Anaerobic Co-digestion of Organic Fraction of Municipal Solid Waste and Municipal Sludge With and Without Microwave Pre-treatment

EFATH ARA

A thesis submitted under the supervision of

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In partial fulfillment of the requirements for the degree of
Master of Applied Sciences in Environmental Engineering

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Abstract

Anaerobic co-digestion of organic fraction of municipal solid waste (OFMSW), with thickened waste activated sludge (TWAS) and primary sludge (PS) has the potential to enhance (biodegradation) of solid waste, increase longevity of existing landfills and lead to more sustainable development by improving waste to energy production. This study reports on mesophilic batch anaerobic biological methane potential (BMP) assays carried out with different concentrations and combinations (ratios) of OFMSW, TWAS (microwave (MW) pre-treated and untreated) and PS to assess digester stability and potential improved specific biodegradability and potential increased specific biogas production by digestion of OFMSW with PS and TWAS in various tri-substrate mixtures. Results indicated improvements in specific biogas production with concomitant improvements in COD and volatile solid (VS) removal for co-digestion of OMSW, TWAS and PS vs. controls. In terms of improvements in biogas production and digester stability the OFMSW:TWAS:PS:50:25:25 ratio with or without TWAS MW treatment was deemed best for further continuous digester studies. At a 15d HRT which is the regulatory policy in the province of Ontario for municipal mesophilic anaerobic TWAS:PS treatment, co-digestion of OFMSW:TWAS:PS, and OFMSW:$TWAS_{MW}$:PS resulted in a 1.38 and 1.46 fold relative improvement in biogas production and concomitant waste stabilization when compared to TWAS:PS and $TWAS_{MW}$:PS digestion at the same HRT and volumetric VS loading rate respectively. Treatment of OFMSW with PS and TWAS provides beneficial effects that could be exploited at MWWTP that are being operated at loading rates less than design capacity.

Acknowledgements

It is my pleasure to say thank you to my supervisor Dr. Kevin Kennedy for his guidance, patience and financial support. I do believe the word “thank you” is way much lighter to go against the love, guide, support and knowledge I received from Dr. Kennedy throughout my entire M.A.Sc. It was amazing, marvelous and wonderful, the time that I spent with him in the lab and in the office discussing about the research. I used to say to my other fellow colleagues who saw me working hard in the lab that all my hard works are just a way to show gratefulness to my supervisor.

I would like to extend a special thanks to lab technician Christine Séguin, and Erica Kennedy and Dean Kennedy for their all help and support in the lab. I do believe I would not have been able to finish my experiment on time without Christine’s help and support to get all the materials and machine ready.

Special and cordial thanks to all of my friends and colleagues both from University of Ottawa and Carleton University for being lovely to me when I need them most. Among them I like to mention the name of Anna Crolla, Bradly Young, Haleh Shahriari, Rania Alqaralleh and Nuno Coelho specially. I truly appreciate your help guys.

Last but not least, I would like to extend my loving thanks to my family and friends for their constant encouragement. My deepest gratitude goes to my beloved Shamiur Rahman for his support, patience, and encouragement throughout the time we are together. Also I definitely would like to convey respect and honor to my mentors, Mahajatak Sahid al Bukhari and Madam Nahar al Bukhari for their all love and wishes and thoughts of the right way of life. And special thank my parents, Daud Ahmed and Mahbuba Ahmed, sister Rabeya Rushi, And I believe without the love and support of these people I likely would not have been able to accomplish what I set out today.

Dedicated to

All the members of the Quantum Family

Nomenclature

BOD	Biological Oxygen Demand, mg O_2/L
$CaCO_3$	Calcium Carbonate, dimensionless
COD	Chemical Oxygen Demand, mg O_2/L
C:N	Carbon – to – Nitrogen Ratio, dimensionless
F/M	Food-to-Microorganism Ratio (g $VS_{substrate}$/g $VSS_{inocumul}$), dimensionless
HRT	Hydraulic Retention Time, d
IA	Intermediate Alkalinity, mg $CaCO_3$/L
$KHCO_3$	Potassium by Carbonate, dimensionless
$NaHCO_3$	Sodium by Carbonate, dimensionless
OLR	Organic loading rate, g Feed/L/d
PA	Partial Alkalinity, mg CaCO3/L
pH	Concentration of Hydrogen Ions (H^+) in a Solution, dimensionless
sCOD	Soluble Chemical Oxygen Demand, mg O_2/L
SRT	Solids Retention Time, d
TA	Total Alkalinity, mg CaCO3/L
TS	Total Solids, % mass
V	Volume, mL or L
VFA	Volatile Fatty Acids, mg/L
VS	Volatile Solids, % mass
wt	Weight, mg, g or kg

List of Abbreviations

AD	Anaerobic Digestion
BMP	Biochemical Methane Potential
CBP	Cumulative Biogas Production
CEB	Calculated Expected Biogas
CH_4	Methane
CO	Carbon monoxide
CO_2	Carbon dioxide
CSBY	Cumulative Specific Biogas Yield
D	Days
DBP	Daily Biogas Production
EPA	Environmental Protection Agency
EPS	Exocellular Polymeric Substances
FVFMSW	Fruit & Vegetable Fraction of Municipal Solid waste
FOG	Fat Oil and Grease
IEWS	Integrated Environmental Waste Services
IF_t	Improvement Factor for Tri-digestion
IF_p	Improvement Factor for Pre-treatment
ISWM	Integrated Solid Waste Management
GC	Gas Chromatograph
MSW	Municipal Solid Waste
MWWTP	Municipal Waste Water Treatment Plant
MW	Micro Wave
OFMSW	Organic Fraction of Municipal Solid Waste
ORWC	Ontario Rural Wastewater Centre
PS	Primary Sludge
TPAD	Temperature Phased Anaerobic Digestion
tpy	ton per year

RPM	Revolutions per Minute
ROPEC	Robert O. Pickard Environmental Center
SS	Steady State
SRR	Stability Ratio of a Reactor
TWAS	Thickened Waste Activated Sludge
$TWAS_{MW}$	Micro waved Thickened Waste Activated Sludge
$TWAS_{ultimate}$	Ultimately Solubilized Thickened Waste Activated Sludge
UK	United Kingdom
USEPA	United States Environmental Protection Agency
WAS	Waste Activated Sludge
WTE	Waste to Energy

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Chapter 1- Introduction

1.1 Back Ground

The annual production of municipal solid waste (MSW) (EPA, 2011) continues to grow while the disposal capacity via traditional land filling is diminishing (Steuteville, 1995) relative to the growth, and other forms of management such as incineration have not been accepted in North America, concomitantly alternative strategies are required to manage MSW in a sustainable and economic manner. Anaerobic biological treatment of the organic fraction of municipal solid (OFMSW) can be an acceptable alternative to current disposal strategies as it reduces the volume of OFMSW, stabilizes OFMSW, produces a residue that can be used for soil conditioning, and recovers energy from OFMSW in the form of methane gas (Tchobanoglous, et al., 1993).

However one of the problems most frequently found during biological processing of OFMSW is the high C: N ratio of these residues (Bujoczek, et al., 2002). To circumvent this problem, several researchers have proposed co-digestion of OFMSW, either with sewage sludge from Municipal Wastewater Treatment Plants (MWWTPs) or residues from livestock farms (Sosnowski, et al., 2003). Therefore, the addition of sewage sludge to OFMSW digestion processes provides the nitrogen, as well as other macro and micro nutrients that are not present at sufficient levels in OFMSW alone. Consequently, co-digestion of OFMSW and municipal sludge may be an attractive alternative for the management of two separate waste streams that are produced in every community (Ahring, 1995). The feasibility of digesting nutrient-deficient OFMSW can be improved through the addition of bio-solids, such as primary sludge (PS) (Schmit, et al., 2001) and/or waste activated sludge (WAS) (Gomez, et al., 2005). While the knowledge base for anaerobic co-digestion has significantly expanded; more research is needed on the effects of various compositions of co-substrates and their influence on anaerobic digestion (AD) process performance and stability (Stroot, et al., 2001). In North America, this option was initially examined in a study evaluating the technical feasibility of the anaerobic co digestion process for typical solid wastes (Hamzawi, et al., 1998). Gomez, et al., (2005) took the process one step farther and studied co-fermentation of readily degradable PS and the fruit and vegetable fraction of MSW (FVFMSW) in standalone anaerobic reactors and the effect of mixing conditions and different organic loading rates on AD system's performance and biogas production. Improvements in specific biogas production and biogas yield due to co-digestion of fruit and vegetable fraction of MSW with primary sludge was reported. However, the impact of combining OFMSW with secondary sludge or combined primary and secondary sludge was not addressed.

Hydrolysis is the rate limiting step for conventional AD of solid wastes and particularly limiting for secondary municipal sludge due to the cell structure of microbial biomass and the extra-cellular polymeric substances that maintain the floc matrix of WAS that resists hydrolysis (Baier and Schmidheiny, 1997). Schmit, et al., (2001) summarized that higher net methane yield and biodegradable volatile solids (VS) reduction were achieved at a faster degradation rate when thickened WAS (TWAS) was co-digested with OFMSW vs. as a single substrate. However, Eskicioglu, et al., (2007a, 2007b) reported enhancement of biodegradability and increase in biogas production due to microwave (MW) pre-treatment of WAS/TWAS.

1.2 Purpose

In existing municipal wastewater treatment plants the solids side of the plant is usually tasked with stabilization of an approximately equal mix of readily degradable PS and more difficult to degrade secondary sludge (WAS/TWAS). In the majority of conventional MWWTP stabilization is carried out by mesophilic AD. In a number of MWWTP that are undergoing capital projects expanding AD the design load of AD facilities for treatment of PS and secondary sludge is not met until the design life of the plant (i.e. approximately 20-25 years). As such the AD stabilization facility is operated at under capacity conditions until near the end of its design life. While this condition results in a stabilized end product, opportunities are missed to maximize energy production from the facility by operating at design capacity. Integrating conventional PS/secondary sludge digestion at MWWTP with co-digestion of OFMSW has a number of potentially positive outcomes for the AD plant: (1) increased stability of the AD process (2) increased biogas production for energy under the Feed in Tariff rates (3) increased specific biogas yields, (4) alternative management plan for OFMSW and (5) reduced use of landfill space. Co-digestion of OFMSW at under loaded PS/secondary sludge digesters at MWWTP can be accomplished via a time sliding diminishing supply contract between the local MWWTP and suppliers of OFMSW. Supply of OFMSW will diminish proportionally with increased PS/secondary sludge generation as the community serviced by the MWWTP grows.

Figure 1-1 shows the simulation of possible mixing combination of OFMSW and TWAS/PS in a real MWWTP. At the beginning when plants are operated under capacity the proportion of OFMSW digested could be higher (for example 90%) and as time goes by and the plants reach their ultimate design capacity the OFMSW fraction decreases in the co-digestion mixture. Additionally, an added benefit of tri-digestion of OFMSW with PS/WAS or TWAS is that OFMSW has a high solids concentration so only small volumes need to be mixed into the PS/WAS or TWAS sludge mix. This means that organic loading rates (OLRs) can be maintained without negatively impacting the AD process hydraulic retention time RT which is set by regulation at 15d by the Ontario, Ministry of the Environment.

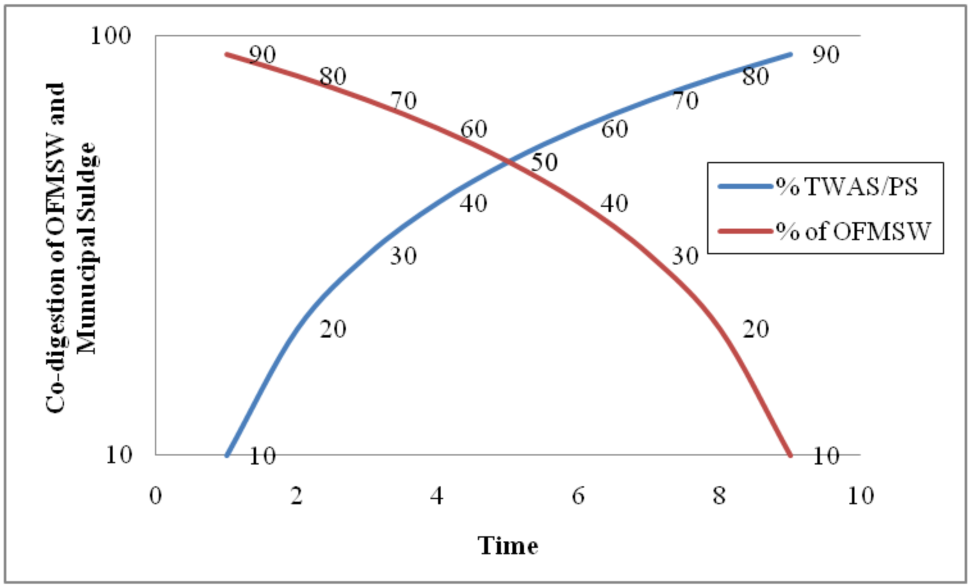


Figure 1-1 A time sliding diminishing supply of OFMSW in AD co-digestion with TWAS/PS in MWWTP

1.3 Objectives

This study evaluates the potential for mesophilic anaerobic co-digestion of OFMSW with PS and/or TWAS. The experimental work is done in two phases divided into batch AD biological methane potential (BMP) assays followed by semi-continuous reactor studies. Objectives of the batch tests are as follows:

1. BMP assays conducted for co-digestion of OFMSW, with TWAS, PS and TWAS plus PS for the evaluation of batch reactor efficiency in terms of biogas production, total solid (TS), VS and total chemical oxygen demand (TCOD) removal.
2. MW pre-treatment of TWAS ($TWAS_{MW}$) to determine the effect of temperature and MW holding time (HT) on solubilization of $TWAS_{MW}$ which will be used as a co-substrate with OFMSW and PS.

3. BMP assays (second set) to evaluate the effect of $TWAS_{MW}$ and HTs on tri-digestion of OFMSW with $TWAS_{MW}$ and $TWAS_{MW}$ plus PS at different substrate mixing ratios on batch reactor efficiency in terms of biogas production, TS, VS and TCOD removal.

The main objective of the semi-continuous flow anaerobic reactor tests (based on the best conditions from the batch BMP study) was to show the potential improvements in mesophilic anaerobic reactor performance while of co-digestion of OFMSW:TWAS:PS and OFMSW:$TWAS_{MW}$:PS compared to PS:TWAS and PS:$TWAS_{MW}$ at various organic loading rates and hydraulic retention times at practical range of conditions that may be possible at a conventional secondary MWWTP with digestion facilities. Again reactor efficiency will be evaluated in terms of biogas production, TS, VS and TCOD removal.

1.4 Thesis Layout

This thesis is divided into 5 chapters, followed by appendices. Chapter 1, the Introduction, is followed by a Literature Review in Chapter 2. Chapter 3 summarizes the Materials and Methods employed for the presented research, detailing the set up and operation of BMP assays and semi-continuous reactors. It also describes the analytical methods used for sample analysis. Research Results are summarized and discussed in Chapter 4, which is followed by Conclusions and Recommendations for Future Work in Chapter 5.

Chapter 2- Literature Review

2.1 Terminologies

2.1.1 Municipal Solid Waste

Unwanted or discarded material with insufficient liquid content to be free flowing is frequently called solid waste or refuse. Waste arising from residential households and apartment buildings, commercial and institutional establishments, construction and demolition waste, municipal services, and treatment plants (sludge) are considered as MSW (Tchobanoglous, et al., 1993).

2.1.2 OFMSW

OFMSW stands for organic fraction of municipal solid waste and is usually defined as waste containing carbon compounds; derived from animal and plant materials. Typical organic components of MSW are food wastes, paper, cardboard, plastics, textiles, rubber, leather, yard wastes, wood etc. (Tchobanoglous, et al., 1993)

2.1.3 Municipal Sludge

Solid, semisolid or liquid waste generated from a municipal or industrial wastewater treatment plant, water treatment plant, or air pollution control facilities are called sludge which is mostly handled in solid waste management systems. In North America 21 kg (47 lb) (approximately) of dry sewage sludge per person is generated annually (Metcaff, et al., 2003). The characteristics of sludge is determined by analytical measurements of TS, VS, pH, nutrients, organic matter, pathogens, metals, organic chemicals and hazardous pollutants.

Figure 2-1 shows a typical flow diagram for a MWWTP. Sludge (residuals) generated from primary, secondary and tertiary treatment are known as primary, secondary and tertiary sludge which needs to be treated before disposal. Sludge treatment and disposal constitute approximately 50% of the operating costs.

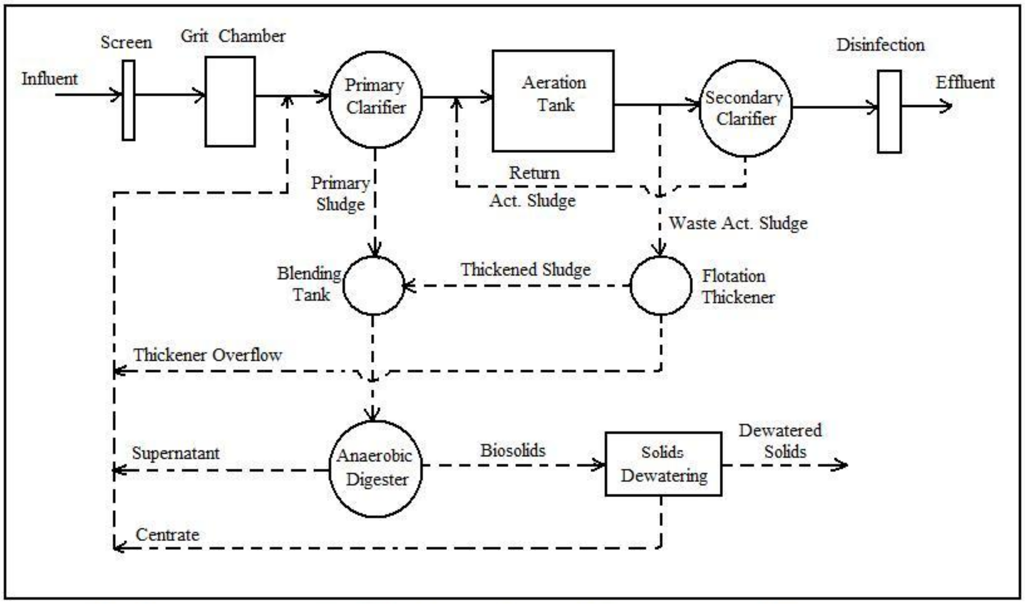


Figure 2-1 Flow diagram of a typical MWWTP (Metcaff, et al., 2003)

2.1.3.1 Primary Sludge

Residue from primary settling tank of a MWWTP is known as primary sludge which contains from 3 to 7% solids and of that is approximately 60-80% organic. PS solids are usually gray in color, slimy, fairly coarse, and with highly obnoxious odor. PS is readily digested under suitable anaerobic conditions. (Metcaff, et al., 2003)

2.1.3.2 Secondary Sludge

Sludge from secondary settling tanks of a MWWTP has commonly a brownish, flocculent appearance and an earthy odor. Secondary sludge consists of mainly microorganisms enmeshed within a matrix of extracellular polymeric material (EPS) (75-90% organic) and inert materials. WAS contains and trickling filter sludge contains 0.5-2%, and 2-5% solids, respectively. The microbial cell and extra-cellular polymeric substances maintains a floc matrix composition that makes it more difficult to degrade than PS. WAS is usually thickened before treatment and disposal. Sludge thickening is used to remove water and increase the solids content. If waste activated sludge with 0.6% solids is thickened to a content of 3% solids, a fivefold decrease in sludge volume can be achieved. Sludge thickening mainly involves physical processes such as gravity settling, flotation, centrifugation or gravity belts. After thickening to (3-6 % TS) WAS is normally called TWAS.

2.2 Sources, Composition and Properties of MSW

2.2.1 Sources

Sources of solid wastes in a community are related to the land use and zoning. Based on typical waste generation facilities, activities and locations associated with each sources can be classified into following categories (Tchobanoglous, et al., 1993)

- Residential
- Commercial
- Institutional
- Construction and demolish
- Municipal services

- Treatment plant sites
- Industrial and
- Agricultural

2.2.2 Composition

Composition is the term used to describe the individual components that make up a solid waste stream and their relative distribution, usually based on percent by weight. (Tchobanoglous, et al., 1993). Table 2-1 shows component distribution of MSW in percentage by weight of a typical community and Table 2-2 illustrates composition of residential MSW based on economical stability of a society; both of the table are adopted from (Tchobanoglous, et al., 1993).Composition of MSW depends on many factors including regional and seasonal variation, population density, income of the average people, culture climate, extents of waste diversion, the existing legislation and frequency of collection of waste.

Table 2-1 Distribution of typical components of MSW in a community (Adapted from Tchobanoglous, et al., 1993)

Waste category	**Percent by weight**
Residential and Commercial, excluding special and hazardous wastes	50-75
Hazardous	0.01-1
Institutional	3-5
Construction and demolition	8-20
Municipal services	6-15
Treatment plants sludge	3-8

Table 2-2 Distribution of components in residential MSW (Adapted from Tchobanoglous, et al., 1993)

Components	Low-income countries	Middle-income countries	Upper-income countries
Organics			
Food wastes	40-85	20-65	6-30
Paper/Cardboard	1-10	8-30	20-45
Plastics	1-5	2-6	2-8
Textiles	1-5	2-10	2-6
Rubber/Leather	1-5	1-4	0-2
Yard wastes/ Wood	1-6	1-10	11-25
Misc. Organics	-		
Inorganic			
Glass	1-10	1-10	4-12
Tin cans/Aluminums/Other metals	1-5	1-5	3-15
Dirt, ash etc.	1-40	1-30	0-10

2.3 Physical, Chemical and Biological Properties of MSW

2.3.1 Physical Properties of MSW

Important physical characteristics of MSW include specific weight, moisture content, particle size and size distribution, field capacity and compacted waste porosity. (Tchobanoglous, et al., 1993)

2.3.2 Chemical Properties of MSW

Chemical properties are known as

- Proximate analysis
- Fusing point of ash
- Ultimate analysis
- Energy content

Information of chemical properties of the component of MSW is very important considering the energy recovery from that waste. (Tchobanoglous, et al., 1993)

2.3.3 Biological Properties of MSW

Excluding plastic, rubber and leather components, organic fraction of most MSW can be classified as follows: (Tchobanoglous, et al., 1993)

- Water-soluble constituents, such as sugars, starches, amino acids and various organic acids
- Hemicelluloses, a condensation product of five carbon sugars
- Cellulose, a condensation product of six-carbon sugar glucose
- Fats, oils and waxes, which are esters of alcohols and long –chain fatty acids
- Lignin, a polymeric material containing aromatic rings with methoxyl groups, the exact chemical nature of which is still not know
- Lignocelluloses, a combination of lignin and cellulose
- Proteins, which are composed of chains of amino acids.

2.4 Engineering Principles of MSW

2.4.1 MSW Generation Rate

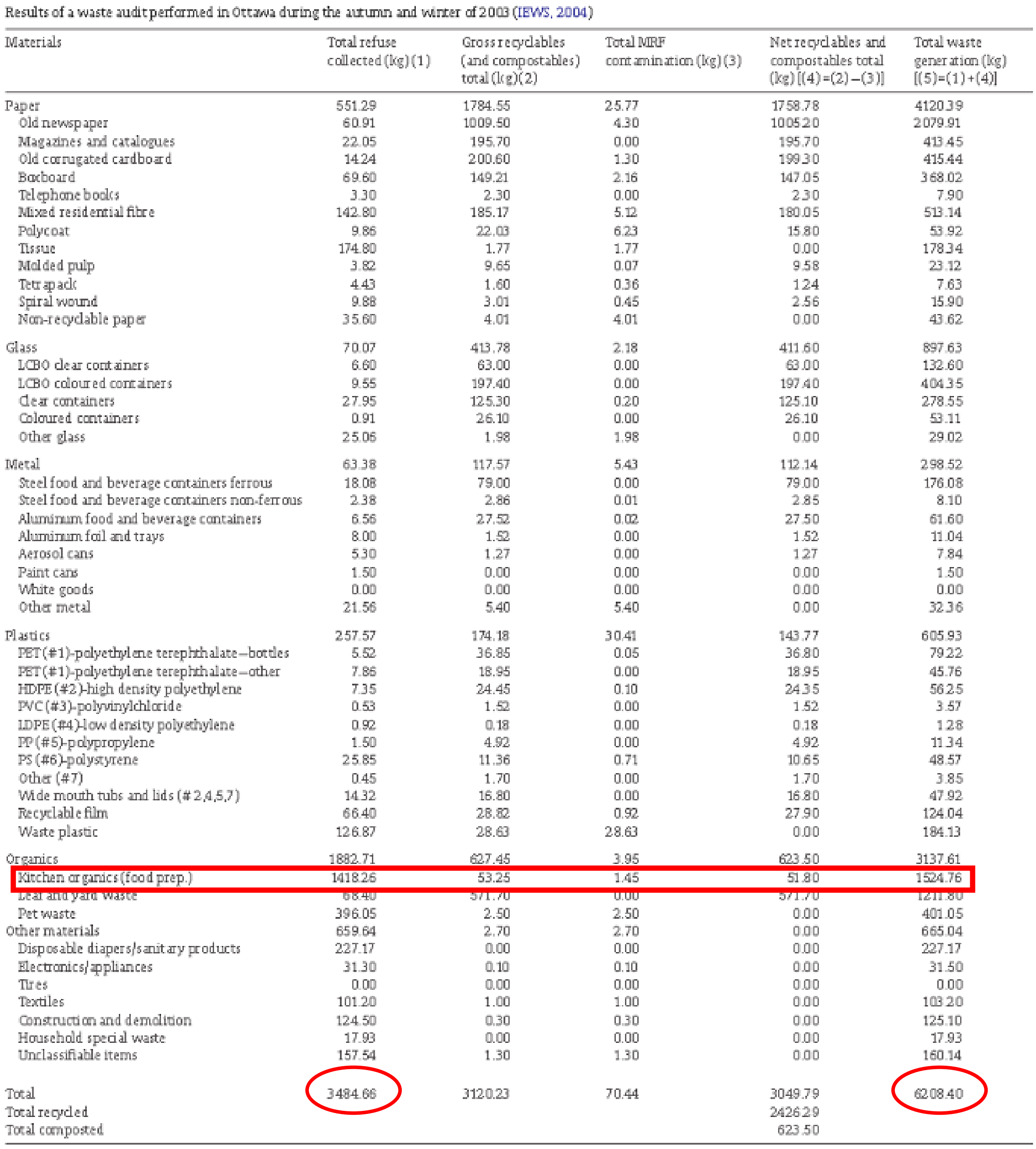
Waste generation rate information is very important for assessing compliance with diversion goals, designing waste collection routes and designing material recovery facilities, resource recovery facilities, landfills, etc. A study done by the waste diversion and processing division of the City of Ottawa during the year 2003 said that residential solid waste generation was 308,609 tonnes the management of which is presented in Table 2-3.

Table 2-3 Waste generation in City of Ottawa during 2003 (Harris, 2004)

Waste Source	Amount (Tonnes)
Residential solid waste	308,609
Sent for disposal to landfill	206,307
Waste sent for recycling	68,835
Sent from MRF to market	66,799
Sent from MRF to disposal	2,036
Leaf and yard waste sent for composting	31,452
Food waste sent for composting	2,015
Compost produced	20,080
Organic waste diverted from backyard	7400

Meanwhile, a waste audit performed by the City of Ottawa in order to determine waste composition and waste disposal patterns during the fall and winter, 2003 (IEWS, 2004) are presented in Table 2-4 and it shows that total kitchen organics that was generated accounts for 1524 Kg of which only 3% is recycled or recovered in MRFs (marked in red box).

Table 2-4 Composition of Ottawa waste [After: IEWS, 2004]



Results of a waste audit performed in Ottawa during the autumn and winter of 2003 (IEWS, 2004)

Materials	Total refuse collected (kg) (1)	Gross recyclables (and compostables) total (kg)(2)	Total MRF contamination (kg) (3)	Net recyclables and compostables total (kg) [(4)=(2)−(3)]	Total waste generation (kg) [(5)=(1)+(4)]
Paper	551.29	1784.55	25.77	1758.78	4120.39
Old newspaper	60.91	1009.50	4.30	1005.20	2079.91
Magazines and catalogues	22.05	195.70	0.00	195.70	413.45
Old corrugated cardboard	14.24	200.60	1.30	199.30	415.44
Boxboard	69.60	149.21	2.16	147.05	368.02
Telephone books	3.30	2.30	0.00	2.30	7.90
Mixed residential fibre	142.80	185.17	5.12	180.05	513.14
Polycoat	9.86	22.03	6.23	15.80	53.92
Tissue	174.80	1.77	1.77	0.00	178.34
Molded pulp	3.82	9.65	0.07	9.58	23.12
Tetrapack	4.43	1.60	0.36	1.24	7.63
Spiral wound	9.88	3.01	0.45	2.56	15.90
Non-recyclable paper	35.60	4.01	4.01	0.00	43.62
Glass	70.07	413.78	2.18	411.60	897.63
LCBO clear containers	6.60	63.00	0.00	63.00	132.60
LCBO coloured containers	9.55	197.40	0.00	197.40	404.35
Clear containers	27.95	125.30	0.20	125.10	278.55
Coloured containers	0.91	26.10	0.00	26.10	53.11
Other glass	25.06	1.98	1.98	0.00	29.02
Metal	63.38	117.57	5.43	112.14	298.52
Steel food and beverage containers ferrous	18.08	79.00	0.00	79.00	176.08
Steel food and beverage containers non-ferrous	2.38	2.86	0.01	2.85	8.10
Aluminum food and beverage containers	6.56	27.52	0.02	27.50	61.60
Aluminum foil and trays	8.00	1.52	0.00	1.52	11.04
Aerosol cans	5.30	1.27	0.00	1.27	7.84
Paint cans	1.50	0.00	0.00	0.00	1.50
White goods	0.00	0.00	0.00	0.00	0.00
Other metal	21.56	5.40	5.40	0.00	32.36
Plastics	257.57	174.18	30.41	143.77	605.93
PET (#1)-polyethylene terephthalate—bottles	5.52	36.85	0.05	36.80	79.22
PET (#1)-polyethylene terephthalate—other	7.86	18.95	0.00	18.95	45.76
HDPE (#2)-high density polyethylene	7.35	24.45	0.10	24.35	56.25
PVC (#3)-polyvinylchloride	0.53	1.52	0.00	1.52	3.57
LDPE (#4)-low density polyethylene	0.92	0.18	0.00	0.18	1.28
PP (#5)-polypropylene	1.50	4.92	0.00	4.92	11.34
PS (#6)-polystyrene	25.85	11.36	0.71	10.65	48.57
Other (#7)	0.45	1.70	0.00	1.70	3.85
Wide mouth tubs and lids (# 2,4,5,7)	14.32	16.80	0.00	16.80	47.92
Recyclable film	66.40	28.82	0.92	27.90	124.04
Waste plastic	126.87	28.63	28.63	0.00	184.13
Organics	1882.71	627.45	3.95	623.50	3137.61
Kitchen organics (food prep.)	1418.26	53.25	1.45	51.80	1524.76
Leaf and yard waste	68.40	571.70	0.00	571.70	1211.80
Pet waste	396.05	2.50	2.50	0.00	401.05
Other materials	659.64	2.70	2.70	0.00	665.04
Disposable diapers/sanitary products	227.17	0.00	0.00	0.00	227.17
Electronics/appliances	31.30	0.10	0.10	0.00	31.50
Tires	0.00	0.00	0.00	0.00	0.00
Textiles	101.20	1.00	1.00	0.00	103.20
Construction and demolition	124.50	0.30	0.30	0.00	125.10
Household special waste	17.93	0.00	0.00	0.00	17.93
Unclassifiable items	157.54	1.30	1.30	0.00	160.14
Total	3484.66	3120.23	70.44	3049.79	6208.40
Total recycled				2426.29	
Total composted				623.50	

2.4.2 MSW Separation

2.4.2.1 Waste Separation at the Source of Generation

Waste separation at the source is usually accomplished by manual means. The number and types of components separated will depend on the waste diversion goals established for a community (Tchobanoglous, et al., 1993) and at present (2012) the City of Ottawa has a three-way source separation program to individually collect kitchen waste and yard waste, recyclable paper and plastic and cans. Figure 2-2 shows the green bin cans which are used to collect kitchen waste.

Figure 2-2 Green bin program in the city of Ottawa for the collection of kitchen waste

2.4.2.2 Waste Separation at MRFs

MRF refers to material recovery facilities. MRFs are used for

- The further processing of source-separated wastes obtained from curb side collection programs and drop-off and buy-back centers without processing facilities
- The separation and recovery of reusable and recyclable materials from comingled MSW and improvements in the quality of the recovered waste materials (Tchobanoglous ,et al., 1993

There are two types of MRFs

1. Manual Separation

Figure 2-3 shows the picture of one of the manual MRFs in Brazil.

Figure 2-3 Manual MRF (Adapted from Tchobanoglous, et al., 1993)

2. Mechanically Separated

Mechanically separated MRFs consist of different unit operations depending on the sophistication of material needed to be separated and the targeted waste diversion goal set. The City of Gatineau in the Ottawa Capital region of Canada operates a combined manual/mechanical MRF.

- Size reduction--- the unit operation in which as collected waste materials are mechanically reduced in size. Shedders, grinders, crushers and millers are used as size reduction equipments (Tchobanoglous, et al., 1993).
- Screening-- the unit operation used to separate mixtures of materials of different sizes into two or more size fractions by means of one or more screening surfaces. There are different types of screens, such as vibrating screen, rotary screen, disc screen (Tchobanoglous, et al., 1993). Figure 2-4 shows a picture of a screen at an MRF.

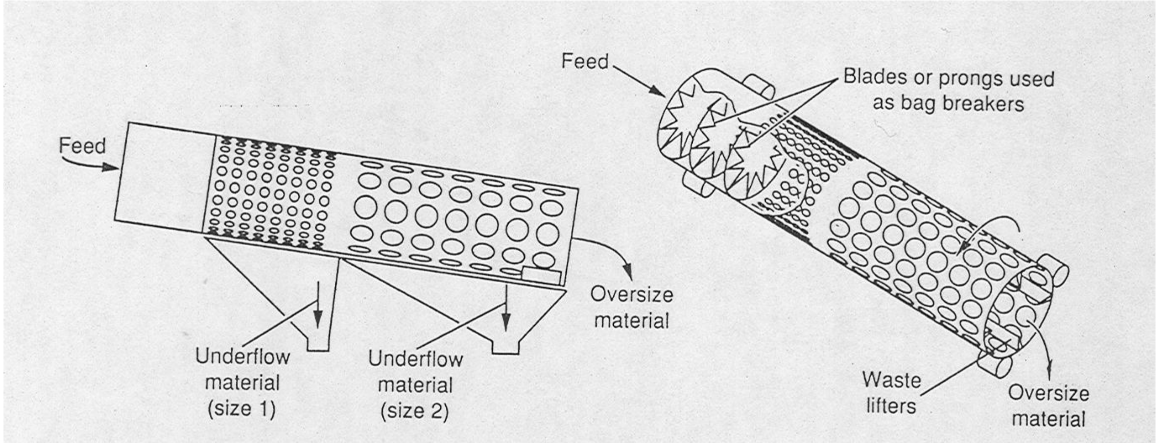


Figure 2-4 Screen operation at MRF (Adapted from Tchobanoglous, et al., 1993)

- Air classification--the unit operation used to separate light materials such as paper and plastic from heavier materials such as ferrous metal, based on the weight difference of the material in an air stream. (Tchobanoglous, et al., 1993).
- Magnetic separator--the unit operation where by ferrous metals are separated from other waste materials by utilizing their magnetic properties and aluminum cans from tin cans. (Tchobanoglous et al., 1993). Figure 2-5 illustrates a magnetic separator.

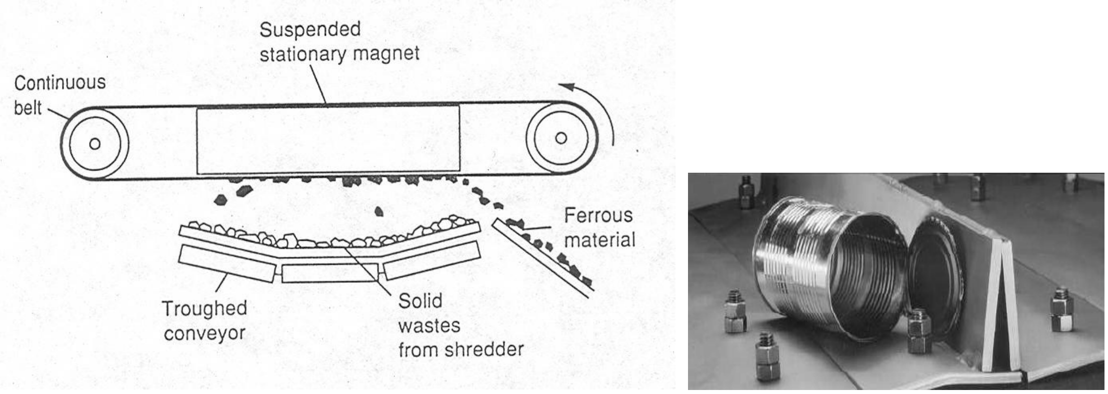


Figure 2-5 Magnetic separator (Adapted from Tchobanoglous, et al., 1993)

MRFs typically use a different process train to separate organic fraction of comingled municipal solid waste by deducting/ separating paper, glass, tin, cans wood etc. Typical flow train for the removal of tin cans, mixed MSW and paper are shown in Figure 2-6, 2-7 and 2-8 respectively.

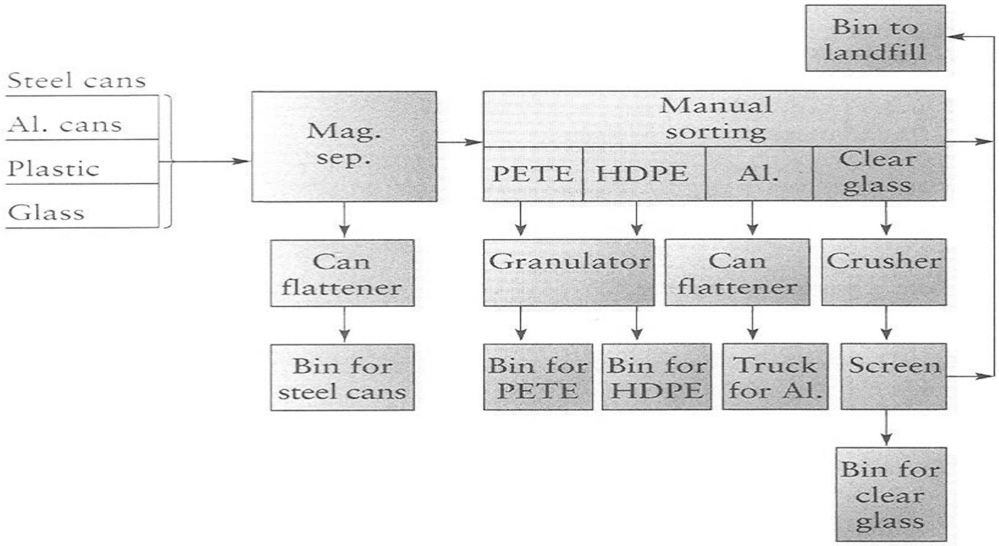


Figure 2-6 Typical process train for tin cans in a MRF (Adapted from Tchobanoglous, et al., 1993)

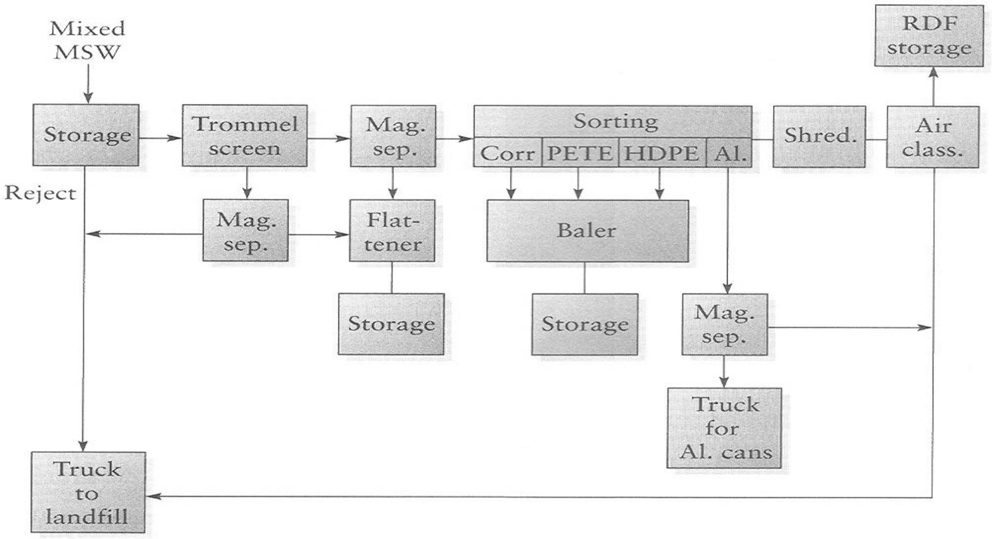


Figure 2-7 Typical process train for mixed MSW in a MRF (Adapted from Tchobanoglous, et al., 1993)

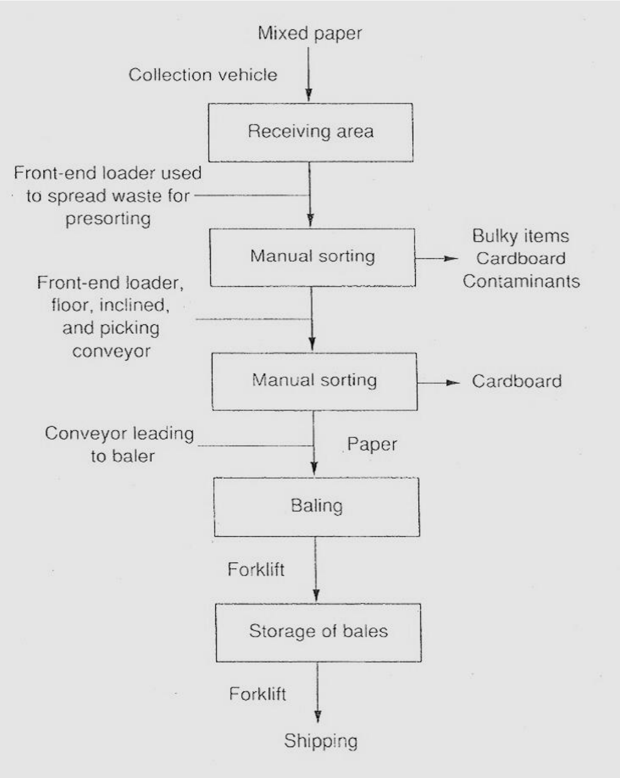


Figure 2-8 Typical process train for mixed paper in a MRF (Adapted from Tchobanoglous, et al., 1993)

2.5 Solid Waste Management

Solid waste management which involves waste generation, on-site storage, collection, transport, and disposal is one of the biggest concerns of civilization as it requires municipalities, provincial and federal government, private waste management firms as well as general public to play their different roles at different stages. Because of its association with public health and environmental consciences solid waste management is essential for a society and it demands an investment of huge amounts of money.

2.5.1 Integrated Solid Waste Management

When all the functional elements of waste management and the connections between all the elements are considered for their effectiveness and economic feasibility, solid waste management is called integrated solid waste management (ISWM). ISWM involves selecting applicable techniques, technologies and management programs to achieve certain waste management goals. These objectives vary from location to location. Different societies attempt to achieve their integrated solid waste management plan by setting up different goals. Ontario and Québec have a goal to achieve 60 % diversion of waste going to the land fill by increasing the reduction, reuse and recycle of waste by 2015. To achieve these goals recent legislation, regulations and government directives have set a hierarchy or ranking according to importance of waste management which is discussed in the next segment.

2.5.2 Solid Waste Management Hierarchy

Figure 2-9 illustrates the steps of solid waste management according to their preference or importance in North America. U.S. Environmental Protection Agency (EPA) has ranked the most environmentally sound strategies for municipal solid waste. Source reduction (including reuse) is the most preferred method, followed by recycling and composting, and, lastly, disposal in combustion facilities and landfills.

Figure 2-9 Solid waste management hierarchy (Adapted from EPA, 2011)

2.5.2.1 Source Reduction and Reuse

Source reduction, also known as waste prevention, means reducing waste at the source. It can take many different forms, including reusing or donating items, buying in bulk, reducing packaging, redesigning products, and reducing toxicity. Source reduction also is important in manufacturing. Light weight packaging, reuse, and re-manufacturing are all becoming more popular business trends. Purchasing products that incorporate these features supports source reduction. (EPA, 2011), Source reduction can:

- Save natural resources;
- Conserve energy;
- Reduce pollution;
- Reduce the toxicity of our waste;
- Save money for consumers and businesses alike

2.5.2.2 Recycling

Recycling is a series of activities that includes the collection of used, reused, or unused items that would otherwise be considered waste; sorting and processing the recyclable products into raw materials; and remanufacturing the recycled raw materials into new products. Consumers provide the last link in recycling by purchasing products made from recycled content. Recycling also can include composting of food scraps, yard trimmings, and other organic materials. Recycling prevents the emission of many greenhouse gases and water pollutants, saves energy, supplies valuable raw materials to industry, creates jobs, stimulates the development of greener technologies, conserves resources for our children's future, and reduces the need for new landfills and combustors. Figure 2-10 illustrates separate collect bins for paper; plastic bottles and aluminum drink cans for recycle. (EPA, 2011)

Figure 2-10 Recycle program in the City of Ottawa

2.5.3 Waste to Energy Generation

Waste to energy (WTE) generation technologies are the thermal, chemical and biological processes that recover energy from the waste. Commonly addressed WTE processes are incineration and anaerobic digestion. WTE plants offer two important benefits, environmentally safe waste management and disposal, as well as the generation of cleaner electric power. WTE facilities produce cleaner, renewable energy through thermal, biochemical and physicochemical methods. The growing use of WTE processes as a method to dispose of solid and liquid wastes and generate power has greatly reduced environmental impacts of ISWM, including emissions of greenhouse gases.

WTE conversion reduces greenhouse gas emissions in two ways. Electricity is generated which reduces the dependence on electrical production from power plants based on fossil fuels. The greenhouse gas emissions are significantly reduced by preventing methane emissions from landfills. Moreover, WTE plants are highly efficient in harnessing the untapped sources of energy from a variety of wastes.

An environmentally sound and techno-economically viable methodology to treat biodegradable waste is highly crucial for the sustainability of modern societies. A transition from conventional energy systems to one based on renewable resources is necessary to meet the ever-increasing demand for energy and to address environmental concerns.

The global market for WTE technologies was valued at US$19.9bn in 2008. This has been forecasted to increase to US$26.2bn by 2014. While the biological WTE segment is expected to grow more rapidly from US$1.4bn in 2008 to approximately US$2.5bn in 2014, the thermal WTE segment is nonetheless estimated to still constitute the vast bulk of the entire industry's worth. This segment was valued at US$18.5bn in 2008 and is forecasted to expand to US$23.7bn in 2014. (EPA, 2011)

Waste-to-energy refers to any waste treatment that creates energy in the form of electricity or heat from a waste source that would have been disposed of in a landfill, otherwise known as municipal solid waste. WTE is a renewable energy because its fuel source, garbage, is sustainable and is not depleted. According to the USEPA, WTE is a clean, reliable, renewable source of energy. (EPA, 2011)

2.5.4 Anaerobic Digestion as WTE

The USEPA defines AD as a process where microorganisms break down organic materials, such as food scraps, manure and sewage sludge, in the absence of oxygen. In the context of ISWM, anaerobic digestion (also called Anaerobic Composting or Biomethanation) is a method to treat source separated organic waste to recover energy in the form of biogas. Biogas consists of methane and carbon dioxide and can be used as fuel or, by using a generator it can be converted to electricity on-site. The ability to recover energy and compost from organics puts AD above aerobic composting on the hierarchy of waste management. It also reduces green house gas emissions by using methane as an energy source which would otherwise be emitted from land filling waste. (EPA, 2011)

2.5.4.1 AD as WTE in Europe

More than 70 plants treating bio-waste or MSW were installed during the last five years (2006-2010) in Europe. The expected installed capacity by the end of 2010 will be about 6 million tons/year divided over 200 plants in 17 European countries. In contrast, 15 plants were installed between 1991 and 1995, with total capacity of about 200,000 tonnes. (Baere, et al., 2010) However, a lot of large-scale AD facilities are planned in countries like the United Kingdom and France. Generally, source separation of household waste is less actively encouraged in these countries, resulting in large MSW-based AD plants. Table 2-5 highlights some of the large scale AD plants which are treating MSW and producing electricity in UK and Ireland. On the other hand, Switzerland, Austria, Sweden and Norway tend to install smaller anaerobic digestion units of 8,000 to 15,000 tpy. Germany is the leader in anaerobic digestion with planning to have a capacity of more than 1.7 million tons by the end of 2010. Spain is in second position (1.5 million tpy), followed by France 800,000 tpy. (Baere, el at., 2010) (www.anaerobic-digestion.com, 2010)

Table 2-5 AD plants in UK and Irelands (Adapted from anaerobic-digestion.com, 2010)

Plant Type	**(Technology Provider)**	**Feedstock.**	**Status.**	**Power MagaWatt**
Holsworthy.	Mesophilic-(Farmatic technology).	Cattle, pig and poultry manure, organic food, waste.	Operational large scale.	2.1
Leicester.	Mesophilic.	Biodegradable Municipal Waste.	Operational large scale.	1.5
Ludlow	Mesophilic-(Greenfinch technology)	Biodegradable Municipal Waste	Under development	Not applicable
Western Isles Scotland	Mesophilic-(Linde technology)	Biodegradable Municipal Waste	Under construction	Not applicable

2.5.4.2 AD as WTE in Asia

In 2005, China with about 10% of the world's rural population was already producing 5.5bn nm^3/biogas annually from 15m units. 56m digesters will be operated till 2020 and

20bn nm^3/yr of biogas will be produced for decentralised energy supply (cooking, lightning), and will make these areas widely independent from central energy supply systems (www.anaerobic-digestion.com, 2010). (It has being summarized in Table 2-6)

Table 2-6 MSW based energy recovery plants in China (Adapted from www.anaerobic-digestion.com, 2010)

Location	Start	Feedstock	Technology Developer	Capacity mt/a	€	Comments
Beijing Dong Cun Taihu Coun.	2007	Restaurant-MSW,manure	Linde Valorga Biomax	0.2	18m Fee13.5/t	Feasibility 2005
Beijing	2010	Restaurant-. MSW.				9 plants anticipated
Shanghai Jinshan	2008	MSW		0.22	32m	Ppublic tender
Shanghai Putuo, Shanghai	2007	MSW	Valorga Biomax	0.18 to 0.29	l30m Fee17/t	Feasibility 2005,
GuangzhouLikeng (Guandong)	2007	MSW	Valorga Biomax	0.36	32m	Preparation
Changsha Huiming (Hunan)	2005	MSW		0.73	11m	Biogas power plant
Mianyang (Sichuan)	2002	MSW	Tunnel type	0.25 AD: 3600t/y		AD as pilot project

2.6 Resource Recovery vs. Landfill

Landfill is the ultimate disposal for solid waste and comes at the bottom of the ISWM hierarchy table. In countries like Canada with a huge available land area landfills are still considered acceptable means to dispose of residues but most of the European countries enforce mandatory resource recovery by all possible means before disposal in a landfill is considered. Land filling can have both advantages and disadvantages considering the location and socio-economic condition of a community. Advantages and disadvantages of land filling are as follows: (Tchobanoglous, et al., 1993)

Advantages:

- Where land is available, it is the cheapest method of disposal
- Low initial investment
- It is a final disposal method
- It is very flexible operational wise
- Sub marginal land may be reclaimed for use as parking lots, playground, ski hills, parks, etc.

Disadvantages:

- In highly populated areas, suitable land may not be available within economical hauling distance
- Proper operation must be maintained or it can result in an open dump
- Sanitary landfills located in residential areas may be opposed by residents
- Completed landfills will settle & require periodic maintenance
- Buildings on a completed landfill must be designed to account for the settling
- Methane gas needs to be controlled to protect air pollution
- The leachate produced must be contained and collected to protect the ground water table contaminated
- Vermin and fire problems are not completely eliminated

However, a landfill lifespan study done in the City of Ottawa, Canada by a private organization “Ottawalandfillwatch” in 2004 for Ottawa’s, Trail Road landfill forecasted the life span of the facility with and without waste diversion. Figure 2-11 shows lifespan predictions of the Trail Road landfill, if all residential MSW are sent to this land fill, indicating that the landfill might only last until 2017 (shown in purple color line) but if the City of Ottawa can achieve 60 % diversion of waste going to the landfill then Trail Road land fill can serve the city till 2038(shown in green line).

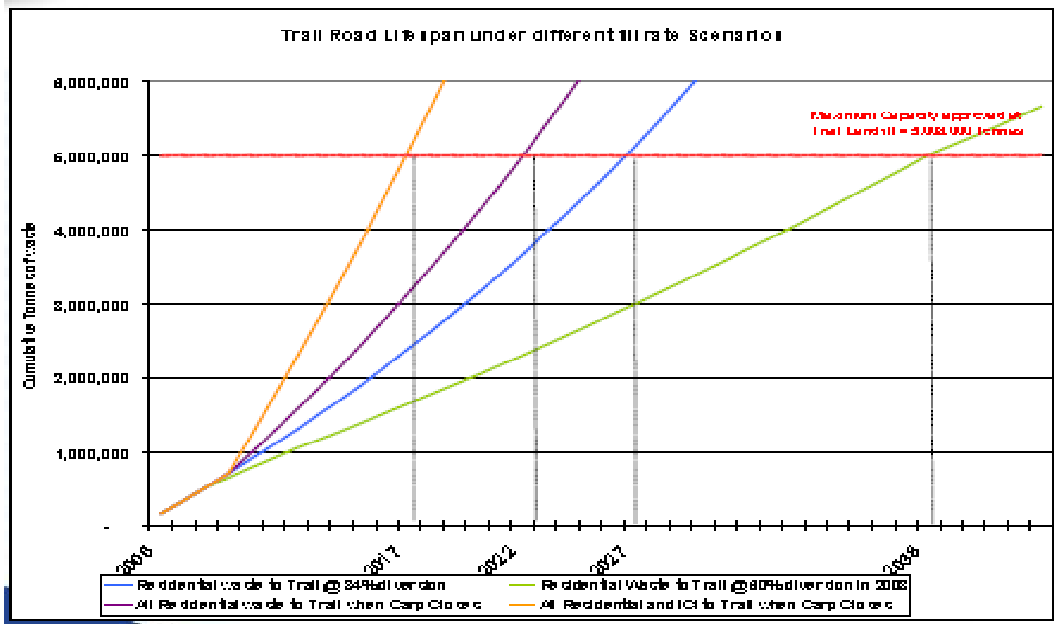


Figure 2-11 Life cycle prediction (After Ottawalandfillwatch)

Meanwhile, several European countries have already achieved a 76 % waste diversion rate by diverting most of their organic waste a major component of MSW to use for renewable energy through anaerobic digestion as it has significant advantages like control over green house gas emissions over other options like aerobic composting. Considering all these facts a conclusion can be drawn that to achieve higher diversion targets (60% in Ontario and Québec) anaerobic digestion or co-digestion of OFMSW with other biodegradable matters can be a good means. In fact to reach the projected 60% waste diversion rate (study done by Ottawalandfillwatch.org), at least 26% of MSW organics needs to be diverted from the main waste stream in the City of Ottawa.

2.7 Anaerobic Digestion

AD has been implemented for sludge stabilization from municipal and industrial wastewater treatment (Manariotis, et al., 2010). However, due to recent demand for renewable energy alternatives and rising energy costs, AD has been re-addressed as a prospective process for useable energy production in the form of biogas (Agler, et al., 2008). Rapid industrialization has also resulted in a large quantity of high-strength organic wastewaters in need of treatment. Additionally, AD has been implemented as a beneficial resource recovery approach in Europe and part of Asia (Rajeshwari, et al., 2000).

Several advantages of AD over conventional aerobic digestion make it attractive for the treatment of high solid substances (Kennedy, et al., 1985). Table 2-7 summarizes the motivation for the use of anaerobic treatment processes. When oxygen is the terminal electron acceptor a significantly greater quantity of energy is available to microorganisms, as is the case in aerobic digestion. Anaerobic pathways on the other hand have been shown to yield seven times less available energy than aerobic processes from the same substrate. Consequently, the greater available energy in aerobic processes results in increased biomass yield, and therefore waste biomass disposal concerns. In comparison, anaerobic systems produce methane gas, which is unavailable for biomass synthesis, resulting in only 5-20% as much waste biomass, significantly reducing financial and disposal site requirements (Speece, 2008).

Table 2-7 Advantages and disadvantages of anaerobic treatment of wastewaters

Advantages	**Disadvantages**
• Less energy required, resulting in lower operating costs • Less biological sludge production due to lower microbial growth rate, also increased sludge stabilization and dewaterability • Less nutrients required • Potential for energy recovery through methane production • Rapid response to substrate addition after periods of dormancy • With acclimation most organic compounds can be transformed • Smaller reactor volume required • Technology requires simple construction, operation and maintenance	• Longer start up period required to develop the necessary biomass inventory • May require alkalinity or specific ion additions • Lower removal efficiency: effluent may require polishing steps following AD to meet discharge requirements • Biological nitrogen and phosphorus removal is not possible • Sensitive to changes in temperature, which can cause reduced reaction rates • May be more susceptible to system upsets due to toxic compounds • Potential production of odors and corrosive H_2S gas

*Table adapted from (Metcaff, et al., 2003), (Manariotis, et al., 2010), and (Speece, 2008)

The small catabolic energy yield in methanogenesis has resulted in highly efficient and synergistic relationships between methanogenic bacteria. The ultimate products of biological metabolism in anaerobic systems are carbon dioxide (CO_2) and methane (CH_4), the latter of which has inherent economic value as a source of energy. Methane production is a major consideration in the selection of anaerobic digestion technology for wastewater treatment.

The following equations illustrate the conversion of hexose and the consequent energy produced, through aerobic and anaerobic processes respectively:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ ($\Delta G° = -2,870 kJ/mol$) Eqn. 2.1

$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4$ ($\Delta G° = -390 kJ/mol$) Eqn. 2.2

Despite a low energy yield, methanogenesis results in a great deal of potential energy stored in the methane gas produced ($\Delta H° = 816 kJ/mol$), which can then be harvested and exploited in the presence of oxygen for human uses and other physical processes (Schink, 1997).

2.7.1Anaerobic Metabolism

AD is a complex interdependent process involving several diverse microbial populations, working collectively to convert organic compounds into CH_4 and CO_2 in absence of oxygen. (Gujer, et al., 1983), described the scheme of anaerobic digestion metabolism by six distinct processes:

1. Hydrolysis of biopolymers
    a. Hydrolysis of proteins
    b. Hydrolysis of carbohydrates
    c. Hydrolysis of lipids
2. Fermentation of amino acids and sugars
3. Anaerobic oxidation of long chain fatty acids and alcohols
4. Anaerobic oxidation of intermediary products such as volatile acids. Conversion of acetate to methane and carbon dioxide
5. Conversion of hydrogen to methane and carbon dioxide

The conversion of complex substrates via AD is obtained due to the collaborative exertion of a minimum of four different bacterial groups. In order to achieve full digestion and formation of end products, these four bacterial groups uptake substrates from one group of microorganisms to another. The bacterial groups involved in AD include primary fermenting bacteria, secondary fermenting bacteria, and two types of methanogens (Mohana, et al., 2010), (Schaefer, et al., 2008). Figure 2-12 and 2-13 illustrates the flow of the anaerobic digestion process from complex organic compounds (lignin and cellulose) to the final products of CH_4 and CO_2. A more detailed description of the four main stages (hydrolysis, acidogenesis, acetogenesis and methanogenesis) of anaerobic digestion follows.

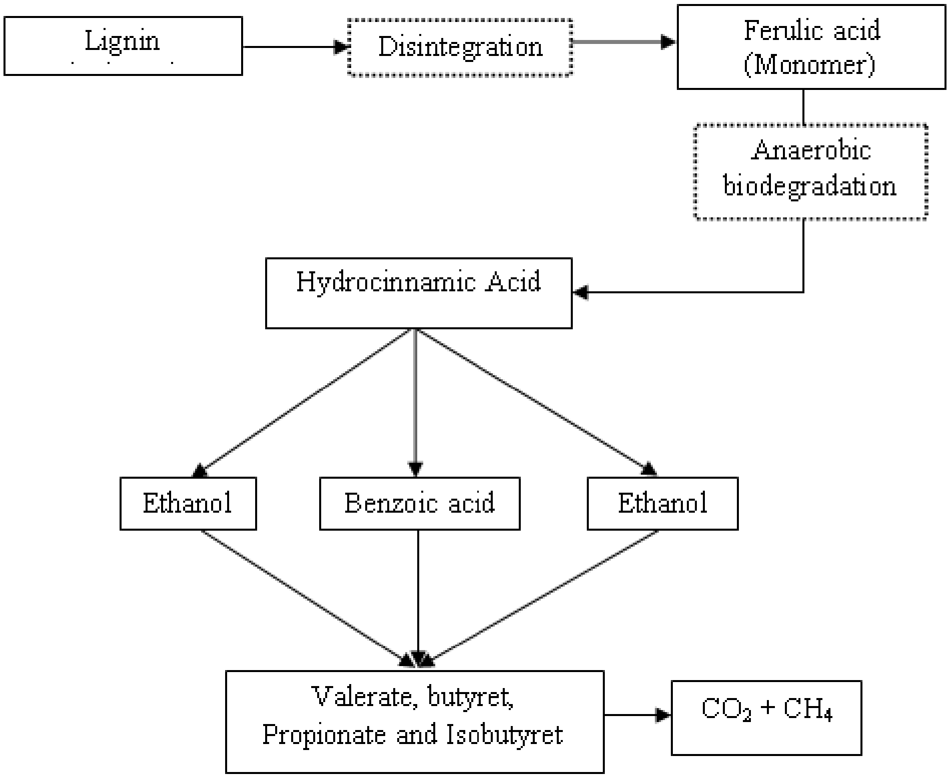


Figure 2-12 Anaerobic metabolism of complex organic material (lipid) (Adapted from Manariotis, et. al., 2010)

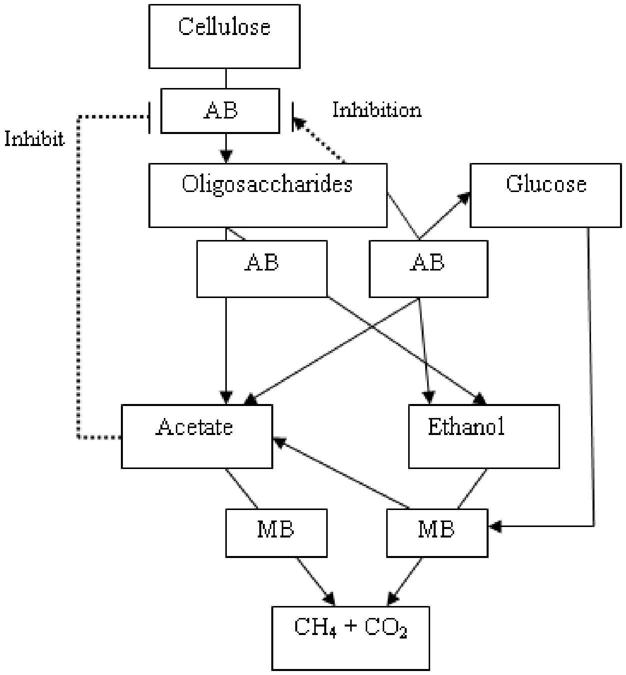


Figure 2-13 Anaerobic metabolism of complex organic material (Cellulose) (Adapted from Manariotis, et. al., 2010)

2.7.1.1 Hydrolysis

The first stage of anaerobic digestion is hydrolysis that converts the complex organic materials into their constituent parts through the work of primary fermentative bacteria. Since methanogenic and acetogenic bacteria are unable to directly use polymers, higher polymers need to be converted first into soluble monomers before the process can proceed farther. Usually polysaccharides are converted into simple sugars such as glucose, proteins are transformed into amino acids, nucleic acids become purines and pyrimidines, and lipids become fatty acids and glycerol by the action of the hydrolytic bacteria (Schaefer, 2006).

However, hydrolysis is catalyzed by the action of extracellular hydrolytic enzymes i.e calluses, proteases, and lipases. These enzymes are excreted by the primary fermentative bacteria in order to break large substrate molecules down. For complex feedstock, the hydrolytic phase is relatively slow and that becomes the time limiting factor for AD.

(Manariotis, et al., 2010). Schaefer, et al., (2008) considered hydrolysis to follow first order kinetics for a typical complex waste.

2.7.1.2 Acid Forming Steps (Acidogenesis and Acetogenesis)

Hydrolysis is immediately followed by the acid forming stage, in which two groups of secondary fermentative bacteria convert the monomers produced by hydrolysis into several intermediary products. Biological and chemical oxygen demand (BOD and COD) is reduced through these acid-generating pathways. The products of hydrolysis are converted into volatile fatty acids (VFAs) (acetic, butyric, propionic, formic, lactic or succinic acids), keytones (methanol, ethanol, glycerol or acetone) as well as alcohols by acidogenic bacteria. Characteristics and concentration of the end products by the secondary fermentative bacteria in the acidogenic phase varies depending on the culture condition such as pH and temperature. The most likely combination of oxidized end products is acetate, carbon dioxide, and hydrogen which are the only precursors that can be used by methanogenic bacteria. (Wilkinson, 2011).

The conversion of organic material to organic acids in the acid forming stages naturally causes the pH of the system to drop. This is beneficial for the acidogenic and acetogenic bacteria that prefer a slightly acidic environment, in the range of 4.5–5.5, but can be problematic for bacteria in the final stage of methanogenesis. (Speece, 2008)

2.7.1.3 Methanogenesis

The next step of AD is methanogenesis which is a ubiquitous process in most anaerobic environments. Rittmann, (2008) reported that the generation of CH_4 is performed by two unique groups of strictly anaerobic *Archaea* with one of them being aceticlastic methanogens, which are responsible for biogas production directly from acetate, and the second group are hydrogen scavenging hydrogenotrophic methanogens that convert H_2 and CO_2 to methane. Acetate is the major methanogenic precursor in most anaerobic ecosystems (Horan, 2003). It has been reported by Sawayama, et al., (2004) that acetoclastic methanogens are responsible for 70% of methane production, while hydrogenotrophic methanogens account for the remaining 30%.

Hydrogen (H_2) also has an intermediary integral role in the whole process of anaerobic digestion. Long chain fatty acids produced by lipid hydrolysis, are oxidized by acetogenic bacteria in the acid forming steps to acetate or propionate and hydrogen gas is produced. Hydrogen present under standard conditions results in inhibition of oxidation, and the reaction (i.e. propionic acid oxidation to acetic acid) can only proceed if the partial pressure of hydrogen is low enough (<100Pa) to thermodynamically allow the reaction to occur (Schink, 1997, Rittmann, 2008). Hydrogen scavenging methanogenic bacteria that consume hydrogen are essential for any anaerobic system. Their action lowers the H_2 partial pressure, ensuring thermodynamically favorable conditions resulting in full oxidation of acids present in the system minimizing the accumulation of longer chain VFAs (propionic and butyric acids). (Wilkinson, 2011)

Methanogens are very sensitive to acidic condition and system changes and mostly prefer a slightly alkaline environment. Preferable pH range for methanogens is 6.5-8.0 (Horan, 2003). For soluble substrates methanogenesis is the rate controlling process as methanogens have much slower growth rates than acidogenic and acetogenic bacteria. Therefore, following hydrolysis of solid wastes the global kinetic rate of the process is driven by the kinetics of the methanogenic bacteria (Davis, et al., 1998)

2.8 Anaerobic Process Parameters

Several digestion parameters affect the physical system and consequently the rate of digestion and production of biogas (CH_4 and CO_2). The following parameters must be monitored and maintained at acceptable levels to ensure process stability: temperature, pH and alkalinity, carbon-to-nitrogen (C:N) ratio, Hydraulic Retention Time (HRT), solids retention time (SRT), OLR, nutrients, and presence of toxic inhibitors (ammonia, sulfate and sulfites). Deviations from the acceptable ranges for these parameters can result in digester failure and it is essential to understand the importance of each parameter. (Wilkinson, 2011)

2.8.1 Temperature

Temperature has a strong effect on the rate of digestion which makes temperature an important parameter. Anaerobic bacteria have the ability to survive in a wide range of temperatures from freezing up to 70°C, (Sakar, et al., 2009) which is typically divided into the following classifications: psychrophilic (0°C to 20°C), mesophilic (20°C to 40°C) and thermophilic (50°C to 65°C) and extreme thermophiles (>65°C) for AD. Normally, anaerobic digestion is carried out within the mesophilic or thermophilic ranges, with optimum temperatures being 35°C and 55°C for mesophilic and thermophilic digestion respectively.

Both mesophilic and thermophilic temperature ranges have advantages and disadvantages associated with them. Thermophilic digestion allows higher loading rates, achieves a higher percentage of pathogen destruction, and greater substrate degradation (Parawira, 2004). However, thermophilic systems are reported to be more sensitive to toxicity and environmental changes than mesophilic systems. From the energy perspective a mesophilic system is a more favorable process over thermophilic, as the thermophilic process requires a greater heat input for the process if the feedstock to be digested is initially cool. This may not be accurate if the feed is incoming at a higher temperature which would not require the addition of heat prior to the addition to a thermophilic digester. (Wilkinson, 2011)

Mesophilic bacteria are thought to be more robust and able to tolerate greater environmental changes in comparison to thermophilic bacteria. Despite the longer retention times required, mesophilic bacteria have demonstrated considerable stability in many different applications, which makes them the bacterial population of choice in most anaerobic digestion facilities. (Wilkinson, 2011)

2.8.2 pH and Alkalinity

pH is a primary indication of a healthy anaerobic digestion system and a stable pH indicates digester equilibrium and stability during the different stages of anaerobic digestion. An acceptable pH range for anaerobic digestion is anywhere between 5.5 and 8.5, however the optimal pH for growth of anaerobic bacteria lies between 6.5 and 7.8 (Sakar, et al., 2009, Horan, 2003). Methanogenic bacteria are especially sensitive to extremes in pH, and in order to maintain efficient methanogenesis a suitable and stable pH should be maintained within a digester. The major chemical and biochemical reactions that influence the pH of a system are (Horan, 2003),

- VFA production and consumption
- Ammonia release and consumption
- Sulphide release due to reduction of sulphate or sulphite

The accumulation of acids in a reactor can lead to inhibition of methanogens, resulting in a declining pH and unstable digester. A consequence of increased availability of volatile solids results in increase in the activity of acidogenic bacteria present in the system. The increased activities of acidogenic bacteria will accordingly tend to produce larger amounts of VFAs and lower the pH of the system. If the pH falls into a range that is inhibitory to methanogens (pH < 6), the system creates a positive feedback loop. As methanogens are responsible for the consumption of volatile fatty acids to maintain a balanced system, a decrease in their population will result in further accumulation of acids. Conversely, profuse methanogenesis can result in an increased pH (pH > 8). An accumulation of ammonia resulting from overactive methanogenesis will result in acidogenesis being inhibited and ultimately methanogenesis will be as well, due to the reduced VFAs available for conversion to biogas (Sawayama, et al., 2004). (Wilkinson, 2011)

Monitoring pH during start up of an anaerobic reactor is highly important for a stable digester system. Fresh waste must go though acidogenesis and acetogenesis stages before methane formation takes place. This results in an initial dip in pH levels, which can be easily combated with the addition of bicarbonate alkalinity to buffer the system.

Alkalinity refers to the ability of a system to resist changes in pH. This is important to an anaerobic system, as when acids are produced or added to the system, alkalinity present will contribute hydroxide ions, helping to neutralize the acids present (Sakar, et al., 2009). Another advantage to adding alkalinity to a digester system is its tendency to cause particulate organics to swell, making them more susceptible to enzymatic activity (Baccay & Hasimoto, 1984). (Wilkinson, 2011)

2.8.3 Carbon-to-Nitrogen Ratio

The carbon-to-nitrogen (C:N) ratio is a measure of the organic carbon and nitrogen present in the feedstock. For an aerobic system, the optimum range for the C:N ratio is between 20-30, however anaerobic systems have much lower biomass yields and consequently have lower nitrogen requirements. The most commonly practiced and recommended C:N ratio for anaerobic digestion is 40:1 however successful AD has been reported at higher ratios of 100-200 to 1 depending on the substrate (Horan, 2003). If there is too much nitrogen present (a low C:N ratio), toxic conditions can result due to the accumulation of ammonia in the system, leading to pH values greater than 8.5. Conversely, too much carbon (or a high C:N ratio), results in the rapid consumption of nitrogen by acetoclastic and methanogenic bacteria and consequently lower biogas production (Hartmann, et al., 2002). (Wilkinson, 2011).

2.8.4 Hydraulic Retention Time

HRT also known as the residence time, is the average time that the digester liquid remains in the reactor (Sakar, et al., 2009). It represents the amount of time the mixed liquor remains in the reactor and is calculated based on the volumetric loading rate at which a reactor is operated. Theoretically, longer retention times yield more complete degradation of a feedstock. Reaction rate, however, is known to decrease with increasing retention time, and an optimum time should be determined based on time and cost effectiveness. Appropriate retention times will differ for each substrate and for most dry processes ranges from 14 to 30 days, while wet processes can be as little as 3 days. The shorter the retention time is, the smaller the digester that can be employed, resulting in cost savings in operation and building materials. Shorter retention times will increase production rate per unit reactor volume, but will eventually result in a lower final waste degradation. Research has indicated that for certain wastes a reduction of 64–85% of volatile solids in a complete mixed reactor could be achieved in as little as 10 hours, but typically retention times of ten days are required for complete digestion and sustainability of the microbial consortia (Horan, 2003). (Wilkinson, 2011)

2.8.5 Solids Retention Time

SRT of a reactor is a measurement of the concentration of bacteria maintained within the system with time. Higher SRTs result in greater populations of biomass being retained within the reactor, and consequently has impacts on the type and size of reactor required (Horan, 2003). In a completely mixed reactor with no solids recycle the SRT is equal to the HRT, while more advanced reactor configurations such as sludge recycle systems like the anaerobic contact reactor, fixed film reactors or upflow anaerobic sludge blanket reactors are able to un-couple the SRT from the HRT.(Wilkinson, 2011)

2.8.6 Organic Loading Rate

OLR of a reactor describes the amount of volatile solids introduced over a period of time. A higher loading rate introduces a greater amount of volatile solids per unit time, and if too high, can stress a digester into shock by a rapid and severe drop in pH. As previously discussed, since methanogenic bacteria reproduce at a rate slower than that of the acid-producing bacteria, there is a danger of rapid production of acids when a greater amount of volatile solids are available for consumption by acid forming bacteria. (Wilkinson, 2011)

2.8.7 Food to Microorganism (F/M) Ratio

Another key factor controlling anaerobic digestion is the food-to-microorganism (F/M) ratio (Burke, 2001). The F/M ratio expresses the ratio of substrate (F) to the amount of inoculum (M) present in a system (Koksoy and Sanin, 2010). It can be described in the units of grams (g) of VS or COD of substrate per g volatile suspended solids (VSS) of the bacterial population (inoculum) present. Pranshanth, et al., 2006 reported F/M ratio as an important parameter for evaluating the potential volatile solids loading and consumption rate of the substrate by the bacteria becomes constant at a given temperature. Higher F/M ratio can end up having inhibiton in AD process (Pranshanth, et al., 2006). (Wilkinson, 2011)

Pranshanth, et al. (2006) also studied the performance of F/M ratios between 0.18 and 2.0 g COD/g VS for the digestion of a synthesized wastewater (cellulose, sucrose, and peptone) and they reported 0.57 and 0.68 g COD/g VS as an optimum ratios for batch mesophilic systems. Koksoy and Sanin (2010) also investigated the effect of F/M ratios at mesophilic temperatures. F/M ratios of 0.5, 2.0, 5 and 10 (g VS_{WAS} /g VS_{INOC}) were examined with sonicated and un-sonicated WAS as the substrate. High F/M ratios initially experienced a lag period, but ultimately the cumulative biogas production surpassed that of the lower F/M ratios. (Wilkinson, 2011)

2.8.8 Nutrients

Methanogenic bacteria have a wide spectrum of micro and macro-nutrients required for healthy growth. Appropriate concentrations and ratios of these nutrients are required for reactor stability and proper bacterial metabolism. (Wilkinson, 2011)

2.8.9 Sources of Inhibition

There are a wide range of sources of potential inhibition for mesophilic anaerobic digestion. These include the accumulation VFA or alcohols, potentially caused by hydraulic or organic overloading, temperature fluctuations or the presence of toxins. The accumulation of VFAs is typically an indication of methanogenic microbial stress, however at a neutral reactor pH, only propionate has been shown to have adverse effects on digestion. Acetic and butyric acids have not been shown to have toxicity effects on methanogenic bacteria up to a total concentration of 10,000mg/L, while propionate concentrations higher than 1000mg/L have been shown to be unfavourable for digestion (Eskicioglu, et al., 2011). (Wilkinson, 2011)

Increased ammonia levels are also reported to inhibit methanogenesis. Sawayama, et al., (2004) studied the effect of ammonium on mesophilic methanogens immobilized on carbon felt in a fluidized bed reactor at pH between 7-8. The authors reported that ammonium concentrations of greater than 6000mg N/L negatively impacted reactor biogas production. Methanogenic activity was reported to decrease by 10% at ammonium concentrations of between 1670 mg N/L and 3720mg N/L and by 50% for 4090 to 5550 mg N/L. Between 5550mg N/L and 6000mg N/L, methanogenic activity was observed to decrease to nearly zero. (Wilkinson, 2011)

Organic or inorganic toxins and changes in substrate are also possible sources of inhibition (Ahring, et al., 1995). Substances that can potentially cause inhibition and reduced reactor performance include sulfate/sulfide, furfural, and phenol (Schaefer, 2009). (Wilkinson, 2011)

2.9 Substrate Pre-treatment in AD

Hydrolysis of TWAS is the rate limiting step of AD due to the presence of large organic molecules associated with microbial cells and EPS matrix. Previous studies have found that activated sludge has a complex floc structure comprised of different groups of microorganisms, organic and inorganic matter agglomerated together in a polymeric network formed by microbial EPS and divalent cations that resist degradation (Li and Ganzarczyk, 1990; Frolund, et al., 1995). According to the most recent WAS-floc agglomeration concept, EPS and divalent cations may be the most important parameter governing WAS hydrolysis. Therefore, external disruption of EPS and cation network by pre-treatment methods followed by subsequent anaerobic stabilization of microbial biomass could result in enhanced WAS biodegradability (Park, et al., 2003) and increase dewaterability (Ormeci, et al., 2001) during and after anaerobic digestion. Pre-treatment of WAS can enhance the methane production for both mesophilic (43–145%) and thermophilic (4–58%) AD processes conditions (Hamer, et al., 2004; Eskicioglu, et al., 2007a; 2007b;(Coelho, 2012)). Figure 2-13 illustrates a schematic of a typical bio-cell.

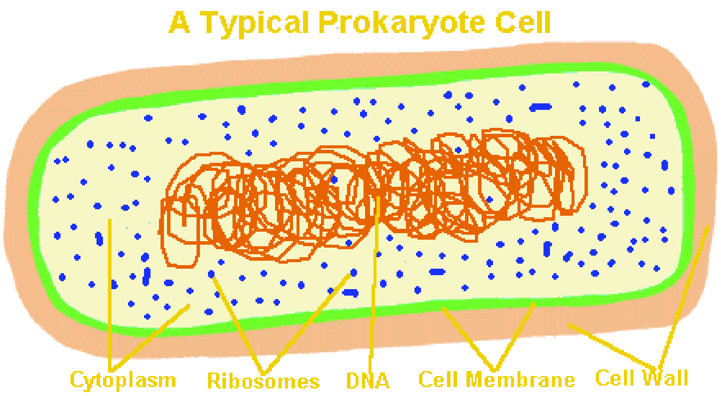


Figure 2-14 A typical bio-solid cell (Coelho, 2012)

From Figure 2-14 one can note that organics in the bio-cell are enclosed by a cell membrane and cell wall surrounded by a complex extracellular polymer all of which resist hydrolysis and circumventing lysis of the cell releasing organics and solubilization of the surrounding EPS matrix. It is a logical approach if the cell wall membrane complex and EPS matix of activated sludge can be destroyed or disrupted internal organics would be released into solution and the rate and extend of the digestion process can be improved. There are different processes for pre-treatment that are being applied in anaerobic digestion process. Major pre-treatment processes are:

- Thermal pre-treatment (conventional)
- Chemical pre-treatment
- Mechanical pre-treatment (conventional)
- Ultrasound pre-treatment
- Microwave pre-treatment

2.9.1 Thermal Pre-treatment

Thermal pre-treatment is the most widely used technique in both lab and pilot scale studies as a way of promoting cell lyses prior to anaerobic digestion. Gavala, et al., 2003 and Haug, (1977) studied thermal pre-treatment of bio-solids and found improvements in VS biodegradability and biogas production and better dewaterability of the treated sludge as well as increased pathogen reduction and odor control.

Bougrier, et al. (2006) studied sludge thermal pre-treatment in the temperature range of 150 ˚C to 170˚C with conventional heating and elongated holding time at desired temperature for periods longer than 30 min. They reported that for sludge pre-treatment at elevated temperature, TS and VS removal was 85 and 74 % respectively which was a few percentage points higher than that of the control and pre-treated sludge at lower temperature.

2.9.2 Chemical Pre-treatment

Another alternative to hydrolyze and decompose lipids and proteins to smaller soluble substances is acid and caustic addition. Chemical pre-treatment of OFMSW was examined by (Rania, 2012) and NaOH and KOH were used as alkaline reagent. Pre-treatment was examined at four pH levels 10, 11, 12 and 14, and two different temperatures 23±1°C and 80±1°C. It was reported that chemical pre-treatment enhances stability in the digestion process, and after hydrolysis pH stabilizes and over all biogas production was improved in both batch and semi-continuous mesophilic processes.

Chiu, et al., (1997) performed an experiment on TWAS with three different combinations of alkaline treatment and ultrasonic treatment: (1) sludge pre-treated with 40 meq/L of NaOH for 23 -24 hours, (2) sludge pre-treated with 40 meq/L of NaOH for 24 hours followed by low frequency ultrasonic vibration (20 kHz, 24 s/mL), (3) simultaneous ultrasound treatment (20 kHz, 14.4 s/mL) applied to samples dosed with 40 meq/L NaOH. Pre-treatment using simultaneous alkaline and ultrasonic treatment (3) was observed to be more effective in releasing both soluble COD and soluble organic nitrogen than alkaline treatment alone (1) and achieved results close to alkaline treatment followed by ultrasonic treatment (2). Pretreatment with NaOH only, for 24 hours was not effective in hydrolyzing the particulate COD. Improved solubility results were reported when low frequency ultrasound (20 kHz, 20 min) treatment was applied to sludge conditioned with a cationic polyelectrolyte flocculent (Chu, et al., 2002)

2.9.3 Mechanical Pre-treatment

Mechanical pre-treatments disintegrate the sludge flocs and destroy cells using shear stress. In mechanical pre-treatment, the disintegration of cells occurs rapidly and intracellular components are released and readily available for biological degradation. Different mechanical techniques have been investigated to create mechanical shear on sludge.

Muller, et al.; (1998) reported on four different pre-treatment methods disintegration by a stirred ball mill, a high pressure homogenizer, an ultrasonic homogenizer and a shear-gap homogenizer. The stirred ball mill and high-pressure homogenizer gave the best improvement in mesophilic AD results. The degree of biodegradation was in the range of 10-20% higher than the untreated sludge. They were able to show that mechanical pre-treatment could increase biogas productions and reduce digester volume. Figure 2-15 shows jetting pre-treatment of WAS and it illustrates that due to this mechanical pre-treatment flocs are converted into smaller particles that enhances solubilization of WAS.

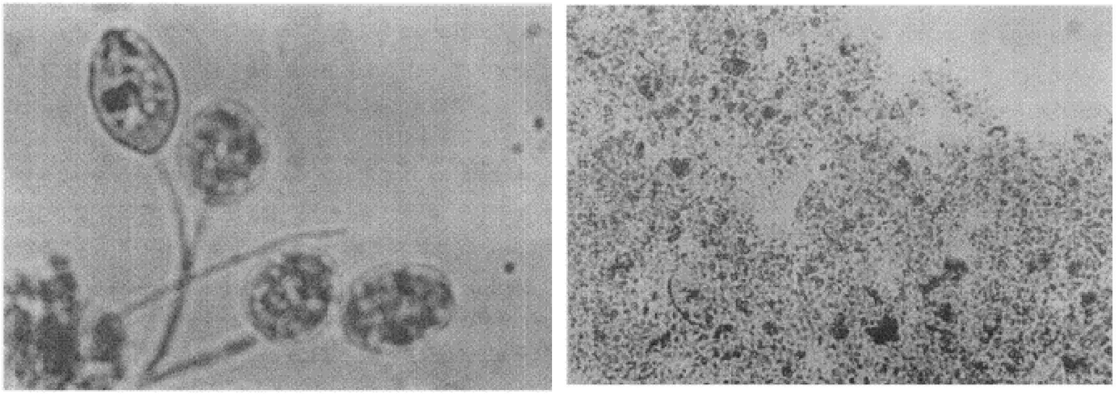


Figure 2-15 Microphotograph of WAS (a) prior and (b) after jetting and colliding in to a collision-plate at 30 bar (Adapted from Nah, et al., 2000).

2.9.4 Ultrasound Pre-treatment

Ultrasound is a sound wave at a frequency above the normal hearing range of an average person (> 20 KHz).Figure 2-16 shows the relative frequencies of sound waves, and the range of ultrasound waves most commonly used in environmental studies. Propagation of ultrasound in the liquid medium such as sludge generates a repeating pattern of compressions and rarefactions called cavitations. Cavitations are normally the formation, growth and sudden collapse of the cells in the sludge. In the rarefaction part of the ultrasonic wave, when the liquid is stretched micro bubbles form because of reduced pressure. According to (Adewuyi, 2001)when the bubbles are collapsed they are capable of producing high local temperature and pressures as illustrated in Figure 2-17. Figure 2-18 shows bio-solid cell destruction due to sonication where cells are destroyed at shear stress after Khanal, et al., (2006).

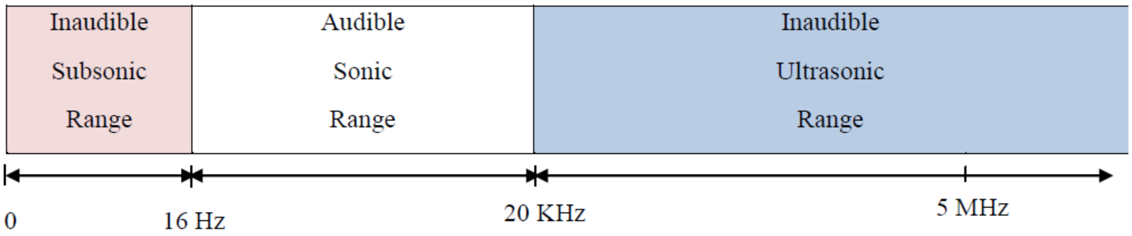


Figure 2-16 Sound waves at different frequencies (adapted from Khanal et.al. 2008)

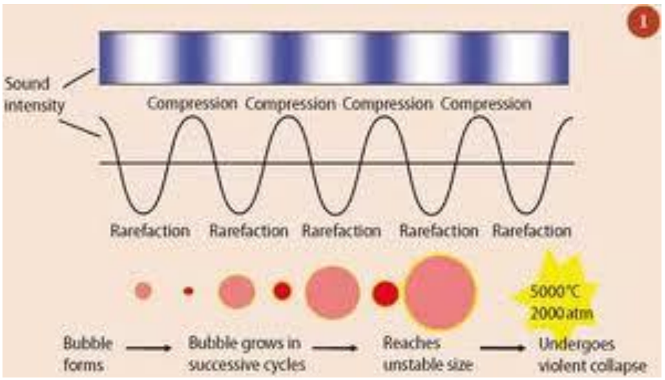


Figure 2-17 Microbubbles collapsing due to cavitation (Adapted from (deafwhale, 2011)

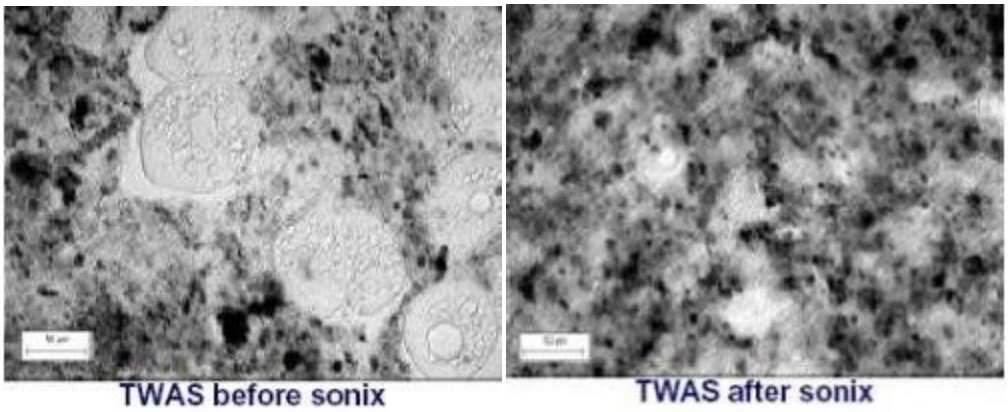
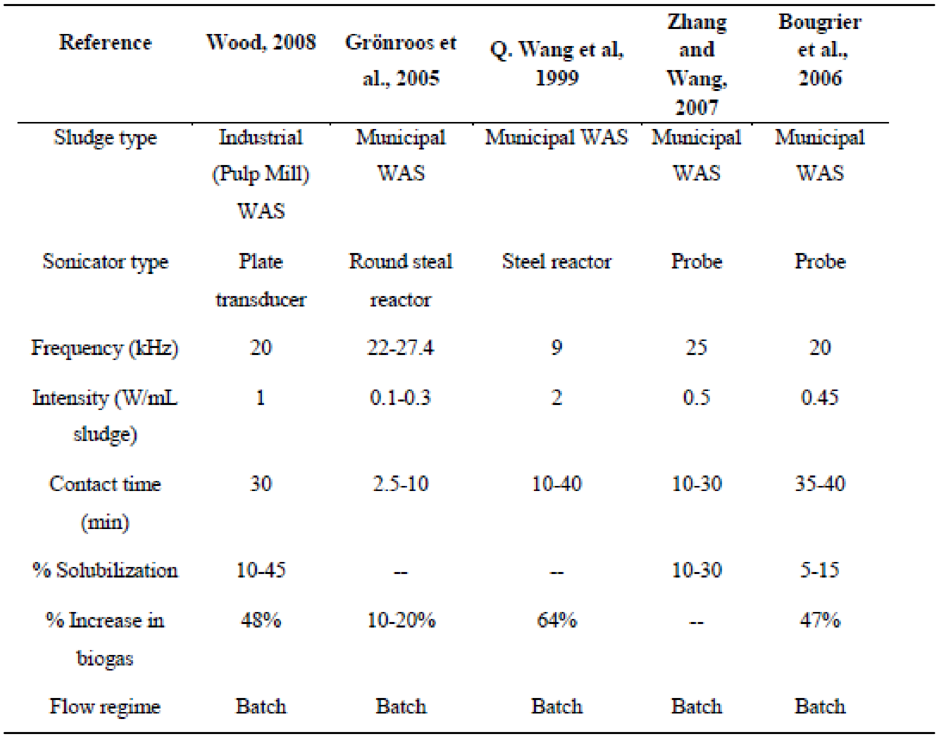

TWAS before sonix TWAS after sonix

Figure 2-18 Cell lysis effect after sonication (Adapted from Khanal et al., 2006)

Ultrasound or sonication was applied for cell lysis in different environmental studies specifically on WAS and it was reported that US pre-treatment of WAS increase solubilization which enhanced VS destruction and ultimate biogas production Saha (2011). Table 2-8 in the nest page summarizes some recent studies where ultrasound was applied in pre-treatment of WAS, table is adapted from (Saha, 2011).

Table 2-8 Summary of different studies on sonication of WAS. (Adapted from Saha, 2011)



Reference	**Wood, 2008**	**Grönroos et al., 2005**	**Q. Wang et al, 1999**	**Zhang and Wang, 2007**	**Bougrier et al., 2006**
Sludge type	Industrial (Pulp Mill) WAS	Municipal WAS	Municipal WAS	Municipal WAS	Municipal WAS
Sonicator type	Plate transducer	Round steal reactor	Steel reactor	Probe	Probe
Frequency (kHz)	20	22-27.4	9	25	20
Intensity (W/mL sludge)	1	0.1-0.3	2	0.5	0.45
Contact time (min)	30	2.5-10	10-40	10-30	35-40
% Solubilization	10-45	--	--	10-30	5-15
% Increase in biogas	48%	10-20%	64%	--	47%
Flow regime	Batch	Batch	Batch	Batch	Batch

2.9.5 Microwave Pre-treatment

2.9.5.1 Theory of Microwave

MW is electromagnetic waves that lie in the region of 0.3 to 300 GHz of the electromagnetic spectrum. Other types of radiation include infrared, ultraviolet, radio waves, X-rays and gamma rays. All these waves travel at the speed of light and the only difference between them is their wavelength, which is inversely proportional to their energy. The shorter the wavelength of the radiation, the greater will be their energy. Each of the wavelengths has specific characteristics and consequently different applications. Low frequency waves are useful in communications, MW and infra-red heating, visible light in photosynthesis, X-rays in the visualization of internal structures, etc. Research on the potential applications of MW started in the Second World War when the first MW generator was produced. Since then the industrial use of MWs has been increasing steadily. MWs are used primarily for communications or to heat materials. MW ovens are designed to produce waves that interact with polar materials. The behaviour of a sample subject to microwave heating is dependent on its chemical and physical properties. The most important properties are the dielectric loss factor, the dielectric constant and the dissipation factor. The materials are then classified according to their characteristics when exposed to MWs. (Coelho, 2012). The materials can be: (Coelho, 2012)

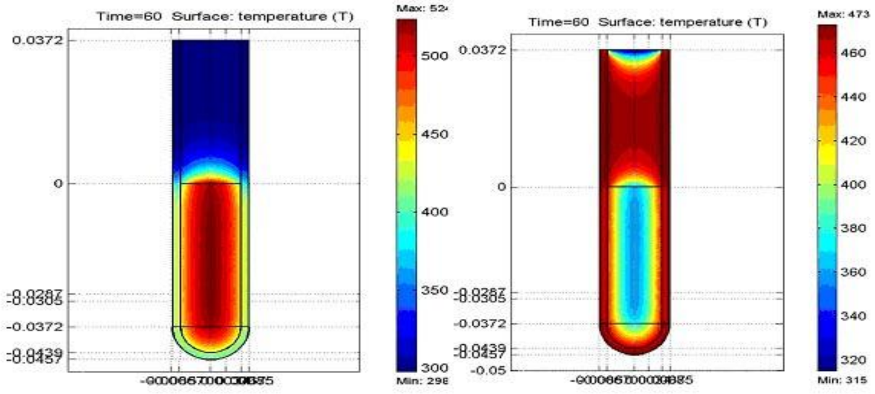
- Absorbers - if they absorb a great amount of the energy irradiated. An example of an absorber material is water. These materials have high dielectric constants.
- Transparent - if they do not absorb energy. An example of this type of material is glass or kerosene. These materials have very low dielectric constants.
- Reflectors- if they reflect the waves that are applied to them. No absorption or transmission occurs in these materials. An example is metals. (Coelho, 2012)

MW ovens are generally comprised of six components, the MW cavity, turntable, magnetron (the device that generates the MWs), wave guide (that directs the waves to the MW cavity), mode stirrer (that distributes the waves inside the MW cavity) and circulator (that directs the lost energy to a dummy load to protect the magnetron). When MWs are adequately used, heating can be accomplished in shorter time and more economically when compared with conventional heating. Some of the advantages of MW heating compared to conventional heating are (Metaxas and Meredith, 1983): (Coelho, 2012)

- Rapid and uniform heating: The heating occurs instantly and throughout the whole sample. Despite some temperature profiles in samples subject to MW treatment, heating can reasonably be considered uniform throughout the sample.
- Heating can be controlled instantly, and the power applied can be regulated accurately.
- Selective heating: The heat will concentrate in the materials that have a high dielectric factor.

At present, MW heating is used in many industries, besides its usual use in domestic households. It has been used in the food industry (baking, thawing, pasteurization, and drying), the forestry industry for drying of lumber and in the medical industry (sterilization) among other areas. (Coelho, 2012)

Figure 2-19 shows thermal image differences of conventional and MW heating. In conventional heating first the vessel consumes the heat and then heats is transferred to the sample but in microwave heating direct activation of molecules are possible and that brings the heat instantly to the sample. As the thermal image shows after conventional heating for a period of 60 minutes most of the heat is located in the exterior portions of the vessel and the interior of the sample is cool as the sample is heated from the outside in. However when the samples are placed in the MW heater the whole sample interior and exterior is heated relatively evenly.



Microwave Heating **Conventional Heating**

Figure 2-19 Thermal image of conventional and MW heating **(**Shahriari, et al., 2011)

2.9.5.2 Application of MW Pre-treatment

As one of the newest sludge pre-treatment technologies, MW pre-treatment has been previously tested for municipal WAS. Recent studies indicate that irradiation at 2.45 GHz can effectively break down the EPS and divalent cation network in bio-solids (Eskicioglu et al., 2007a). In addition to thermal effects, MWs can also cause an athermal effect by polarized parts of macromolecules aligning with the electromagnetic field poles that can cause the possible breakage of hydrogen bonds (Eskicioglu et al., 2007b; (Coelho, 2012). As a result of this enhanced disintegration and hydrolysis, MW irradiations increases the rate/extent of AD as well as dewaterability (Chia-Jung, et al., 2011); Eskicioglu, et al., 2007a, 2007b; Coehlo, 2012) and inactivate fecal coliforms and *salmonella spp*. to produce Class A sludge

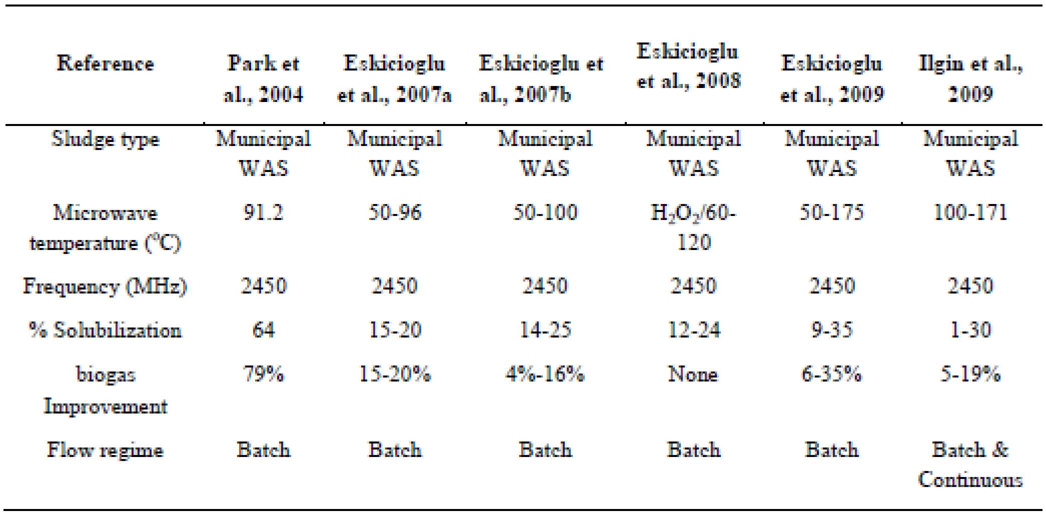
MW irradiation of secondary sludge was studied by Chia-Jung, et al., (2011) and they reported that the ratio of sCOD to TCOD improved from 2% to 22% after microwaving to 91˚C. Mesophilic AD with pre-treated sludge was run at HRTs of 8, 10, 12 and 15 days in the study and it was observed that due to pre-treatment of sludge successful AD can be achieved at lower HRTs of 8 days which is very impressive for commercial application

Pre-treatment of TWAS by MW was studied by Toreci, et al., (2009) who found that solubilization of TWAS increased with increase of temperature and there is an inverse relation of intensity on TWAS solubilization. They reported that at 175˚C improvement in soluble COD was 2.5 fold higher than that of untreated TWAS. However, enhancement in solubilization was higher at higher sludge concentration than at low sludge concentration.

About 10-12% improvement in biogas production was reported by Toreci, et al., (2010) when TWAS was pre-treated at temperature higher than 100°C prior to mesophilic BMP assays. Again similar improvements were observed by the same authors when pre-treated TWAS was used in semi-continuous mesophilic AD. Importantly, it was noticed that MW pre-treatment of TWAS enables digesters to operate at lower HRTs of 5 days and the effluent after digestion was classified as Class A sludge which can be land applied directly.

Similar outcomes were observed by (Coelho, 2012)who worked with thermophilic AD MW treated TWAS producing effluent after AD with zero pathogens while enhancing their rate of digestion as well as biogas yield. Table 2-9 shows a summary of different studies of MW pre-treatment of WAS (Saha, 2011)

Table 2-9 Summary of different studies of MW pre-treatment of WAS. (After Saha, 2011)



Reference	Park et al., 2004	Eskicioglu et al., 2007a	Eskicioglu et al., 2007b	Eskicioglu et al., 2008	Eskicioglu et al., 2009	Ilgin et al., 2009
Sludge type	Municipal WAS	Municipal WAS	Municipal WAS	Municipal WAS	Municipal WAS	Municipal WAS
Microwave temperature (°C)	91.2	50-96	50-100	H_2O_2/60-120	50-175	100-171
Frequency (MHz)	2450	2450	2450	2450	2450	2450
% Solubilization	64	15-20	14-25	12-24	9-35	1-30
biogas Improvement	79%	15-20%	4%-16%	None	6-35%	5-19%
Flow regime	Batch	Batch	Batch	Batch	Batch	Batch & Continuous

However, MW pre-treatment of OFMSW was studied by (Shahriari, et al., 2011)who reported that although MW heating improved solubilization it did not result in enhancement in rate of biogas production or biogas yield in either batch or continuous mesophilic studies. Shahriari, et al., (2011) explained these results by pointing out that the model OFMSW which had been used as substrate for evaluating MW pre-treatment effects on biogas production was a rapidly degradable substrate compared to TWAS and concomitantly no advantage was derived from the pre-treatment Similar results and explanations have been reported for primary sludge (Jian, et al., 2009).

2.10 Anaerobic Digestion vs. Co-digestion

Anaerobic treatment that involves more than one type of waste together is called co-digestion. AD of single substrates can sometimes be found to have several problems or drawbacks that limit AD and which have lead to interest in combining different kinds of substrates together. Combining different types of wastes for anaerobic digestion can potentially lead to several advantages, allowing treatment of a broader range of organic waste types. One of the most frequently found problems during biological processing especially with digestion of OFMSW is the high C:N ratio which is problematic for AD. On the other hand anaerobic digestion of TWAS which has a lower C: N ratio has been reported to be time consuming considering the slow hydrolysis rate of WAS. To circumvent this problem, several researchers have proposed co-digestion of the OFMSW, either with sewage sludge from WWTPs or residues from livestock farms (Sosnowski, et al., 2003). Hartmann, et al., (2003) reported that improved process economics and positive effects on the anaerobic digestion process, including improved methane yields, and improved reactor stability are the main advantages of co-digestion. In most cases, co-digestion improved biogas yields due to synergy developed in the digestion medium and a source of potentially missing nutrients provided by the co-substrate (Mata-Alvarz, et al., 2000).

The key to successful co-digestion lies in maintaining a balance of several parameters in the mixture of substrates. While some qualities of each substrate can be beneficial, there are also other properties that may hinder or be inhibitory for the anaerobic digestion process. Other drawbacks include slurry transportation costs when co-digesting solid waste and problems arising from varying policies of the waste-generators (Mata-Alvarez, et al., 2000). As previously discussed, an appropriate C:N ratio and pH are integral for efficient anaerobic digestion and co-digestion offers the potential to solve these issues in a practical and economical way. An inappropriate C:N ratio can be corrected for by digestion with a nutrient rich waste that will help to reach the desired ratio. The pH of a system can also be addressed by co-digestion with a waste that offers a higher pH and high buffering capacity, which will help to protect the system from failure due to a drop in pH if VFA concentrations begin to rise. Other advantages include offsets in the inhibitory effects of ammonia and sulfide, for substrates co-digested with clay and iron compounds respectively and the potential to lower the TS concentration of a waste by co-digestion with another waste that has a lower TS concentration (Hartmann et al., 2002).

2.11Studies Regarding Anaerobic Co-digestion

Anaerobic co-digestion of undiluted cow manure WAS and their mixtures in different ratios were conducted at 35°C in the laboratory-scale single-stage batch reactors for 63 days by (Jianzheng, et al., 2011). The specific biogas production reported for the manure and sludge ratios of 1:0, 4:1, 3:2, 2:3, 1:4 and 0:1 were 56.94, 58.51, 61.64, 63.12, 59.30 and 55.39 L/kg, with methane yield were 32.01, 33.14, 35.31, 36.91, 34.76 and 32.63 L/kg respectively in this study. Co-digestion at a ratio of 2:3 of manure and sludge produced the greatest total biogas production of 63.12 L/kg, methane yield of 0.328

m^3/kgVS and TS, VS, COD, total organic carbon reductions of 34.24, 54.80, 55.22 and 70.71% compared to other co-digestion ratios and single substrate digestion controls. VFA accumulation in the batch reactors with higher percentages of more readily degradable manure was not found to be significant in this study which reveals the potential stability offered by two different types of substrates co-digested together.

Co-digestion of cattle manure slurries and a range of agricultural wastes has been evaluated in batch BMP studies by (Callaghana, et al., 2002). They were able to show that based on VS reduction, total methane production and methane yield, co-digestion of cattle slurry with FVFMSW and with chicken manure were among the more promising combinations. In a second study the authors used continuously stirred tank (18 litres) mesophilic (35◦C) anaerobic reactors and examined the effect of addition of the FVFMSW and chicken manure to the cattle manure slurry system. The continuous test was done at a HRT of 21 days and the OLR was maintained in the range of 3.19–5 kg VS/ m^3 /d. Increasing the proportion of FVFMSW was varied from 20% to 50% in the mixture and the results demonstrated that improvement in FVFMSW percentage enhanced the methane yield from 0.23 to 0:45 m^3 CH_4/ kg VS added, and caused the VS reduction to decrease slightly. However the authors also reported that increasing the proportion of chicken manure in the feed which is high in ammonia caused a steady deterioration in biogas and VS reduction digester performance.

However, anaerobic co-digestion of algal sludge and waste paper (which is high source of carbon but tougher to degrade) was reported by (Yen, et al., 2007)Algal sludge (low C:N ratio) usually appears to have unbalanced nutrients and it is regarded as an important limitation factor to anaerobic digestion process. Adding the high carbon content of waste paper into the algal sludge feedstock to have a balanced C:N ratio was undertaken prior to addition to semi-continuous AD at HRT of 10 days and OLR 2, 4 and 6 gVS/L/d. The results reported that adding 50% (based on VS) of waste paper into an algal sludge feedstock increased the methane production rate 2 fold to 1170±75mL/L day, as compared to 573± 28mL/L day of algal sludge digestion alone, when operated at 4 g VS/L day, 35 °C and 10 days HRT. The maximum methane production rate of 1607±17 mL/L day was observed at a combined 5 g VS/L day loading rate with 60% (VS based) paper and to 40% algal sludge feedstock. While it appears high an optimum C:N ratio for co-digestion of algal sludge and waste paper in the range of 20–25/1 was suggested in this study. Anaerobic co-digestion of OFMSW (diluted pet food) and fat oil and grease (FOG) coming from food industry was conducted in a pilot plant semi-continuous mesophilic reactor in the range at an HRT 17 days by (Fernandez, et al., 2005). Fat content in the feedstock was gradually increased up to 28% (VS basis) during the test until reactors came to a steady state. No accumulation of VFA was detected by the authors due to co-digestion of a readily degradable OFMSW with the potentially higher organic content of the FOG. After a short adaptation period, total fat removal throughout the experiment was reported at over 88%, although biogas and methane yields were very similar to those of simulated OFMSW. At steady state the digester, treating a simulated OFMSW had the highest VS removal (73%), with typical yields of biogas and methane generation (0.8m^3biogas/kg of TVS degraded, 0.5m^3 methane/ kg of VS degraded, 58% methane in biogas). The authors concluded that as both fats from animal and vegetable origin were almost completely degraded at high percentages (94% for animal fat and 97% for vegetable fat), which confirmed that anaerobic co-digestion technology was suitable for treatment of OFMSW and FOG.

Sosnowski, el at., (2003) investigated methane production of sewage sludge and OFMSW under thermophilic and mesophilic conditions. Co-digestion experiments were performed both in batch and semi-continuous reactors at different mixing condition of OFMSW and sewage sludge. Average OLRs were in the range of 0.39-3 gVSS/dm^3/d while results biogas yield were 0.019- 0.023 dm^3/ dm^3 per gVSS respectively over that OLR range. The effect of co-digestion of sludge and OFMSW improved the C:N ratio of the blend mixture over that of the single substrates sludge which resulted in enhancement in biogas production from the co-digestion. The authors reported that biogas production was achieved more slowly at higher rather than at lower organic load but also reported that the cumulative biogas production(CBP) of the mixtures of sewage sludge and the OFMSW increased with increasing proportions of the OFMSW. The biological efficiency of methane production in the semi-continuous reactors was 82% which is much greater than that of batch test at 49.3%. Although this research concluded that anaerobic co-digestion of sewage sludge and OFMSW seems to be an attractive method for environmental protection and energy savings one of the key issues was the determination of the correct proportion of co-substrates which was not suggested in this study's conclusions.

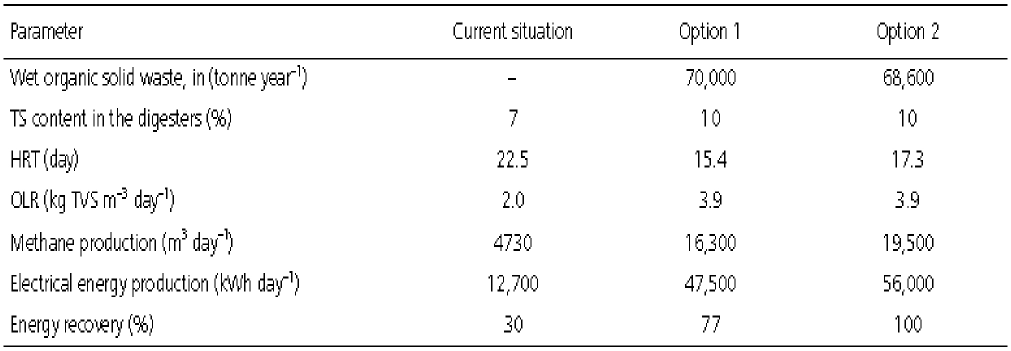
The effect of proportional difference between different substrates and AD phase separation was described in a study performed by Schmit, et al., (2001) using synthetic OFMSW (50% office paper, 10% newspaper, 26% grass clippings, and 14% dog food that contained 21.0% minimum crude protein, 9.0% minimum crude fat, 4.0% crude fiber, and 12.0% maximum moisture by weight. 30-mm pieces of bleached paper and newspapers were blended before feeding). Proportional effects of substrate co-digestion ratios of 0:100, 20:80, 40:60, 60:40, and 80:20 OFMSW-PS on a total solids (w/w) basis were digested in semi continuous phased digesters. Temperature Phased Anaerobic digesters (TPAD thermophilic/mesophilic) were used in this study. Results demonstrated that the temperature-phased system had VS destruction ranging from 47.5% to 71.6 % which was greater than the two-phase system, 39.6 % to 69.3% at OFMSW weight percentages up to 60% while at 60 and 80% OFMSW, the systems had comparable VS removal efficiencies. The authors found the maximum methane production rate was 0.42 to 0.02 L/g VS fed for the temperature phased system and 0.33 to 0.01 L/g VS fed for the two-phase system. Despite the fact that the TPAD system consistently had higher specific hydrolysis rates, differences in overall system performance were less evident at the 60:40 and 80:20 feed ratios. The study indicates co-digestion of OFMSW and sludge in a temperature phased system was better than a two phase reactor system has a better AD process performance.

Anaerobic co-digestion performance of the FVFMSW with sludge was evaluated by Gomez, et al., (2005) at different mixing conditions and different OLRs. At OLR of 2.5 gVS/d daily average biogas production was 0.5 and 0.8 L/day when mixing was done at high and low rate respectively. Under low mixing conditions, the systems were able to absorb the disturbance of a shock load compared to the high mixing condition. Again according to the authors the application of a sudden increase in the organic load of the co digestion systems led to higher gas production, accompanied by a downgrading of the performance of the digesters.

A similar study done by Stroot, et al., (2001) concluded that anaerobic sludge effluent served as an excellent inoculum to accomplish a fast start-up of co digesters treating OFMSW and sewage sludge in continuously mixed AD systems operated at low OLR of 3.5–3.7 g VS/L active volume/day or for minimally mixed systems at higher organic loading rates of 9.4 g VS/L active volume/day. Furthermore, start-up without an appropriate inoculum was found to be slower, but resulted in more resistance and resilience during short-term disturbances. They also demonstrated that minimally mixed, high solids digestion of OFMSW and pre-thickened sewage sludge resulted in excellent performance at a 17-day HRT and an OLR of 11.2 g VS/L active volume/day. High gas production rates of 5.5 L biogas/L active volume/day and specific gas productions of 0.49 L biogas/gVS$_{added}$/day were observed for those operational conditions. Continuous, vigorous mixing was found to be inhibitory for reactors operated at a high OLR, possibly due to the disruption of syntrophic anaerobic microbial relationships and spatial juxtaposition. The above studies highlight that co-digestion of OFMSW and sewage sludge is beneficiary to reduce mixing costs.

Full scale anaerobic co-digestion of PS with OFMSW was carried out in Kayseri Turkey MWWTP at two operational conditions. One reactor co-digested PS and mechanically separated OFMSW and other PS and source separated OFMSW (Dereli, et al., 2010). AD in both cases was carried out with 70,000 ton of wet organic waste per year having similar TS content and concomitant similar OLRs. Results shown in Table 2-10 indicated that digestion of PS and mechanically separated OFMSW and PS and source separated OFMSW produced four and five times more methane (m^3 day^{-1}) respectively compared to the control treating only PS again indicating the advantages of co-digestion.

Table 2-10 Results field study done in Turkey :(After Dereli et al.,2010)]



Parameter	Current situation	Option 1	Option 2
Wet organic solid waste, in (tonne year^{-1})	–	70,000	68,600
TS content in the digesters (%)	7	10	10
HRT (day)	22.5	15.4	17.3
OLR (kg TVS m^{-3} day^{-1})	2.0	3.9	3.9
Methane production (m^3 day^{-1})	4730	16,300	19,500
Electrical energy production (kWh day^{-1})	12,700	47,500	56,000
Energy recovery (%)	30	77	100

The authors commented that the additional biogas due to co-digestion increased electrical energy by a factor of 3 over the year. In other words additional energy can be recovered from co-digestion of SS-OFMSW and municipal sludge which could be a good example for cities like Toronto and Ottawa that collect source separated OFMSW and have unused digester capacity at MWWTP due to recent capital expansions.

Chapter 3- Materials and Methods

3.1 Experimental Design

This study which focused on co/tri-digestion of OFMSW, TWAS and PS was performed in six graduated steps from waste characterization to continuous anaerobic performance. Steps are illustrated in Figure 3-1 and summarized below.

- First step, sample collection and characterization to obtain the fundamental and basic information about the nature of the substrates to be used.
- Second step, initial BMP test were conducted to obtain preliminary results for the entire research by first determining the biodegradability of the individual substrate and their feasibility for potential anaerobic waste stabilization improvements via their co/tri-digestion.
- Third step, BMP assays were conducted for the evaluation of co/tri-digestion of OFMSW, TWAS/PS and batch reactor efficiency in terms of TS, VS, TCOD removal.
- Fourth step MW pre-treatment of TWAS to determine the extent of improvements of solubilization of TWAS which will be used as a co-substrate with OFMSW and PS.
- Fifth step, BMP tests (second set) to demonstrate the effect of MW pre-treatment of TWAS while tri-digested with OFMSW and PS at different mixing ratios and batch reactor efficiency in terms of TS and VS removal.
- Sixth step, semi-continuous flow anaerobic reactor test (based on the optimum condition of batch study) to explain potential improvements in the reactor

performance while illustrating a practical scenario of a MWWTP where the effect of tri-digestion of OFMSW, TWAS and PS vs. co-digestion of TWAS and PS was evaluated and along with the MW pre-treatment effect of TWAS on anaerobic co/tri-digestion.

3.2 Materials

TWAS and PS were used in this study as co-substrates to evaluate their effect on the co/tri-digestion of OFMSW. Among all these three substrates, OFMSW was prepared in the lab while TWAS and PS were collected from the local MWWTP. Sample collection, preparation and storage procedure is discussed in the following sections.

3.2.1 TWAS and PS collection

TWAS (approx. 5% w/w solid) and PS (approx. 5% w/w solid) were obtained from the Robert O. Pickard Environmental Center (ROPEC) located in Gloucester, ON Canada. ROPEC is a waste water treatment plant with preliminary and primary treatment facilities followed by a conventional activated sludge plant with an average SRT of 5-7 days. ROPEC uses centrifugation to thicken waste activated sludge from approximately 1% to 5% solids concentration. Ferric chloride is added to WAS for phosphorus removal prior to thickening. TWAS and PS are blended in a 58:42 (v/v) ratio and undergo mesophilic anaerobic digestion to produce a stabilized bio-solids product for disposal. TWAS and PS were transported to the lab in 5 L plastic tanks. The containers were not fully filled (75%) to leave some headspace in the tanks for any gas production. TWAS and PS were collected prior to the tests and stored in the lab at 4^{o}C.

3.2.2 TWAS and PS Storage

TWAS and PS were stored in side arm volumetric tanks and the mouth of the tanks was not closed tightly enough not to initiate anaerobic condition while they were stored. TWAS and PS were stored at 4˚C. Storage tanks were mixed vigorously by hand prior to their use to get representative samples every time considering the potential for thicker sludge at the bottom of the tanks due to settlement. Sludge samples were brought to room temperature every time before their use as feed for BMP assays, semi-continuous reactor or for sample characterization.

3.2.3 Preparation of OFMSW

Model OFMSW was prepared in the lab with constituents which simulate green bin waste or kitchen waste. Instead of using actual kitchen waste, a model OFMSW was used in this study in order to maintain a consistent homogeneous waste composition throughout the whole study. The OFMSW includes components from the major food groups that are representative of food used in Canadian kitchens and was derived based on the Canadian Food Guide (Shahriari, et al., 2011). The components of the OFMSW used in this study are shown in Table 3-1. The model waste was prepared the day before experiments were performed. The waste was mixed in a kitchen food processor to generate a particle size range between 1-5 mm and the mixture almost looked like a paste. The resulting mixture was stored in zipper-lock storage bags at 4°C.The waste was allowed to reach room temperature prior to the testing.

Rice and macaroni pasta was boiled for 20 minutes in a rice cooker prior to the preparation of the waste and all the vegetable and fruits were used as fresh. 1% of the waste combination consisted of biodegradable/compostable plastic garbage bags which were cut into 2.5x2.5 cm (approximate) size squares. BPI compostable bags were used in this study with the product code mark of 972-CTCOMP20. Characterization of the model OFMSW has been done and is described in chapter 4.

Table 3-1 Model -OFMSW composition

Composition	**Percentage (%w/w)**
Cooked rice	17.5
Cooked pasta	17.5
White cabbage	11
Carrot	11
Banana	11
Apple	11
Cooked ground beef	10
Dog food	10
Biodegradable Plastic bags	1
Total	100

3.2.4 Anaerobic Mesophilic Inoculum

Anaerobic inoculum used in this study was obtained from the ROPEC. The anaerobic inoculum was maintained in a 5-L side-armed volumetric flask reactor. The flask was placed on a shaker agitated on a New Brunswick Scientific Controlled Environment Incubator Shaker model G-25 at rotating at 90 rpm at 35°C. Characterization of inoculum was performed before it was used for BMP and semi-continuous flow reactor experiments. It had a specific methanogenic activity of 0.05 gVS/gVS.d. (Based on plant data)

3.3 Experimental Setup and Protocols for BMP Assays

BMP batch assays (Owen, et al., 1979), were conducted in order to determine the suitability of OFMSW, TWAS and PS for anaerobic co-digestion and biogas production for energy recovery. Prior to the start of each batch test, characterization of inoculum and substrates were conducted, verifying TCOD, TS,VS, ammonia concentration, alkalinity and pH, as described in the following section, 3-6 Analytical Methods.

Identical experimental set-up and sampling procedures were carried out for each set of batch tests. Five sets of BMP tests were conducted in duplicates, evaluating different conditions for the co/tri-digestion of OFMSW and municipal sludge. The BMP assays conducted were:

- An initial suitability study to evaluate the digestion of OFMSW, TWAS and PS as single substrate.
- An investigation into the effects of co-digestion of OFMSW and TWAS in terms of biogas production and reactor organic removal efficiency.
- A study of tri-digestion of OFMSW, TWAS and PS was evaluated by a third BMP test in terms of biogas production and reactor organic removal efficiency.
- Three sub-sets BMP assays identical to the BMP assay at step two, to evaluate the effects of MW pre-treatment of TWAS at different pre-treatment conditions on co-digestion OFMSW and $TWAS_{MW}$ vs. OFMSW and $TWAS_{no\text{-}pretreatment}$.
- Three more sets of BMP assay identical with the BMP assay in step three to examine the potential of $TWAS_{MW}$ at different pre-treatment conditions on tri-digestion of OFMSW, $TWAS_{MW}$ and PS vs. OFMSW, $TWAS_{no\text{-}pretreatment}$ and PS.

3.3.1 Experimental Set-up

500 mL Kimax glass bottles capped with butyl rubber stoppers were used to perform mesophilic BMP assay test. Total working volume of substrate in each bottle was 360 mL including 60 mL of mesophilic anaerobic inoculum biomass. Equal portion of $NaHCO_3$ and $KHCO_3$ were added to each bottle to achieve an alkalinity concentration of between 4000 and 6000mg/L as $CaCO_3$. Experiment bottles were subsequently sparged with nitrogen gas for 2 minutes to produce anaerobic conditions and to prevent exposure to air and then sealed. Assay bottles were brought to atmospheric pressure prior to incubation by inserting a BD 21G1½ needle connected to a u-tube manometer and allowing the bottle pressure to equilibrate with atmospheric pressure.

Bottles were incubated at 35 ± 1°C and agitated in a New Brunswick Scientific Controlled Environment Incubator Shaker model G-25 at 100 rpm, in order to keep bacteria and substrate in suspension. Biogas production was monitored daily with a BD 21G½ needle connected to a u-tube manometer, while pH, VFAs and biogas composition (methane and carbon dioxide) were typically monitored weekly until the conclusion of the experiment. The experiments were deemed complete when the biogas production rates began to approach zero.

Final characterization of the assay contents was conducted at the conclusion of each assay. Analysis of total and soluble COD, TS and VS, final pH, VFA concentrations, as well as ammonia and alkalinity of the experiment bottles were conducted. All experiments and analyses were carried out in duplicate.

3.3.2 Initial BMP Assay

Initial BMP assays were carried out using OFMSW, TWAS and PS as individual substrate. Characterization tests of OFMSW, TWAS and PS found that their TS was 23, 5.3 and 3.7 % solids respectively. Prior to feeding OFMSW into assay bottles the solid content of OFMSW was diluted to aprox.5% by adding distilled water. Table 3-2 shows the contents of the three initial assays tested.

Table 3-2 Contents of the initial BMP assays

Substrate	**Weight of the Substrate (g)**	**Distilled Water Added (mL)**	**Anaerobic Inoculum (mL)**
OFMSW	50	250	60
TWAS	300	-	60
PS	300	-	60

Experimental bottles were prepared in duplicate and monitored as described in section 3.3.1 Experimental Set-up. Biogas production and pH was monitored daily during the BMP test and pH was always kept in a range of 6.8-7.6. To keep the pH in the desired range alkalinity in terms of an equal mix of $NaHCO_3$ and $KHCO_3$ in liquid form was added as necessary. BMP assays were carried out until biogas production was stopped.

3.3.3 Co/tri-digestion BMP Assay

The first set of co-digestion BMP assays were carried out at three different concentrations of OFMSW and TWAS. Table 3-3 shows the combination and concentration of the substrates. All the BMP assays were identical in terms of substrate volume, initial organic load and initial anaerobic inoculum charge. Experimental bottles were prepared in duplicate and monitored as described in section 3.2.1 Experimental Set-up. Biogas production and pH was monitored daily during the BMP test and pH was always kept in a range of 6.8-7.6. To keep the pH higher in the desired range alkalinity was added to the assay in terms of an equal mix of $NaHCO_3$ and $KHCO_3$ in liquid form was added on requirement necessary. BMP assays were carried out until biogas production was stopped.

Table 3-3 Contents of the co-digestion BMP assays of OFMSW and TWAS with and without TWAS pre-treatment

Substrate	**Co-digestion combination (w:w)**	**OFMSW (g)**	**Distilled Water Added(mL)**	**TWAS (mL)**	**Anaerobic Inoculum (mL)**
OFMSW:TWAS	25:75	12.5	62.5	225	60
OFMSW:TWAS	50:50	25	125	150	60
OFMSW:TWAS	75:25	37.5	187.5	75	60

The second set of tri-digestion BMP assays were carried out at three different concentrations of OFMSW, TWAS and PS. Table 3-4 shows the combination and concentration of the various substrates. All the BMP assays were identical in terms of substrate volume, initial organic load and initial anaerobic inoculum charge. Experimental bottles were prepared in duplicate and monitored as described in section 3.2.1 Experimental Set-up. Biogas production and pH was monitored daily during the

BMP test and pH was always kept in a range of 6.8-7.6. Alkalinity in terms of an equal mix of $NaHCO_3$ and $KHCO_3$ in liquid form was added in the assay to keep the pH higher and in the desired range was added as necessary. BMP assays were carried out until biogas production stopped.

Table 3-4 Contents of the tri-digestion BMP assays of OFMSW, TWAS and PS with and without TWAS pre-treatment

Substrate	Tri-digestion combination (w:w)	OFMW (g)	Distilled Water Added(mL)	TWAS (mL)	PS (mL)	Anaerobic Inoculum (mL)
OFMSW:TWAS:PS	25:37.5:37.5	12.5	62.5	112.5	112.5	60
OFMSW:TWAS:PS	50:25:25	25	125	75	75	60
OFMSW:TWAS:PS	75:12.5:12.5	37.5	187.5	37.5	37.5	60
TWAS:PS	50:50	0	0	150	150	60

3.3.4 Co-digestion of OFMSW with TWAS after MW Pre-treatment

To evaluate effect of MW pre-treatment of TWAS while co-digested with OFMSW another set of BMP assays were conducted in three sub-sets. Each subset was different than the others based on the MW pre-treatment condition of TWAS. TWAS was pre-treated with MW from room temperature to 135˚C at a constant intensity (temperature ramp) of 2.5˚C/ min. Once the pre-treatment temperature was reached that temperature was held for three different holding times (HT). HT used was 1, 10 and 20 minutes. Procedure of MW pre-treatment has been described in the section 3.4. Despite having different pre-treatment conditions for TWAS all the BMP assays were conducted at similar amount of VS content and inoculum charge and assays were carried at pH range of 6.8-7.6. Substrates combinations and concentrations of substrates were identical in each subset and as described in Table 3-3. pH adjustment was done adding an equal mix of $NaHCO_3$ and $KHCO_3$ in liquid form as necessary. BMP assays were carried out until biogas production stopped. Characterization of co-substrate was done before and after digestion and results are discussed in chapter 4.

3.3.5 Co-digestion of OFMSW, TWAS and PS

To evaluate effect of MW pre-treatment of TWAS while digested with OFMSW and PS, a final set of BMP assays were conducted in three sub-sets. Each subset was different than the others based on the pre-treatment conditions of TWAS. As mentioned in section 3.3.4 TWAS was pre-treated with MW from room temperature to 135˚C at a constant intensity of 2.5˚C/ min followed by the 3 different HTs. Co-substrate combinations and concentration of substrates were identical in each subset and were identical to what was described in Table 3-4. And assays were carried at pH range of 6.8-7.6. pH adjustment was done adding an equal mix of $NaHCO_3$ and $KHCO_3$ in liquid form as necessary. BMP assays were carried out until biogas production stopped. Characterization of co-substrate assays was done before and after digestion to evaluate the biological methane potential efficiency in terms of biogas production and VS, TCOD removals and results are discussed in chapter 4.

3.4 MW Pre-treatment

MW pre-treatment of TWAS was carried out with a Mars 5© (MW Accelerated Reaction System; CEM Corporation) MW oven. Mars 5© has a frequency of 2450 MHz and can deliver 1200 W ± 15% of the MW energy at full power with complete MW penetration of the sample vessels. With its temperature and pressure it is possible to monitor and control operating conditions up to 250˚ C and 34.5 bars.

3.4.1 Experimental Set-up for MW Pre-treatment

MW heating of TWAS was done in 40 Teflon vessels and each vessel was filled with 40 g of TWAS so that each of the vessels gets equal amount of pressure. Vessels were rotating on the carousel. Figure of the MW oven is shown in the appendix A. Sludge samples were brought to room temperature (25˚C) before MW heating. After the targeted temperatures and HTs were reached, samples were removed from the heating source and let them cool down to room temperature in closed vessels to avoid evaporation of organics. MWed samples were stored in plastic containers at 4˚C until need for other experiment.

A multilevel factorial design was used to investigate the variables affecting efficiency of pre-treatment which were chosen to be: (1) pre-treatment temperature, and (2) exposure/ HT at desired temperature. (Table 3-5 and Figure 3-1).Intensity of microwaving (the MW intensity was programmed such that the temperature change per unit time is constant) was kept as low as the intensity of conventional heating (2.5 ˚C/ min). Figure 3-2 illustrates relation between temperature and intensity function in the pre-treatment test.

Table 3-5 Variables and their levels used in the statistical design

Variables → **Levels ↓**	**Temperature ˚C**	**HT at Targeted Temperature min**
1	95	1
2	115	10
3	135	20

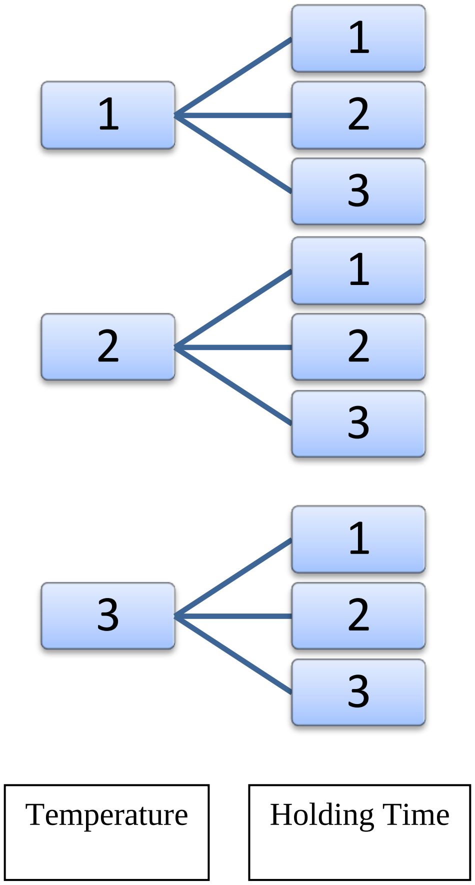


Figure 3-1 Illustration of the multilevel factorial design used

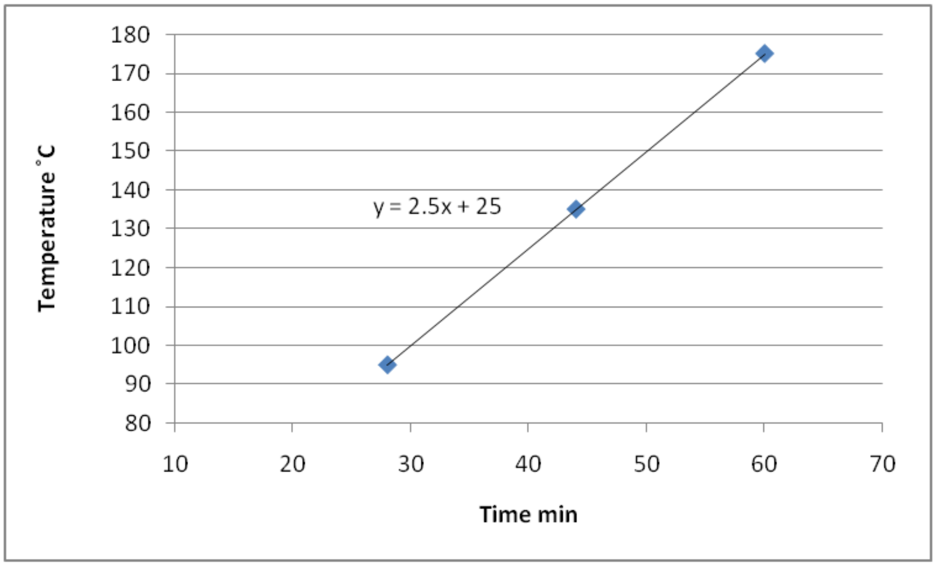


Figure 3-2 Time and temperature relationship at a constant intensity

Figure 3-2 shows the relationship between final pre-treatment temperature and time requirement to reach the desired temperature by the MW oven when intensity was programmed at 2.5 ˚C/ min. With starting point sludge sample temperature of 25˚C the required time and targeted temperature can be expressed by the following equation 3.1

$$\boxed{\text{Temperature} = 2.5 \times \text{Time} + 25 \qquad \text{Eqn.3.1}}$$

The effect of elevated temperatures and elongated holding time was measured in terms of solubilization of TWAS by determining the sCOD/TCOD ratio of the micro-waved samples. Analytical measurement in terms of sCOD, TCOD, pH, VS and TS were performed before and after MW heating of TWAS (procedures are described in the next segments)

3.4.2 Ultimate Solubilization of TWAS

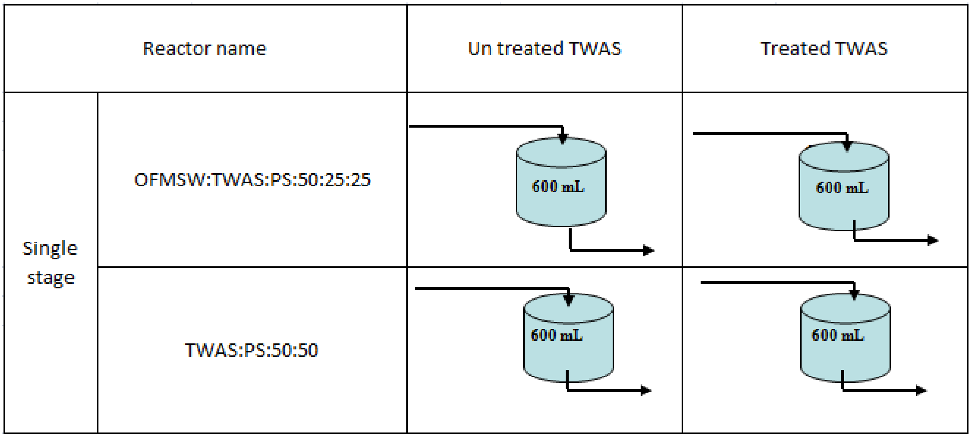
Ultimate solubilization of TWAS was performed by addition of NaOH to the raw TWAS based on the procedure of (Bougrier, et al., 2005).In a 1L glass bicker 10 mL TWAS was submersed into 100 ml NaOH (1N) for 24 hours in room temperature and the sCOD and TCOD was measured after 24 hours.

3.5 Semi-Continuous Anaerobic Digestion Reactors

3.5.1 Semi-continuous Reactor Combinations

Mesophilic anaerobic semi-continuous single stage reactors were carried out at four different conditions and ran at three different HRTs and OLRs to evaluate the effect of tri-digestion of OFMSW,TWAS and PS and effect of MW pre-treatment of TWAS in co/tri-digestion. Three SRTs 15, 10 and 7 days (SRT was equal to HRT) were examined (Mata-Alvarez, J., 2003) in this study. OLRs at HRTS of 15, 10 and 7 days were 2, 3.5

and 4.5 gVS/L/d respectively. Reactors were always operated by sampling first followed by feeding. Figure 3-3 shows the combination of the semi-continuous reactors operated. There were four reactors in total. One of the first two reactors was treating OMSW:$\text{TWAS}_{\text{MW:}}$:PS at 50:25:25 combination while other one was fed with OMSW:TWAS:PS at 50:25:25 ratio. Feed for the other two reactors was TWAS_{MW}:PS:50:50 and TWAS:PS:50:50 respectively. Reactor set-up for the semi-continuous flow is discussed in next segment.



Reactor name		Un treated TWAS	Treated TWAS
Single stage	OFMSW:TWAS:PS:50:25:25	600 mL	600 mL
	TWAS:PS:50:50	600 mL	600 mL

Figure 3-3 Semi-continuous mesophilic anaerobic reactor combinations

3.5.2 Semi-continuous Reactor Set-up

Schematics of the reactors have been shown in Figure 3-4.1-L Erlenmeyer volumetric flasks were used as semi-continuous flow reactors (Figure is shown in the appendix A). Flasks were tightly closed with a rubber stopper. Two holes were made in the stoppers; one of the holes was used for feeding the reactor by attaching a 10-cm piece of tubing. A 5-cm piece of hard plastic tube was inserted in the other hole which was connected with tubing to a 1-L Tedlar bag for the collection of biogas. Holes were made slightly smaller than the diameter of the tube used to ensure that no gas leakage occurred from these ports. A bigger port was also used to withdraw the effluent samples from the reactor; effluent tubing was tightly closed with a castaloy screw compressor clamp. Before starting, the reactors were tested for air-tightness by pressurizing the reactors with nitrogen gas and immersing them in water. Any apparent leaks are indicted by bubbles in the water. A Leak-Tec® leak detector liquid was also used to test the air-tightness of the reactors when not submerged in water but it was not as effective. The working volume in the reactors was 600 mL and (of which) 500 mL of anaerobic inoculum mixture were added to each reactor. Daily flow rate to the reactors were different based on the HRT of the reactor. Daily flow rates were 40, 60 and 86 mL/d at HRTs of 15, 10 and 7 days respectively. A mixture with equal amounts of $KHCO_3$ and $NaHCO_3$ was added prior to the start of each reactor to ensure an alkalinity concentration of 4000 mg/L alkalinity as $CaCO_3$. The reactors were purged for about 3-4 minutes with nitrogen gas before finally sealed with the stoppers and adding a layer of silicone all around the connecting areas between the glass and the stopper, stopper with tubes and tubes with tubing to ensure gas-tightness of the reactors.

All reactors were kept at 35°C ±1°C in a (New Brunswick Scientific Co. Inc., NB, Canada) incubator model G-25 which was set at a 90 rpm rotational speed.

To maintain the desired HRT a constant volume effluent sample was withdrawn and the same volume of feed was added daily for each reactor. The effluent was extracted using a 50 mL syringe that was adapted to fit tightly into the sampling tubing. After connecting the syringe to the sampling tubing the screw compressor clamp was opened, then the desired volume of effluent/ feed was withdrawn/ added. Finally the compressor clamp was closed tightly before removing the syringe. The mixture of OFMSW, TWAS/PS as feed samples for reactors was prepared prior to feeding every day; samples were brought to room temperature before the feeding process. All the reactors were run at controlled pH of 6.8-7.6.To maintain pH range of 6.8-7.6, buffer in form of $KHCO_3$ and $NaHCO_3$ was added with the feed when needed. Reactors were considered achieving steady state when stabilization of the daily biogas production occurred (biogas production fluctuate within a 10% range).

The biogas production was measured daily by emptying the Tedlar bags using a u-tube manometer and gas composition was measured every two weeks. Effluent TCOD, sCOD, pH, TS, VS, alkalinity and ammonia were determined.

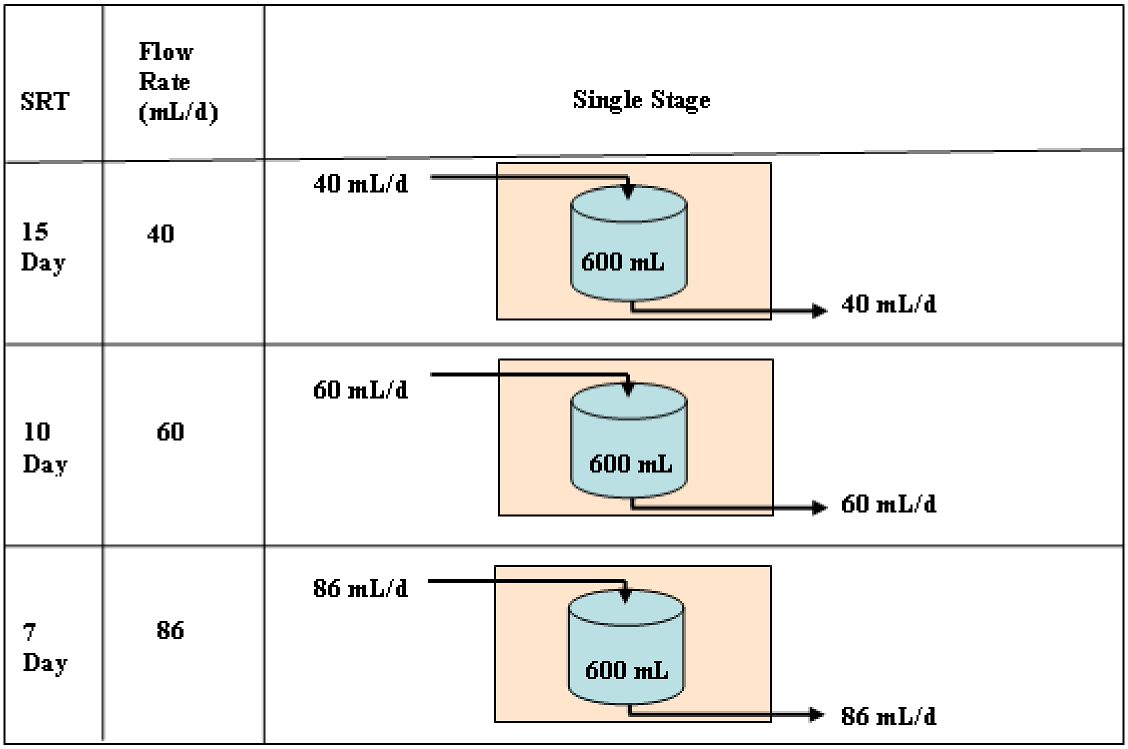


Figure 3-4 Schematics and flow rates of semi-continuous reactors

3.6Analytical Methods

3.6.1 Frequency of Analytical Experiments

Table 3-6 illustrates the frequency of the analytical experiments done before/after characterization of influent/effluent of the reactors as well as for samples.

Table 3-6 Frequency of the analytical experiments

Parameter	Before MW pre-treatment of TWAS	After MW pre-treatment of TWAS	Influent BMP assay	Effluent BMP assay	Influent Semi-continuous flow reactors	Effluent Semi-continuous flow reactors
pH	Before Test	After Test	Before Test	Daily	Once/ month	Daily
TS	Before Test	After Test	Before Test	After Test	Once/ month	Thrice /week
VS	Before Test	After Test	Before Test	After Test	Once/ month	Thrice /week
Ammonia	Before Test	After Test	Before Test	After Test	Once/ month	Twice /week
VFA	Before Test	After Test	Before Test	After Test	Once/ month	Twice /week
Alkalinity	Before Test	After Test	Before Test	After Test	Once/ month	Twice /week
TCOD	Before Test	After Test	Before Test	After Test	Once/ month	Twice /week
SCOD	Before Test	After Test	Before Test	After Test	Once/ month	Twice /week
Biogas production	n.a.	n.a.	n.a.	Daily	n.a.	Daily
Biogas composition	n.a.	n.a.	n.a.	Once /week	n.a.	Once /week

3.6.2 pH

The pH of all samples, substrates and inoculum was determined using a Fisher Accumet model XL25 dual channel pH/ion meter equipped with a glass electrode (serial number 498442). The pH electrode was stored in a pH 7 buffer solution and was removed, rinsed with distilled water, and dried with Kimwipes task wipers before sample pH was measured. The electrode was returned to the buffer solution, rinsed again with distilled water and dried between each pH measurement.

Sample pH was typically measured at room temperature, with the exception of continuous reactor effluent samples. The pH of continuous reactor samples was measured immediately after being removed from the reactors in order to reduce the extent of pH change due to the escape of carbon dioxide from solution. It is important to know the pH of the continuous reactors at the operating temperature, which was maintained at 35 ± 1°C, as it gives a more accurate representation of reactor performance. Therefore pH was measured while the continuous reactor samples were still warm, as the pH at room temperature would be less representative of the actual reactor operating conditions. (Wilkinson, 2011)

3.6.3 Alkalinity

Alkalinity determination was carried out according to Standard Method 2320B. Samples were centrifuged in a Thermoscientific Sorvall Legend T+ model centrifuge at 10,000 rpm (relative centrifugal force of 11,292) for between 45 and 80 minutes depending on the type of sample. Following centrifugation, samples were poured onto and filtered through 47mm diameter sterile 0.45μm filters by applying a vacuum using a Fisher Scientific pump. (Wilkinson, 2011)

A known volume of filtered sample was then poured into a Pyrex beaker along with a magnetic stirring rod and was placed on a Thermix stirrer model 120MR, set at speed 5. Sample pH was then measured with the Fisher Accumet XL25 dual channel pH/ion meter. Titration of the sample was carried out using 0.1N sulfuric acid dispensed through a 25 mL Kimax burette, and the volume of acid required to reach a pH of 5.4 and 4.25 was recorded for partial alkalinity (PA) and total alkalinity (TA) respectively. The pH electrode was thoroughly rinsed with distilled water and dried with Kimwipes between each sample. Alkalinity in terms of mg/L was the determined based on the results of the titration with the following equation:

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A\ x\ N\ x\ 50000}{mL\ sample\ \ titrated} \qquad \text{Eqn. 3.2}$$

Where:

A= mL standard acid used

N = normality of standard acid

Reactor stability test was performed using the data obtained from PA and TA.

Calculations:

$$IA = TA - PA \qquad \text{Eqn.3.3}$$

Where IA = Intermediate alkalinity, mg $CaCO_3/L$

IA performed on effluent from the reactors which represents the amount of VFA concentration in the reactor. (ORWC, 2011)

SR= IA/ TA Eqn.3.4

Where SR = Stability Ratio of reactor, mg $CaCO_3$/L

Notes:

SR should not be greater than 0.3 for a stable digester. A SR range between 0.1 and 0.2 is acceptable .A range of 0.07 to 0.08 is a good working ratio for the ractor. (ORWC, 2011)

3.6.4 Ammonia

Standard method 4500D was used to determine the dissolved ammonia (NH_3 and NH_4^+) concentration present in sample supernatant. Samples were centrifuged with an RCF of 11,292 for between 45 and 80 minutes in a Thermoscientific Sorvall Legend T+ model centrifuge, equipped with a Heraeus rotor #3334. Following centrifugation, samples were poured onto and filtered through 47 mm diameter sterile 0.45μm filters by applying a vacuum using a Fisher Scientific pump. A Fisher Accumet pH/ion meter model 750 equipped with an Accumet ammonia ion selective electrode was used for sample measurement. The electrode was stored in a 10 mg NH_3-N/L solution when not in use, and was placed in a 10mg NH_3-N/L solution in between sample measurements. (Wilkinson, 2011)

A three-point calibration curve was prepared each time the probe was used. One millilitre of 10N NaOH solution was added to each standard solution (10, 100 and 1000mg NH_3-N/L) to ensure a pH of greater than 11 before ammonia measurement was carried out. Likewise, a drop of 10N NaOH was also added to sample supernatant used for analysis, to ensure a pH greater than 11. Samples were then poured into Pyrex beakers with magnetic stir rods, and placed on a Thermix stirrer 120MR set at speed 5.The ammonia electrode was thoroughly rinsed with distilled water and dried with Kimwipes between each sample. When possible, measurements were carried out in duplicate. Measurements were taken in ± millivolts (mV), and converted to mg NH_3-N/L with the use of the calibration curve equation. (Wilkinson, 2011)

3.6.5 Total and Soluble Chemical Oxygen Demand

TCOD/sCOD was carried out according to the closed reflux, colorimetric Standard Method 5220D. Procedures for preparation of the digestion solution and catalyst required for COD determination can be found in the Standard Methods. (Wilkinson, 2011)

An eight-point calibration curve was prepared using an 850mg/L stock solution of potassium hydrogen phthalate, which has a theoretical COD of 1000mg O_2/L. Standard tubes were prepared by diluting the stock solution to achieve the following chemical oxygen demand concentrations: 0, 100, 200, 300, 400, 500, 600 and 700 mg/L. Once the appropriate dilutions had been made, 10mL of each standard was dispensed along with 6 mL of COD digestion reagent and 14mL of the sulfuric acid catalyst to prepare each standard tube. All standard tubes were prepared in duplicate, with the exception of the 0 mg/L and 500mg/L standards, which were prepared in triplicate. Tubes were then capped and vortexed for four seconds using a Fisher vortex Genie 2^{TM} and placed in a Precision mechanical convection oven set at 150 degrees Celsius for three hours (plus or minus 15 minutes). After three hours, the tubes were removed from the oven and allowed to cool in a dark place overnight. The next day, the outside of the tubes were cleaned with distilled water and dried with Kimwipes prior to absorbance readings. The absorbance of the standard tubes was measured at a 600nm wavelength with a Coleman Spectrophotometer model 295.Corresponding absorbance were recorded and used to generate a standard curve to determine the COD concentration (in mg O_2/L) of prepared experiment samples. (Wilkinson, 2011)

TCOD determination made use of a well mixed sample, while sCOD required the use of filtered sample supernatant, and as such, sCOD samples were prepared in the same manner as previously described for alkalinity determination. Once filtration of all supernatant samples was complete, dilution of both fully mixed samples (for TCOD) and filtered supernatant (for sCOD) were carried out to ensure sample absorbance would fall within the range of the calibration curve. Dilution was conducted with the use of 5 and 10mL serological pipettes, as well as a 10mL Pyrex graduated cylinders. Volumes were weighed to ensure accurate measurements. Samples were diluted in a 100mL volumetric flask for TCOD and in a 10mL volumetric flask for sCOD. Once the appropriate dilutions had been made, 10mL of sample (for both total and soluble COD) was measured and dispensed into Kimax tubes, which were then prepared and digested as described above for the standard curve. All experiment samples were analyzed for TCOD and sCOD in duplicate. (Wilkinson, 2011)

Once the sample tubes were digested, cooled overnight and cleaned, the absorbance of the solution in the tubes were measured following the calibration of the spectrophotometer with the prepared standard curve. Sample absorbance was recorded and subsequently used in the calibration curve equation to determine the corresponding concentration of COD (total or soluble) in mg O_2/L. (Wilkinson, 2011)

3.6.6 Total and Volatile Solids

TS and VS were analyzed according to Standard Method 2540G and all samples were analyzed in duplicate. Prior to solids determination, porcelain evaporating dishes were prepared by scrubbing with soap and water, followed by soaking in a 10% (v/v) sulfuric acid solution over night. The dishes were removed from the sulfuric acid solution the following morning, rinsed with distilled water and subsequently placed in a Thermolyne 62700 muffle furnace model F62730 at 550 degrees Celsius, to ensure no organic residues remained. After 60 minutes the evaporating dishes were removed from the muffle furnace and allowed to cool for 15 minutes in a Precision mechanical convection oven model 23 maintained at 105 degrees Celsius. The dishes were then transferred to desiccators to cool completely for another 60-minute period. (Wilkinson, 2011)

Following desiccation, each dish was placed on a Mettler Toledo Classic Plus Model AB204 – s/fact analytical balance and the weight (W) was recorded. A well-mixed sample was then transferred into the dish and the weight of the dish with the sample (X) was recorded. The dish containing the sample was subsequently placed in the Precision mechanical convection oven, set at 105°C, until all the water in the sample had evaporated. Samples were typically left in the oven overnight, requiring a minimum of 12 hours for full evaporation. Once oven dried, the dishes were transferred to desiccators for 60 minutes, and then weighed on the analytical balance and the new mass (Y) was recorded. The oven-dried dishes were then placed in the muffle furnace set at 550°C and were ignited for 60 minutes. The dishes were again transferred to the 105°C oven to cool for approximately 15 minutes and subsequently placed in desiccators for another 60 minutes. The final weight of the dishes after desiccation (Z) was noted, and the percentage total and volatile solids were determined using the recorded weights (W, X, Y, Z) and the following equations:

$$\%\ \text{Total Solid (TS)} = \frac{(Y-W)}{(X-W)} \text{ x } 100\ \% \qquad \text{Eqn. 3.5}$$

$$\%\ \text{Volatile Solid (VS)} = \frac{(Y-Z)}{(X-W)} \text{ x } 100\ \% \qquad \text{Eqn. 3.6}$$

3.6.7 Volatile Fatty Acids

Measurement of VFAs was accomplished with the use of an Agilent 6890 Series Gas Chromatography (GC) system equipped with a flame ionization detector, an Agilent 7683 Series auto-sampler and injector, and an Innowax splitless column (30m x 0.25mm ID capillary column, coated with 0.5μm film thickness). HP ChemStation (Rev. 06.03 [509]) chromatographic software was used for sample component separation and identification. The oven temperature was programmed to ramp up to a final temperature of 250°C according to the following sequence: 80°C for 0.2 minutes, followed by an increase from 80 to 120°C at 20°C per minute and held at 120°C for 1 minute, an increase from 120 to 250°C at 25°C per minute and then a final temperature of 250°C maintained for 0.10 minutes. The inlet and detector temperatures were set at 250°C and 300°C respectively. The carrier gas was helium, flowing at a rate of 1.7 mL per minute, and a linear velocity of 43cm/s. Hydrogen and air, at 30 and 400ml/min correspondingly, were used in the GC/FID with N_2 flowing at 25ml/min, as the make-up gas. (Wilkinson, 2011)

A VFA standard mixture was prepared containing 2000mg/L of each acetic, butyric and propionic acid, and an internal standard was prepared with a concentration of 2000mg/L isobutyric acid. Calibration of the GC was accomplished by adding 0.5mL of the standard mixture and 0.5mL of the internal standard to an auto-sampler vial, vortexing followed by placing the vial into the auto-sampler, which injected 0.1μL of sample into the GC for analysis. Injection and re-calibration was iterated until integration resulted in a measured concentration of 2000 ± 50mg/L for acetic, propionic and butyric acids.

Experiment samples were prepared for VFA analysis by centrifugation in 1.5 mL eppendorf tubes in a Brinkmann Eppendorf centrifuge model 5415 at 14,000 rpm for 10 – 15 minutes (RCF of 8984). Centrifuged samples were filtered through an Acrodisc® LC 13 mm diameter 0.2 µm PVDF membrane syringe filter, and 0.5 mL was dispensed into auto-sampler vials, along with 0.5 mL of the internal standard solution. Vials were then vortexed on a Fisher vortex Genie 2^{TM} and placed in the auto-sampling tray and analyzed by the GC. Results were reported after integration by the software in mg/L of acetic propionic and butyric acids. (Wilkinson, 2011)

3.6.8 Biogas Measurement

3.6.8.1 Batch Experiments

Biogas production was measured with the use of a manometer water displacement apparatus for batch bottles. Assay bottles were removed from the incubator and allowed to cool to room temperature prior to biogas volume measurement. A BD 21G1½ needle connected to a U-tube manometer was inserted through the rubber stopper sealing each batch bottle, and were allowed to equilibrate until the pressure in the assay bottle reached atmospheric pressure. The volume of biogas produced was determined by noting the starting and ending point of the water in the burette connected to the manometer. The difference between these two values provided the volume of biogas produced in mL. (Wilkinson, 2011)

3.6.8.2 Continuous Reactors

Biogas produced in the semi-continuous flow reactors was collected in 1 L-Tedlar bags (Chromatographic Specialties Inc., ON, Canada). Daily biogas production (DBP) was measured using a Cole Palmer easy-load pump model 75553-02 with a Masterflex speed controller to pump the contents of the Tedlar bags into a customized U-tube manometer. The biogas volume was calculated by multiplying the height of water column displaced by the biogas and the cross-sectional area of the manometer tube. (Wilkinson, 2011)

3.6.9 Biogas Composition

Biogas composition (consisting of methane and carbon dioxide) was determined with a Hewlett Packard 5712A GC equipped with a metal packed column (Chromatographic Specialties Inc. Brockville, ON, Canada, Porapak T, packing mesh size: 50/80, column length x OD: 304.8 x 0.635cm) a 5705A thermal conductivity detector (oven, inlet and outlet temperatures: 70, 100 and 150°C, respectively) and a 3380A model integrator. Carrier gas was maintained at 25mL of helium per minute. Samples for composition analysis were collected by inserting a BD 21G1½ needle tip attached to a 1mL syringe through the stopper of the batch bottles or the septum of the gas sampling port on the continuous reactors. Gas samples were collected and discharged with the syringe two to three times before a sample was collected for analysis, in order to ensure a representative sample. A 1mL of biogas was taken for analysis, of which 0.5mL was wasted into the air and the remaining 0.5mL was injected manually into the GC. National Instruments™ LabVIEW version 6.0 was installed on the computer connected to the GC. Biogas composition was evaluated by the computer software. (Wilkinson, 2011)

3.7 Sample Preservation

If analysis could not be completed within an acceptable time period, samples were preserved according to the following instructions summarized in Table 3-7, for analysis at another time.

Table 3-7 Sample preservation techniques

Analyses	**Sample Type**	**Preservation**	**Bottle Type**	**Maximum storage time**
pH	Sludge	Refrigerated, 4°C	Plastic or glass	14 days
Alkalinity	Filtered (0.45µm) supernatant	Refrigerated, 4°C	Plastic or glass	14 days
Ammonia	Filtered (0.45µm) supernatant	pH to less than 2, with concentrated H_2SO_4 Refrigerated, 4°C	Plastic or glass	28 days
VFAs	Filtered (0.2µm) supernatant	Frozen, -18°C	Plastic micro-centrifuge tube	
TS/VS	Sludge	Refrigerated, 4°C	Plastic or glass	7 Days
TCOD	Sludge	pH to less than 2, with H_2SO_4 Refrigerated, 4°C	Amber glass	28 days
SCOD	Filtered (0.45µm) supernatant	pH to less than 2, with H_2SO_4 Refrigerated,4°C	Amber glass	28 days

Chapter 4- Results and Discussion

4.1 Characterization Studies

Initial characterization of TWAS, PS and OFWSW substrates and the anaerobic inoculum used for this research was completed prior to the start of each experiment and results are shown in Table 4-1.The results shown are the arithmetic means of a minimum of 4 samples with associated standard deviation. The PS and TWAS was in the range of 3.4 and 5.3% TS respectively as produced at ROPEC while the OFMSW used in the study was much more concentrated at about 23.3% TS. It should be noted that while the VS/TS ratio of the PS and TWAS were similar at about 75% the OFMSW had a much higher organic content at approximately 94%.Consequently the ratio of OFMSW to PS or TWAS when blending various mixtures was very low on a volume basis meaning that a large increase in load was achieved with a small volume of OFMSW. The anaerobic inoculum used for all studies was an active heterogeneous consortium of anaerobic bacteria acclimated to PS and TWAS and had a specific anaerobic activity of approximately 0.05±0.01 gVS/gVS/d.

Table 4-1 Characteristics of TWAS, PS and OFWSW and anaerobic inoculum

Property	TWAS	OFMSW	PS	Anaerobic Inoculum
TS%	5.36±0.11	23.34±0.23	3.42±0.15	3.1±0.10
VS%	3.90±0.12	21.87±0.22	2.64±0.17	1.75±0.11
VS/TS	0.73±0	0.94±0	0.77±0.05	0.57±0.09
TCOD g/L	62.7±0.38	226.6±0.3	25.2±0.3	24.3±0.31
sCOD g/L	13.3±0.3	86.1±0.2	2.00±0.24	0.41±0.26

4.2 Initial BMP Assay

BMP and biodegradability of OFMSW, TWAS and PS as single substrates were evaluated using mesophilic BMP assays with acclimated anaerobic inoculum. Figure 4-1 shows the CBP during the digestion of OFMSW, TWAS and PS. All three BMP assays were designed identically, providing initial anaerobic inoculum charge hence the same initial specific VS OLR so that differences in biogas yield can be used as an indication of bio-degradability and differences in biological methane potential of the substrates when digested individually. One can first notice that no lag phase was evident likely because the inoculum was acclimated to TWAS and PS which are difficult and easy to degrade respectively. No lag phase was observed for the OFMSW but this was not too unexpected as this material has a large component of readily degradable organics.

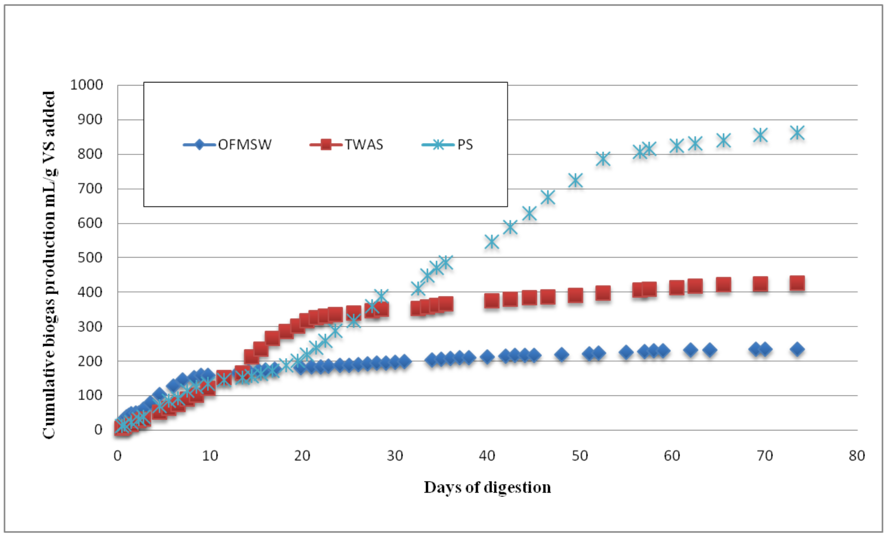


Figure 4-1 Cumulative biogas production from individual substrates (Average of duplicate runs)

Two different and distinct waste degradation patterns could be determined from the individual substrate CBP curves of OFMSW, TWAS and PS. Among these 3 substrates OFMSW which has a high organic fraction (94%) composed of various food type wastes was readily degradable and a rapid CBP phase of 18 mL biogas/gVS$_{added}$/day which produced about 67% of its ultimate biogas production within the first 10 days of digestion. On the other hand, TWAS which is composed of microbial cells with protective cell wall and membrane within a matrix of EPS is a comparatively tougher material to hydrolyze and biodegrade. Based on CBP curve biogas production of TWAS was slower than the OFMSW at a rate of 14 mL/gVS$_{added}$/day requiring about 23 days for achieving about 70% of its ultimate biogas production. Additionally, PS which had about the same VS/TS ratio of TWAS but a high volatile suspended solid content of about 80% of VS seemed to demonstrate a CBP pattern of both the easily degraded OFMSW and more difficult to degrade TWAS during its digestion. Early in the assay, the portion of PS substrate which was readily degradable (similar to the OFMSW) produced biogas rapidly for approximately 10-12 days at about 13 mL/gVS$_{added}$/day followed by a plateau then a slower biogas production rate of 19 mL/gVS$_{added}$/day for the remainder of the assay as the more recalcitrant to degrade material was used for biogas production. VFA concentrations in the BMP assays are presented in Figure 4-2. VFA results show that during the first week of digestion VFA accumulation in the OFMSW BMP assay was 6750 mg/L which is almost 2 and 1.5 fold higher than the maximum accumulated VFAs for digestion of TWAS and PS respectively. Rapid accumulation of VFA indicates that OFMSW was readily biodegradable which initially promoted the growth of fast growing acidogenic bacteria (acid formers) and potential instability in the assay if acidification was not in balance with methanogenesis which would result in an unbalanced anaerobic microbial consortia (i.e. greater proportion of acidifiers than methanogens). Knowing the characteristic make up of TWAS and OFMSW (more easily hydrolyzed than the TWAS) greater VFA accumulation with readily degradable substrates is a problem with various substrates and has also been reported by Sosnowski, et al., (2003). The fact that PS produced greater VFA accumulation than TWAS but less than OFMSW in the first week is an indication that this waste exhibited an intermediate level of degradation likely moderated by the more difficult to degrade component that produced the second stage of biogas production. One can also ascertain from Figure 4-2 that once VFA accumulation concentrations peaked and began to decrease that methanogenesis began to dominate over acidogenesis as VFAs were converted to biogas.

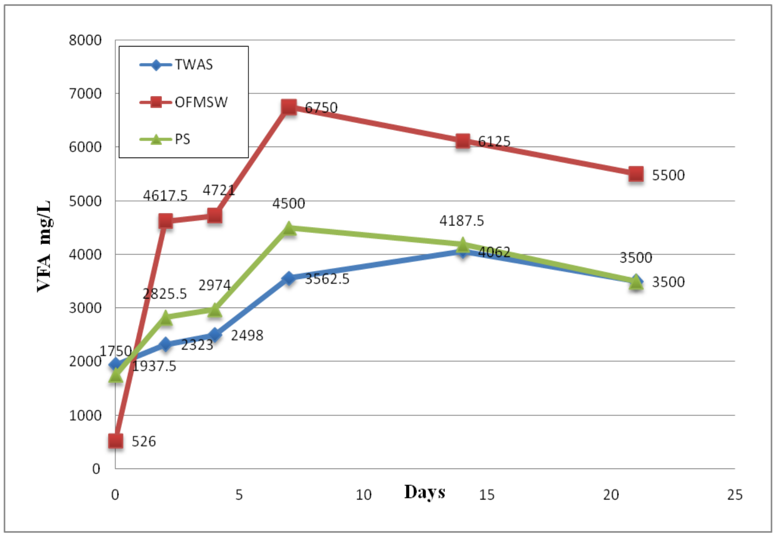


Figure 4-2 VFA accumulation in initial stage of BMP assay (Average of duplicate runs)

Figure 4-1 also shows cumulative specific biogas yield (CSBY) for OFMSW, PS and TWAS were 235, 845 and 426 mL/gVS$_{added}$ respectively indicating the VS digested in the samples have different unit methane potentials. To conclude the comparison between OFMSW, PS and TWAS; OFMSW is concentrated (23.3% TS) readily degradable substrate with a potential for rapid biogas production as well as process instability if rapid acidification is allowed to occur without appropriate pH control. On the other hand, TWAS and PS are less concentrated but have more specific biogas potential than OFMSW. The microbial cell and EPS matrix composition of TWAS that makes it more difficult to degrade does not promote detrimental rapid acidification and coupled with higher associated alkalinity a more stable pH promotes more balance between the various classes of anaerobic microbes that make up the anaerobic microbial consortia. PS found to have a high specific biogas yield that is related to the initial characteristics of the wastewater produced by a community but generally contains simple and complex carbohydrates and proteins with low to average specific yields but potentially also FOG in the form of primary scum or suspended FOG that agglomerated on suspended solids that are known to have very high specific biogas yields (Speece, 2008). The different positive and negative characteristics of OFMSW, PS and TWAS related to their anaerobic degradability suggests that potential advantages may be demonstrated in terms of promotion of a balanced anaerobic microbial consortia that promotes AD stability and/or increased waste biodegradation if OFMSW is co-digested with one or both of the two substrates that are produced at MWWTP. As the objective of this study is to evaluate the benefits of mesophilic tri-digestion of OFMSW with PS and TWAS using BMP assays and semi-continuous reactors, results of assays and reactor runs are discussed sequentially in the next segments.

4.3 Co-digestion and Tri-digestion BMP Assays

The mesophilic anaerobic BMP assays of OFMSW, TWAS and PS as individual substrates was found to have some disadvantages or drawbacks for each that may lead to instability in the digester, less biogas production, or longer digestion times. Based on the known characteristics of the 3 wastes as well as preliminary individual BMP assay it is possible that the rapid acidification and biodegradation characteristics exhibited by OFMSW vs. TWAS/PS might counter balance each other. Co/tri-digestion of OFMSW with TWAS and/or PS may provide conditions for more balanced microbial consortia interaction resulting in a microbial ecology that enhances process stability, biogas yield and overall organic removal efficiency. To evaluate the effect of co/tri-digestion of OFMSW and TWAS and/or PS, mesophilic BMP assays were designed with different combinations of OFMSW with TWAS and/or PS. Mixing ratios tested have been presented in chapter 3 for co-digestion of OFMSW:TWAS and tri-digestion of OFMSW:TWAS:PS in Tables 3-3 and 3-4.While Table 4-1 describes the characteristics of the individual substrates Table 4-2 shows the characteristics for the co-substrate and tri-substrate mixtures used for the BMP assays. It should be emphasized that each BMP assay bottle (single, co- or tri-substrate mixtures) had the same initial volumetric VS load and specific VS load (i.e. identical initial inoculum charge). Additionally while the volumetric and specific VS loads were identical depending on the mixture due to the various characteristics of the individual substrates (Table 4-1) the TCOD and initial alkalinity concentrations in various BMP bottles was different as shown in Table 4.2. For example sCOD for the 25:75, 50:50 and 75:25 mixtures of OFMSW:TWAS were 5.3, 15.2 and 19.2 g/L respectively.

Table 4-2 Characteristics of co- and tri substrate mixtures used for BMP assays **(**Average of duplicate runs)

Property	**OFMSW:TWAS 25:75**	**OFMSW:TWAS 50:50**	**OFMSW:TWAS 75:25**	**OFMSW:TWAS:PS 25:37.5:37.5**	**OFMSW:TWAS:PS 50:25:25**	**OFMSW:TWAS:PS 75:12.5:12.5**
TCOD g/L	51.6±0.33	56.1±0.53	47.05±0.4	49.75±0.36	48.85±0.29	46.85±0.82
sCOD g/L	5.3±0.12	15.2±0.12	19.2±0.13	9.56±0.6	12.5±0.23	18.5±0.09
sCOD/ TCOD	0.1±0.03	0.27±0.04	0.4±0.03	0.192±0.06	0.25±0.07	0.39±0.01
VS g/g	3.53±0.05	3.58±0.01	3.5±0.07	3.41±0.01	3.4±0.02	3.4±0.019
TS g/g	4.4±0.04	4.48±0	4.2±0.07	4.17±0.04	4.2±0.01	4.17±0.025
VS/TS	0.8±0.02	0.8±0.01	0.83±0	0.82±0	0.81±0.01	0.82
Alkalinity, mg $CaCO_3$/L	1800±33	1450±82	950±190	1500±81	900±189	550±128

Results of co-digestion OFMSW:TWAS and tri-digestion of OFMSW:TWAS:PS are discussed in the two following sections.

4.4 Co-digestion BMP Assay of OFMSW and TWAS

To evaluate the effect of co-digestion of OFMSW and TWAS, mesophilic BMP assays were carried out at OFMSW:TWAS mixing at ratios of 25:75, 50:50 and 75:25. Although the mixing combinations of these two substrates were different in each assay, the entire assays were designed at equal volumetric VS loading and initial inoculum concentration. Additionally each assay was started with an equal volume mixture of potassium and sodium bicarbonate as buffer solution equivalent to 6000 mg/L of bicarbonate alkalinity. Figure 4-3 shows that VFA accumulation was different in the different BMP assay. In the first 3 days of anaerobic digestion VFA accumulation in the assays with 25:75, 50:50 and 75:25 of OFMSW:TWAS was in the range of 4600 to 5200 mg/L indicting rapid acidification that resulted in a decrease in pH to as low as 5.6-6 which can inhibit the methanogenic bacteria. Methanogenic bacteria require a favorable pH range of 6.5-7.6 (Mohan, et al., 2008). To maintain the BMP assays in an optimal ecologically balanced condition for anaerobic bacterial consortia, additional buffer solution in the form of an equal mix of potassium and sodium bicarbonate was added as needed and pH was maintained in the range of 6.8-7.6. In addition Figure 4-3 illustrates that VFA accumulation was comparatively higher in the assay with the higher OFMSW component (i.e. 75:25) For the OFMSW:TWAS:75:25 combination during the first 2 weeks VFA concentrations were much higher than the other combinations tested. After seven days VFA accumulations were 6563, 5813 and 5687 mg/L for the 75:25, 50:50 and 25:75 OFMSW:TWAS mixtures, respectively. As one would expect the VFAs for TWAS alone was low in relation to the OFMSW alone or the OFMSW:TWAS mixtures. VFAs in the OFMSW alone were about the same as the mixtures. Higher VFA accumulation simply indicates the rapid acidification which seemed to be increased with the higher percentage of OFMSW in the mixture requiring greater amounts of buffer to maintain pH in the desired range. Irrespective of the potential pH problem issues performance of the various mixtures with pH adjustment in terms of biogas production and organic removal efficiency are highlighted in the next segment.

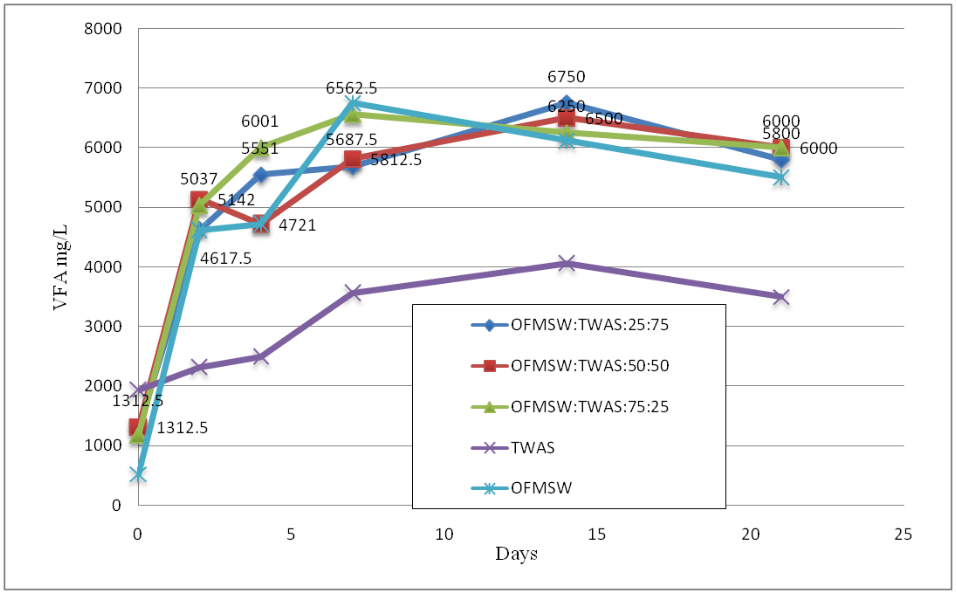


Figure 4-3 VFA accumulation in co-digestion BMP assay of OFMSW:TWAS (Average of duplicate runs)

Figure 4-4 shows CBP from co-digestion of OFMSW:TWAS:25:75 compared with CBP from OFMSW and TWAS individually. By the end of the BMP assay the CSBY for 25:75 of OFMSW:TWAS was 560 mL/gVS$_{added}$ while OFMSW and TWAS individually produced 225 and 425 mL biogas/gVS$_{added}$ respectively. A hypothetical term called calculated expected biogas (CEB) production was introduced to evaluate the effect of co-digestion here. CEB was calculated based on the weighted average of the biogas production from each of the individual single substrates and the portion of each waste co-digested in the assay. If there is no effect of co-digestion in the assay the CEB value represents the minimum amount of biogas that can be produced when accounting for the potential biogas produced from each portion of waste. More specifically if there is no effect of co-digestion the CSBY as measured for the particular mixture should be the same as CEB value. For OFMSW:TWAS:25:75, CSBY was 48 % higher than the CEB of the respective co-substrates indicating a positive influence of co-digestion of OFMSW:TWAS:25:75. Co-digestion of OFMSW:TWAS:25:75 not only produced more biogas than when considering the proportion attributable to the individual substrates but the biogas production rate for the OFMSW:TWAS:25:75 was 22 mL/gVS$_{added}$/d which was 23and 56 % higher than the rate of biogas produced for the individual substrates which was at an 18 and 14 mL/gVS$_{added}$ /d for OFMSW and TWAS respectively. It should be noted that little or no pH adjustment was required for the OFMSW:TWAS:25:75 mixture concomitantly the higher CBP and higher biogas production rate from the co-digestion of OFMSW:TWAS:25:75 indicates that co-digestion of these two substrates at this particular ratio had sufficient buffering capacity and nutrient availability that promoted a balanced acidogenic and methanogenic microbial consortia allowing for greater stabilization potential from this particular mixture.

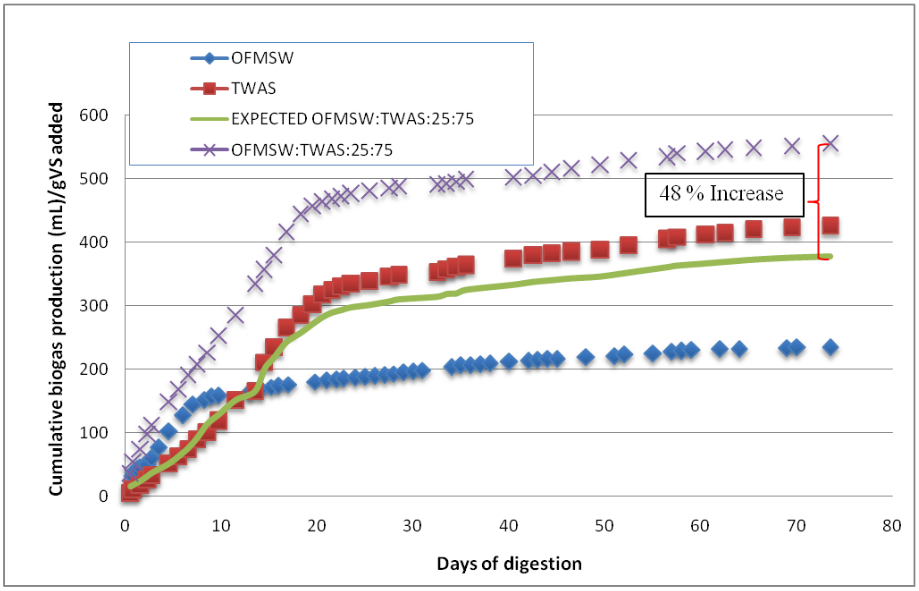


Figure 4-4 Cumulative biogas production from OFMSW:TWAS:25:75 and single substrate (Average of duplicate runs, solid line is the calculated CEB**)**

Since all BMP assays were conducted with the same initial volumetric VS and inoculum load (i.e. volumetric and specific VS loads were identical) and favorable pH conditions for the methanogenic bacteria, differences in ultimate biogas production are indicators of different substrate biodegradability and different specific methane yield potentials (i.e. L biogas/gVS$_{added}$).Figure 4-5 and 4-6 illustrates cumulative specific biogas yield from OFMSW:TWAS at 50:50 and 75:25 respectively compared with OFMSW and TWAS individually as well as the CEB of each particular mixture. CSBY for OFMSW:TWAS:50:50 was 583 mL/gVS$_{added}$ which was 76% higher than the CEB again indicating a positive influence for co-digestion of OFMSW:TWAS:50:50 keeping in mind this was attained with additional pH stabilization.

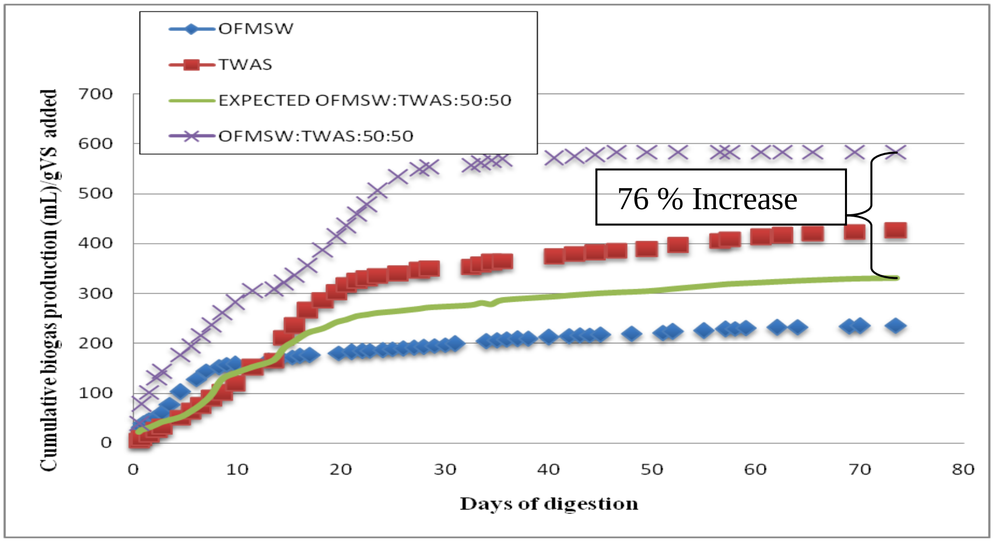


Figure 4-5 Cumulative biogas production from OFMSW:TWAS:50:50 and single substrates **(**Average of duplicate runs, solid line is the calculated CEB**)**

On the other hand, Figure 4-6 illustrates the improvement in CSBY for OFMSW:TWAS:75:25 was 1.4 fold when compared to the CEB of that particular co-substrate mixture. The CBP curve from OFMSW:TWAS:75:25 shown in Figure 4-6 along with the individual curves for OFMSW and TWAS provides an interesting observation in the biodegradation pattern of OFMSW:TWAS:75:25. Unlike the other substrates, this particular mixture had two biogas production phases, in the first phase biogas was produced at a rate of 19 mL biogas/gVS$_{added}$/d and in the second phase the rate was 11 mL biogas/gVS$_{added}$/d. To recap, the rate of biogas production from OFMSW and TWAS (individually) was 18 and 14 mL/gVS$_{added}$/d. So it is interesting to note that the first phase biogas production rate for OFMSW:TWAS:75:25 was similar to that of OFMSW and the second phase biogas rate was a similar rate for TWAS itself. This dual phase biogas production was not discernible during from the CBP of OFMSW:TWAS:50:50 and certainly not for the OFMSW:TWAS 25:75 either. To make a conclusion from the exceptional behavior for the digestion scenario from OFMSW:TWAS:75:25, it may be suggested that the microbial consortia preferred to digest the readily degradable OFMSW (present in a larger proportion compared to TWAS) preferentially to the more difficult to degrade TWAS for the first portion of the assay and followed by extended biogas production segment to digest the more difficult to biodegradable material present in the assay.

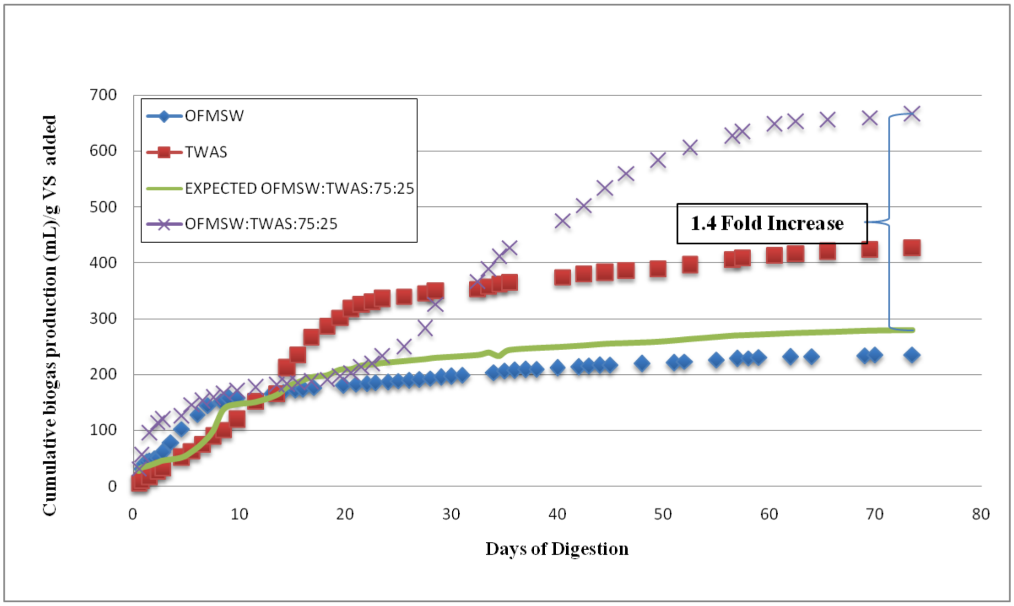


Figure 4-6 Cumulative biogas production from OFMSW:TWAS:75:25 and single substrates **(**Average of duplicate runs, solid line is the calculated CEB**)**

Table 4-3 shows the properties of the effluent from the BMP assays for all OFMSW:TWAS at the conclusion of the BMP assays. A higher percentage of OFMSW in the OFMSW:TWAS mix resulted in two biogas production phases as well as higher VFA accumulations. Higher VFA concentrations in the BMP assay required greater amounts of external buffer addition in order to circumvent a more acidic and less stable environment for the anaerobic microbial consortia in order to allow it to stabilize the waste. However, with pH adjustment the BMP assay indicated that OFMSW:TWAS:75:25 produced comparatively better performance in terms of overall biogas production, VS and TCOD removal with values of 6327 mL, 80 and 77% respectively. This is not too unexpected as OFMSW which is relatively more biodegradable than TWAS was present in greater proportion while the initial VS organic load in each assay was constant.

Table 4-3 Properties of effluent from OFMSW:TWAS co-digestion BMP assays

Property	**OFMSW:TWAS 25:75**	**OFMSW:TWAS 50:50**	**OFMSW:TWAS 75:25**
TCOD g/L	18.8±0.82	16.5±0.36	10.7±0.4
TCOD removal %	63	70	77
Specific VS removal g /g	1.55±0.04	1.26±0.08	0.70±0.05
VS removed %	56	65	80
Specific TS removal g/g	3.03±0.23	2.83±0.19	2.46±0.8
TS removed %	35	37	39
Biogas mL	5896±180	6689±210	6327±200
CH_4 composition, %	58	60	60
Actual CH_4 production mL	3420	4015	3795
Theoretical CH_4 production mL	3455	4155	3815

Ultimate biogas production from OFMSW:TWAS 25:75, 50:50 and 75:25 was 5896±280, 6689±210 and 6327±200 mL while methane percentage in the biogas was relatively constant at 58, 60 and 60 % respectively. Considering the methane percentage, methane production for OFMSW:TWAS 25:75, 50:50 and 75:25 was 3419, 4013 and 3796 mL respectively. Meanwhile, TCOD removal rate was 63, 70 and 77 % for OFMSW:TWAS 25:75, 50:50 and 75:25 respectively. COD conversion to methane based on the theoretical conversion of 1g COD removed per 350mL methane produced at STP (Speece, 2008) was considered for the comparison of actual and theoretical methane production. The total conversion of COD is known (ΔCOD), so theoretical CH_4 production was calculated based on ΔCOD and found to be close to the observed values as shown in Table 4-3.

4.5 Tri-digestion BMP Assay of OFMSW, TWAS & PS

Despite different mixtures of OFMSW:TWAS having different alkaline and acidic potentials, inherent buffering capacities and nutrient availability, co-digestion of these two substrates (with some buffer addition as needed) eventually promoted a balanced acidogenic and methanogenic microbial consortia allowing for greater stabilization potential per mass of organic waste treated. In a more practical field scenario most MWWTPs with secondary treatment facilities such as ROPEC operate anaerobic sludge treatment reactors with a blend of PS and TWAS at an approximately 1 to 1 volumetric ratio (ROPEC local MWWTP for the City of Ottawa, Canada actually uses a 52:48 PS:TWAS volumetric ratio). To evaluate the effect of digestion of OFMSW with municipal sludge tri-digestion BMP assays were also carried out at different combinations of OFMSW, TWAS and PS (described in Table 3-4 in chapter 3) where in all cases the ratio of PS and TWAS in the tri-mixtures was 1 to 1 which is a fixed ratio similar to ROPEC and most other secondary MWWTPs with anaerobic sludge digestion. Although it has been shown that each of the three substrates had different organic characteristics (Table 4-1), all the BMP assays were again tested at identical initial organic VS loading of 3.5% VS/assay and initial inoculum dose. Since all BMP assays were conducted with the same initial VS load and inoculum dose (i.e. volumetric and specific VS loads were identical) differences in ultimate biogas production are indicators of preferential treatment, different substrate biodegradability and different specific biogas yield potentials (i.e. L biogas/gVS_{added}). CSBY as shown in Figure 4-7 indicates that the mixture of OFMSW:TWAS:PS:25:37.5:37.5 was able to produce 690 mL/gVS_{added} biogas which was 25 % more than the CEB if the substrates were digested individually. To recall, CEB was calculated based on the weighted average of the biogas production from each of the individual single substrates and the portion of each waste co-digested in the assay. If there is no effect of co-digestion in the assay the CEB value represents the minimum amount of biogas that can be produced when accounting for the potential biogas produced from each portion of waste. More specifically if there is no effect of tri-digestion the CEB value should be the same as the CSBY as measured for the particular mixture. CSBY for the other combinations of OFMSW:TWAS:PS tested showed lower increases over their respective CEB's.

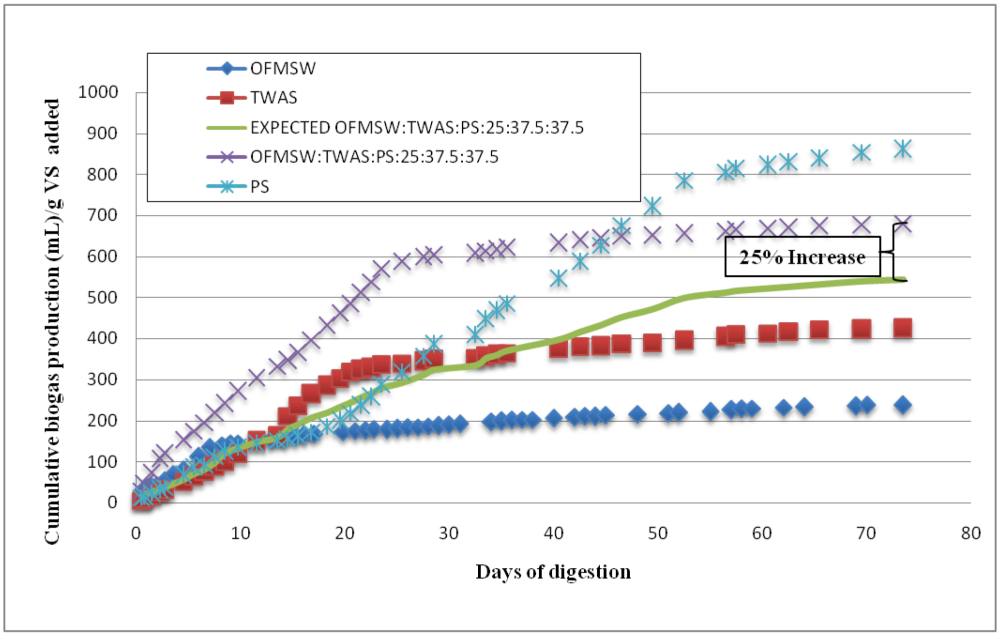


Figure 4-7 Cumulative biogas production from OFMSW:TWAS:PS:25:37.5:37.5 and single substrates **(**Average of duplicate runs, solid line is the calculated CEB**)**

Figures 4-8 and 4-9 shows for OFMSW:TWAS:PS:50:25:25 the CSBY was 720 mL/gVS$_{added}$ which was 62% higher than the corresponding CEB while Figure 4-9 shows for OFMSW:TWAS:PS 75:12.5:12.5 the CSBY was 770 mL/gVS$_{added}$ which was 1.4 fold higher than the CEB. CSBYs at all OFMSW:TWAS:PS combinations was found to be higher than the CEBs indicating that tri-digestion of OFMSW with municipal sludge (PS+TWAS 1 to 1 ratio) which is a more realistic MWWTP scenario has positive effects in terms of biogas yield.

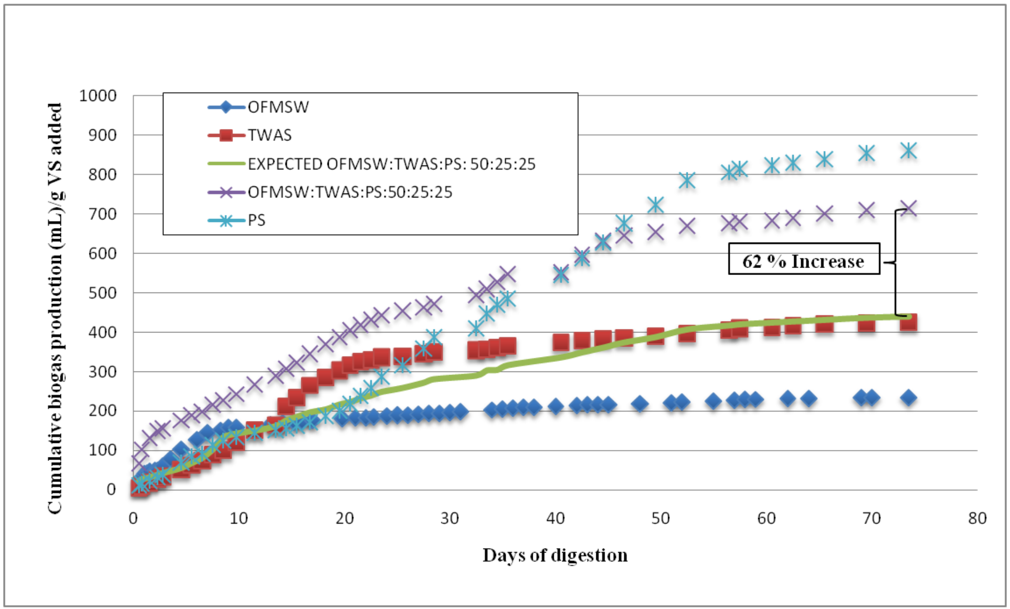


Figure 4-8 Cumulative biogas production from OFMSW:TWAS:PS:50:25:25 and single substrates **(**Average of duplicate runs, solid line is the calculated CEB**)**

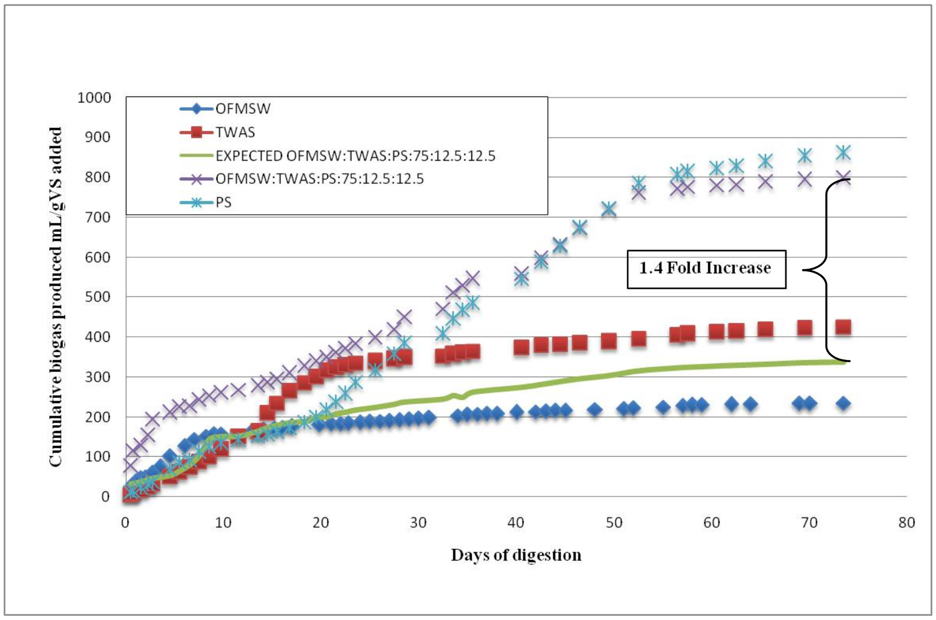


Figure 4-9 Cumulative biogas production from OFMSW:TWAS:PS:75:12.5:12.5 and single substrates (Average of duplicate runs, solid line is the calculated CEB**)**

4.6 Comparison of Co- and Tri-digestion BMP Assays

4.6.1 OFMSW:TWAS:PS versus OFMSW:TWAS

Figure 4-10 shows the comparison in cumulative biogas yield between OFMSW:TWAS and OFMSW:TWAS:PS at all waste combinations tested under identical initial volumetric and specific organic loads. CSBY of OFMSW:TWAS:PS:25:37.5:37.5 tri-mixture was found to be 24 % higher than that of the corresponding co-mixture of OFMSW:TWAS:25:75. Meanwhile, improvement in ultimate biogas production of OFMSW:TWAS:PS:50:25:25 was 22% higher when compared to the CSBY of OFMSW:TWAS:50:50 and improvement was 41% higher for OFMSW:TWAS:PS:75:12.5:12.5 compared with OFMSW:TWAS:75:25. Enhancement in biogas yield for all OFMSW:TWAS:PS combinations were improved compared with the corresponding binary OFMSW:TWAS mixtures indicating that although tri-substrate mixtures were made the feed for the assay more complex but tri-digestion of OFMSW:TWAS:PS provided more stability and promoted a higher balanced acidogenic and methanogenic microbial consortia allowing for greater stabilization potential per mass of VS treated when compare to single substrates or binary mixtures.

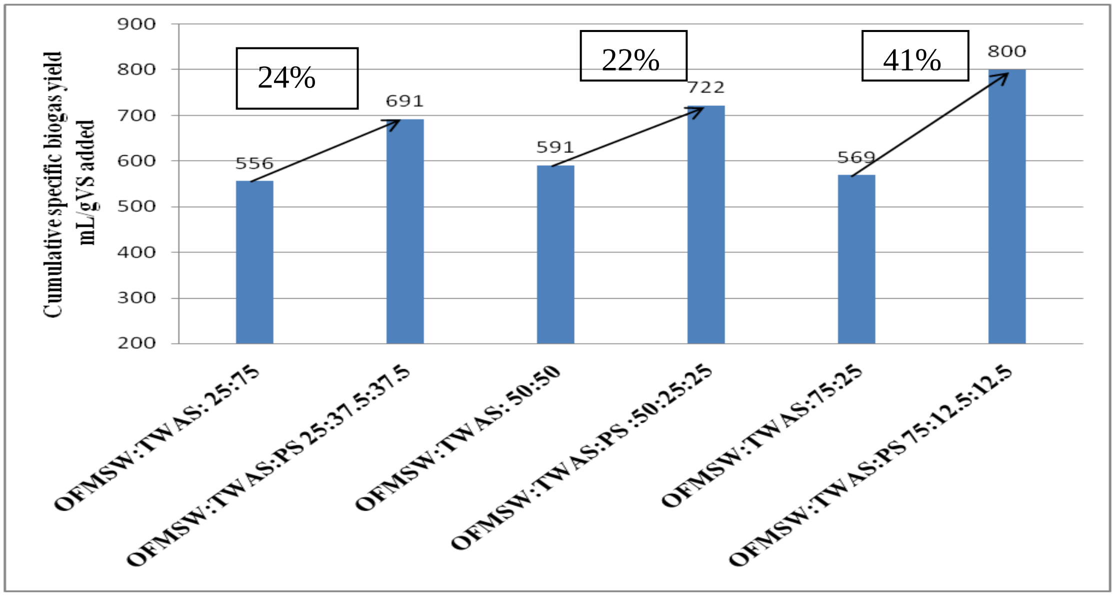


Figure 4-10 CSBY OFMSW:TWAS:PS vs. OFMSW:TWAS (Average of duplicate runs)

Figure 4-11 illustrates the VFA accumulation curve in the BMP assays at different tri-mixture combinations of OFMSW:TWAS:PS. As can be seen in Figure 4-11, after the first week of AD VFA concentrations in the 25:37.5:37.5, 50:25:25 and 75:12.5:12.5 OFMSW:TWAS:PS mixtures were 4200, 5200 and 5500 mg/L respectively which were considerably lower than VFA concentrations shown in Figure 4-2 that were 5687, 5813, and 6563 mg/L for the 25:75, 50:50 and 75:25 of OFMSW:TWAS assays respectively. The combination of higher biogas yield with the more complex OFMSW:TWAS:PS mixtures over OFMSW:TWAS and lower VFA accumulations in the tri-mixture BMP assays is another indicator that tri-digestion of OFMSW:TWAS:PS provides more the suitable mesophilic AD conditions for the microbial consortia. It should be noted that digestion of TWAS:PS (50:50) resulted in maximum VFA accumulation of approximately 4200 mg/L which was lower than the OFMSW:TWAS or OFMSW:TWAS:PS mixtures which also indicates the rapid acidification of OFMSW is problematic and is likely a limiting factor in the amount of OFMSW that can be digested with PS and TWAS without pH control.

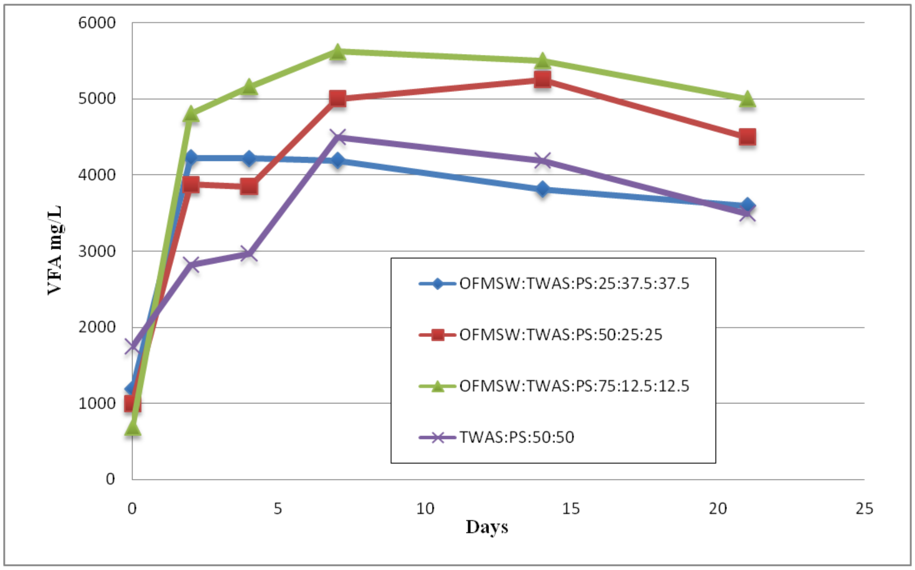


Figure 4-11 VFA accumulation in tri-digestion BMP assays of OFMSW:TWAS:PS (Average of duplicate runs)

4.6.2 Comparison of OFMSW:TWAS:PS with TWAS:PS

Figure 4-12 shows the benefit of tri-digestion (OFMSW:TWAS:PS) when compared to the MWWTP status quo co-digestion of TWAS:PS at 50:50 ratio which is the mixture at ROPEC and at most secondary MWWTPs. The more complex OFMSW:TWAS:PS results in a better CSBY when compared to the measured gas production of TWAS:PS at the same initial load condition. Although all of the BMP assays had the same initial organic loading, the more complex mixture of OFMSW:TWAS:PS in the ratio of 75:12.5:12.5 produced 22 % more biogas than that produced by the 50:50 TWAS:PS mix. For the other two OFMSW:TWAS:PS combinations (50:25:25 and 25:37.5:37.5) the biogas improvements when compared to the 50:50 TWAS:PS was approximately 13%. This finding in conjunction with the sections discussed above provides additional evidence to support the benefit of tri-digestion of OFMSW with municipal sludge. The use of tri-digestion strategies that incorporate OFMSW not only provide alternatives for sustainable integrated solid waste management but may also provide additional options to improve ultimate biogas production at real MWWTPs operating below their design loading capacity.

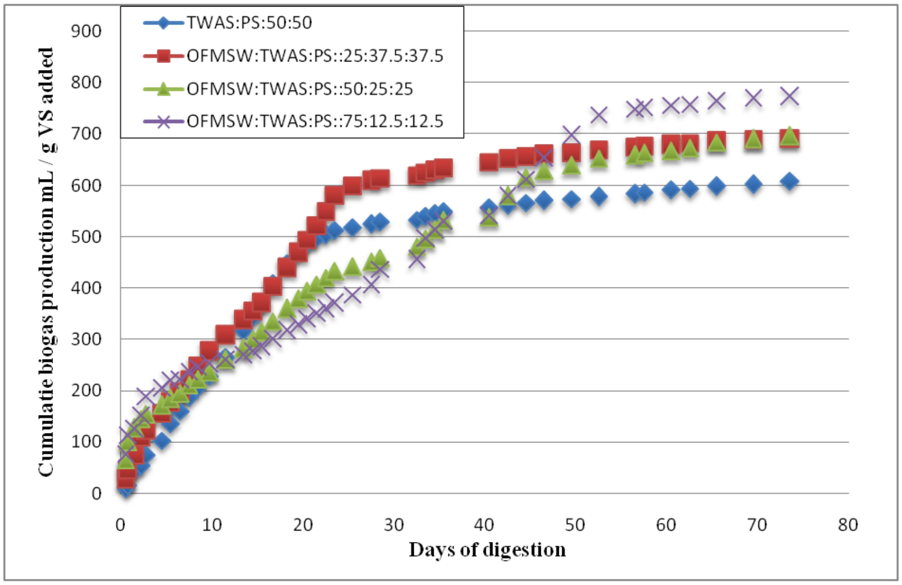


Figure 4-12 Cumulative biogas production OFMSW:TWAS:PS vs. TWAS:PS (Average of duplicate runs)

Table 4-4 shows the effluent characteristics of all combinations of OFMSW:TWAS:PS as well as TWAS:PS at the conclusion of the BMP assays. Tri-digestion of OFMSW:TWAS:PS showed comparatively better performance not only in terms of ultimate biogas production but also in terms of TS, VS, TCOD removal. TS removal for the TWAS:PS at 1:1 mixture was found to be 25 % while TS removal was higher for any of the combinations of OFMSW:TWAS:PS. TS removal was 32, 31 and 35 % for OFMSW:TWAS:PS mixtures of 25:37.5:37.5, 50:25:25 and 75:12.5:12.5 respectively. Similar improvements in VS removal were also observed for OFMSW:TWAS:PS. Despite having the same initial organic VS and inoculum load OFMSW:TWAS:PS 25:37.5:37.5, 50:25:25 and 75:12.5:12.5 resulted in 60, 69 and 73 % VS removal compared to 53% VS removal from TWAS:PS 50:50 which again indicates a positive significance for application of co-digestion of OFMSW with TWAS:PS in secondary MWWTPs having anaerobic sludge digestion facilities.

Table 4-4 Properties of effluent from OFMSW:TWAS:PS and TWAS:PS BMP assays (Average of duplicate runs)

Property	OFMSW:TWAS:PS 25:37.5:37.5	OFMSW:TWAS:PS 50:25:25	OFMSW:TWAS:PS 75:12.5:12.5	TWAS:PS 25:75
TCOD g/L	16.968±0.53	16.075±0.6	11.163±0.4	11±0.86
TCOD removal %	66	67	76	75
VS g/g	1.3±0.1	1.044±0.07	0.9±0.03	1.5±0.098
VS removed %	60	69	73	53
TS g/g	2.82	2.75	2.5	3.3
TS removed %	32	31	35	25
Biogas mL	6966±129	7225±321	8245±657	5857±781
CH_4 composition, %	54	54	56	56
Actual CH_4 production	3762	3902	4617	3280
Theoretical CH_4 production	3775	3885	4620	3454.5

It is important to highlight that among the three substrates, OFMSW and PS are readily biodegradable (Shahriari, et al., 2011) while TWAS which is a mixture of microbial cells within a matrix of EPS is known to be difficult to biodegrade with hydrolysis being the limiting factor for AD. The CSBY curve for the OFMSW:TWAS:PS 75:12.5:12.5 (Figure 4-10) indicates that almost 40% of the ultimate biogas was produced in the first 8-10 days at a rate of 31 mL biogas/gVS$_{added}$/d most likely from the preferential rapid degradation of the readily degradable substrates (OFMSW and PS) then taking an additional 47 days to produce the remaining biogas at a much lower rate of about 11 mL biogas/gVS$_{added}$/d as the more recalcitrant substrates were digested. Similar results were also observed from the co-digestion of OFMSW:TWAS. These results tend to indicate that although the percentage of the more difficult to degradable substrate (TWAS) was small, it has a prominent role in the overall degradation. Other studies have shown that MW pre-treatment that lysis and solubilized TWAS thereby decreasing the impact of TWAS hydrolysis on AD not only improves the rate of anaerobic degradation but can result in enhancement in ultimate specific biogas yield (Toreci, et al., 2009). MW pre-treatment of TWAS (TWAS$_{MW}$) was pursued in this study to evaluate the effect on co-digestion of $TWAS_{MW}$ with OFMSW:PS. The next segment discusses the effect of MW pre-treatment on TWAS solubilization.

4.7 MW Pre-treatment of TWAS

The EPS that maintains and stabilizes the aerobic microbial-floc matrix also protects the activated sludge microbial consortia and slows the hydrolysis of TWAS (Baier, et al., 1997). While rates may vary dependent on the type of solid waste hydrolysis is usually the rate limiting step for conventional AD of solid wastes. In our particular scheme of OFMSW:TWAS:PS it is the TWAS that is the most resistant to hydrolysis, hence only TWAS was pre-treated with MW in this study. Little or no benefits of various pre-treatments have been shown for PS and OFMSW (Shahriari, et al., 2011) which are composed of more readily degradable suspended organics when compared to TWAS which is composed of a heterogeneous mixture of obligate and facultative aerobic microbes enmeshed in an EPS matrix. A multilevel factorial design was used to investigate variables affecting efficiency of TWAS microwave pre-treatment which were chosen to be: (1) MW pre-treatment temperature and (2) MW exposure/holding time (HT) at desired temperature as presented in Table 4-5. The MW intensity was programmed so that the temperature increase per unit time from room temperature was constant at 2.5 ºC/ min which are deemed a low intensity following (Toreci, et al., 2010) who reported that solubilization of TWAS increases at lower conventional and MW heating rates.

Table 4-5 Variables and their levels used in the statistical design

Variables		
Levels	Temperature ºC	HT at Targeted Temperature (min)
1	95	1,10,20
2	115	1,10,20
3	135	1,10,20

MW pre-treatment of TWAS was performed at three different temperatures (95, 115 and 135˚C) and to evaluate the effect of HT at the desired temperature each temperature was held for three different periods of 1, 10 and 20 minutes. In each case, raw TWAS sludge of 5.3% TS sampled from ROPEC (sludge age 5d) was used. MW pre-treatment was evaluated in terms of COD solubilization as MW irradiation converts the solid suspended materials into soluble matter (Eskicioglu, et al., 2006); (Coelho, et al., 2011). Solubilization of the organic suspended material of TWAS was measured in terms of the sCOD/TCOD ratio.

4.7.1 Results of Solubilization of TWAS

Figure 4-13 shows the change in the sCOD/TCOD ratio of $TWAS_{MW}$ due to the various MW pre-treatments. The results illustrate that improvements in $TWAS_{MW}$ sCOD/TCOD ratio vs. the TWAS control (sCOD/TCOD = 0.21) increased with elevated temperatures as well as longer holding times at targeted temperature.

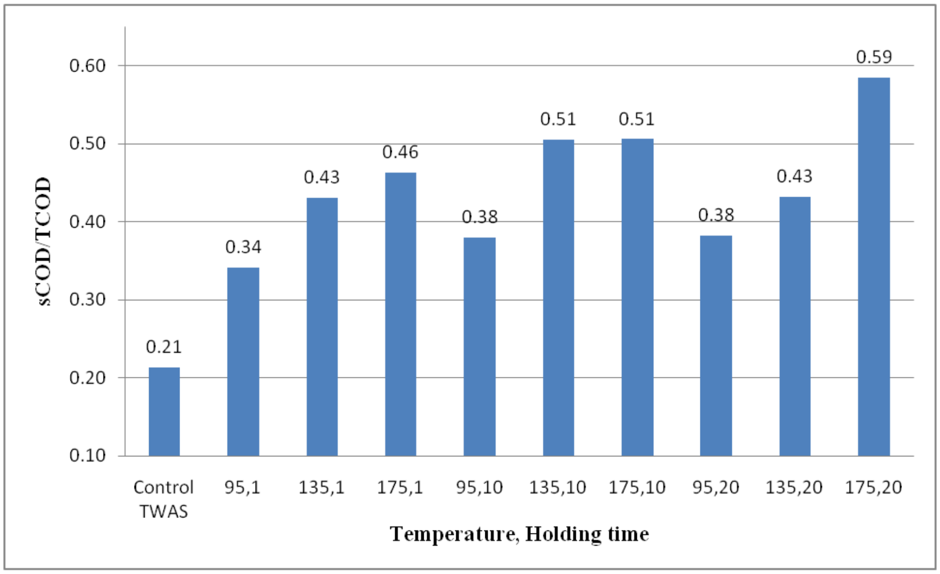


Figure 4-13 sCOD/TCOD ratios of TWAS treated at different temperatures and temperature HT (Average of duplicate runs)

At 95, 135 and 175 ºC and 1 min HT sCOD/TCOD were 0.34±0.04, 0.43±0.06 and 0.46±0.03 which are 1.6, 2 and 2.2 fold higher respectively than that of the TWAS control illustrating increases of sCOD due to increased MW pre-treatment temperature. Similar improvement in $TWAS_{MW}$ solubilization were observed at temperatures 95, 135 and 175 ºC and HT of 10 minutes with sCOD/TCOD ratios of 0.38±0.04, 0.51±0.01 and 0.51±0.03 which were 1.78, 2.37 and 2.37 fold higher respectively than that of the TWAS control and slightly higher than at the same temperatures but with a HT of 1 minute. For 95, 135 and 175 ºC and HT of 20 minutes sCOD/TCOD was 0.38±0.04, 0.43±0.06 and 0.59±0.03 which was 1.8, 2 and 2.75 fold greater respectively than that of TWAS control. The study tends to indicate that sCOD/TCOD was not only improved due to increase in pre-treatment temperature but also improved with an increase of the HT at the desired temperature up to a point as little benefit was observable when the HT was increase to 20 minutes as per the data shown in Figure 4-13.

Ultimate solubilization of $TWAS_{Ultimate}$ was performed by addition of NaOH to the raw TWAS based on the procedure of Bougrier, et al., (2005) and described in chapter 3 to provide an ultimate benchmark for $TWAS_{MW}$. sCOD/TCOD of $TWAS_{Ultimate}$ after 24 hours of contact with NaOH resulted in a ratio of 0.73±0.02 which indicates that 73 % of the total organic material of TWAS as determined by COD can be ultimately be ultimately solubilized. Figure 4-14 illustrates percentage of solubilization at different pre-treatment conditions compared to TWAS$_{Ultimate}$.

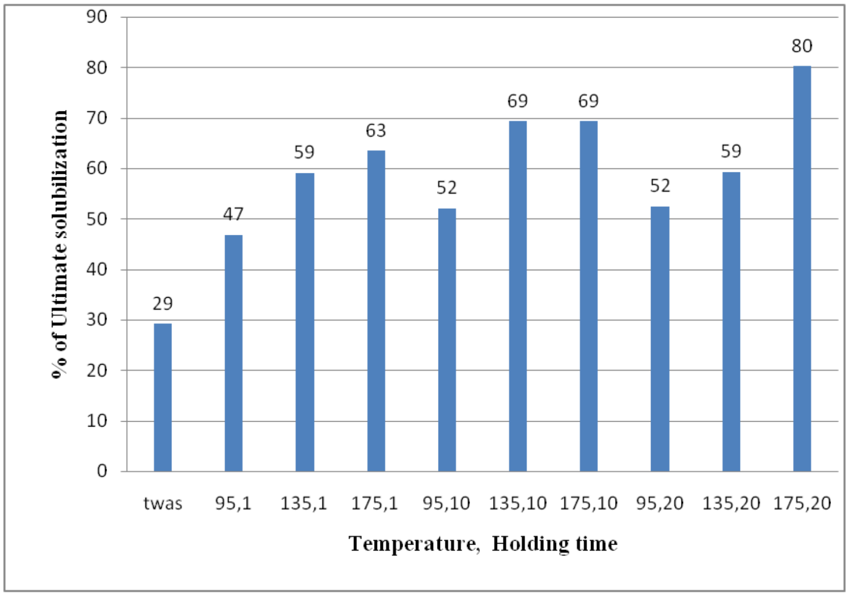


Figure 4-14 Percent of ultimate solubilization after MW pre-treatment of TWAS at different conditions (Average of triplicate runs)

For the control situation, the natural soluble component of TWAS as produced at the MWWTP was found to be only 29 % of TWAS$_{Ultimate}$ which was ten improved due MW pre-treatment. As might be expected based on the above section on TWAS solubilization the percentage of solubilization relative to TWAS$_{Ultimate}$ increased with increase in temperature and HT (upto a point). The sCOD/TCOD ratios relative to TWAS$_{Ultimate}$ were 47, 59 and 63 % for 95, 135, 175ºC and HT of 1 min indicating the positive effect of temperature but also indicating that ultimate TWAS solubilization was not achieved. On the other hand, at temperature 175 ºC and HT of 1, 10 and 20 min 52, 59 and 80 % of TWAS$_{Ultimate}$ obtained illustrating the positive effect of HT on TWAS solubilization but again indicating that ultimate solubilization of TWAS was not achieved even at the highest MW temperature and HT. These results suggest that to achieve the ultimate solubilization of TWAS in reasonable times and energy requirements combinations of various pretreatment technologies may have to be combined.

4.7.2 Statistical Empirical Model

Although the results presented in Figure 4-14 illustrates positive influence of elevated temperature and longer HT on solubilization of TWAS, the relative interaction of temperature increase and increase in HT is not straight forward. To evaluate relative effect of these two factors empirical models were developed to characterize percentage of COD solubilisation over the control as a function of both MW pre-treatment temperature and temperature HT in the area bordered by the conditions evaluated (Table 4-5). Different model structures (from simple zero-order to more complicated third-order) were tested and evaluated and all factors that were statistically significant using ANOVA were included in the model expression. Model complexity and adequacy of its function in relation to the amount of variability of the measured response is a function of variables included in the model. (Coelho, 2012)The coefficient of determination (R^2) is sometimes used to evaluate the quality of the model in terms of its ability to explain the variability of measured data. However, the coefficient of determination is positively correlated with the number of variables included in the model, so this criterion is not adequate to evaluate competing models for the same data set. Adding additional terms without checking its significance to the model increases R^2 and can lead to a model that is *overfitted* (inclusion of too many unnecessary model terms). A more useful evaluation tool is the adjusted coefficient of determination (R^2_{adj}):

$$R^2_{adj} = 1 - \frac{n-1}{n-(k+1)}(1-R^2) \qquad \text{Eqn. 4-1}$$

R^2_{adj} is formulated as a variation of R^2 that provides an adjustment for degrees of freedom of the model. R^2 cannot decrease with the addition of model terms and consequent decrease of degrees of freedom, but R^2_{adj} decreases its value when non-significant terms are added to the model expression and, in conjunction with F-tests and t-tests can be used as a tool to detect the best subset of parameters that minimize the use of unimportant terms that can cause the variance of the estimated response to increase (Coelho, 2012).

Forward Stepwise regression method was used to evaluate the improvement of solubilization of $TWAS_{MW}$ through the following regression model along with simple first order regression:

- Multiple regression to analyze the first-order effect of MW temperature and MW temperature HT
- Factorial regression to analyze the higher-order interactive effects of MW temperature and MW temperature HT
- Polynomial regression to analyze quadratic, cubic and higher-order effects of MW temperature and MW temperature HT
- Response surface regression to analyze interactive polynomial effects of MW temperature and MW temperature HT

The algorithm used for the forward stepwise regression is as follows (Walpole, et al., 2011):

1. The variable (x_1) with the largest increase in R^2 is chosen from the pool of initial variables, and its significance is tested using an F-test. If the variable is not significant the algorithm is terminated.

2. A second variable (x_2) is chosen that returns the largest R^2 increase with the presence of (x_1) over the R^2 found in step 1. The variable (x_2) is the one, from the remaining pool of variables, that has the highest value of regression sum of squares adjusted for the other variables, that is, that maximizes expression (4.2).

$$R(\beta_2|\beta_1) = R(\beta_2, \beta_1) - R(\beta_1) \qquad \text{Eqn. 4.2}$$

3. The model with (x_1) and (x_2) is fitted, and (x_2) is tested for significance, as well as the increase in R^2 using the appropriate F-test as expressed in (4.3):

$$f = \frac{R(\beta 2, \beta 1)}{s^2}, f_{crit} = f_{\alpha}(1, n-k) \qquad \text{Eqn. 4.3}$$

If f fails the significance test, the algorithm is terminated. s^2 is the mean square error of the model containing the variables added so far.

4. Since it is possible that the addition of a new variable might render a previous existent and significant variable redundant and unimportant because of interactions between it and other variables entering at later stages, an F-test is performed for all the variables added at this stage, and the ones that do not show a significant -value are deleted. The procedure is continued until a stage is reached where no additional variables can be deleted.

5. A third variable is added (x_3) that maximizes expression (4):

$$R(\beta_3 | \beta_2 | \beta_1) = R(\beta_3, \beta_2, \beta_1) - R(\beta_2, \beta_1) \qquad \text{Eqn. 4.4}$$

The same tests as described in 5 steps are performed and the process is repeated until the last variable added fails to induce a significant increase in the explained regression.

In the cases where more than one subset of variables was chosen by the algorithm (algorithm depends partially on the initial pool of available variables defined), the final model expression was chosen using the R^2_{adj} criteria (highest R^2_{adj}), since it favours models with lower complexity and equivalent predictive potential (Coelho, 2012) Table 4-6 in the next page shows the results of forward step regression analysis for the full model.

Table 4-6 Results of forward step regression analysis for the full TWAS solubilization model

Effect	**Comment**	**Solubilization Parameter**	**Solubilization Std. Err**	**t**	**p**
Intercept		**105.6**	6.6	15.8	0.000004
Temp	Pooled				
$Temp^2$		**0.0064**	0.0003	19.8	0.000001
Temp HT	Pooled				
$T\ HT^2$	Pooled				
Temp x T HT		**0.01**	0.002	6.2	0.000807
Total Model					
Multiple R^2	**Adjusted R^2**	**SS_{model}**	**MS_{model}**	**F**	**p**
0.989569	0.98	424.9	70.8	284.6	0.000001

Figure 4-15 depicts the empirical response surface regression model based on the data obtained from the pre-treatment of TWAS at three different temperatures and three different pre-treatment temperature holding times, boundary conditions are given in Table 4-5. Among all the models tested polynomial and response surface regression models were very close in representing the data. Results of the total model for polynomial regression analysis are found in the appendix with the profile. The response surface regression model showed the second order effect temperature and interactive effect of temperature and temperature holding time are significant terms that describe TWAS solubilization results due to MW pre-treatment and statistical checks (adjusted R ^{2} , t, p and F values) were satisfactory. It is interesting to notice that in the polynomial regression analysis model the second order term for temperature was found to be significant along with first order term of temperature HT showing positive influence of temperature and HT but when the extra term interaction between temperature and HT was considered in the response surface regression model it was found that the first order term describing effect of temperature HT became insignificant and the interactive term for MW temperature and MW temperature HT factor was dominant in the model and was the best fit to the data.

So the best fit model was expressed by the equation 4-5:

$$\boxed{\text{Solubilization improvement \%} = 105.6 + 0.0064 \times \text{Temp}^{2} + 0.0163 \times \text{Temp-Temp HT}}$$

.... Eqn. 4.5

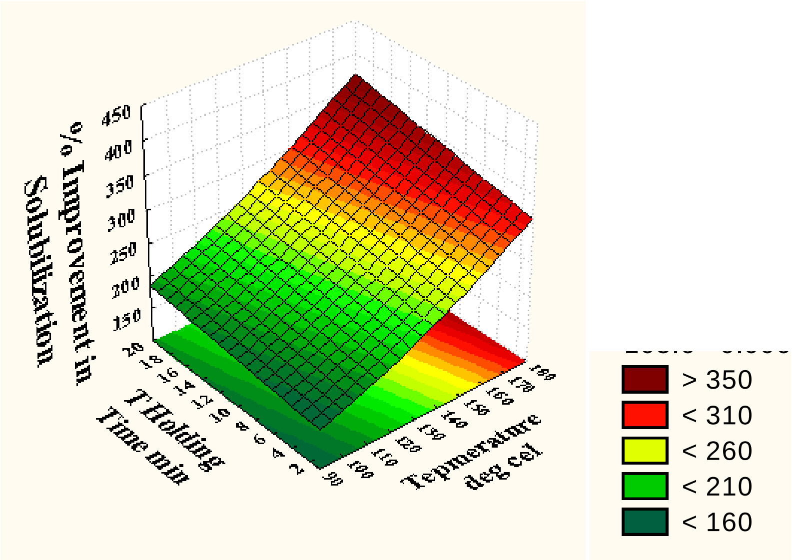


Figure 4-15 Solubilization improvement factor as a function of pre-treatment temperature and holding time

4.8 Co-digestion of OFMSW and TWAS$_{MW}$

To evaluate the effect of MW pre-treatment of TWAS on co-digestion, OFMSW and TWAS$_{MW}$ BMP assays at various conditions as describe in chapter 3 were performed. To recall, MW pre-treatment of TWAS brought from room temperature to 135˚C at an intensity of 2.5˚C/ min and final temperature was held for 1 min. Coelho, et al., (2011) reported that higher MW pre-treatment temperature (175^{o}C) increases the extent of

TWAS solubilization (in term of sCOD). It was emphasized by previous studies that if pretreatments at high temperature (>175^{o}C), were applied for a longer time than necessary, enzymes can be inactivated, nitrogenous organic materials become less biodegradable and inhibitory concentration of ammonia, can be formed (Stuckey, et al., 1984). Again Hodge, 1953 observed the production of melanoidins (brown nitrogen copolymers) and humic acids at high temperature. (Shahriari, et al., 2011) It has been reported that MW pre-treatment to 135˚C not only improves solubilization but causes less acute inhibition of the digestion process (Toreci, et al., 2009). Concomitantly, for this study a more conservative pre-temperature of 135˚C was chosen. While the BMP assays were set up so they all had the same VS OLR of 3.5%VS/assay, due to MW pre-treatment of TWAS the initial sCOD and alkalinity concentrations of the mixtures were different in the BMP assays. An interesting observation can be made from Figure 4-16 in which a comparison of the CBP profiles for BMP assays for co-digestion of OFMSW with either TWAS or TWAS$_{MW}$. Unlike BMP assays for co-digestion of OFMSW:TWAS 25:75 and 50:50 which exhibited a single biogas production phase co-digestion of OFMSW:TWAS$_{MW}$ at 25:75 and 50:50 produced biogas in two phases. It has already been described in the previous section that co-digestion of OFMSW:TWAS 75:25 exhibited a two biogas phases over time on the way to its ultimate biogas yield. A similar observation was also made for OFMSW:TWAS$_{MW}$:75:25 as can be seen in Figure 4-16 (in green circle).These bi-phase types of CBP curve and hence concomitant organic degradation pattern can only occur if the anaerobic bacteria consortia preferentially metabolize one class of substrates over another. It is likely that the anaerobic bacterial consortia chose to preferentially digest the readily degradable OFMSW to the more difficult to degrade TWAS for a certain period of time until readily degradable substrate started to become limited. As readily degradable material became limited the microbial consortia had a slight lag phase before a second period of elevated biogas production was exhibited as more difficult to degrade biodegradable material remaining in the assay bottles was metabolized and stabilized.

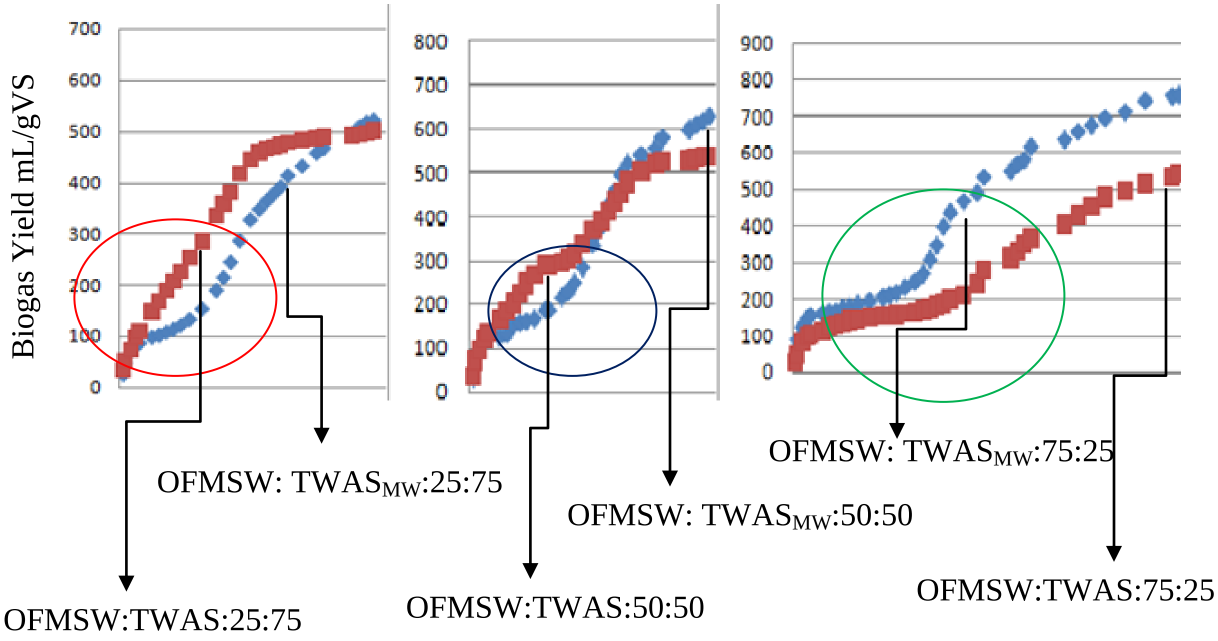


Figure 4-16 Cumulative biogas yield curves for OFMSW:TWAS and OFMSW:TWAS$_{MW}$ at different co-digestion conditions (Average of duplicate runs; Figures do not use same scale).

Red and purple circles marked in Figure 4-16 illustrate the dual biogas production phases of bacterial substrate utilization for OFMSW:TWAS$_{MW}$ 25:75 and 50:50 mixtures respectively. This unusual behavior vs. the respective control suggests the possibility that cell lysis and solubilisation of TWAS$_{MW}$ produced more readily degradable substrate (making it behave similar to OFMSW:TWAS:75:25 and OFMSW:TWAS$_{MW}$:75:25) that the bacterial consortia preferentially consumed. In other words the bacterial consortia always seem to preferentially digest readily degradable substrate in a heterogeneous mix first leaving the more difficult to degrade material until readily degradable substrate became limiting. This preferential degradation of readily degradable substrate can have negative effects on digestion if its concentration is too high and system alkalinity is low as rapid acidification could result in depressed pH that would inhibit methanogenesis. Table 4-7 shows the rate of biogas production (considering both phases) for the co-digestion of OFMSW:TWAS and OFMSW:TWAS$_{MW}$. Biogas production rates were 21.8 and 13 mL/gVS/d in the first and second biogas phases for co-digestion of

OFMSW:TWAS$_{MW}$ 25:75, and were 23.7 and 15.7 mL/gVS/d during the first and second biogas stages for OFMSW:TWAS$_{MW}$:50:50. Co-digestion of OFMSW:TWAS 25:75 and 50:50 resulted in only one biogas phase and it is difficult to comment on the comparative difference in rates of biogas production with OFMSW:TWAS$_{MW}$. However, improvement in biogas production rates were observed in both biogas phases for co-digestion of OFMSW:TWAS$_{MW}$:75:25 relative to OFMSW:TWAS:75:25. Rates of biogas production were 35 and 11 mL/gVS/d during the first and second biogas production phases for OFMSW:TWAS$_{MW}$:75:25 which were 40and 30 % higher than the two rates with OFMSW:TWAS 75:25 which were 22.7 and 8.6 mL/gVS/d respectively. The improvement in biogas production rates gives an indication of improvements in biodegradability of substrate in the mixed liquor due to the MW pre-treatment of TWAS$_{MW}$.

Table 4-7 Biogas production rates for OFMSW:TWAS and OFMSW:TWAS$_{MW}$ at different co-digestion conditions (Average of duplicate runs)

Co-digestion combination		**Exponential Phase**	**Biogas production rate mL/gVS/d**
OFMSW:TWAS:25:75	TWAS	1st phase	21.8
		2nd phase	-
	TWAS$_{MW}$	1st phase	21.7
		2nd phase	13
OFMSW:TWAS:50:50	TWAS	1st phase	19
		2nd phase	-
	TWAS$_{MW}$	1st phase	23.7
		2nd phase	15.7
OFMSW:TWAS:75:25	TWAS	1st phase	22.7
		2nd phase	8.6
	TWAS$_{MW}$	1st phase	35
		2nd phase	11

As all BMP assays had same VS organic loading and initial inoculum charge differences in biogas production are indicators of different substrate biodegradabilies, different specific methane potentials and concomitantly differing specific methane yields (i.e.

L biogas/gVS$_{added}$). CSBY from OFMSW:TWAS$_{MW}$ and OFMSW:TWAS at different combination tested are shown in the Figure 4-17. CSBY for OFMSW:TWAS:25:75 was 556 mL biogas/gVS$_{added}$ while for OFMSW:TWAS$_{MW}$ 25:75 CSBY was 628 mL biogas/gVS$_{added}$ which was 13% higher. Similarly as shown in Figure 4-17 CSBY from the OFMSW:TWAS$_{MW}$:50:50 was 729 mL biogas/gVS$_{added}$ which 23% higher than without TWAS microwave treatment which for OFMSW:TWAS:50:50 was 591 mL biogas/gVS$_{added}$. Similarly, OFMSW:TWAS$_{MW}$:75:25 produced a biogas yield of 787 mL biogas/gVS$_{added}$ which was substantially better (38%) CSBY of OFMSW:75:25. This large improvement suggests that not only did MW enhance digestibility of TWAS but likely improved the characteristics of the mixed liquor (i.e. possible increase in alkalinity or available N) that enhanced digestion of both substrates. Enhancement in biogas production rates and CSBY from co-digestion of OFMSW:TWAS$_{MW}$ provides additional support suggesting that improvement in TWAS solubilisation due to MW pre-treatment resulted in better microbial-substrate interaction that improved biogas yield since all assays had identical initial volumetric and specific VS organic loads. It should be noted that while the proportion of TWAS$_{MW}$ used in the mixtures increased to 75 % while the proportion of OFMSW decreased the expected enhancement in CSBY did not increase proportionally to the increase in TWAS$_{MW}$. Toreci, et al., (2009) reported that high temperature TWAS$_{MW}$ not only increases solubilisation but also the accumulation of substances that are inhibitory to methanogenesis. It was believed that as the proportion of TWAS$_{MW}$ increased from 25 to 75 % the benefits of cell lysis and solubilisation were counteracted by the accumulation of inhibitory compounds. And improvement in biogas production was more predominantly effected by the OFMSW percentage in the mixture rather than the effect of TWAS$_{MW}$ percentage.

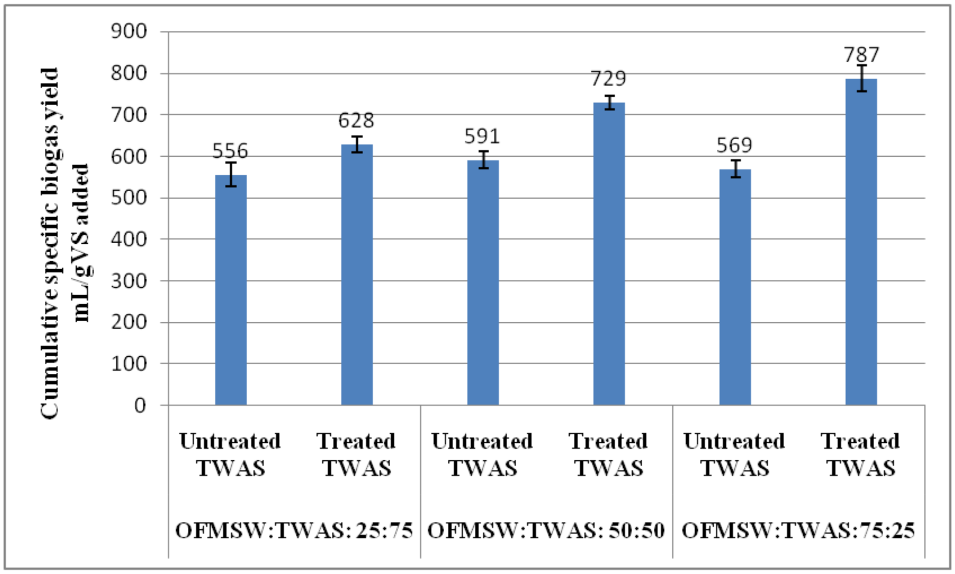


Figure 4-17 CSBY for OFMSW:TWAS and OFMSW:TWAS$_{MW}$ at different co-digestion conditions (Average of duplicate runs)

Table 4-8 shows the characteristics of the effluent after anaerobic mesophilic digestion from all the BMP assays at different combinations of OFMSW:TWAS and OFMSW:TWAS$_{MW}$. In all assays the percentage of methane in the biogas was in range of 57-60%. Doing a balance considering the methane percentage and amount of biogas produced from OFMSW:TWAS$_{MW}$ at 25:75, 50:50 and 75:25 cumulative methane production were calculated to be 3992, 4965 and 5082 mL respectively and 3420, 4013 and 3606 mL for OFMSW:TWAS at 25:75, 50:50 and 75:25 respectively again showing the benefits of co-digestion as well as pretreatment of TWAS.

Table 4-8 Properties of final effluent from BMP assays for OFMSW:TWAS and OFMSW:TWAS$_{MW}$ at different co-digestion conditions (Average of duplicate runs)

Properties	**OFMSW:TWAS 25:75**		**OFMSW:TWAS 50:50**		**OFMSW:TWAS 75:25**	
	Untreated TWAS	**Treated TWAS**	**Untreated TWAS**	**Treated TWAS**	**Untreated TWAS**	**Treated TWAS**
Initial TS %	4.6±0.2	4.7±0.25	4.6±0.23	4.5±0.1	4.2±0.19	4.0±0.3
Final TS %	3±0.1	3.19±0.1	2.8±0.15	3±0.05	2.5±0.2	2.44±0
TS % removal	35	32	37	33	39	39
Initial VS %	3.54±0.04	3.55±0.01	3.58±0.02	3.56±0	3.5±0.08	3.60±0
Final VS %	1.55±0.02	1.22±0.03	1.26±0.02	1.2±0.1	0.87±0.04	0.8±0.0
VS % removal	56	65	65	66	75	77
Biogas mL	5896	6654	6689	8275	6327	8762
CH_4 %	58	60	60	60	57	58
CH_4 mL	3420	3992	4013	4965	3606	5082

4.9 Tri-digestion BMP Assay of OFMSW, TWAS$_{MW}$ and PS

Three different combinations of OFMSW, TWAS$_{MW}$ and PS were tested to evaluate the effect of MW pre-treatment of TWAS on the tri-digestion as described in chapter 3. In all cases the binary combination of TWAS$_{MW}$:PS or TWAS:PS in the tri mixture were combined at 1:1 ratio by mass simulating the actual feed ratio at ROPEC and other secondary treatment MWWTP facilities. To recall, TWAS$_{MW}$ was heated from room temperature to 135˚C at an intensity of 2.5˚C/ min and temperature held for 1 min.

Despite having the same volumetric and specific VS OLR of 3.5%VS/assay, due to the characteristics of $TWAS_{MW}$ initial sCOD and initial alkalinity of the tri-mixtures was different in the different BMP assays. Impact of tri-digesting OFMSW:$TWAS_{MW}$:PS is shown in Figure 4-18. Improvements in CSBY of 11, 13 and 5 % were observed for OFMSW:$TWAS_{MW}$:PS when compared to their respective OFMSW:TWAS:PS controls at 75:12.5:12.5, 50:25:25 and 25:37.5:37.5 respectively. It should be noted that the proportion of $TWAS_{MW}$ used in the mixtures were increased from 12.5 to 25 to 37.5 % but the expected enhancement in CSBY did not increase proportionally. As discussed above Toreci, et al., (2009) reported that high temperature $TWAS_{MW}$ not only increases solubilisation but also possible accumulation of substances that are acutely inhibitory to methanogenesis. It is again believed that similar to the studies with binary digestion mixtures of $TWAS_{MW}$:OFMSW, as the proportion of $TWAS_{MW}$ increased from 12.5 to 37.5 % the benefits of lysis and solubilisation were in part potentially counteracted by the accumulation of inhibitory compounds. Therefore the overall effect of pretreatment decreased the positive impact on digestion of the OFMSW:$TWAS_{MW}$:PS with the greatest proportion of $TWAS_{MW}$. It is possible that inhibitory effects to methanogenesis with increased $TWAS_{MW}$ were exacerbated by the decrease in the percentage of OFMSW in the mixture. As the percent of $TWAS_{MW}$:PS (50:50 ratio) in the tri-mixture increased the percentage of OFMSW which is known to have beneficial effects for co- or tri-digestion decreased.

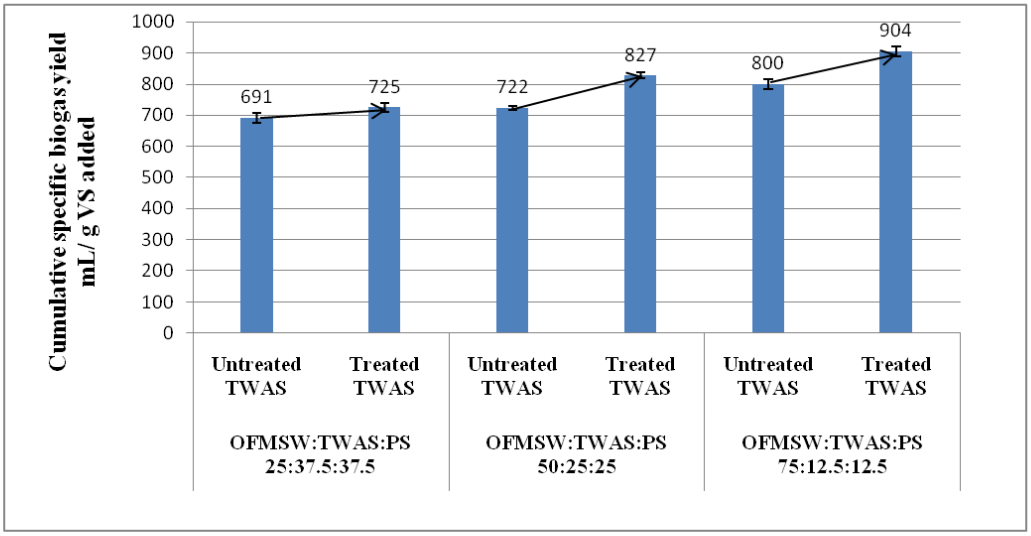


Figure 4-18 CSBY of OFMSW:TWAS:PS and OFMSW:TWAS$_{MW}$:PS at different tri-digestion conditions (Average of duplicate runs)

Table 4-9 shows the properties of the final effluent after anaerobic mesophilic BMP assays for OFMSW:TWAS:PS and OFMSW:TWAS$_{MW}$:PS at different tri-digestion conditions. The percentage of methane in the biogas was relatively constant and was in the range of 52-56% for all the combinations tested. Again considering the methane percentage and overall biogas produced the amount of methane produced from OFMSW:TWAS$_{MW}$:PS at 25:37.5:37.5, 50:25:25 and 75:12.5:12.5 was 4183,4546 and 5225 mL which were 12, 21 and 13 % higher than the methane from OFMSW:TWAS:PS 25:37.5:37.5, 50:25:25 and 75:12.5:12.5 respectively. Definitely MW pre-treatment of TWAS proved to be beneficial for the co-digestion of the tri-substrate mixture.

Table 4-9 Properties of final effluent from BMP assays for OFMSW:TWAS:PS and $OFMSW:TWAS_{MW}:PS$ at different tri-digestion conditions (Average of duplicate runs)

Properties	**OFMSW:TWAS:PS 25:37.5:37.5**		**OFMSW:TWAS:PS 50:25:25**		**OFMSW:TWAS:PS 75:12.5:12.5**	
	Untreated TWAS	**Treated TWAS**	**Untreated TWAS**	**Treated TWAS**	**Untreated TWAS**	**Treated TWAS**
Initial TS %	4.2±0.28	4.0±0.29	4.0±0.21	4.4±0.23	4.0±0.2	4.2±0.19
Final TS %	2.8±0.15	2.8±0.1	2.5±0.1	2.67±0.15	2.44±0.19	2.33±0.2
TS % removal	32	30	31	39	35	44
Initial VS %	3.42±0.01	3.54±0.04	3.37±0.04	3.58±0.02	3.44±0.09	3.5±0.08
Final VS %	1.31±0.03	1.17±0.02	1.04±0.1	0.96±0.025	0.93±0.02	0.82±0.04
VS % removal	62	65	69	73	73	77
Biogas mL	6970	7470	7225	8270	8245	9330
CH_4 %	54	56	52	55	56	56
CH4 mL	3760	4180	3760	4545	4615	5225

4.10 Effect of MW Pre-treatment HT

4.10.1 Effect on BMP Assay of $OFMSW:TWAS_{MW}$

Results presented in section 4-7 indicated that there is a positive effect on the extent of waste solubilisation of $TWAS_{MW}$ in terms of sCOD/TCOD due to increased temperature and interaction of increased holding time and increased temperature. To recall, BMP assays were repeated at the same combinations OFMSW:TWAS and $OFMSW:TWAS_{MW}$ with $TWAS_{MW}$ being MW from room temperature to 135˚C at an intensity of 2.5˚C/ min and temperature held for 1 min, 10 min or 20 min (described in chapter 3). Like all the other situations when $TWAS_{MW}$ was used the BMP assays started with same initial volumetric VS organic load of 3.5% VS/assay and specific VS organic load but $TWAS_{MW}$ increased the initial sCOD and initial alkalinity based on the mixing ratios. Since all the assays were loaded at the same initial VS organic load and inoculum charge and carried out at a neutral pH condition (minimize pH inhibition of the methane producing bacterial consortia) differences in ultimate biogas production are indicators of the benefits of pre-treatment in term of improve substrate biodegradability, and specific biogas yields (i.e. L biogas/gVS_{added}). Figure 4-19 shows the CSBY of OFMSW:$TWAS_{MW}$ held at 135˚C for 1 min, 10 min and 20 min. Although solubilization increased due to MW heating and longer HT, enhancement in sCOD was not found to result in excess biogas production. Basically biogas yield was higher in all the cases vs. the untreated control. However OFMSW:$TWAS_{MW}$ biogas yield did not improve with increased HT relative to their respective HT controls (HT 1 min). Taking into account the additional energy input with longer HT, improvements in biogas production were not proportional. Figure 4-19 clearly indicates that there was no advantage to extending the HT at any waste mixture combination. In fact for all cases pretreatment at extended times of 20 and 10 min resulted in the same or lower biogas yields than that of HT 1 min. It was not clear what exact relation existed between the effect HT and biogas production so experimental design to determine the empirical effect is described in the next segment.

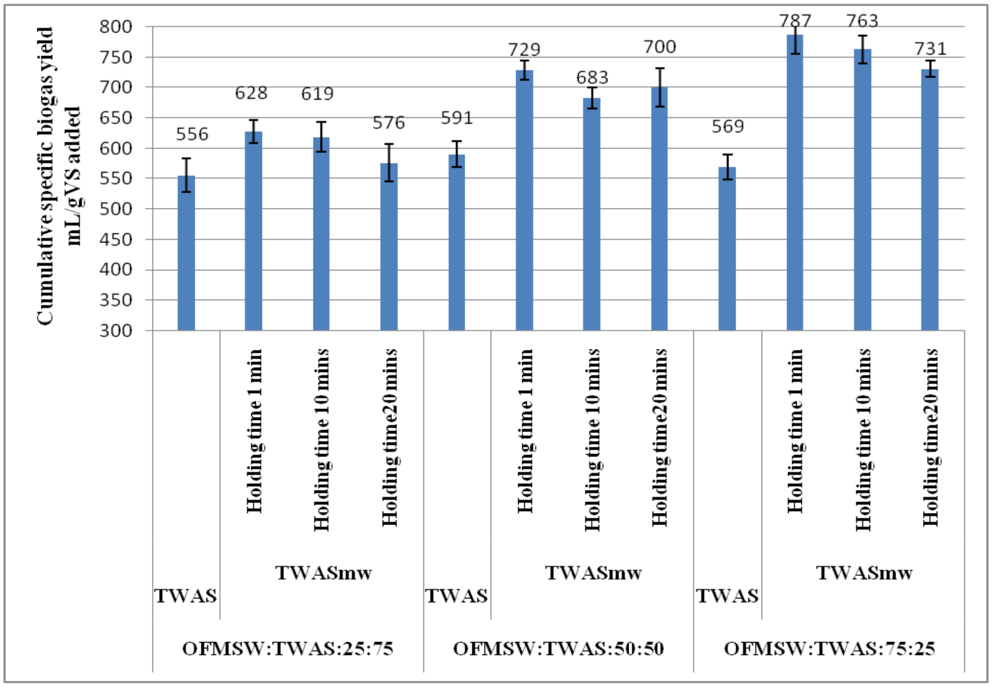


Figure 4-19 CSBY of OFMSW:TWAS and OFMSW:$TWAS_{MW}$ when $TWAS_{MW}$ was treated at three different HT (Average of duplicate runs)

Among all models used to fit the data of ultimate BMP biogas production from assays as of OFMSW:$TWAS_{MW}$ treated by MW at 135˚C at three different holding times and the percentage of OFMSW in the mix response surface regression analysis was found to adequately fit the data. Statistical checks of $R^2_{adj,}$ t, p and F values were found satisfactory in this model and results are shown in Table 4-10.

Complete model can be represented by the following Eqn. 4.6:

$$\boxed{\text{Biogas Production}=3559+140 \times \%\text{OFMSW in Mix} - 0.9 \times (\%\text{OFMSW in Mix})^2}$$

……………Eqn. 4.6

Table 4-10 Results of forward step regression analysis for the full biogas production model from OFMSW:TWAS as a function of OFMSW percentage and temperature HT

	Comment	**Biogas production Parameter**	**Biogas production Std. Err**	**Biogas production t**	**Biogas production p**
Intercept		**3559.00**	715.72	4.972	0.002
% OFMSW in the mix		**140.25**	32.50	4.3140	0.005
% OFMSW in the mix^2		**-0.998**	0.3218	-3.1004	0.021
T HT	Pooled				
T HT^2	Pooled				
% OFMSW in the mix x T HT	Pooled				

Whole Model

Multiple R^2	**Adjusted R^2**	**SS**	**MS**	**F**	**p**
0.93	0.91	6924393	3462196	42.8	0.0002

Results shown in Table 4-10 in conjunction with Figure 4-20 indicate that within the range studied increased biogas production from the BMP assay treating OFMSW:TWAS$_{MW}$ is a function of increased OFMSW percentage in the mix (increasing the component of OFMSW and decreasing the TWAS$_{MW}$) while temperature holding time has little to minimal effect on biogas production.

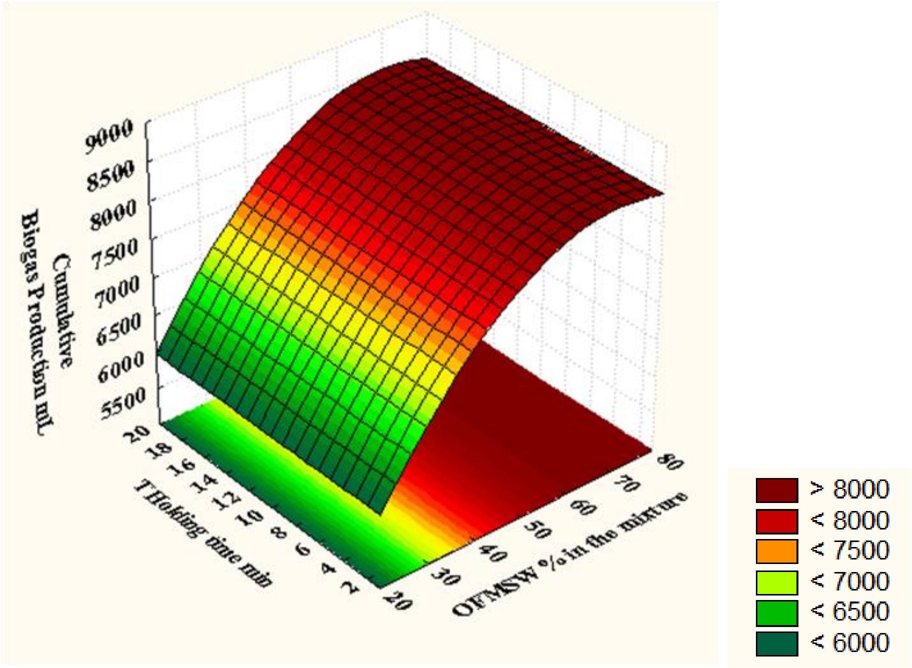


Figure 4-20 Surface response of biogas produced from OFMSW:TWAS$_{MW}$ as a function of OFMSW percentage and MW temperature holding time

4.10.2 Effect on BMP Assay of OFMSW:TWAS$_{MW}$:PS

Figure 4-21 shows the ultimate biogas production from the BMP co-digestion of OFMSW:TWAS$_{MW}$:PS when TWAS$_{MW}$ was treated at three different HTs. From Figure 4-21, biogas production results due to tri-substrate mixtures (same VS organic load) and MW pre-treatment HT are not straight forward. Concomitantly, statistical regression analysis was performed on the data (discussed above) and the results for the best fitted model have been shown in Table 4-11. The simple regression model was found to fit the data more adequately than other higher order models as R^2_{adj} decreased when additional higher order terms were added to the model. (Data shown in appendix B)

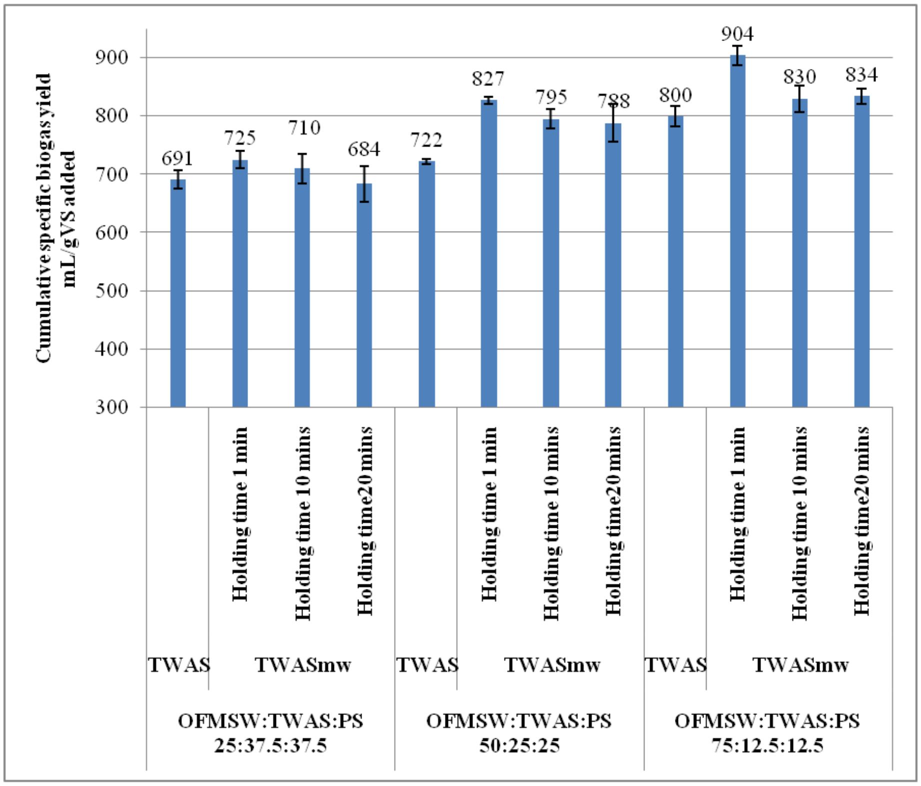


Figure 4-21 CSBY at different combination of OFMSW:TWAS:PS when TWAS was MW treated at three different holding time

The results shown in Table 4-11 and Figure 4-21 illustrate that ultimate biogas yield from the tri-digestion BMP assay of the more complex but more realistic mixtures of OFMSW:TWAS$_{MW}$:PS that would be generated at municipal secondary MWWTP. Ultimate biogas is again a function of increasing OFMSW percentage in the tri-mixture (increasing the component of OFMSW and decreasing PS and TWAS$_{MW}$) and again there is little or minimal effect of HT on biogas production similar to what was found for the biogas yield from co-digestion of OFMSW:TWAS$_{MW}$.

The model that represents the data obtained can be described by the following Eqn 4.7:

$$\text{Biogas production}=5637.3+40.5 \text{ x } (\% \text{ OFMSW in mix})$$

……Eqn. 4.7

Table 4-11 The results of the complete model of biogas produced from OFMSW:TWAS:PS as a function of OFMSW percentage and temperature HT

	Comment	**Biogas production Parameter**	**Biogas production Std. Err**	**Biogas production t**	**Biogas production p**
Intercept		**5637.3**	374.5818	15.1	0.000001
% OFMSW in the mix		**40.48**	6.9359	5.8	0.0006
T HT	Pooled				
% OFMSW in the mix x T HT	Pooled				
		Whole Model			
Multiple R^2	**Adjusted R^2**	**SS**	**MS**	**F**	**p**
0.82	0.80	6146888	6146888	34.1	0.0006

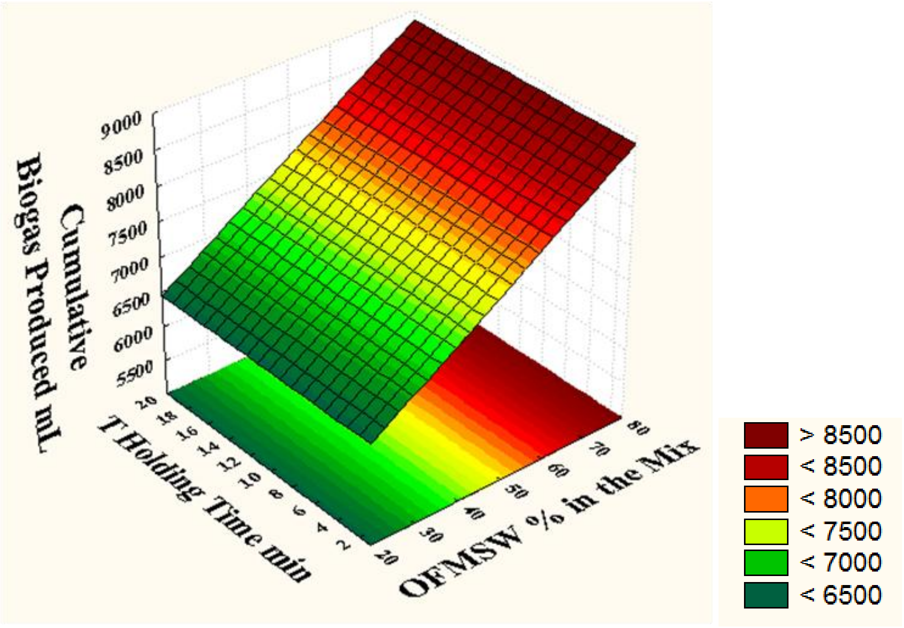


Figure 4-22 Surface response of biogas produced from OFMSW:TWAS$_{MW:}$PS as a function of OFMSW percentage and MW temperature HTs

Among all the OFMSW:TWAS$_{MW}$:PS and OFMSW:TWAS:PS combinations tested 75:12.5:12 produced the highest CSBY of 0.9 and 0.8 L/gVS$_{added}$ respectively although care had to be taken to regulate the pH. However, from a practical and likely regulatory application position, MWWTPs will most likely not allow such a high percentage of OFMSW to be added to digester especially with the potential of pH control issue. Considering a more practical scenario to study the potential benefits or problems related to co- and tri-digestion of TWAS:PS and OFMSW:TWAS:PS semi-continuous mesophilic anaerobic digesters were operated with a lower proportion of OFMSW (50% rather than 75% in the mixtures) at HRTs of 7, 10 and 15 days (OLRs 2.0, 3.0 and 4.5 gVS/L/d) with either TWAS or TWAS$_{MW}$. In all cases the ratio of PS:TWAS and PS:TWAS$_{MW}$ was maintained at 1 to 1 which is a fixed ratio at ROPEC and most MWWTPs. Results are discussed in the following section.

4.11 Results of Semi-Continuous Digestion

Four single stage semi-continuous mesophilic AD reactors were operated at HRTs of 15, 10 and 7 days and three different OLR to evaluate the comparative effect of digestion of tri-mixtures of OFMSW:TWAS$_{MW}$:PS and OFMSW:TWAS:PS over binary mixture of TWAS and PS. Moreover, two of the semi-continuous reactors were operated using TWAS$_{MW}$ in the binary or tri-mixtures that were digested. Reactor configurations and operational details have been discussed in chapter 3. Figure 4-23 recalls the name of the reactors.

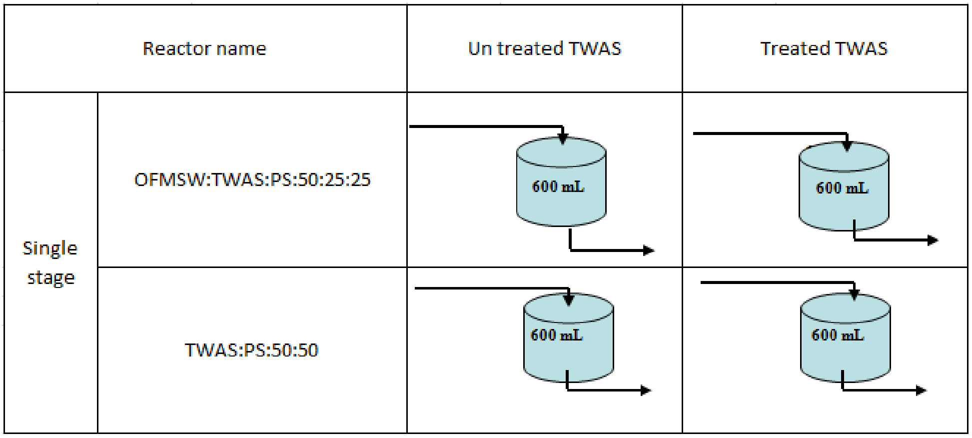


Figure 4-23 Reactor configurations for semi-continuous studies

4.11.1 Reactor Stability Test

All four reactors were started at HRT of 15 days with an OLR of 2.0 gVS/L/d. None of the 4 semi-continuous reactors had adaptation problem as they were already acclimated to PS and TWAS and were started at a high HRT of 15 day and low OLR resulting in a pH which was normally stable in the range of 6.8-7.6 which is favorable for the methane producing bacteria. (Jash and Ghosh, 1996). Besides regular monitoring of pH, IA and TA (mg $CaCO_3$/L) was measured. The SR of the reactors was defined as the ratio of IA/TA tested of the reactor effluent. The IA represents VFAs and TA is the total alkalinity or buffering capacity of the reactor. (Speece, 2008). The SR should be less than or equal to 0.3 to consider a reactor as stable, as this ratio indicates the presence of sufficient buffering capacity in the reactor to maintain it in the neutral range without the possibility of rapid decreasing pH change (ORWC, 2011). Additional data showed that during the entire test all four reactors were able to maintain the stability ratio at/ less than 0.3. However, at the highest OLR (4.5 gVS/L/d) and HRT of 7 days the reactors treating OFMSW:$TWAS_{MW}$:PS and OFMSW:TWAS:PS had some problem maintaining a neutral pH which decreased to approximately a pH of 5 before pH was adjusted manually. In order to circumvent the low pH problem, sodium and potassium buffer solutions were added as needed to maintain the pH between 6.8-7.4 which is suitable for methane forming bacteria (Jash, et al., 1996). Table 4-12 shows the results of the stability ratio check of all four reactors at the three different HRTs tested. VFA concentrations in the reactor treating OFMSW:TWAS$_{MW}$:PS:50:25:25 was 1300-1800 mg/L which was higher than that of OFMSWLTWAS:PS:50:25:25. It was believed that MW pre-treatment increased solubilisation of TWAS which in combination with the OFMSW resulted in more readily degradable substrate for the bacterial consortia that contributed to faster acidification in the reactors while at the same time likely produced some MW heat related inhibitory substances that would tend to inhibit methanogens. This waste combination leads to an accumulation of VFAs especially at higher OLR when it was believed that the acetoclastic methogens could not maintain their rate of conversion. In conjunction with this discussion, higher SR were observed at all HRTs by the reactor treating OFMSW:TWAS$_{MW}$:PS and OFMSW:TWAS:PS that were in general about 3 fold higher than reactors treating TWAS:PS or TWAS$_{MW}$:PS suggesting that the more readily degradable substances in the OFMSW and not the MW treatment were responsible for the accumulation of VFAs and decrease in reactor stability as measured by the higher SR values. However, Table 4-12 also shows that while the SR for reactor treating OFMSW:TWAS$_{MW}$:PS and OFMSW:TWAS:PS were in the range 0.17-0.3 which was higher than that of TWAS:PS and TWAS$_{MW}$:PS reactors. But there still should have been enough buffering capacity in the reactors for the bacterial consortia and to prevent the reactor from decreasing even at higher OLRs of 4.5 gVS/L/d. Reactors treating TWAS:PS and TWAS$_{MW}$:PS had lower stability ratios of 0.07-0.1 at HRTs of 15, 10 and 7 days. Reactor with a SR of 0.1-0.2 is considered ideal working conditions for methanogenesis. (ORWC, 2011). To conclude the observations made on reactor stability, it can be mentioned that digestion of OFMSW with TWAS:PS and TWAS$_{MW}$:PS might cause some problems in terms of maintaining neutral pH if loading rates are increased however given time tri-digestion eventually should come to a stable condition acceptable for the anaerobic consortia in the system.

Table 4-12 Results of reactor stability test

Reactor name	HRT days	OLR gVS/L/d	IA mg /L as CaCO3	TA mg/L as CaCO3	TVFA mg/L	Stability Ratio(=IA/TA)
OFMSW:TWAS$_{MW}$:PS	15	2	1400±112	4700±291	1750±88	0.3
OFMSW:TWAS:PS			1050±104	4250±125	1312±178	0.25
TWAS$_{MW}$:PS			350±50	3750±490	437±70	0.09
TWAS:PS			400±58	4350±619	500±90	0.09
OFMSW:TWAS$_{MW}$:PS	10	3.5	900±82	5200±296	1125±114	0.17
OFMSW:TWAS:PS			500±26	4600±325	625±119	0.3
TWAS$_{MW}$:PS			450±37	4350±102	360±69	0.1
TWAS:PS			150±19	3950±562	188±96	0.09
OFMSW:TWAS$_{MW}$:PS	7	4.5	1450±238	5700±523	1812±63	0.25
OFMSW:TWAS:PS			1400±127	5600±342	1750±142	0.25
TWAS$_{MW}$:PS			400±140	5000±237	320±57	0.08
TWAS:PS			350±98	4900±459	437±101	0.07

4.11.2 Biogas Study from Semi-continuous Reactors

Daily biogas production (DBP) data obtained at different HRTs are presented in Figure 4-24 and 4-25. Figure 4-24 and 4-25 shows the SS data and indicates substantial improvement in biogas production from reactors treating OFMSW:TWAS$_{MW}$:PS and OFMSW:TWAS:PS compared with TWAS:PS and TWAS$_{MW}$:PS while being operated at the same OLR. If daily biogas production varied within 10 % after 3 HRTs that phase was considered at SS at a given HRT. Average biogas production at SS from the reactor treating OFMSW:TWAS$_{MW}$:PS was 1106, 1367 and 1677 mL/L.d while for reactor treating TWAS$_{MW}$:PS was 825, 978 and 1278 mL/L.d at OLRs of 2.0, 3.0 and 4.5

gVS/L/d respectively. On the other hand, at the same OLRs of 2.0, 3.0 and 4.5 gVS/L/d, reactor treating OFMSW:TWAS:PS without MW pretreatment produced biogas at average rates of 970, 1270 and 1500 mL/L.d while from TWAS:PS 750, 827, 1200 mL/L.d biogas was produced.

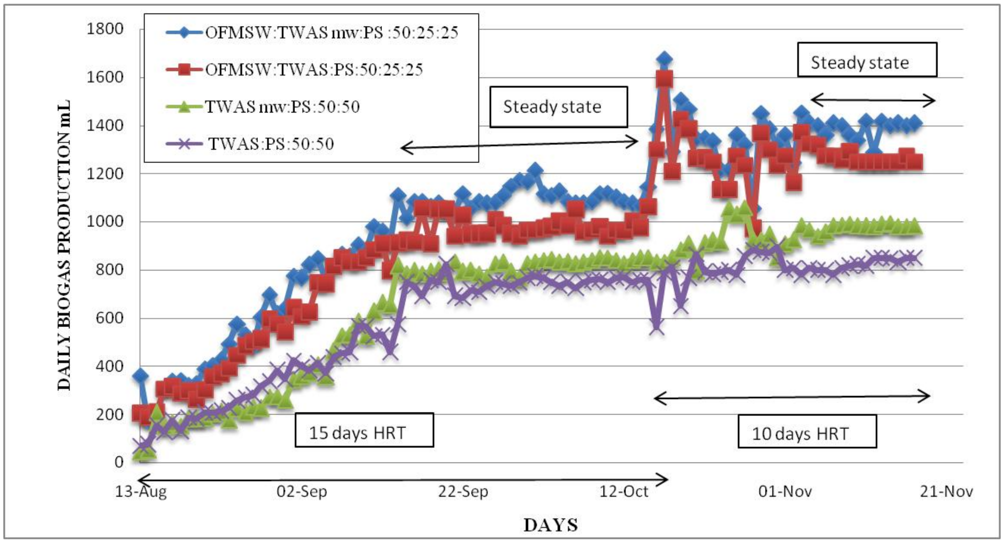


Figure 4-24 Daily biogas production at 15 days and 10 days HRTs

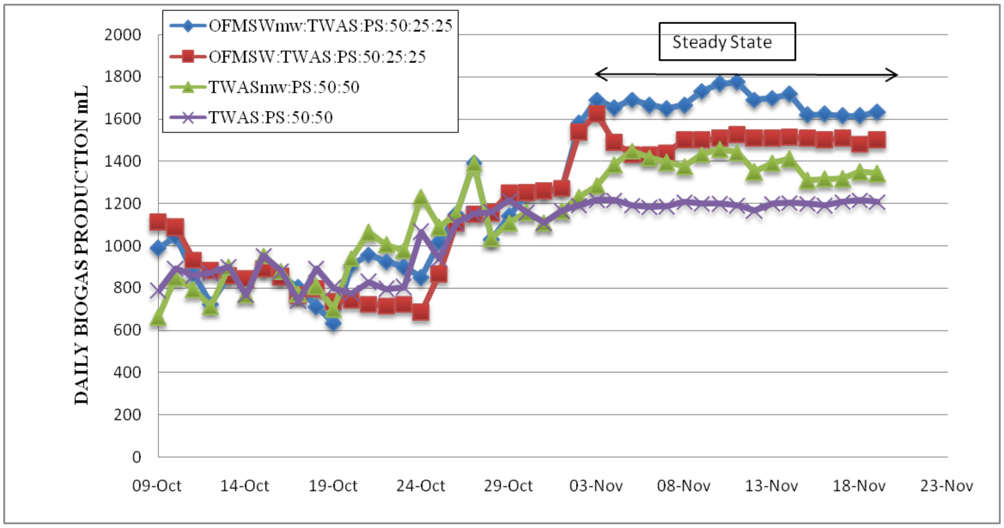


Figure 4-25 Daily biogas production at 7 days HRT

A comparative evaluation of average DBP at different SS from the different reactors in Figure 4-26 shows the comparison between DBP of OFMSW:TWAS$_{MW}$:PS over TWAS$_{MW}$:PS and OFMSW:TWAS:PS over TWAS:PS at SS. Improvement in DBP at SS for OFMSW:TWAS$_{MW}$:PS was 26, 29 and 18 % higher than that of TWAS$_{MW}$:PS at HRTs of 15, 10 and 7 days respectively. On the other hand, increase in average DBP for OFMSW:TWAS:PS at SS was 23, 35 and 20 % greater than that of TWAS:PS at HRTs of 15, 10 and 7 days respectively. Improvement in semi-continuous reactor biogas production supports the results of the previous BMP assays and gives additional evidence that tri-digestion of OFMSW with TWAS:PS and TWAS$_{MW}$:PS provides a positive influence on AD regardless if pre-treatment of TWAS is or is not used.

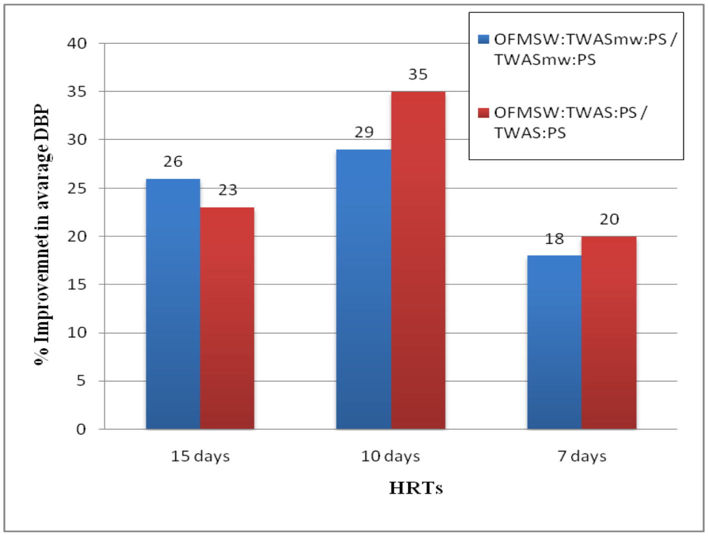


Figure 4-26 Improvement in daily biogas production in semi-continuous reactors

Figure 4-27 illustrates that in general, biogas production/gVS$_{destroyed}$ had an inverse relation with increasing OLR as might be expected as the contact and reaction time required for stabilization of the waste mixtures by the microbial consortia becomes limiting. However, if reactor OFMSW:TWAS:PS and reactor TWAS:PS are being compared (no MW pre-treatment), higher biogas yields can be noted for the tri-digestion of OFMSW:TWAS:PS which went from 1884 to 1251 mL/gVS$_{destroyed}$ vs 1369 to 949 mL/gVS$_{destroyed}$ for TWAS:PS as the HRT decreased from 15 to 7 days. A similar result was observed by comparing reactor OFMSW:TWAS$_{MW}$:PS and reactor TWAS$_{MW}$:PS. Biogas yields decreased from 1761 to 1484 mL/gVS$_{destroyed}$ and from 1205 to 1121 mL/gVS$_{destroyed}$ for OFMSW:TWAS$_{MW}$:PS and TWAS$_{MW}$:PS respectively for decreasing HRTs from 15 days to 7 days and increasing OLRs from 2 gVS/L/d to 4.5 gVS/L/d. These results again exemplify the advantages of tri-digestion of OFMSW:TWAS$_{MW}$:PS or OFMSW:TWAS:PS over TWAS$_{MW}$:PS or TWAS:PS and additionally indicates that increasing the OLR (shorter HRTs) provides an abundance of organic substances for digestion and the complete mixed AD process has insufficient reaction times to use the substrate efficiently. These results are in agreement with results reported by Hartmann and Ahring, (2003). On the other hand, (Coelho, et al., 2011)used TWAS$_{MW}$ pre-treated to 96º C and reported that gas production/mass VS added or destroyed decreased with decreasing HRT with or without pre-treatment. (Toreci, et al., 2009) also evaluated TWAS$_{MW}$ at pre-treatment temperatures above 100º C and found that biogas production/mass VS added for controls decreased with shorter HRTs but it remained relatively constant for HRT of >10days suggesting that pre-treatment increased biodegradability of the TWAS.

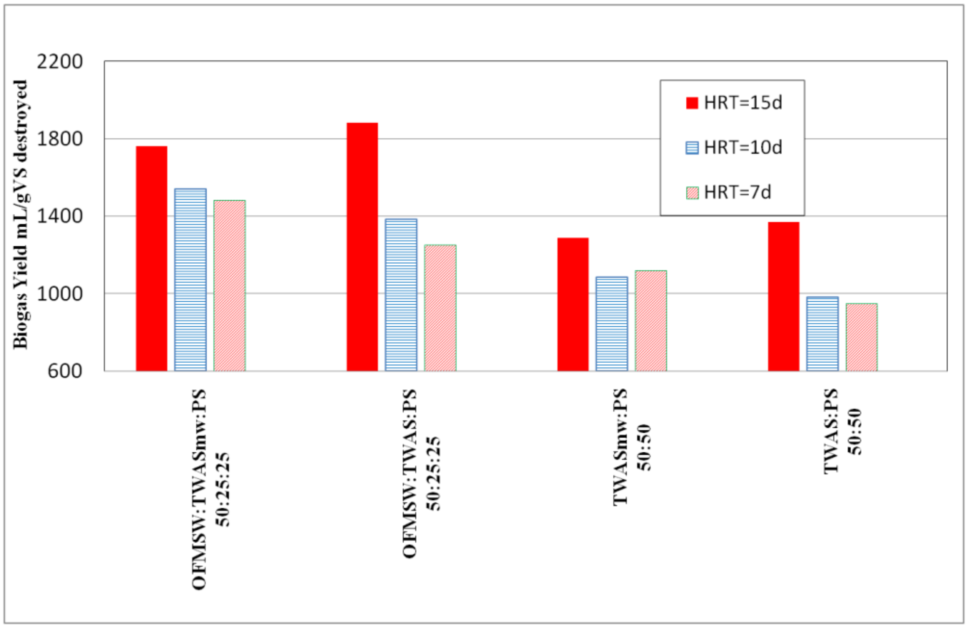


Figure 4-27 Biogas yield at various HRTs and co/tri-digestion scenarios

4.12 Performance of Semi-Continuous Reactors

As the objective of this study was to evaluate the effect of anaerobic co-digestion on biogas production and reactor performance as well as the effect of MW pre-treatment of TWAS on anaerobic co-digestion the overall been are presented in the following two segments.

4.12.1 Effect of Tri-digestion on Biogas Yield

To better evaluate the effect of anaerobic tri-digestion at various HRTs for OFMSW:TWAS:PS and OFMSW:TWAS$_{MW}$:PS a relative improvement factor for tri-digestion (IF$_t$) was determined by the comparison of biogas yields of the TWAS:PS or TWAS$_{MW}$:PS reactors at the same HRT conditions and is presented in Figure 4-28. Figure 4-28 indicates that the IF$_t$ for OFMSW:TWAS:PS and OFMSW:TWAS$_{MW}$:PS increases with increasing HRT regardless of the use of TWAS or TWAS$_{MW}$ in the mixture.

At 15 days HRT anaerobic tri-digestion of OFMSW:TWAS:PS and OFMSW:TWAS$_{MW}$:PS had IF$_t$ values of 1.37 and 1.46 respectively indicating a positive impact of tri-digestion of OFMSW:TWAS:PS and OFMSW:TWAS$_{MW}$:PS over TWAS:PS or TWAS$_{MW}$:PS. However as the OLR was increased (shorter HRT) the IF$_t$ decreased and at the highest OLR of 4.5 gVS/L/d and the shortest HRT of 7 days the IF$_t$ for OFMSW:TWAS:PS and OFMSW:TWAS$_{MW}$:PS decreased to 1.31 and 1.32 respectively demonstrating that operation of longer HRTs produced the best conditions to benefit from the co-digestion of OFMSW with TWAS:PS or TWAS$_{MW}$:PS. When the reactors were operated at high OLRs (short HRT) improvement decreased likely due to insufficient reaction time for the more difficult components in the substrate to be degraded. Additionally, possible inhibition due to accumulation of VFAs since methanogens grow slower than acidogenic bacteria at higher OLR causes imbalance in the anaerobic consortia and eventual reduction in methane production. (Mohan, et al., 2008)

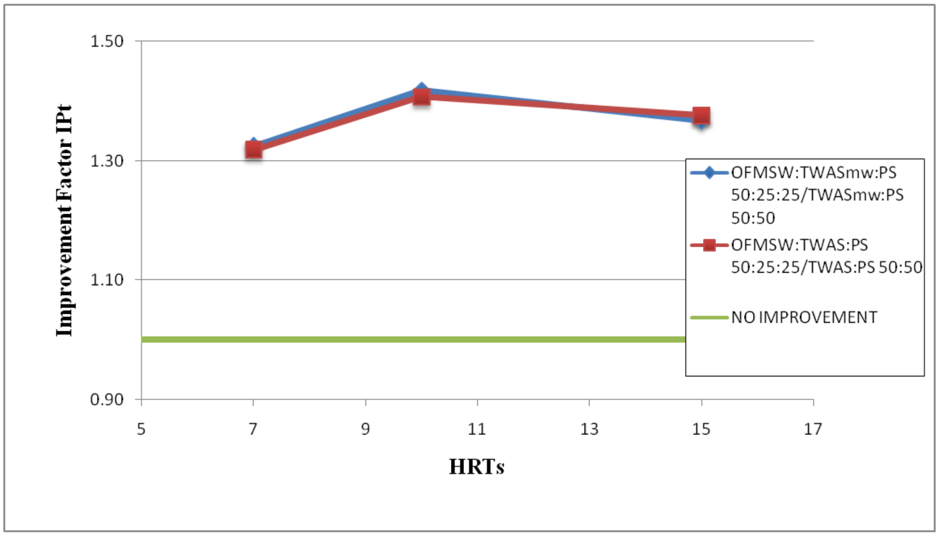


Figure 4-28 Improvement factors at different co-digestion scenario

4.12.2 Effect of MW Pre-Treatment on Biogas Yield

To evaluate the effect of MW pre-treatment a comparative evaluation between reactor which treated OFMSW:TWAS$_{MW}$:PS over reactor treating OFMSW:TWAS:PS as well as reactor treating TWAS $_{MW}$:PS over reactor treating TWAS:PS was done based on the biogas production/gVS$_{destroyed}$. A relative improvement factor for pretreatment (IF$_p$) was determined from the comparison of biogas yields and is presented in Figure 4-29. Figure 4-29 shows that the IF$_p$ of OFMSW:TWAS$_{MW}$:PS and TWAS$_{MW}$:PS reactors decreased inversely with increase in HRT. At short HRT of 7 days IF$_p$ was 1.19 and 1.18 while IF$_p$ decreased to 1.11 and 1.10 for OFMSW:TWAS$_{MW}$:PS and TWAS$_{MW}$:PS reactors respectively at HRT of 10 days. Although the improvement in biogas yield was not that significant at the highest HRT tested (15 days), these results are evidence that MW pre-treatment of TWAS provides some improvement in biogas production/gVS$_{destroyed}$ at certain OLRs regardless of co-digestion of OFMSW, TWAS and PS or TWAS and PS. Similar findings were reported by Coelho, et. al., (2011) for digestion of TWAS. It is important to note that for anaerobic digestion of TWAS improved COD removal and higher biogas production compared to control has been reported (Toreci, et al., 2010)but the improvement was observed only at lower HRTs. Two interesting points that come from the present study are that MW pre-treatment enhances biodegradability of TWAS components which are tougher to degrade at HRTs of less than 10 days and additionally, increase in biogas yield was not proportional with the higher percentage of TWAS (by weight) in the mixture as the co-substrate. For example the IF_p was 1.19 and 1.18 for OFMSW, $TWAS_{MW}$ and PS and $TWAS_{MW}$ and PS reactors respectively at OLR of 4.5 gVS/L/d (HRT 7 days), and the IF_p was 1.11 and 1.10 for OFMSW, $TWAS_{MW}$ and PS and $TWAS_{MW}$ and PS respectively at HRT of 10 days, however the respective percentage of $TWAS_{MW}$ was 25 and 50 % in the mixture respectively suggesting the possibility of inhibition due to MW irradiation (Toreci, et al., 2009) as well as indirect inhibition of acetocalastic methanogens resulting from $TWAS_{MW}$ components that result in accumulation of VFAs since methanogens grow slower than acidogenic bacteria, which causes imbalance in the anaerobic consortia and eventual reduction in methane production. (Mohan, et al., 2008)

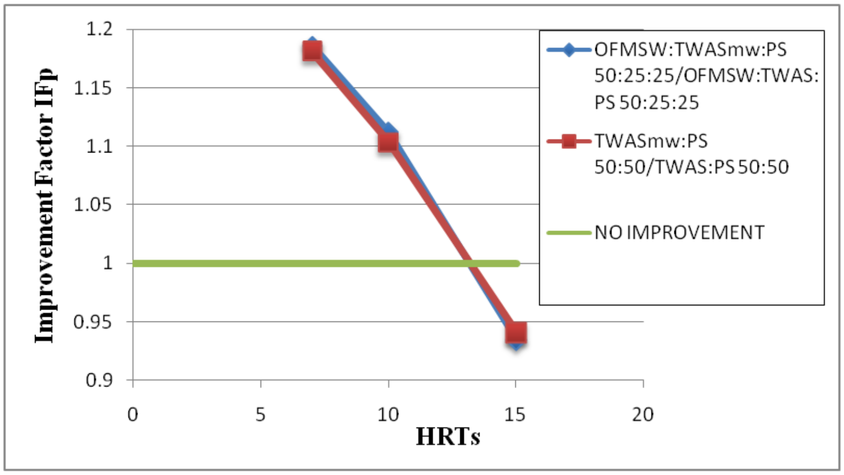


Figure 4-29 Effect of MW pre-treatment on semi-continuous reactors

Table 4-13 summarizes the SS properties of the reactors at different HRTs. All the data are the arithmetic means of measured values and standard deviations are indicated in the parenthesis below each data.

Treatment performances of all four reactors are evaluated in terms of TCOD and VS removal in this study. It was shown in Figure 4-30 that TCOD removal efficiency decreased with the decrease of the HRTs as might be expected as the contact and reaction time required for stabilization of the waste mixtures by the microbial consortia becomes limiting. (Shahriari, et al., 2011). TCOD removal rate for the OFMSW:TWAS$_{MW}$:PS reactor decreased from 47 to 44 to 40 % with the increase in OLR from 2 to 3 to 4.5 gVS/L/d, meanwhile in reactor treating TWAS$_{MW}$:PS, TCOD removal rate was 40, 40 and 34 at the same corresponding OLRs. Higher TCOD removal in the OFMSW:TWAS$_{MW}$:PS reactor (tri-mixture with TWAS$_{MW}$) compared to reactor OFMSW:TWAS:PS (tri-mixture without MW pre-treatment of TWAS) indicates a positive interaction for tri-digestion of OFMSW, TWAS$_{MW}$ and PS which might be consideration for real MWWTPs that they can achieve a better organic removal efficiency in treatment facilities by introducing co-digestion of OFMSW (with vs. without MW pre-treatment) with their conventional feed (TWAS and PS). A similar observation was drawn from the comparison of TCOD removal efficiency of reactor OFMSW:TWAS:PS which were 53, 46 and 41% at HRTs of 15, 10 and 7 days respectively while at the same HRTs were 34, 37(exceptionally higher removal rate at 10 days HRT than 15 days HRT) and 30 % from reactor TWAS:PS.

Table 4-13 SS effluent properties of the semi-continuous reactors at different HRTs

	15 Days HRT				10 Days HRT				7 Days HRT			
	OFMSW: TWAS:PS:50:25:25		TWAS:PS:50:50		OFMSW: TWAS:PS:50:25:25		TWAS:PS:50:50		OFMSW: TWAS:PS:50:25:25		TWAS:PS:50:50	
Properties	TREATED TWAS	UNTREATED TWAS	TREATED TWAS	UNTREATED TWAS	TREATED TWAS	UNTREATED TWAS	TREATED TWAS	UNTREATED TWAS	TREATED TWAS	UNTREATED TWAS	TREATED TWAS	UNTREATED TWAS
OLR gVS/L/d	2.03(0.03)	1.97(0.06)	2(0.03)	1.92(0.08)	3.05(0.01)	3(0)	3.1(0.05)	3.01(0)	4.5(0.04)	4.5	4.5(0)	4.5(0.01)
OLR g tCOD/L/d	3.85(0.09)	3.67(0.07)	3.8(0.03)	3.73(0.7)	5.5(0.5)	5.4(0.07)	5.3(0.03)	5.1(0.09)	8	7.9(0.08)	7.3(0.08)	7.8(0.09)
VS removal %	52(1.1)	43(1.5)	51(2.3)	47(1)	49(2)	42(1.01)	48(1)	46(2)	41(1.5)	41(1.7)	35(0.5)	34(0.7)
tCOD removal %	47(4)	52(2)	40(1)	34(1)	44(0)	46(1)	40(2)	37(1.2)	40(2)	41(0)	34(3)	30(2.4)
Biogas prod. mL/L/d	1105(40)	970(25)	825(48)	750(16)	1387(37)	1269(26)	978(52)	827(24)	1677(50)	1500(43)	1378(24)	1200(13)
Biogas yield mL/ gVS destroyed	1761	1883	1204	1368	1540	1385	1090	984	1484	1251	1120	949
CH$_4$ %	61	58	59	60	60	62	62	60	61	62	59	60
Actual CH4 yield mL/L/d	652	563	486	435	832	786	596	496	1022	870	799	720
Theoetical CH$_4$ yield mL/L/d	637	676	536	443	851	869	746	665	1156	1137	746	725

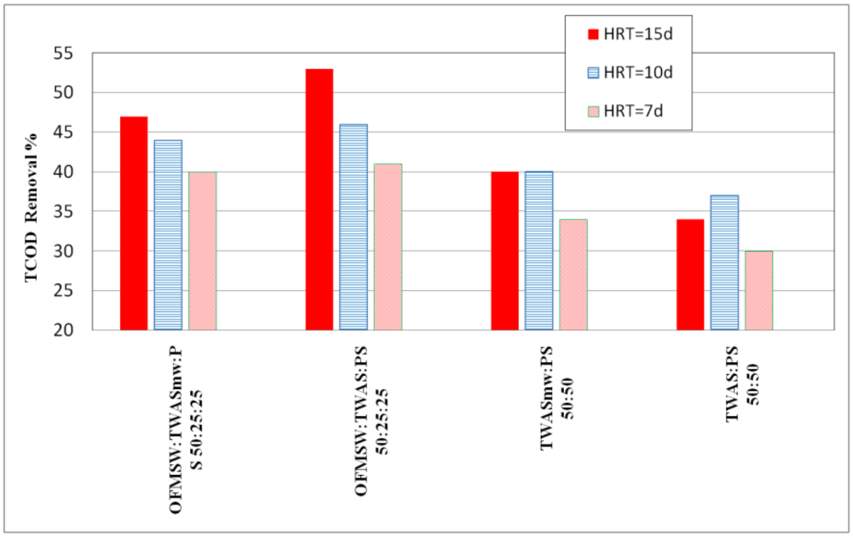


Figure 4-30 TCOD removal efficiency of semi-continuous reactors

On the other hand, overall VS removals from all the four reactors were in the range of 35-48% (Figure 4-32). An advantage of anaerobic tri-digestion has been in terms of increased biogas yield per gVS destroyed. VS removal rate from all four reactors decreased with decrease of HRTs as the contact and reaction time required for stabilization of the waste mixtures by the microbial consortia becomes limiting. (Shahriari, et al., 2011). VS removal rates from reactor OFMSW:TWAS$_{MW}$:PS were 51,49 and 41% at OLR of 2, 3 and 4.5 gVS/L/d respectively. It is interesting to notice that while the OLR was more doubled at HRT of 7 days vs. 15 days reactor OFMSW:TWAS$_{MW}$:PS was still capable of achieving 41 % VS removal at HRT of 7 days. Although removal percentage decreased, overall mass of VS removal (total amount of VS removed in g/L/d) was higher at shorter HRT which is as consideration for real MWWTPs. Similar observations were made for the reactor treating the tri-mix blend without TWAS pre-treatment (OFMSW:TWAS:PS). VS removal was 44, 42 and 41% from reactor OFMSW:TWAS:PS at HRTs of 15, 10 and 7 days respectively. Meanwhile 51, 48 and 35 % VS removal from the reactor TWAS$_{MW}$:PS and 47,46 and 34 % VS removal from reactor TWAS:PS were achieved at the HRT of 15, 10 and 7 days respectively.

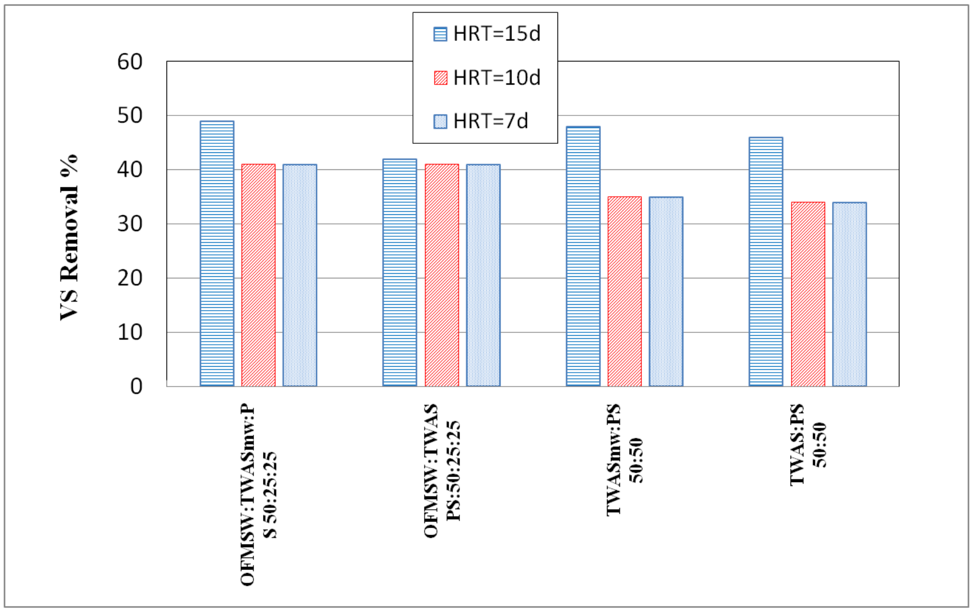


Figure 4-31 VS removal efficiency of semi-continuous reactors

4.12.3 Mass Balance in Semi-Continuous Test

To evaluate the overall performance for all four semi-continuous reactors a COD mass balance was completed and is presented in Table 4-13. COD conversion to methane was based on the theoretical conversion of 1g COD removed per 350ml methane produced at STP (Speece, 2008). The daily conversion of COD is known (ΔCOD), so CH_4 production can be calculated and compared with the observed values (Table 4-13). While most of the calculated results are in agreement with the experimental observations it could be argued that the observed values were slightly higher than predicted. However based on daily fluctuations in feed concentration, determination of COD values and errors in measurement of gas the data comparison shows a very close correlation and gives us added confidence in our evaluation of the various processes and pre-treatments.

Chapter 5- Conclusions

5.1 Conclusions

5.1.1 Conclusions for Batch BMP Assay Study

Digestion of tri-mixtures of OFMSW:TWAS:PS at various ratios consistently produced CSBY that were markedly improved vs. digestion of binary mixtures of OFMSW:TWAS. Although all BMP assays had the same initial organic loading, the more complex mixture of OFMSW:TWAS:PS in the ratio of 75:12.5:12.5 produced 22 % more biogas than that produced by the 50:50 TWAS:PS mix. For the other two OFMSW:TWAS:PS combinations (50:25:25 and 25:37.5:37.5) the biogas improvements when compared to the 50:50 TWAS:PS was approximately 13%. However it was recognized that increasing the fraction of OFMSW in the tri-mixture led to increase acidification (accumulation of VFAs) in the BMP assays that in cases required pH increase intervention. The best tri-mixture combination in terms of improved biogas yield and balanced methanogenesis selected for use for continuous reactor testing was OFMSW:TWAS:PS:50:25:25 (with or without $TWAS_{MW}$).

MW pre-treatment of TWAS was found to increase COD solubilization increasing 1.6, 2 and 2.2 fold higher than TWAS control when pre-treatment temperatures increased from 95 to 135 to 175 ºC and held for a 1 min HT. Improvements in TWAS solubilization were mainly effected by temperature and interaction between increased temperature and increased HT. However the greatest improvements in biogas production and CSBY were observed at 135 ºC with 1 min HT and no additional improvements were realized by increased temperature or by extending the MW HT.

Based on the batch BMP assays the range of variables to be tested in semi-continuous reactors was narrowed to what was felt to be the best conditions to realize increased performance from tri-digestion (OFMSW:TWAS:PS:50:25:25 and OFMSW:$TWAS_{MW}$:PS 50:25:25 vs. binary (TWAS:PS and $TWAS_{MW}$:PS 50:50) co-digestion.

5.1.2 Conclusion for the Semi-continuous Study

Semi-continuous reactor studies demonstrated that per kg of VS treated, considerably more biogas could be produced by digesting a tri-mixture of OFMSW:$TWAS_{MW}$:PS or OFMSW:TWAS:PS (50:25:25) vs. the standard method of TWAS:PS (50:50) digestion at MWWTPs. For example digestion of OFMSW:TWAS:PS produced 36% more biogas than digestion of TWAS:PS (no MW pre-treatment) at an HRT of 15 days. These improved biogas yields were realized at HRTs of 10 and 15 days and OLRs of 3.5-2 kg VS/m^3/d both of which are loadings that are used at secondary MWWTP. The high concentration of organics in the OFMSW allows for increased loading without effecting HRT. In fact until the design load of the MWWTP is approached OLR rather than HRT will be the deciding design operating factor. The development of a business case for co-digesting of OFMSW with PS and TWAS should be developed so that other bottlenecks either technical (size reduction of OFMSW, use of alternative sources of organics i.e. kitchen waste or FOG) or economic (tipping fees) can be addressed.

5.2 Recommendations for Future Work

The following recommendations are intended to build upon the research presented in this thesis, in order to assess the full potential for the co-digestion of OFMSW and TWAS/PS:

- BMP assays in this research were run at comparatively higher organic loading (3.5 % VS/assay) that in some cases lead to VFA inhibition in the assay that resulted in a portion of TCOD not being converted into methane. To evaluate the full potential of the substrates BMP co-digestion assays could be run at lower organic loads; OLR for similar future BMP assays should be reduced to 2 % VS/assay. BMP assays run at lower organic loadings may still provide differences in biogas yield but reactor acidification in the batch test can be minimized which would potentially circumvent the need for pH control especially when co-digestion of OFMSW and municipal sludge is performed with a greater proportion of OFMSW in the mixture.
- This research was carried out considering the co-digestion philosophy that ultimate biogas production capacity of existing MWWTPs can be improved by co-digesting with OFMSW. So in that context other issues that may arise that need to be looked at might include effects on pathogen concentrations impact of material present in Sludge that may have a negative effect on co-digestion such as heavy metal or scum concentrations in the PS and TWAS sludge. Future work might not only stress waste stabilization of the effluent but also pathogen removal and possible pathogen re-growth if sludge is used for direct land.
- pH adjustment was always a tricky issue in this co-digestion study especially when co-digestion of OFMSW and municipal sludge is performed with greater proportion of OFMSW in the mixture. Separated 2 phase (acid and methane phase reactors) digester study would be interesting and more representative and beneficial to simulate practical scenario.

- Batch and/or continuous pilot scale anaerobic co-digestion reactors could be performed that better represent actual field conditions. These studies could be used as part of an overall economic feasibility analysis on co-digestion of OFMSW and municipal sludge needs from the curb to the digester to get an actual profit and loss analysis for a community.

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Appendix A

OFMSW Preparation

Model OFMSW used in this research was prepared in the lab consisted of cooked rice, pasta, banana, banana, apple, carrot, cabbage, dog food , ground beef and shredded biodegradable plastic bags. The waste was mixed in a kitchen food processor to generate a particle size range between 1-5 mm and the mixture almost looked like a paste. Figure A-1 shows the photographic of prepared was.

Figure A-1 Model OFMSW

Microwave Pre-treatment of TWAS

MW pre-treatment of TWAS was carried out with a Mars 5© (MW Accelerated Reaction System; CEM Corporation) MW oven. Mars 5© has a frequency of 2450 MHz and can deliver 1200 W ± 15% of the MW energy at full power with complete MW penetration of the sample vessels. Figure A-2 shows the photograph of MW oven and the MW vessels that was used in this research.

Figure A-2 Microwave oven

Biological Methane Potential Assay

500 mL Kimax glass bottles capped with butyl rubber stoppers were used to perform mesophilic BMP assay test. Total working volume of substrate in each bottle was 360 mL including 60 mL of mesophilic anaerobic inoculum biomass. Figure A-3 shows the photograph of BMP assays.

Figure A-3 BMP assay bottles prior to biogas measurement

Semi-continuous Flow Reactor

1-L Erlenmeyer volumetric flasks were used as semi-continuous flow reactors. Photograph of the reactors are shown in Figure A-4.

Figure A-4 Semi-continuous flow reactors

Appendix B

B.1 Substrates Characterization Data

Table B-1 Solid analysis data of the substrates and inoculum

Sample name	Pan wt	Pan +Sample wt	Wt after 105˚c	Wt after 550˚c	TS	Avg. TS	VS	AVG VS
OFMSW	2.1585	22.4127	6.8377	2.3498	23.1024	23.3	22.1579	21.87
	2.1420	30.7773	8.7689	2.8925	23.1424		20.5215	
	2.1129	31.9275	9.2015	2.3648	23.7756		22.9307	
TWAS	2.1356	25.0189	3.303	2.6579	5.1015	5.3	2.8190864	2.90
	2.1958	24.6869	3.3534	2.7084	5.1469		2.867801	
	2.1515	19.6694	3.1192	2.593	5.5241		3.0037847	
PS	2.1682	18.8057	2.743	2.3008	3.4548	3.4	2.6578512	2.63
	2.2117	23.7412	2.9482	2.3812	3.4209		2.6335958	
	2.2018	20.1649	2.8124	2.3416	3.3992		2.6209285	
Biomass	2.3101	17.0084	2.7627	2.5051	3.0793	3.1	1.7525836	1.75
	2.2843	17.5807	2.7483	2.4843	3.0334		1.7258963	
	2.2949	12.7971	2.6201	2.4334	3.0965		1.7777228	

B.2 TWAS Solubilization Data

COD Analysis Data

Table B-2 TCOD analysis data after MW pre-treatment of TWAS

Temp, HT	Dilution	Reading	TCOD	Avg.TCOD g/L
Raw TWAS	100	0.49	62073.2	62.71
	100	0.5	63340	
90,1	100	0.41	60975.2	60.98
	100	0.41	60975.2	
90,10	100	0.42	62462.4	61.72
	100	0.41	60975.2	
90,20	100	0.4	59488	60.23
	100	0.41	60975.2	
135,1	100	0.4	59488	59.49
	100	0.4	59488	
135,10	100	0.44	65436.8	66.18
	100	0.45	66924	
135,20	100	0.44	65436.8	66.18
	100	0.45	66924	
175,1	100	0.4	59488	60.98
	100	0.42	62462.4	
175,10	100	0.41	60975.2	61.72
	100	0.42	62462.4	
175,20	100	0.41	60975.2	60.98
	100	0.41	60975.2	

Table B-3 sCOD data of TWAS after MW pre-treatment

Temp, HT	**Dilution**	**Reading**	**sCOD**	**Avg.sCOD g/L**
Raw TWAS	100	0.2	13020	13.35
	100	0.21	13671	
90,1	100	0.14	20820.8	20.82
	100	0.14	20820.8	
90,10	100	0.15	22308	23.46
	100	0.1655	24613.16	
90,20	100	0.16	23795.2	23.05
	100	0.15	22308	
135,1	100	0.175	26026	25.65
	100	0.17	25282.4	
135,10	100	0.21	31231.2	33.46
	100	0.24	35692.8	
135,20	100	0.19	28256.8	28.63
	100	0.195	29000.4	
175,1	100	0.19	28256.8	28.26
	100	0.19	28256.8	
175,10	100	0.2	29744	31.23
	100	0.22	32718.4	
175,20	100	0.24	35692.8	35.69
	100	0.24	35692.8	

Statistical Analysis Data

Table B-4 Regression analysis data

Polynomial Regression Analysis

Effect	Comment	Solubilization Parameter	Solubilization Std. Err	t	p
Intercept		93.6751221	8.926006371	10.4946	4.4E-05
Temperature	Pooled				
Tepmerature^2		0.007015484	0.000365727	19.1823	1.3E-06
HT		2.164821648	0.434115451	4.98674	0.00248
HT^2	Pooled				

Total Model

Multiple R^2	Adjusted R^2	SS$_{model}$	MS$_{model}$	F	p
0.984955905	0.97994	40124.69591	20062.34796	196.414	3.4E-06

Factorial Regression Analysis

Effect	Comment	Solubilization Parameter	Solubilization Std. Err	t	p
Intercept		2.176651306	14.85797875	0.1465	0.88833
Temp		1.6844538	0.113747811	14.8087	6E-06
HT	Pooled				
Temp -HT		0.016295611	0.00350189	4.65338	0.00349

Total Model

Multiple R^2	Adjusted R^2	SS$_{model}$	MS$_{model}$	F	p
0.981416265	0.97522	39980.4996	19990.2498	158.431	6.4E-06

B.5 BMP Data

Table B-5 Solid analysis data of BMP test after digestion (Untreated TWAS)

Sample Name	Pan wt g	Pan +Sample wt g	wt after 105˚C g	Wt after 550˚C g	TS	AVG TS	VS	AVG VS
OFMSW:TWAS 25:75	2.173	16.097	2.597	2.394	3.04	3.04	1.46	1.55
	2.148	17.013	2.598	2.355	3.03		1.63	
OFMSW:TWAS 50:50	2.167	16.725	2.575	2.394	2.80	2.83	1.24	1.26
	2.118	16.491	2.530	2.346	2.86		1.28	
OFMSW:TWAS 75:25	2.337	16.455	2.686	2.584	2.47	2.46	0.72	0.70
	2.135	14.269	2.432	2.351	2.45		0.67	
TWAS	2.302	15.630	2.769	2.546	3.50	3.51	1.68	1.68
	2.319	16.687	2.824	2.582	3.51		1.68	
OFMSW:TWAS:PS 25:37.5:37.5	2.310	13.314	2.622	2.474	2.84	2.82	1.34	1.31
	2.324	14.544	2.664	2.509	2.79		1.28	
OFMSW:TWAS:PS 50:25:25	2.142	15.437	2.505	2.383	2.73	2.75	0.92	1.04
	2.128	11.791	2.396	2.283	2.77		1.17	
OFMSW:TWAS:PS 75:12.5:12.5	2.146	14.289	2.432	2.334	2.35	2.50	0.81	0.91
	2.324	14.162	2.636	2.515	2.64		1.02	
TWAS:PS 50:50	2.158	16.208	2.619	2.404	3.28	3.25	1.53	1.50
	2.138	15.038	2.554	2.364	3.23		1.48	

Table B-6 Solid analysis data of BMP test after digestion (MW Pre-treated TWAS for HT of 1 min)

Sample Name	Pan wt g	Pan +Sample wt g	wt after 105˚C g	Wt after 550˚C g	TS	AVG TS	VS	AVG VS
OFMSW:TWAS 25:75	2.0965	18.535	2.6207	2.412	3.19	3.20	1.27	1.20
	2.3301	16.509	2.7847	2.6243	3.21		1.13	
OFMSW:TWAS 50:50	2.3347	17.641	2.7949	2.6087	3.01	3.04	1.22	1.23
	2.3338	15.676	2.7435	2.5783	3.07		1.24	
OFMSW:TWAS 75:25	2.1722	17.726	2.5455	2.4128	2.40	2.44	0.85	0.88
	2.1993	18.142	2.5952	2.4513	2.48		0.90	
TWAS	2.1323	16.584	2.6352	2.3986	3.48	3.48	1.64	1.64
	2.3381	14.714	2.7692		3.48		1.64	
OFMSW:TWAS:PS 25:37.5:37.5	2.1259	18.184	2.5764	2.3931	2.81	2.80	1.14	1.17
	2.1505	18.013	2.5943	2.4038	2.80		1.20	
OFMSW:TWAS:PS 50:25:25	2.1574	17.504	2.5662	2.4285	2.66	2.68	0.90	0.96
	2.3102	15.841	2.6742	2.536	2.69		1.02	
OFMSW:TWAS:PS 75:12.5:12.5	2.1121	15.857	2.4384	2.3225	2.37	2.34	0.84	0.82
	2.3328	16.642	2.6617	2.5472	2.30		0.80	
TWAS:PS 50:50	2.1023	14.635	2.5397	2.3617	3.49	3.48	1.42	1.42
	2.1606	15.291	2.616	2.4285	3.47		1.43	

Table B-7 Solid analysis data of BMP test after digestion (MW Pre-treated TWAS for HT of 10 min)

Sample Name	Pan wt g	Pan +Sample wt g	wt after 105˚C g	Wt after 550˚C g	TS	AVG TS	VS	AVG VS
OFMSW:TWAS 25:75	2.3147	15.443	2.7433	2.5617	3.26	3.2721	1.3833	1.4098
	2.1503	13.979	2.5382	2.3683	3.28		1.4363	
OFMSW:TWAS 50:50	2.3456	15.669	2.7089	2.5722	2.73	2.7396	1.026	1.0363
	2.2989	14.375	2.6313	2.5049	2.75		1.0467	
OFMSW:TWAS 75:25	2.311	13.493	2.585	2.5018	2.45	2.4988	0.744	0.8124
	2.2969	13.979	2.5945	2.4916	2.55		0.8808	
TWAS	2.3184	18.188	2.883	2.6371	3.56	3.6057	1.5496	1.5865
	2.2858	14.698	2.7393	2.5378	3.65		1.6234	
OFMSW:TWAS:PS 25:37.5:37.5	2.3172	14.053	2.6618	2.5448	2.94	2.6779	0.997	0.9534
	2.1399	13.78	2.4215	2.3156	2.42		0.9098	
OFMSW:TWAS:PS 50:25:25	2.2903	13.774	2.6718	2.4687	3.32	3.322	1.7686	1.7686
OFMSW:TWAS:PS 75:12.5:12.5	2.1316	16.043	2.4393	2.335	2.21	2.2346	0.7497	0.7576
	2.315	13.421	2.5657	2.4807	2.26		0.7654	
TWAS:PS 50:50	2.3085	13.481	2.6718	2.528	3.25	3.2271	1.2871	1.2595
	2.2939	14.609	2.6883	2.5366	3.20		1.2318	

Table B-8 Solid analysis data of BMP test after digestion (MW Pre-treated TWAS for HT of 20 min)

Sample Name	Pan wt g	Pan +Sample wt g	wt after 105˚C g	Wt after 550˚C g	TS	AVG TS	VS	AVG VS
OFMSW:TWAS 25:75	2.3494	13.433	2.6872	2.5355	3.05		1.3687	
	2.1414	12.103	2.4485	2.312	3.08	3.0653	1.3703	1.37
OFMSW:TWAS 50:50	2.1762	13.895	2.4938	2.3712	2.71		1.0462	
	2.1439	15.268	2.5089	2.3533	2.78	2.7457	1.1856	1.12
OFMSW:TWAS 75:25	2.3081	15.365	2.5717	2.5032	2.02		0.5246	
						2.0189		0.52
TWAS	2.304	17.015	2.8301	2.5866	3.58		1.6552	
	2.2962	15.363	2.7606	2.5469	3.55	3.5652	1.6355	1.65
OFMSW:TWAS:PS 25:37.5:37.5	2.1302	12.482	2.3742	2.2789	2.36		0.9206	
	2.1092	14.748	2.3998	2.286	2.30	2.3281	0.9004	0.91
OFMSW:TWAS:PS 50:25:25	2.132	16.09	2.5119	2.3762	2.72		0.9722	
	2.1517	15.241	2.5086	2.3798	2.73	2.7242	0.984	0.98
OFMSW:TWAS:PS 75:12.5:12.5	2.1633	14.925	2.4837	2.3733	2.51		0.8651	
	2.1331	19.655	2.5538	2.4158	2.40	2.4558	0.7876	0.83
TWAS:PS	2.144	18.915	2.6687	2.4653	3.13		1.2128	
	2.1395	15.254	2.5568	2.3942	3.18	3.1553	1.2398	1.23

Statistical Model Analysis of BMP Assays

Table B-9 Regression analysis data for co-digestion assay of OFMSW and TWAS as a function of OFMSW percentage in the mix and TWAS pre-treatment temperature holding time

Polynomial Regression Analysis

Effect	Comment	Solubilization Parameter	Solubilization Std. Err	t	p
Intercept		3559.000	715.7237	4.97259	0.002520
OFMSW%		140.247	32.5096	4.31401	0.005015
OFMSW%^2		-0.998	0.3218	-3.10043	0.021105
T HT	Pooled				
T HT^2	Pooled				

Total Model

Multiple R^2	Adjusted R^2	SS $_{model}$	MS $_{model}$	F	p
0.934505	0.912673	6924393	3462196	42.80488	0.000281

Factorial Regression Analysis

Effect	Comment	Solubilization Parameter	Solubilization Std. Err	t	p
Intercept		5829.606	651.9185	8.942231	0.000291
OFMSW%		41.306	12.0712	3.421875	0.018800
T HT		-18.607	50.4470	-0.368843	0.727348
OFMSW%*T HT		-0.079	0.9341	-0.084894	0.935640

Total Model

Multiple R^2	Adjusted R^2	SS $_{model}$	MS $_{model}$	F	p
0.867034	0.787254	6424454	2141485	10.86785	0.012493

Multiple Regression

Effect	Comment	Solubilization Parameter	Solubilization Std. Err	t	p
Intercept		5637.333	374.5818	15.0496716	1.3739E-06
OFMSW%		40.487	6.9359	5.83725586	0.00063882
T HT	Pooled				

Total Model

Multiple R^2	Adjusted R^2	SS $_{model}$	MS $_{model}$	F	p
0.829574045	0.80523	6146888.2	6146888.2	34.073556	0.00063882

Table B-9 Regression data for tri-digestion of OFMSW, TWAS and PS as a function of OFMSW percentage in the mix and TWAS pre-treatment temperature holding time

Response Surface Regression Analysis

Effect	Comment	Solubilization Parameter	Solubilization Std. Err	t	p
Intercept		6380.556	417.1529	15.2954	1.2302E-06
OFMSW%		36.967	7.7242	4.78584	0.00199872
OFMSW%^2	Pooled				
T HT	Pooled				
T HT^2	Pooled				
OFMSW%*T HT	Pooled				

Total Model

Multiple R^2	Adjusted R^2	SS_{model}	MS_{model}	F	p
0.765920	0.7324799	5124504.17	5124504.17	22.9042	0.0019987

Polynomial Regression Analysis

Effect	Comment	Solubilization Parameter	Solubilization Std. Err	t	p
Intercept		6380.555556	417	15.2954	1.2302E-06
OFMSW%		36.96666667	8	4.78584	0.00199872
OFMSW%^2	Pooled				
T HT	Pooled				
T HT^2	Pooled				

Total Model

Multiple R^2	Adjusted R^2	SS_{model}	MS_{model}	F	p
0.765919896	0.73247988	5124504.167	5124504	22.9042	0.00199872

Factorial Regression Analysis

Effect	Comment	Solubilization Parameter	Solubilization Std. Err	t	p
Intercept		6380.555556	417	15.2954	1.2302E-06
OFMSW%		36.96666667	8	4.78584	0.00199872
T HT	Pooled				

Total Model

Multiple R^2	Adjusted R^2	SS_{model}	MS_{model}	F	p
0.765919896	0.73247988	5124504.167	5124504	22.9042	0.00199872