@aub.edu.lb*

Abstract: Long startups are often considered a financial and technical burden in large scale anaerobic digestion of food waste; whereas exceedingly short startup strategies can be deemed too risky and may not establish a proper microbial community. In this paper, a biomass acclimation strategy that involved gradual and incremental increase of inoculum and loading rate over 20 weeks was contrasted with a faster 12-week startup. The substantial (30%) reduction in startup time was achieved through shortened (one-step) inoculation and sharp (step-wise) increase in OLR. The reactor with fast startup showed an operational stability comparable to the one with slower startup, and produced: average methane yield of 0.34 l.gVS^{-1}, Intermediate-to-Partial alkalinity ratio below 0.4, COD removal of 81% and VS removal of 79%. Furthermore, in order to check the effectiveness of the short startup in insuring stable steady-state performance, the reactor was operated and monitored for 15 additional weeks (= 3.5 HRT) at a steady loading rate of 2.1 gVS/L/d.

B. 1. INTRODUCTION

Anaerobic digestion is a naturally occurring process that is carried out in four stages: hydrolysis, acidogenesis, acetogenesis and finally the methanogenesis (Park et al. 2008). Each stage is accomplished by a distinct set of microorganisms with different growing rate and resilience to upsets (Mata-Álvarez 2003). Thus, during sensitive periods such the startup, the acid's production rate can be higher than the methanogens' consumption rate, leading to accumulation of VFAs thus inhibiting methanogenesis and reducing the quality of the organic matter destruction and the biogas production (Charles et al. 2009; Martín-González et al. 2013; Mata-Álvarez 2003).

In fact, startup in anaerobic digesters (AD) is a critical step for establishing an efficient microbial community that could ensure effective organic matter removal, rapid process stabilization and proper methane generation (Angelidaki et al. 2006). However, rapid

production and accumulation of volatile fatty acids (VFA) and a loss in the system's buffering capacity (Charles et al. 2009; Martín-González et al. 2013) can lead to technical difficulties and tediously long startups resulting in limited full scale application of thermophilic anaerobic digestion in the past (Griffin et al. 1998; Maroun and El Fadel 2007; Suwannoppadol et al. 2011). Nevertheless, the thermophilic anaerobic technology has been taking momentum slowly in the last few years due to its advantages with regard to mesophilic AD, namely: superior loading rate, higher biogas yield, enhanced hydrolysis, better organic matter removal, considerable pathogen reduction and resistance to foaming (Angelidaki et al. 2006; Palatsi et al. 2009).

Various startup strategies has been proposed. For instance, using a dry- thermophilic anaerobic CSTR to treat the OFMSW, Fdez-Güelfo et al. (2010) began feeding the digester from the first day of inoculation to reduce the time required for stabilization to 110 days. Griffin et al. (1998) also pursued an aggressive startup strategy to treat simulated OFMSW where they started a thermophilic AD with mesophilic anaerobic sewage sludge and cattle manure as inoculum, the feeding and wasting also started immediately after inoculation and the startup period only lasted 20 days (1 HRT), the digester was monitored for an additional 70 days after the startup.

In contrast, Angelidaki et al. (2006) has proposed an innovative concept targeting the gradual build-up of microbial competence by coordinating the daily loading to the amount of "activated biomass". The importance of maintaining a low food-to-microorganism ratio during startup has been reported and demonstrated (Angelidaki et al. 2006; Bolzonella et al. 2003). In this respect, gradual increase in loading that is proportional to the concomitant increase in biomass has been adopted by Angelidaki et al. 2006 resulting in a food-to-microorganism ratio of 1:10. The rationale behind this strategy is that daily feeding of the reactor in the early stages should be proportional to

the “activated biomass” volume and not the total volume of the liquor. This approach was tested through the slow incremental loading in reactor 1, which necessitated 20 weeks to reach the design OLR of 2.1 $gVS.l^{-1}.d^{-1}$. In comparison, with fast (step-wise) incremental loading in reactor 2, the same design load was reached in only 14 weeks resulting in 30% reduction in startup period (Figure 2). In order to test the impact of fast incremental loading on startup, the stability (in terms of pH) and performance (in terms of methane generation) of reactor 2 during startup was assessed and compared to slow (gradual) startup of reactor 1.

In this context, a biomass acclimation strategy similar to that of Angelidaki et al. (2006), involving a gradual and incremental increase of the inoculum and the OLR, was applied on reactor 1 and compared to a faster strategy where the loading rate in reactor 2 was instantaneously (step-wise) increased. In order to check the effectiveness of the shorter startup, reactor 2 was operated and monitored for 15 weeks (= 3.5 HRT), after startup, at a steady loading rate of 2.1 gVS/L/d.

B.1.1. MATERIAL AND METHODS

B.1.2. Experimental design

The experiment was conducted using two 9 l working volume, Bioflo 110, New Brunswick Scientific Co. anaerobic digesters (**Error! Reference source not found.**) running at 80 rpm under thermophilic conditions (55°C). The digesters were heated using thermostatically controlled electric heating jackets, and the pH was monitored using built-in probes and adjusted using peristaltic pumps connected to NaOH (5M) solution. Feeding was done by fed batch mode once a day, six times a week. The digesters were flushed with nitrogen gas and then thoroughly mixed for 3 minutes at 200 rpm after feeding to insure anaerobic conditions in the reactors and proper integration of the feed in the liquor.

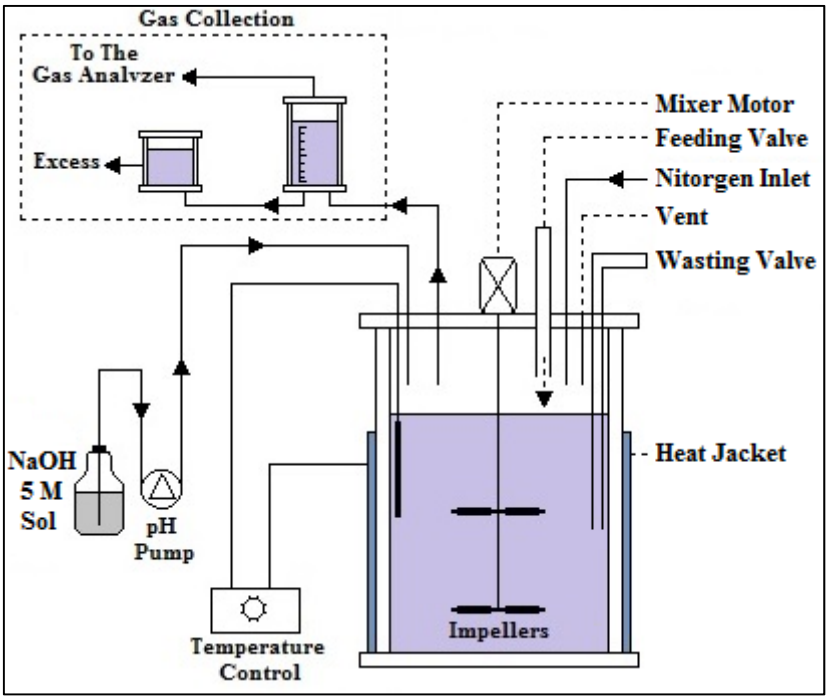


Figure B. 1 Schematic diagram of an individual digester

B.1.3. Substrate sources

In order to maintain a homogeneous feed, the waste was collected, ground, mixed and frozen at -20°C at the start of the experiment. The feed was prepared and frozen in bulk before the start of the experiment to ensure its stable characteristics; then thawed and diluted shortly prior to feeding. The substrate was prepared to mimic SS-OFMSW using raw fruit and vegetable market waste supplemented with meat residue.

B.1.4. Start-up procedure

Reactor 1 was filled with inoculum up to 30% of its working volume and was gradually filled with diluted FW and additional inoculum to reach full capacity, without wasting (except for sampling). The organic loading rate was gradually increased from 0.5gVS/L/d to 2.1gVS/L/d by adding 1 g of FW on a daily basis. On the other hand,

reactor 2 was inoculated with 4.5 liters of inoculum and directly filled with distilled water to full capacity (9L). A step-wise (fast) incremental loading rate was adopted whereby the initial OLR (0.5 gVS/L/d) was maintained for 3 weeks then doubled in 3 weeks to reach 1.1gVS/L/d. Similarly, the latter OLR was kept constant for 3 weeks than doubled in another 3 weeks to reach 2.1gVS/L/d. The impact of fast startup on system performance was assessed by running reactor 2 for 3.5 HRTs at steady-state conditions during which system stability, removal efficiency and methane production were determined.

B.1.5. Analysis

The biogas volume was monitored, on a daily basis, by the water displacement method and gas composition (methane and carbon dioxide) was determined using a dual wavelength infrared cell with reference channels (GEM-2000 monitor, Keison Products, UK). To monitor the solids content, total and volatile solids (TS and VS) were determined by drying at 110^{o}C and igniting at 540°C, respectively (APHA 2012). Soluble samples, for detection of volatile fatty acids, soluble COD and ammonia, were obtained by centrifuging digestate samples at 13000 rpm, then passing the supernatant through 1.2 μm pore-size filtersand 0.45 μm syringe filters. Alkalinity and total COD testing were performed on raw digestate samples according to Ripley et al. (1986) and the modifiedStandard Methods 5220D procedure (HACH HR and HR+ kits, HACH Company, Loveland, Colorado), respectively.

B. 2. RESULTS AND DISCUSSION

B.2.1. Slow vs. fast startup

B.2.1.1. Reactor 1 (slow startup)

The pH in reactor 1 (slow startup) was closely controlled, through addition of an alkaline solution (NaOH, 5M), to maintain a pH above 7.0 during the first few hours after feeding, when VFAs generation is at its max (**Error! Reference source not found.**– A). In contrast, in reactor 2 (fast startup), pH control was needed only during the first 7 weeks. It was self-sustaining for the rest of the run with a gradual increase in pH value to around 7.7 to 7.8 at steady state (OLR=2.1 $gVS.l^{-1}.d^{-1}$, Figure 2 – B).

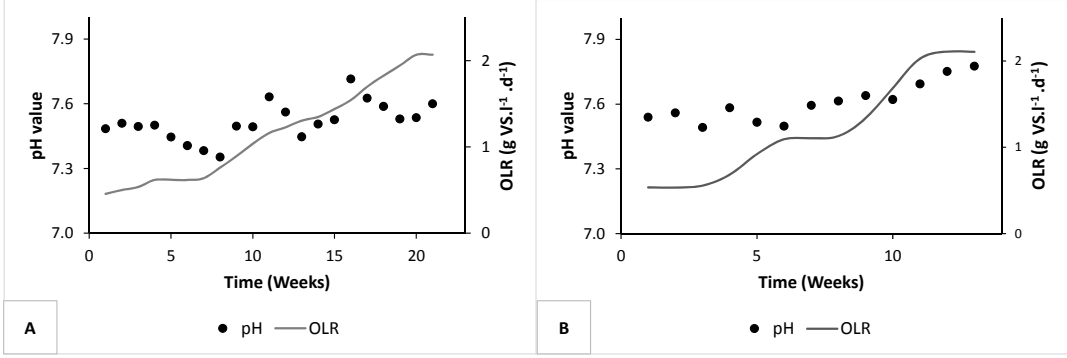


Figure B. 2 pH values for (A) slow stratup in reactor 1 and (B) fast startup in reactor 2 during startup

In reactor 1, the specific methane yield was 0.19$l.gVS^{-1}$, with a methane content of 23% and a CO_2/CH_4 ratio of 0.35, during the early stages of the startup (OLR ≤ 0.6 $gVS.l^{-1}.d^{-1}$).Methane generation increased continuously with the increasing loading rate to reach a specific yield of 0.3 $l.gVS^{-1}$, with an average CO_2/CH_4 ratio of 0.49, for OLR between 0.8 and 1.9 $gVS.l^{-1}.d^{-1}$(**Error! Reference source not found.** – A and B). On average, the methane content was stabilized at 45% starting the 12th week of operation, with an average CO_2/CH_4 ratio of 0.54 and an average specific biogas yield of 0.87 $l.gVS^{-1}$. During this period, the specific methane yield was around 0.33 $l.gVS^{-1}$, on average, reaching its highest value of 0.44 $l.gVS^{-1}$ at OLR = 2.1 $gVS.l^{-1}.d^{-1}$ during week 21.

B.2.1.2. Reactor 2 (Fast startup)

The average biogas production in digester 2 was similar to that of digester 1 (about 0.5 $l.l^{-1}.d^{-1}$) throughout the startup period. In comparison, the specific methane yield was comparable in both digesters (about 0.2 $l.gVS^{-1}$) only during the first 3-7 weeks of the experiment (OLR < 0.6 $gVS.l^{-1}.d^{-1}$). Afterwards, the rapid increase of OLR in digester 2 (up to OLR = 2 $gVS.l^{-1}$) seemed to affect the methane content (average of 35% in reactor 2 versus 40% in reactor 1) (**Error! Reference source not found.**) indicating that methanogenic growth was not fast enough to cope with the increase in substrate level. Concomitantly, ammonia accumulation was observed with a steady increase from 0.6 $g.l^{-1}$ (week 7) to reach the near threshold value of 0.96 $g.l^{-1}$ (week 11) (**Error! Reference source not found.** – D), which might have caused a slight inhibition effect and reduced the methane producing potential (Fotidis et al. 2013a). While the IA/PA average level doubled from 0.11 (week 2 to 6, average OLR = 0.76 $gVS.l^{-1}.d^{-1}$) to 0.22 (week 7 to 11, average OLR = 1.46 $gVS.l^{-1}.d^{-1}$) (**Error! Reference source not found.**) suggesting a parallel increase in free VFA concentrations, which may have caused inhibition in the hydrolysis/acidogenesis stages (Fotidis et al. 2013b). This inhibition phenomenon was also observed during the start of this incremental OLR period with a drop in methane yield to below 0.2 $l.gVS^{-1}$ in both reactors and a decrease in soluble COD (SCOD) in reactor 2. The decrease in the readily available organic matter produced by substrate hydrolysis (i.e.: SCOD) (Eastman and Ferguson 1981; Hartmann and Ahring 2005) could indicate an overloaded hydrolysis (**Error! Reference source not found.** – A, **Error! Reference source not found.** – B). Nonetheless, reactor 2 seemed to recover by the start of the two weeks of OLR = 2.1 $gVS.l^{-1}$ with seemingly higher but stable ammonia concentrations. At the same time, methane content and specific yield increased and stabilized from 33±8% and 0.24±0.05 $l.gVS^{-1}$ during

startup, to 46±1% and 0.27±0.01 l.gVS^{-1} during the first two weeks of the steady OLR, respectively.

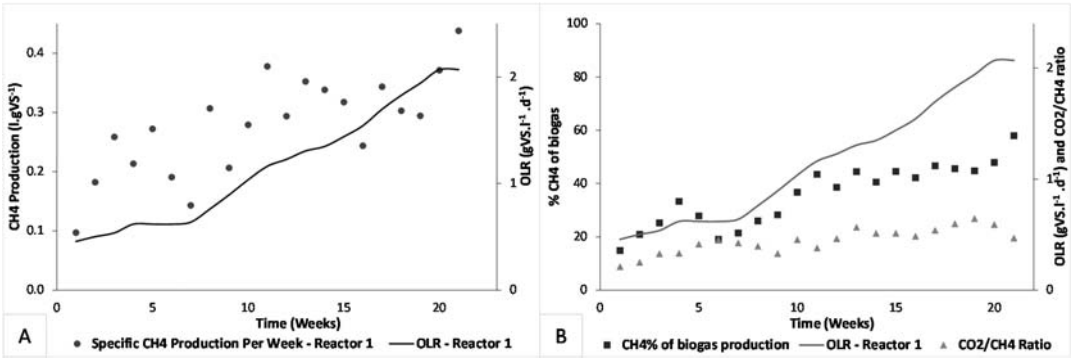


Figure B. 3 Methane generation in reactor 1: (A) specific CH4 yield; (B) CH4 content and CO2/CH4 ratio

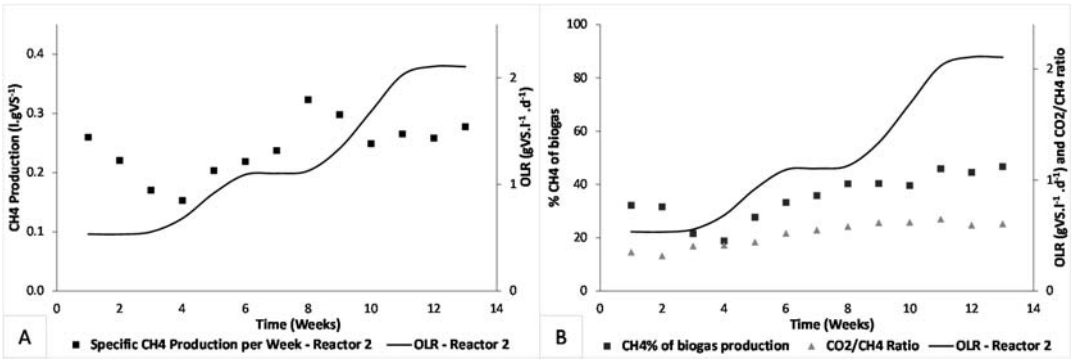


Figure B. 4 Methane generation in Reactor 2 during the startup phase: (A) specific CH4 yield; (B) CH4 content and CO2/CH4 ratio

Table B. 1 Biogas production and composition in reactor 1 and 2 at different OLR (Average ±

Standard Deviation)

OLR $gVS.l^{-1}.d^{-1}$	Week number	Biogas Production ($l.l^{-1}.d^{-1}$)	CH4 content in biogas (%)	Specific CH4 Yield ($l.gVS^{-1}$)
Reactor 1				
≤0.6	1-7	0.49±0.06	23±6	0.19±0.06
0.6 to 2.0	8-19	1.02±0.21	40±6	0.30±0.05
2.1 (First two weeks)	20-21	1.69±0.05	53±5	0.40±0.03
Reactor 2				
≤0.6	1-3	0.48±0.03	28±5	0.22±0.04
0.6 to 2.0	4-11	0.94±0.24	35±8	0.24±0.05
2.1 (First two weeks)	12-13	1.53±0.14	46±1	0.27±0.01
2.1 (Steady state)	14-25	1.61±0.25	47±1	0.34±0.02

B.2.1.3. Startup analysis

The overall methane content in both startups was low (<45%), signaling that the loading rate increase was disturbing for both digesters; coupled with ammonia-N levels below $1.2g.l^{-1}$, this implies that hydrogenotrophic methanogenesis was limited.(Fotidis et al. 2013a). In fact, non-acclimated aceticlastic methanogens thrive more in environments with lower ammonia-N concentration ($<2g.l^{-1}$) than hydrogenotrophic methanogens ((Fotidis et al. 2013a; Karakashev et al. 2006; Yenigün and Demirel 2013). In addition, Amani et al. (2010) concluded that during periods of increased loading, hydrogenotrophs, which are known to have slower growth rates than aceticlastic methanogens (Yenigün and Demirel 2013), are more likely to get overwhelmed with increased amounts of hydrogen produced from hydrolysis. Therefore a longer and slower startup period is not a guarantee for a less disturbed process.

B.2.2. Stability of reactor 2 (Fast startup)

At the end of the fast startup, the loading rate of reactor 2 was stabilized at 2.1 $gVS.l^{-1}.d^{-1}$ and steady-state performance was assessed over a period of 3.5 HRTs. Despite the

fact that specific methane yield was lower than reactor 1, it was close to reported values for reactors treating similar waste under thermophilic temperatures: 0.37 $l.gVS^{-1}$ (Browne et al. 2014), 0.35 $l.gVS^{-1}$ (Gómez et al. 2010), 0.33 $l.gVS^{-1}$(Ghanimeh et al. 2012) and 0.32 $l.gVS^{-1}$(Angelidaki et al. 2006).

Partial alkalinity started off at 2798 mg $CaCO_3.l^{-1}$, then increased to an average of 4845 $mg.l^{-1}$ of $CaCO_3$ between week 2 and 9, showing reasonable stability and buffering capacity during the first OLR increase (**Error! Reference source not found.** – B), this is concurring with the low intermediate-to-partial alkalinity ratio (IA/PA) which averaged 0.14 during that period; well below the recommended 0.4 threshold for stable anaerobic digesters (Ripley et al. 1986). The intermediate alkalinity, which is the difference between total and partial alkalinity, is representative of the VFA's level and IA/PA is often used as an indication of VFAs to buffering capacity of the system (Martín-González et al. 2013). Following the second increase of loading rate, between week 11 and 14 (to OLR = 2 g $VS.l^{-1}.d^{-1}$), IA/PA ratio increased to 0.3, despite still being in the recommended range, this increase is indicative of a rise in free VFAs in the digester. VFAs are known to be inhibitors of the methanogenic bacterial community (Garcia-Peña et al. 2011), thus their increase upon reaching the OLR of 2 g $VS.l^{-1}.d^{-1}$could be the cause of the slight dip in the specific methane generation.

Despite these fluctuation, partial alkalinity remained within or above the recommended range for stable digesters treating similar type of waste (2000-4000 $mg.l^{-1}$ of $CaCO_3$) (Bouallagui et al. 2009; Velmurugan and Ramanujam 2011). According to Bouallagui et al. (2009), high partial alkalinity levels could be caused by high amounts of protein in the feed which lead to an increase in NH_4^+. In fact, the feed used in this experiment has low C:N ratio of 11 and the high amount of protein could be the cause of high partial alkalinity and ammonia-N levels. The latter remained above 0.6 $g.l^{-1}$and averaged 0.8

g.l^{-1} during the startup period and 1.3 g.l^{-1}during steady-state operation; thus, reaching borderline reported ammonia inhibition thresholds of 0.6, 1.0 and 1.2 g.l^{-1}(Hartmann and Ahring 2005; Mata-Álvarez 2003; Yenigün and Demirel 2013) (**Error! Reference source not found.** – D). Usually, high alkalinity concentrations coupled with the high pH range (7.5 – 7.9) (**Error! Reference source not found.**) and inhibitory ammonia levels can cause toxicity conditions and hamper the bacterial community (Yenigün and Demirel 2013).Yet, the reactor seemed to tolerate these circumstances and remain stable and efficient, evident by adequate specific methane generation during steady-state (0.34 l.gVS^{-1}) and high total COD removal rates of 81%(**Error! Reference source not found.Error! Reference source not found.** – C, **Error! Reference source not found.** –A).

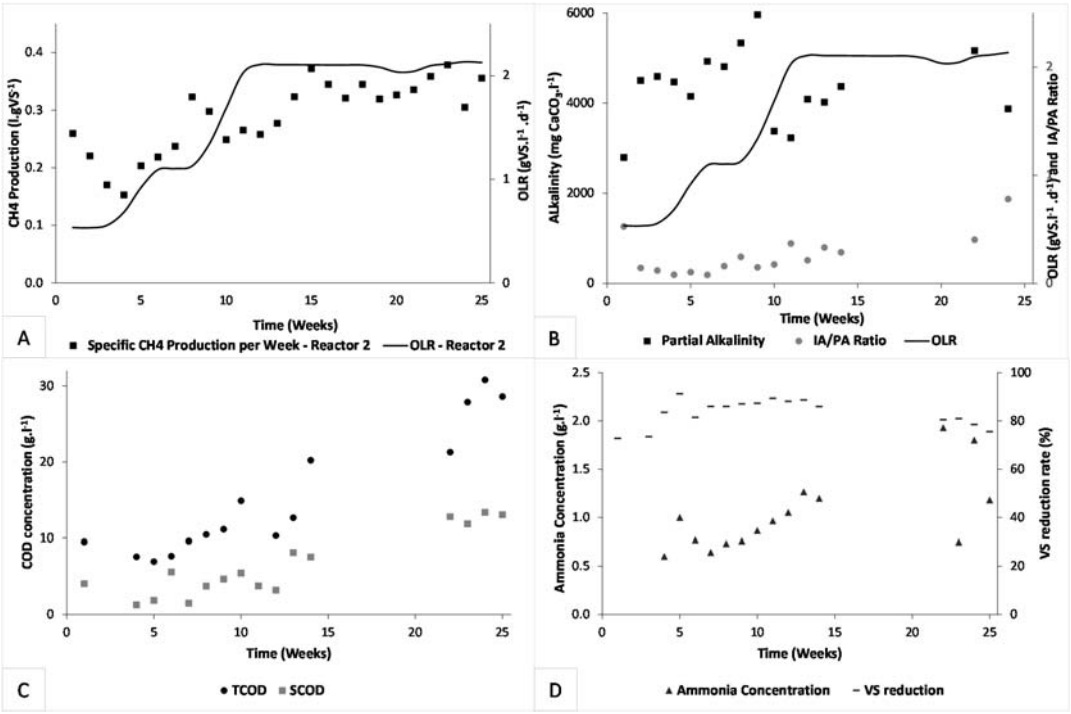


Figure B. 5 Parameters for reactor 2: (A) Specific methane production, (B) partial alkalinity concentration and intermediate-to-partial alkalinity ratio, (C) COD concentrations, (D) ammonia and volatile solid reduction (D).

The VS removal rate was stable at about 87 % during the incremental OLR increase of the startup period, showing proper hydrolysis of the organic material, and decreased during steady operation to an average of 79 %, showing slower hydrolysis of more recalcitrant organic matter (**Error! Reference source not found.**). On average, the VS removal rate was 79% during steady state, which is comparable or higher to other reported values in the literature: 70% for a pilot scale thermophilic anaerobic digester treating food waste (Banks and Stringfellow 2008), 81% for a CSTR treating canteen food under mesophilic temperatures (Browne et al. 2014), 54% for an aggressive startup of a thermophilic digester (Griffin et al. 1998), and 73% for the co-digestion of Fruit and vegetable waste mixed equally with meat residue (Garcia-Peña et al. 2011).

Table B. 2 Volatile solid removal throughout different OLR stages in reactor 2

OLR	Average % VS removal
OLR≤ 0.6	73
0.6<OLR<1.1	87
OLR=1.1	84
1.1<OLR≤2.11	88
OLR=2.1	79

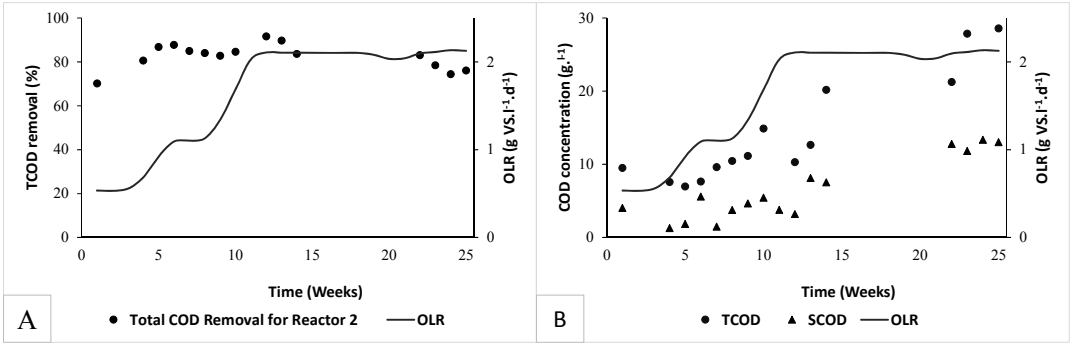


Figure B. 6 Total COD removal for reactor 2 (A), total and soluble COD concentrations (B)

B. 3. CONCLUSION

The fast startup strategy showed a 30% decrease in startup period with about 15 % reduction in final methane generation (0.4 l. gVS^{-1} in reactor 1); yet the resulting average specific methane yield during steady state (0.34 l. gVS^{-1}) was within the reported range for digesters treating a similar type of waste and under similar operating conditions. Overall the reactor with fast startup exhibited efficient organic matter removal with adequate VS and TCOD removal, good system stability with high alkalinity concentration and an IA/PA ratio within recommended values. In addition, it was able to withstand borderline ammonia concentration levels. The faster strategy proved to be effective in providing a stable system over a steady-state period equivalent to 3.5 HRT. It can be concluded that the startup time can be reduced by adopting a strategy consisting of (1) immediate filing of the digester with immediate initiation of the wasting/feeding process, and (2) adopting a step-wise loading consisting of doubling the loading rate at each step and allow for a subsequent short period (~ ¾ HRT) of stabilization.

B. 4. REFERENCES

Amani T, Nosrati M, Sreekrishnan TR. 2010. Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects-a review. Environmental Reviews 18:255-278.

APHA. 2012. Standard methods for the examination of water and wastewater.

Banks CJ, Stringfellow A. 2008. A pilot-scale trial comparing mesophilic and thermophilic digestion for the stabilisation of source segregated kitchen waste.

Bolzonella D, Innocenti L, Pavan P, Traverso P, Cecchi F. 2003. Semi-dry thermophilic anaerobic digestion of the organic fraction of municipal solid waste: Focusing on the start-up phase. Bioresource technology 86:123-129.

Bouallagui H, Lahdheb H, Ben Romdan E, Rachdi B, Hamdi M. 2009. Improvement of fruit and vegetable waste anaerobic digestion performance and stability with co-substrates addition. Journal of environmental management 90:1844-1849.

Browne JD, Allen E, Murphy JD. 2014. Assessing biomethane production from the organic fraction of municipal solid waste in batch and continuous operation. Biomethane production from food waste and organic residues:157.

Charles W, Walker L, Cord-Ruwisch R. 2009. Effect of pre-aeration and inoculum on the start-up of batch thermophilic anaerobic digestion of municipal solid waste. Bioresource technology 100:2329-2335.

Eastman JA, Ferguson JF. 1981. Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. Journal (Water Pollution Control Federation):352-366.

Fdez-Güelfo LA, Alvarez-Gallego C, Sales Márquez D, Romero García LI. 2010. Start-up of thermophilic–dry anaerobic digestion of ofmsw using adapted modified sebac inoculum. Bioresource technology 101:9031-9039.

Fotidis IA, Karakashev D, Angelidaki I. 2013a. The dominant acetate degradation pathway/methanogenic composition in full-scale anaerobic digesters operating under different ammonia levels. International Journal of Environmental Science and Technology:1-8.

Fotidis IA, Karakashev D, Kotsopoulos TA, Martzopoulos GG, Angelidaki I. 2013b. Effect of ammonium and acetate on methanogenic pathway and methanogenic community composition. FEMS microbiology ecology 83:38-48.

Garcia-Peña EI, Parameswaran P, Kang DW, Canul-Chan M, Krajmalnik-Brown R. 2011. Anaerobic digestion and co-digestion processes of vegetable and fruit residues: Process and microbial ecology. Bioresource technology 102:9447-9455.

Hartmann H, Ahring BK. 2005. Anaerobic digestion of the organic fraction of municipal solid waste: Influence of co-digestion with manure. Water Research 39.

Karakashev D, Batstone DJ, Trably E, Angelidaki I. 2006. Acetate oxidation is the dominant methanogenic pathway from acetate in the absence of methanosaetaceae. Applied and Environmental Microbiology 72:5138-5141.

Maroun R, El Fadel M. 2007. Start-up of anaerobic digestion of source-sorted organic municipal solid waste in the absence of classical inocula. Environmental science & technology 41:6808-6814.

Mata-Álvarez J. 2003. Biomethanization of the organic fraction of municipal solid wastes:IWA publishing.

Park Y, Hong F, Cheon J, Hidaka T, Tsuno H. 2008. Comparison of thermophilic anaerobic digestion characteristics between single-phase and two-phase systems for kitchen garbage treatment. Journal of bioscience and bioengineering 105:48-54.

Ripley LE, Boyle WC, Converse JC. 1986. Improved alkalimetric monitoring for anaerobic digestion of high-strength wastes. Journal (Water Pollution Control Federation):406-411.

Suwannoppadol S, Ho G, Cord-Ruwisch R. 2011. Rapid start-up of thermophilic anaerobic digestion with the turf fraction of msw as inoculum. Bioresource technology 102:7762-7767.

Velmurugan B, Ramanujam RA. 2011. Anaerobic digestion of vegetable wastes for biogas production in a fed-batch reactor. IntJEmergSci 1:478-486.

Yenigün O, Demirel B. 2013. Ammonia inhibition in anaerobic digestion: A review. Process Biochemistry 48:901-911.