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Anaerobic Digestion and Co-digestion of Thickened Waste Activated Sludge (TWAS) and Food Waste (FW)

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Abstract

This study aimed at assessing the impact of ultrasonication on the anaerobic digestibility of thickened waste activated sludge (TWAS) in anaerobic fluidized bed reactor (AnFBR), determining the unbiodegradable fraction of TWAS, and exploring co-digestion of food waste (FW) and TWAS. Two lab-scale AnFBRs treating TWAS were studied to explore the impact of ultrasonication (US) in the break down and reuse of scum for methane production. At an organic loading rate (OLR) of 4.7 kg COD/m^3-d and 480 kJ of US energy, COD and VSS destruction efficiencies were 65% and 63%, respectively roughly 20% higher than the control reactor without US. To explore the specific methanogenic bacterial activity (SMA), the SMA test was conducted during OLR of 9.7 kg COD/m^3-d and showed that the activity-based sludge retention time is higher for the ultrasonicated AnFBR (U-AnFBR) (7.1 days) compared to AnFBR (5.1 days). The investigation also indicated that the unbiodegradable fractions of PCOD and VSS were 0.28 based on PCOD and 0.26 based on VSS. However, to investigate the co-digestibility of FW as co-substrate, five semi-continuous flow anaerobic digesters were operated to explore the co-digestion performance treating a mixture of FW and municipal biosolids (primary sludge and TWAS) at an HRT of 20 days. Sixty days of steady-state operation at organic loading rates of 2.2 kgCOD/m^3-d to 3.85 kgCOD/m^3-d showed that COD removals were higher for the three co-digesters than for the two municipal biosolids digesters i.e. 61%-69% versus 47%-52%. Specific methane production per influent CODs were 1.3-1.8 folds higher in co-digestion than mono-digestion. Additional methane production through synergism accounted for the minimum 18%-20% of the overall methane production.

Keywords

Anaerobic fluidized bed bioreactor, TWAS, specific methanogenic activity test, unbiodegradable fraction of particulate COD, food waste.

Co-Authorship Statement

Dr. George Nakhla and Dr. Jesse Zhu provided supervision and guidance to the research project. All experimental works involving operation and testing of the CSTR digestion was the responsibility of the author, under the supervision of Dr. Nakhla and Dr. Zhu and with help from Dr. Mingu Kim for co-digestion in data interpretation and paper writing.

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Nomenclature

C_p	Particle concentration
L_f	Biofilm thickness
S_0	Influent COD concentration
S_e	Effluent COD concentration
S_s	Scum layer COD concentration
V_d	VSS destruction
$Q_{feeding}$	Flow rate of feeding
Q_{scum}	Accumulating rate of scum layer
$Q_{effluent}$	Flow rate of effluent
S_{bp}	Biodegradable particulate COD
S_{ti}	Influent particulate COD
S_{te}	Effluent particulate COD
Wp	Weight of total clean particle carriers
$f_{as,up}$	Unbiodegradable particulate COD fraction
R	Sludge retention time
Y_{AD}	Biomass yield
b_{AD}	Decay constant
C/N	Carbon to nitrogen ratio
kJ	KiloJoule
MJ	MegaJoule
kg	Kilogram
H	Specific cumulative biogas production
R_m	Maximum specific biogas production rate
λ	Lag time

List of Abbreviations

ABR	Anaerobic baffled reactor
AD	Anaerobic digestion
ADS	Anaerobic digester sludge
AF	Anaerobic filter
AFB	Anaerobic fluidized bed
AnFBR	Anaerobic fluidized bed reactor
AnMBR	Anaerobic membrane bioreactor
AnMBBR	Anaerobic moving bed biofilm reactor
AnSBR	Anaerobic sequencing batch reactor
AS	Activated sludge
BOD5	5-day biochemical oxygen demand
COD	Chemical oxygen demand
CSTR	Completely stirred tank reactor
C/N	Carbon to nitrogen
FFB	Fixed film bioreactor
EGSB	Expanded granular sludge bed
FID	Flame ionization detector
F/M	Food to microorganism ratio
FW	Food waste
FWD	Food waste disposer
FBR	Fluidized bed reactor
HDPE	High-density polyethylene
HRT	Hydraulic retention time
KW	Kitchen waste
MW	Municipal wastes
MBR	Membrane bioreactor
OLR	Organic loading rate
OFMSW	Organic fraction of municipal solids waste
PCOD	Particulate chemical oxygen demand
PS	Primary sludge
SAdA	Specific acidogenic activity

SAtA	Specific acitogenic activity
SCOD	Soluble chemical oxygen demand
SMA	Specific methanogenic activity
SN	Soluble nitrogen
SP	Soluble phosphorous
SRT	Sludge retention time
S/X	substrate-to-biomass ratio
TCD	Thermal conductivity detector
TCOD	Total chemical oxygen demand
TDS	Total dry solids
TSS	Total suspended solids
TP	Total phosphorous
TN	Total nitrogen
TWAS	Thickened waste active sludge
U-AnFBR	Ultrasonicated anaerobic fluidized bed reactor
UASB	Upflow anaerobic sludge blanket
UBF	Anaerobic upflow bed filter
UPO	Unbiodegrable particulate organics
US	Ultrasonication
VFA	Volatile fatty acids
VSS	Volatile suspended solids
WAS	Waste activated sludge
WW	Wastewater
WWTP	Wastewater treatment plant

Chapter 1

Introduction

1.1 General Background

1.1.1 Anaerobic Treatment of TWAS

Biological treatment is key for both municipal and industrial wastewater. Since its inception over 100 years ago, the technologies for biological wastewater treatment have experienced enormous innovation. There are two major categories of wastewater treatment bioreactors, depending on the way in which microorganism grow: suspended in the liquid or attached to a solids support (Grady et al., 2011; Metcalf & Eddy, 2003). The simplest form of a suspended growth bioreactor is the continuously stirred tank reactor (CSTR). On the other hand, attached growth cultures grow as biofilm on a solids support. Packed towers, rotating disc and fluidized bed reactors are the three major types of attached growth bioreactors (Grady et al., 2011).

Anaerobic treatment is predominantly used for the treatment of high strength industrial wastewater due to its capability of supporting higher volumetric loadings (Heijnen et al., 1989), possibility of high degree of waste stabilization, ability to grow in absence of oxygen, low nutrient removal, and additional biogas (methane and hydrogen) production (Chan et al., 2009). Since waste activated sludge (WAS) is biodegradable, the anaerobic system turns out to be an ideal bio-process for treating thickened waste activated sludge (TWAS) (Metcalf & Eddy, 2003).

1.1.2 Co-digestion of Food Waste

Food waste (FW) is considered the single largest component of waste. Many countries dispose of FW in landfills, which exerts a negative impact on the environment due to filtration of leachate to the groundwater and release of hazardous gases to the atmosphere. Environmental regulations have been undertaken to decrease landfill use for biodegradable municipal wastes (MW) such as FW that constitute a large proportion of MW of up to about 60% (Iacovidou et al., 2012). As one of the alternative waste management methods, household food wastes disposers (FWDs) have been implemented to divert FW from landfills to wastewater treatment plants. However, food waste is highly variable, and its composition can differ reportedly from one place to other, which might be a hindrance to efficient digestion (Zhang et al., 2007). To minimize this issue, utilization of food waste as co-substrate in anaerobic digestion, which is known as co-digestion, is a well-established process in many European countries (Iacovidou et al., 2012). Due to the high organic carbon content, extensive studies investigated the anaerobic digestion of food wastes to generate biogas (Curry & Pillay, 2012). Furthermore, due to excess capacity in anaerobic digesters in many wastewater treatment plant, utilization of food waste as co-substrate can be a good source to enhance energy generation. For example, in California, there are almost 140 wastewater treatment facilities that utilize anaerobic digesters, with an estimated excess capacity of 15%-30% (Anon , 2007).

1.2 Problem Statement

AD of biosolids was often limited due to its long retention times (20 days-30 days) and low overall degradation efficiency of the organic dry solids (30%-50%) (Appels et al.,

2008). Therefore, different sludge disintegration methods have been studied as a pre-treatment: thermal, mechanical, chemical, and ultrasound. Firstly, in thermal pre-treatment, WAS is generally subject to temperature and pressure in a range of 15^0C-200^0C and 600kPa-2500kPa (Barlindhaug & Ødegaard, 1996). Cell components are solubilised during the heating because of the chemical bonds of the disruption of the cell wall and membrane. Though positive impacts of thermal pre-treatment were reported in many studies (Valo et al., 2004; Appels et al., 2010; Bougrier et al., 2006), duration and temperature of the optimum pre-treatment varied due to the nature of the sludge (Appels et al., 2008). Secondly, several strategies including high-pressure homogenization, and compressing the sludge to 60MPa (Harrison, 1991), were applied in mechanical treatment to improve the biosolids digestion.

Acid and alkaline hydrolysis and oxidative sludge pre-treatment are two common chemical pre-treatments. Although acid and alkaline hydrolysis were shown as an effective method (Neyens & Baeyens, 2003), sludge solubilisation was noted as a major obstacle since extreme pH levels and subsequent neutralization were required (Appels et al., 2008). On the other hand, although effective solubilization of sludge was achieved in oxidative sludge pre-treatment (Zimpro, 1993), problems associated with odor, corrosion, and high energy cost limited the application of this process. Finally, ultrasound was considered the most powerful pre-treatment method of disrupting the cell of sludge, despite the high power consumption (Weemaes & Verstraete, 1998).

Different types of anaerobic digesters are applied in biosolids digestion: standard-rate digesters, high rate digesters, and two-stage digesters. In the standard-rate digester, stratification occurs in four zones during the biogas generation: (i) a scum layer, (ii) a

liquid layer (or supernatant) which is withdrawn and recycled to the wastewater treatment, (iii) a layer of digesting solids, and (iv) a layer of digested solids which (Appels et al., 2008). However, generation of scum layer and long retention time (30 days – 60 days) were noted as major drawbacks. An improvement of standard-rate digester is known as high-rate digester. Completely mixed and heated sludge was used in high-rate digester. However, two important issues including uniform feeding, and reducing shock load because of sensitive methanogenic bacteria were noted as problematic (Qasim, 1999). In the two stage-digesters, a high-rate digester coupled with second unheated unmixed tank. This type of digesters is rarely used currently because of poor settling commonly associated with an incomplete digestion (Qasim, 1999).

In view of the above mentioned issues, a new technology such as an fluidized beb reactor (FBR) has gained a lot of success because of several advantages: (i) sustaining natural, mixed microbial communities that can operate in synergy, (ii) excellent treatment can be achieved even at high hydraulic loading rates due to immobilization of biomass, and (iii) the process can be operated at high biomass concentration in the reactor, without the need for settlers for biomass retention and recirculation (Nicolella et al. 2000).Although long start-up times, and control of biofilm thickness were noted as negative effects on system performance (Heijnen et al., 1989), FBRs have been successfully used by Nakhla and coworkers for anaerobic digestion of municipal and industrial biosolids (Andalib et al., 2014; Wang et al., 2016). However, one of the major problems of this technology and conventional digestion systems is the generation of scum in the digester. Scum has 6% solids which causes operational and maintenance problem, and also reduces overall

efficiency (Wang et al., 2016). Hence the challenge of this process is to minimize the scum generation in the reactor as well as increase the overall performance.

In order to enhance digestibility, FW as a co-substrate plays a vital role in anaerobic digestion because of its high potential for methane production (Neves et al., 2009). However, despite the potential benefit, digestion stability can be hampered when FW is used as single substrate because of potential nutrients imbalance such as insufficient trace metals (Zn, Fe, Mo, etc), excessive macronutrients (Na, K), high carbon to nitrogen (C/N) ratio, and lipid content (5 g/L) as well as due to the high variability of its composition depending on its source (Zhang et al., 2014). The positive effect of FW on sludge digestion performance could be related to the increased C/N ratio and enhanced kinetics due to the addition of food wastes. In fact, the optimal C/N ratio for anaerobic digestion is 15:1-30:1, much greater than the 6:1 to 9:1 of wastewater sludges (Iacovidou et al., 2012; Koch et al., 2016). More pronounced C/N effect on the continuous co-digestion process can be found in a study by Dai et al. (2013) who reported linear increases in VS destruction (38%-68%) with elevated C/N ratios of feed (7.8- 14.8) although the authors did not elaborate on the relationship. Additionally, the characterization of the microbial activity of different anaerobic microbes such as acidogenes, acetogenes, and methanogenes which may explain effectiveness of co-digestion is lacking, despite the availability of information on microbial speciation (Kim & Oh, 2011).

1.3 Research Objectives

The specific goals of the research are:

- Evaluation of the impact of sonication on the scum layer floating on the top of the fluidized bed reactor (FBR)
- Investigating the effects of organic loading rate (OLR) on FBR performance
- Determination of the unbiodegradable particulate COD fraction for TWAS
- Assessment of the synergistic effects of co-digestion microbial activity

1.4 Thesis Organization

This thesis mainly focuses on the anaerobic digestion of TWAS using anaerobic fluidized bed bioreactor (AnFBR), AnFBR with sonication, and continuous stirred tank reactor (CSTR). Chapter 2 provides a critical literature review on the AD of biosolids, biofilm attachment and detachment, basic applications of anaerobic fluidized bed, and anaerobic co-digestion of food waste as co-substrate.

Chapter 3 discusses the operation and performance of the AnFBR in digesting TWAS. Detailed data of the VSS destruction efficiency, mass balances, optimization of scum, energy balance, and operational conditions are presented and discussed in this section.

Chapter 4 presents the performance of CSTR digestion of TWAS. Detailed data of VSS destruction efficiency, mass balances, operational conditions, and the unbiodegradable fraction of particulate COD are discussed.

Chapter 5 shows the detailed anaerobic co-digestion of FW with municipal biosolids as well as performance analysis of CSTR. Additionally, the characterization of the microbial activity of different anaerobic microbes such as acidogenes, acetogenes, and methanogenes is presented.

Chapter 6 summarizes the main research findings and provides a direction for future research.

1.5 Research Contribution

The current works developed a novel anaerobic fluidized bed digestion incorporating ultrasonication for enhanced biogas production to break down and reuse of scum. The main concept was to utilize the high scum COD to enhance overall performance. The utilization of ultrasound in the anaerobic fluidized bed reactor successfully reduced the scum production as well as minimized the discharge of stabilized solids, thereby reducing transportation costs, landfill disposal as well as environmental impacts. It also developed a quick, simple, and reliable model for assessing the unbiodegradable particulate fraction ($f_{as'up}$) of TWAS from which performance limits of AD with respect to volatile solids reduction and methane production rates can be deduced. Finally, this research explored in detail different co-digestion performance of five lab-scale semi-CSTR digesters fed with biosolids and various FW blend ratios. Moreover, specific methanogenic activity (SMA), specific acetogenenic activity (SAtA), and specific acidogenic activity (SAdA) tests were also conducted to evaluate different microbial behaviors between mono-digestion and co-digestion.

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Chapter 2

Literature Review

2.1 Introduction

Nowadays, anaerobic digestion is gaining significant attention, both as an energy resource for biogas production and also as a solution to environmental concerns (Asam et al., 2011). Bioenergy is estimated as a fourth largest energy resource in the world (Mao et al., 2015). It is also considered as a mitigation source of greenhouse gases as well as alternative of fossil fuels due to its widely applicable characteristics and abundancies. It is reported that at least 25% of all bioenergy was derived from biogas (Nielsen et al., 2009). Biogas production from anaerobic digestion represents an integrated system of physiological process of microbial and energy metabolism as well as materials processing under specific conditions (Mao et al., 2015).

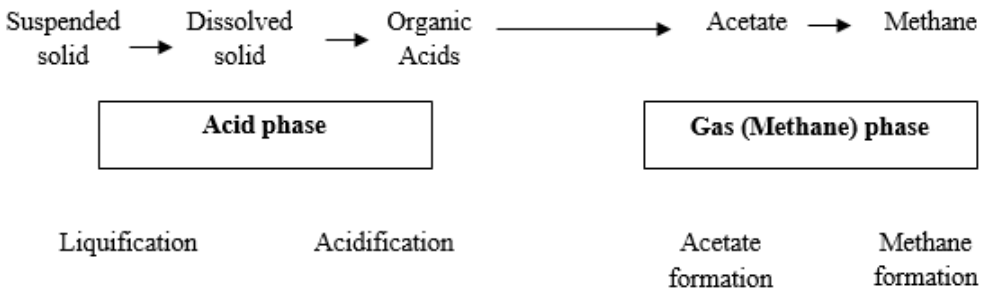
2.2 Anaerobic Digestion Pathways

Anaerobic digestion (AD) is a complex process mainly based on a reduction process consisting of a number of biochemical reactions taking place under anoxic conditions (Aslanzadeh, 2014). As illustrated in Figures 2.1, biogas formation in anaerobic digestion involves four different steps carried out by various microbial groups that exist both in suspended phase and attached biofilm phase in biofilm reactors: hydrolysis (digesting large polymers into small monomers), acidogenesis (converting monomers into volatile fatty acids), acetogenesis (degrading volatile fatty acid into acetic acid, CO_2, and H_2), and methanogenesis (consuming acetate acid and producing CH_4) (Metcalf & Eddy, 2003). The complex organic matter is destroyed and biogas, comprising primarily H_2, CH_4, and CO_2

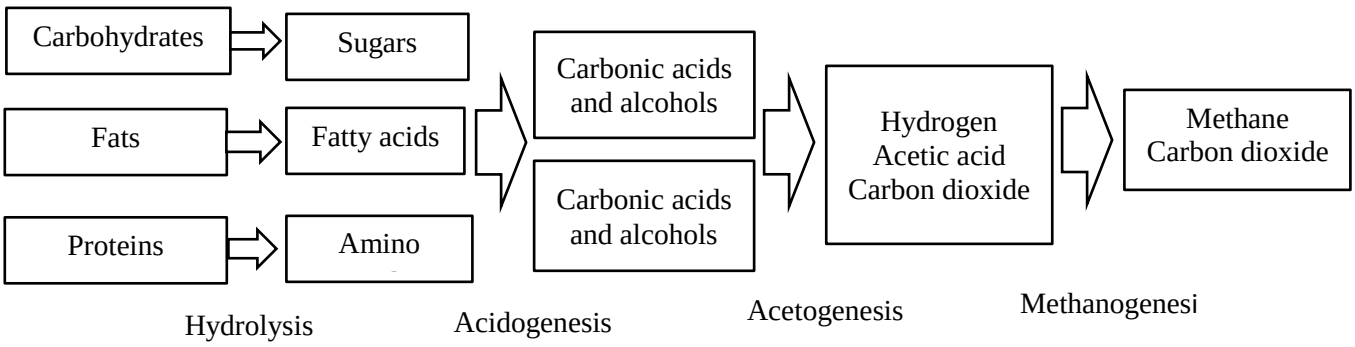
is generated. Hydrolysis is the first step in AD involving the enzyme-mediated transformation of insoluble organic materials and higher molecular mass compounds such as lipids, polyssacharides, proteins, fats, nucleic acid etc. into soluble organic materials i.e. to compounds suitable for the use as source of energy and cell carbon such as monosaccharides, amino acids and other simple organic compounds (Adekunle & Okolie, 2015). Hydrolysis is normally carried out by exo-enzymes excreted by fermentative bacteria and it is a relatively slow process which limits the rate of overall anaerobic digestion process. Acidogenesis or acidification is the second steps in anaerobic digestion process. In this process, the hydrolysis products are converted into simple molecules with low molecular weight, such as volatile fatty acids (e.g. acetic-, propionic- and butyric acid), alcohols, aldehydes and gases like CO_2, H_2 and NH_3. In the third phase, acetogenesis, products formed from acidogenesis are converted into acetic acids, hydrogen, and carbon dioxide by acetogenic bacteria. It is noted that the organisms which carry out the anaerobic oxidation reactions collaborate with the next group, the methane forming microorganisms; this collaboration depends on the partial pressure of the hydrogen present in the system (Adekunle & Okolie, 2015). The final stage in anaerobic digestion is methanogenies, which involves the conversion of the intermediate products by methanogenic bacteria under strict anaerobic conditions (Aslanzadeh, 2014) to CO_2 and CH_4.

However, the rate limiting processes in AD are crucial since they can limit performance and cause failure (Aslanzadeh, 2014). Many researchers reported that the rate-limiting for complex particulate (insoluble) organic substrates is the hydrolysis step (Ma et al., 2011; Izumi et al., 2010; Rafique et al., 2010; Miah et al., 2005) due to the formation of toxic byproducts (complex heterocyclic compounds) or non-desirable volatile fatty acids (VFA)

formed during hydrolysis step whereas methanogenesis is the rate limiting step for readily biodegradable soluble substrates (Adekunle & Okolie, 2015). Figure 2.1 shows that anaerobic processes are mainly divided into two phase: acid phase and gas phase (US EPA Factsheet, 2006). The degradation reactions in the two phases differ substantially because of the microorganisms carrying out the reactions which depend on four different factors: physiology, nutritional needs, growth kinetics, and sensitivity to the environment. It is even difficult to keep a subtle balance between



(a)



(b)

Figure 2.1 (a) Phase separation of the anaerobic digestion system (Adekunle & Okolie, 2015) (b) Key process stages of anaerobic digestion (Seadi et al., 2008)

these two groups: the acid forming and the methane forming microorganisms, which lead to reactor instability and consequently low methane yield (Demirel & Yenigun, 2002).

Different techniques like membrane separation, kinetic control, and pH control are normally employed to accomplish the phase separation (Adekunle & Okolie, 2015).

2.3 Factors affecting digestion

Anaerobic digestion depends on a number of parameters like pH, temperature, mixing, substrate, carbon-to nitrogen (C/N) ratio, and sludge retention time (SRT), to achieve the optimum performance. Unlike aerobic system, anaerobic systems are very temperature sensitive (Wang, 2014). Temperature influences the growth rate and metabolism of micro-organisms and hence the population dynamics in the anaerobic reactor (Appels et al., 2008). Anaerobic process, generally, utilize mesophilic (20-40ºC) and thermophilic (50-60 ºC) conditions. The thermophilic processes is believed to be able to provide a higher metabolic rate according to the Arrhenius equation as well as a larger degree of pathogen deactivation, although energy consumption is relatively high compared with mesophilic systems (Wang, 2014). It is reported that mesophilic condition (35 ºC) is good for anaerobic digestion since richer bacterial species are observed (Guo et al., 2014). Temperature phased anaerobic digestion processes with a thermophilic acidogenic fermenter and a mesophilic methanogenic fermenter have been shown to enhance the biosolids reduction by 5% and biogas production in acidogenic fermenter by 100% for both food waste and municipal biosolids digestion (Wang, 2014).

pH is an important parameter for anaerobic digestion. Neutral pH is favorable for optimum performance, since most of methanogens bacteria grow in the range of pH 6.5-7.2 (Boe, 2006; Adekunle & Okolie, 2015), and the optimal pH is 7 (Zhang et al., 2009). The growth rate of methanogens is significantly decreased at low pH (below 6.6) and higher pH (above

7.2). However, some fermentative microorganisms are found less sensitive to pH and can function in a wider range of pH from 4.0-8.5 (Hwang et al., 2004). Digester pH is controlled by CO_2 concentration in gas phase and HCO_3-alkalinity of the liquid phase (Turovskiy & Mathai, 2005), as well as ammonia formed by the degradation of proteins. Alternatively, pH can be controlled by reducing ammonia toxicity since free ammonia concentration increases during AD (Mao et al., 2015).

The C/N ratio is highly sensitive in digestion system with high C/N ratio inducing a low protein solubilization rate and leading to low ammonia-nitrogen and fatty acids (Mao et al., 2015). The aforementioned study also indicated that high C/N ratio provides insufficient nitrogen to maintain cell biomass and leads to fast nitrogen degradation, resulting in lower biogas production while low C/N ratio increase the risk of ammonia inhibition, which is toxic to methanogens. The optimum C/N ratio for anaerobic digestion is reported between 20 to 30 or 20 to 35, with a ratio of 25 commonly used in many studies (Zhang et al., 2013; Punal et al., 2000; Yen & Brune, 2007).

Proper mixing in anaerobic digestion maintains the solids in suspension and homogenizes the incoming feed with the active microbial community (Lindmark et al., 2014). Anaerobic digestion is reliant on mixing for nutrition and microorganism distribution, inoculation of fresh feed, material homogenization, and removal of end products of metabolism (Deublein & Steinhauser, 2010). Different types of mixing modes such as mechanical mixing, hydraulic mixing, and pneumatic mixing were generally used in AD industry. In mechanical mixing, different type of propellers and agitators are used to homogenize the digester content. Hydraulic mixing uses a pump located outside the digester, which recirculates the AD sludge. Gas is utilized in pneumatic mixing bycompression and release

of biogas to create a horizontal mixing action as the bubble column rises to the surface (Lindmark et al., 2014). The mixing effect on anaerobic digestion of manure was investigated by Kaparaju et al. (2008) and found the intermittent mixing (withholding mixing for 2 h prior to extraction/feeding) and minimal mixing (mixing for 10 min prior to extraction/feeding) strategies increased the methane production by 1.3% and 12.5%, respectively compared to continuous mixing. Karim et al. (2005) have evaluated the effect of mixing in AD of animal waste and noted that external mixing produced about 10%–30% more biogas than the unmixed digester.

Methane-forming microorganisms grow slowly, with a doubling time of around 5 - 16 days. Therefore, the hydraulic retention time should be at least 10 - 15 days, unless these bacteria are retained by, for example, entrapment (Adekunle & Okolie, 2015). The subsequent steps of the digestion process are directly related to SRT (Appels et al., 2008). An increase in SRT increases the extent of reactions and vice versa. The schematic representation of SRT vs specific biogas production (m^3/kg organic dry solids) is shown in Figure 2.2 confirms that (i) retention times shorter than 5 days are insufficient for a stable digestion due to VFA increase as a result of washout of methanogenic bacteria, (ii) VFA concentrations are still relatively high at SRT of 5–8 days as there is an incomplete breakdown of compounds, especially of the lipids, (iii) stable digestion is obtained after 8–10 days: low VFA concentrations, the breakdown of lipids starts, and (iv) the breakdown curve stabilizes at SRT >10 days; all sludge compounds are significantly reduced.

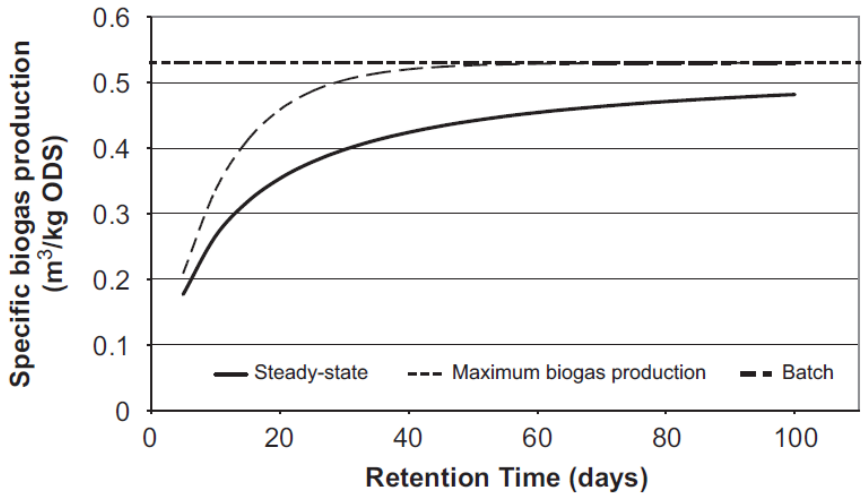


Figure 2.2 Specific biogas production vs. SRT (Appels et al., 2008)

2.4 Anaerobic Digestion of TWAS

2.4.1 Anaerobic Suspended Growth Process

A number of studies have been done on the anaerobic suspended growth processes. These process are adopted widely for biological treatment of industrial wastewaters as well as a reliable method of digesting municipal biosolids (Lyberatos et al., 2010; Heijnen et al., 1989; Sutton et al., 1982). The typical types of anaerobic suspended growth process are used in wastewater field: Completely Stirred Tank Reactor (CSTR), anaerobic baffled bioreactor (ABR), anaerobic sequencing batch reactor (AnSBR), and anaerobic membrane bioreactors (AnMBR).

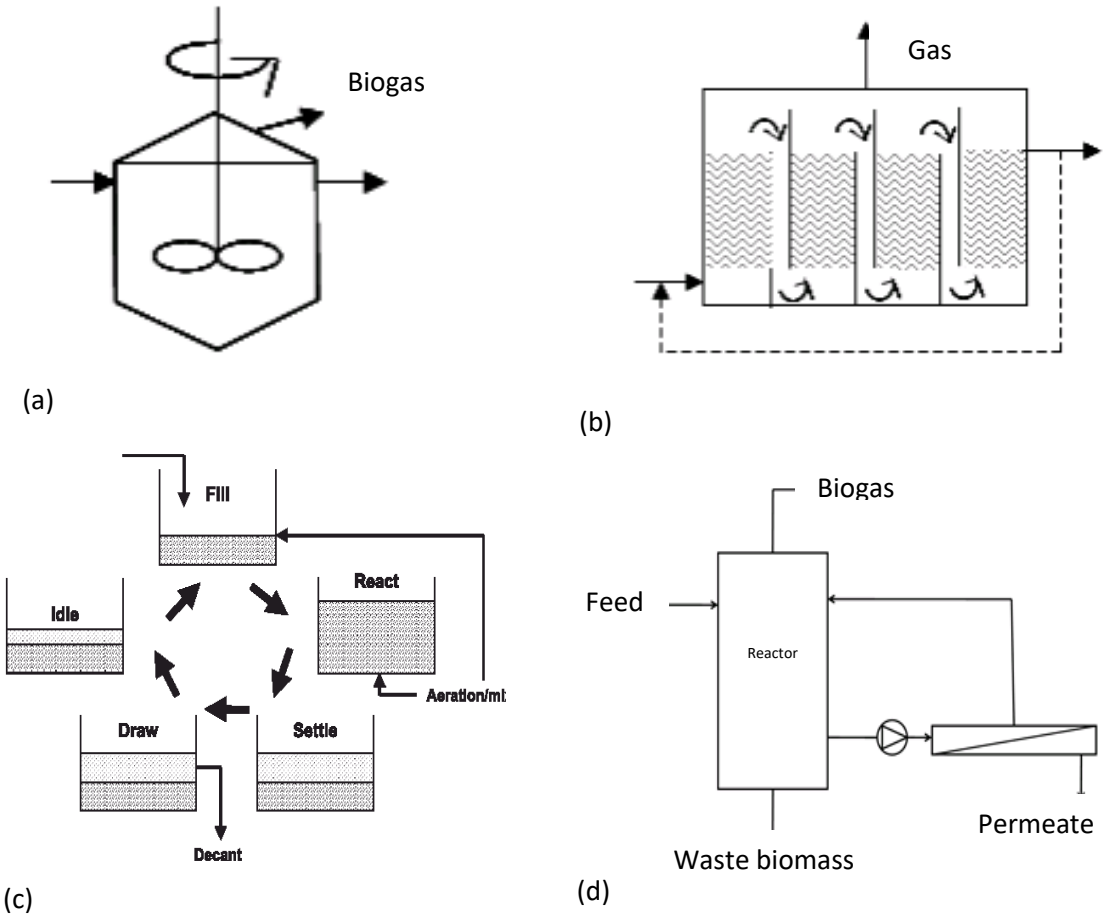


Figure 2.3 Reactors of anaerobic suspended growth treatment (a) CSTR (b) ABR (c) AnSBR (d) AnMBR(Wang, 2014)

The ABR is a series of upflow anaerobic sludge blanket (UASB) divided into a few compartments (McCarty, 1981). Vertical baffles arranged in series forces the wastewater to flow under and over them as it passes from the inlet to the outlet (Hassan & Dahlan, 2013). The main driving force is to enhance the sludge retention capacity (Hassan & Dahlan, 2013). ABR is considered one of the high rate anaerobic bioreactors since the organic loading rate (OLR) can reach a maximum of 40 kg COD/m^3-day (Wang, 2014). Li & Li (2010) have conducted a study for synthetic brewery wastewater with varied OLR from 1.2 to 5.6 kg COD/ m^3-day and found it can remove up to 92% of COD. The

aforementioned study also indicates that the rate of biogas production also increased with increasing feed flow from 6.1 to 24 L/day. Barber & Stuckey (1999) have observed 93% COD removal efficiency for protein carbohydrate wastewater (WW) at an OLR of 2.7 kg COD/m^3-d and HRT of 71h.

In an AnSBR, the reactor is operated on a fill and draw basis in a sequential manner under anaerobic conditions. During feed, the vessel is mixed by biogas or liquid circulation The main advantage of the AnSBR is that it can sustain a higher OLR due to a high SRT, and a high food-to-microorganism (F/M) ratio at the beginning of the react phase that ensures a high reaction rate and biogas production (Ndegwa et al., 2008). AnSBR can remove up to 99% of COD at OLR of 0.2-2 kg COD/m^3-d, and HRT of 12 h-48 h (Table 2.1).

In the last two decades, anaerobic membrane reactors (AnMBR) have evolved from aerobic MBR, with the membrane either external or submerged within the reactor. It can remove up to 95% of high strength COD (20 g/L) at HRT of 10 days and OLR of 1.76 kg COD/m^3-d (Table 2.1). However, membrane fouling remains the major obstacle limiting the AnMBR (Wang, 2014). Table 2.1 gives a summary anaerobic suspended growth system on both industrial waste and municipal wastewater biosolids (Wang, 2014).

2.4.2 Anaerobic Attached Growth Process

The typical types of attached growth bioreactor such as upflow anaerobic sludge blanket (UASB), anaerobic filter (AF), AnFBR, and, expanded granular sludge bed (EGSB) are used in wastewater treatment plant (Figure 2.4).

A combination of biological and physical processes occur in the UASB. The basic principle of physical process is the solids and gas separation from liquid (Bal & Dhagat,

2001). The main differences between UASB and anaerobic filter or fixed film reactor types is the lack of loss of reactor volume by filter or carrier media (Bal & Dhagat, 2001). Usually, high biomass concentration allows the UASB to sustain a high OLR of 10 to 15 kg COD/m^3d at a fairly short HRT of less than 2 days (Nicolella et al., 2000). For achieving the required sufficient contact between sludge and wastewater, the UASB system relies on the agitation brought about by the natural gas production and on an even feed inlet distribution at the bottom of the reactor (Bal & Dhagat, 2001).

Initially, the anaerobic filter (AF) was developed to ensure the support medium for the contact between bacterial mass and influent, thus allowing lower HRT than the biomass retention time (Mao et al., 2015). The main features of the AF design are a distributor in the bottom of the column, a media support structure, inert packing material, a free board above the packing material, effluent draw-off, and optional features such as recycle facilities, backwashing facilities or a sedimentation zone below the packing material (Switzenbaum, 1983). The AF can be operated either as down-flow or up-flow. It proved better performance compared to anaerobic contact process due to elimination of mechanical mixing. However, the AF is usually applied for treating suspended solids (SS) wastewater to prevent the filter from clogging (Tilley et al., 2014).

Table 2.1 Performance of anaerobic suspended growth reactors

Reactor type	Substrate type	reactor Volume (L)	Influent COD (g/L)	OLR (kg COD/m^3d)	HRT(h)	Temp	COD removal (%)	Reference
ABR	synthetic brewery wastewater		1.3-3.5	1.2-5.6	15	35	92	Li Hui et al. (2010)
ABR	Domestic WW		0.68	0.67-2.1	24	25	68-82	Nasr et al. (2009)
ABR	Textile WW		4.2-4.4	7.96	1.75		91.7	Bhuvaneswaria & Ashab (2015)
ABR	Protein carbohydrate wastewater	13	8	2.7	71	35	93	Barber & Stuckey (1999)
ABR	Synthetic greywater	8	0.48	0.1-0.4	48-84	25-33	63-84	Barber & Stuckey (1999)
ABR	Carbohydrate-protein	6.3	8	2.5-36	4.8-71	35	55-93	Barber & Stuckey (1999)
ABR	Carbohydrate-protein		4	1.2-4.8	20	15,25,35	75-83, 93-97, 96	Barber & Stuckey (2000)
ABR	Carbohydrate-protein		5	4.8-9.6	20	35	90-98	Barber & Stuckey (2000)
ABR	Carbohydrate-protein		6	4.8-18	20	35	52-98	Barber & Stuckey (2000)
ASBR	Synthetic milk WW		0.4-1	0.2-2	12 to 48	35	93-99	James Ndon (1995)
ASBR	Synthetic milk WW		0.4-1	0.2-2	12 to 48	25	90-99	James Ndon (1995)
ASBR	Synthetic milk WW		0.4-1	0.2-2	12 to 48	20	86-99	James Ndon (1995)
ASBR	Synthetic milk WW		0.4-1	0.2-2	12 to 48	15	87-99	James Ndon (1995)
ASBR	Lechate	12				35	85	Zaiat et al. (2001)
ASBR	Glucose+fatty acid	1.2				22	60-70	Zaiat et al. (2001)
AnMBR	Starch	1	20.15	1.76	10d	30	95	Roh et al. (2006)
AnMBR	Meat	3	0.45		6	35	95	Aquino et al. (2006)
AnMBR	Glucose+peptone+yeast extract	4.5	27	4	6.5d	45-56	78.5-84.4	Lin et al. (2011)
AnMBR	VFA	2	10	10 to 55	120 d (SRT)	55		Lin et al. (2013)
AnMBR	Molasses+glucose+VFA	0.6	25	2.5	14d	35	99.6	Jeong et al. (2010)
AnMBR	Molasses	0.5	10.2	5.6-14.9	0.5	55	78-81	Wijekoon et al. (2011)

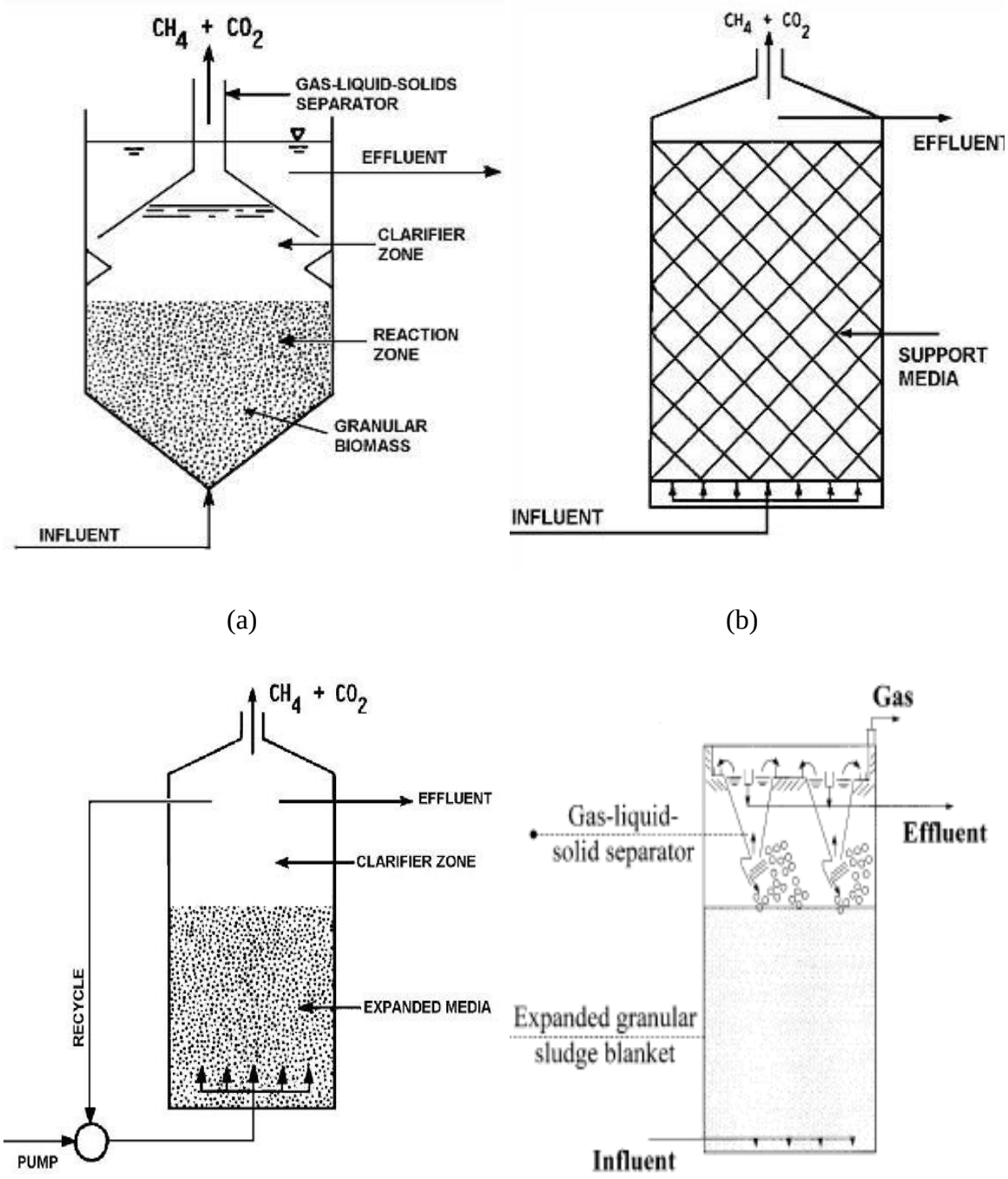


Figure 2.4 Anaerobic attached growth reactors: (a) UASB (b) AF (c) AnFBR, and (d) EGSB (Wang 2014).

A combination of the UASB with a fluidized bed reactor (FBR) is known as expanded EGSB. It is generally used when the volumetric gas production rate is low and UASB mixing by up-flow velocity alone is not sufficient (Mao et al., 2015). EGSB contain granular bioparticle and operate at a slightly higher superficial liquid velocity (5-10 m/hr) (Nicolella et al., 2000). As a family of the UASB, the EGSB is used to treat low strength soluble and complex wastewaters. Several advantages of EGSB including (i) offers a smaller footprint, higher mixing due to higher up-flow velocities and consequently improved mass transfer, biomass activity and better transport of substrate into sludge aggregate; (ii) work under higher organic and hydraulic loadings, (iii) more suitable for soluble pollutant treatments, especially for low strength wastewater (Mao et al., 2015). Table 2.2 shows a comparison between UASB and anaerobic filter on the basis of HRT, OLR, and COD removal. Detailed of anaerobic fluidized bed bioreactors are discussed in Section 2.5.2.

Table 2.2 Performance of UASB and AF

Reactor Type	Type of WW	Influent COD (g/L)	OLR (kg COD/m^3d)	HRT (h)	COD removal (%)	References
UASB	Cotton textile mill	0.6-1	0.48-0.96	30	Sep-51	Isik & Sponza (2004)
UASB	Wool acid dying	0.5-1.9	0.71-2.85	17	51-84	Işık & Sponza (2006)
UASB	Textile	4.2	1 to 15	29.3	91-97	Işık & Sponza (2006)
UASB	Food waste leachate	5.4-20	4.3-15	30	58-79	Ağdağ & Sponza (2005)
UASB	Pulp and paper	5.5-6.6	16	5	85	Tezel et al. (2001)
UASB	pharmaceutical	3	3.6		68-89	Sponza & Demirden (2007)
UASB	Olive mill WW+MWW	1.8-4.4	3 to 7	14.7	70-90	Gizgis et al. (2006)
UASB	Starch industry	20	15	24	77-93	Skylar et al. (2003)
UASB	MWW	0.3-1		4	69-84	Sperling et al. (2001)
UASB	Synthetic textile WW	2.7	4.8	10	50	Yu et al. (2000)
UASB	Synthetic wastewater	>0.1	18	17	95	Kennedy et al. (1989)
UASB	Synthetic wastewater	0.41	28	2	90	Noyola et al. (1988)
UASB	Brewery		14.1	4.9	86	Switzenbaum (1983)
UASB	Starch		11	47	85	Switzenbaum, (1983)
UASB	Sugar		13.3	24	94	Switzenbaum, (1983)
UASB	Alcohol		16	8	90	Switzenbaum, (1983)
AF	Domestic sewage	0.23	3.1	4	55	Elmitwalli et al.(2002a)
AF	Domestic sewage	0.43-0.53	0.9	12	71	Elmitwalli et al. (2002b)
AF	Municipal wastewater	0.35-0.45	0.8	12	91	Bodkhe (2009)
AF	Synthetic domestic sewage	0.71	1-1.7	10 to 17	80	Marttin et al. (2010)
AF	Starch gluten		3.8	22	64	Switzenbaum, (1983)
AF	Guar gum		16	24	60	Switzenbaum, (1983)
AF	Domestic WW	0.29	0.32	24	73	Kobayashi et al. (1983)
EGSB	Slaughterhouse WW		15	5	67	Nunez & Martinez (1999)

EGSB	Short chain organic acid (mixture of maleic, oxalic, fumaric, acetic and formic acids)	2.5	10	6	98	Dinsdale et al. (2000)
EGSB	Short chain organic acid (mixture of acetic, propionic, butyric, maleic, glyoxylic and benzoic acids)		3	24	90	Dinsdale et al. (2000)
EGSB	Domestic WW	0.4-0.85	1.6-4.5	3.5-5.7	83-94	Chu et al. (2005)
EGSB	Domestic WW	0.4-0.85	1.6-4.5	3.5-5.7	76-81	Chu et al. (2005)

2.5 Unbiodegradable Organic Matter in Anaerobic Digestion

Since 1982, the unbiodegradable organic matter in activated sludge system remained a major obstacle limiting anaerobic digestion efficiency (Gossett & Belser, 1982). Several researches were conducted to identify the unbiodegradable particulate fraction (Ikumi et al., 2014; Gossett & Belser, 1982). Gossett and Belser (1982) found that the endogenous residue of the ordinary heterotrophic organisms (OHO) remained unbiodegradable in the AD whereas endogenous residue fraction of OHO (0.37) varied highly from activated sludge (AS) model for real wastewater (0.20) in endogenous respiration-based steady-state models. The aforementioned study concluded that about 15% of the OHO endogenous residue was degraded in AD. Ekama et al. (2006) mentioned that unbiodegradable particulate organics (UPO) from the influent wastewater and the OHO endogenous residue remain unbiodegradable in the AD which is justified by Ekama (2009) that organics which are unbiodegradable in the AS system remain unbiodegradable in AD. Ekumi et al. (2014) have investigated the biodegradability of activated sludge organics in AD and found that UPO fractions for PS and WAS were 0.31 and 0.25, respectively using an AD model.

2.6 Configuration of Continuous Process

2.6.1 Continuous Stirred Tank Reactor (CSTR)

Reactants are well mixed in CSTR so that properties like temperature, density, etc. are uniformly distributed. The performance of the CSTR is mainly dependent on hydraulic retention time (HRT) and the degree of contact between the incoming substrate and a viable bacterial population (Karim et al., 2005). In this reactor, bacteria, substrates and liquid are mixed continuously where SRT and HRT are the same. The main factors that

affect the digester performance are mixing strategy, intensity, and duration (Kaparaju et al., 2008b).

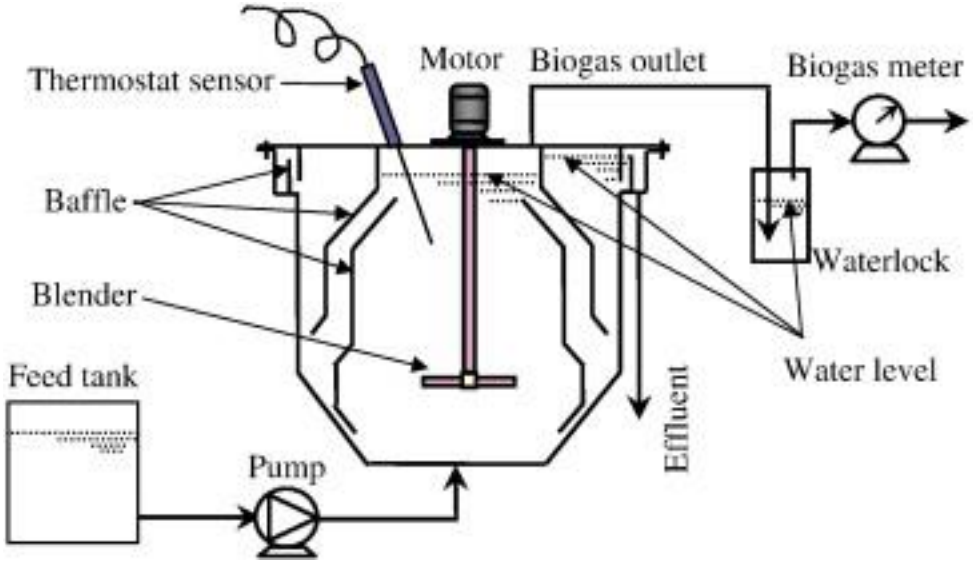


Figure 2.5 Schematic diagram of CSTR

2.6.2 Anaerobic Fluidized Bed Reactor (AnFBR)

Since the beginning of the 1980s, anaerobic technology started to be used for treating the high-strength wastewater, and sewage sludge stabilization (Wang, 2014; Saravanane & Murthy, 2000). Although, this new technology which introduced bio-energy production with low sludge yield in the absence of oxygen, low sludge activity, low reactor capacity, the unsustainability of the process, and inhibitory effects, remain the major obstacles for widespread application of anaerobic processes. To overcome these issues, the anaerobic biofilm technology has been developed The AnFBR has many advantages including enhanced mass and heat transfer rates, stability under shock loadings, achieving high treatment efficiency with low support media, and a uniform distribution within the liquid phase (Wang, 2014). AnFBR have been used in the treatment of industrial and municipal

wastewater: high-strength distillery wastewater (Fernandez et al., 2008), high concentrations of non-ionic surfactant (Motteran et al., 2014), and food-processing, wastewater (Heijnen et al., 1989). In the AnFBR, fine carrier particles are used for biofilm development. The media are fluidized by high up-flow fluid velocities using influent and recirculated effluents. However, some of the disadvantages include long start-up times due to biolayer formation on the carrier, difficulties in liquid biofilm thickness, high energy consumption due to very high liquid recirculation ratio, and high investment cost for liquid distribution to obtain uniform fluidization especially in large-scale applications (Saravanane & Murthy, 2000).

2.6.3 Factors Affecting AnFBR Performance

A detailed study of start-up process, inoculation, biomass and biolayer formation, microbial population dynamics, process stability with respect to shock loads and inhibition, are essential to evaluate the performance of AnFBR process (Saravanane & Murthy, 2000).

Biofilms development is influenced by the liquid flux rate, reactor scale, gas flux and organic loading (Hickey et al., 1991). Biofilm thickness is also influenced by shear at both macro-scale and micro scales (Saravanane & Murthy, 2000). Inoculation is one of the significant parameters impacting reactor performance. Various kinds of inoculum sources have been used as seed for digesting sludge in AnFBR. Supernatant from municipal or animal manure digesters (Hickey et al., 1991), sludge from a full-scale UASB reactor used in swine manure (Motteran et al., 2014), municipal secondary anaerobic digested sludge (Andalib et al., 2014; Wang et al., 2016) were used.

Carrier media characteristics such as size, porosity, density, surface roughness and specific surface area impact reactor performance (Hickey et al., 1991). COD removal efficiencies in the ranges of 27-60% were observed when sand, zeolite, and activated carbon were used for treating sewage sludge (Heijnen et al., 1989). The aforementioned study mentioned that the diameter of carrier particles had some influence with 0.35mm diameter out performing the 0.75mm diameter. Good performance of AnFBR with rougher surface area rather than larger surface area was achieved during the investigation of support media for microbial adhesion (Kida et al., 1990). The aforementioned study also noted that although cristobalite as a carrier media had a much smaller surface area (50 m^2/g) than that of the granular active carbon (1,125 m^2/g), it had a very rough surface with many tubercular processes, resulting in maximum total organic carbon (TOC) loading rate of 8 kg/m^3-d during the treatment of synthetic wastewater. In order to investigate the impact of specific surface area, AnFBR with a specific surface area of 800 m^2/m^3 successfully removed soluble COD at higher OLR (29.59 kg COD/m^3-d) than a smaller specific surface area of 320 m^2/m^3 (18.43 kg COD/m^3-d) (Sheli et al., 2014). Recently, plastic media was used as carrier media (Eldyasti et al., 2012; Wang et al., 2016). Compared to sand media, Yee et al. (1992) observed that start-up times were reduced by more than 50% when using porous support in AnFBR fed with acetic acid at an OLR of 6 $kgTOC/m^3$-d.

Anaerobic digester performance can also vary depending on organic loading rates. Table 2.3 shows the COD removal efficiencies for anaerobic, anaerobic-aerobic fixed film system at different OLR. Wang et al. (2016) investigated the treatment of TWAS and primary sludge (PS) using AnFBR and found that performance varied with OLR. The aforementioned study indicated that the AnFBR can sustain up to 12 kg COD m^{-3} d^{-1} of

OLR for TWAS with 56% COD removal and 18 kg COD m^{-3} d^{-1} for PS with 62% COD removal. During the evaluation of AnFBR performance (Wang et al. 2016), a continuous generation of scum was found, which remains as a major obstacle for enhancing removal performance.

Table 2.3 Treatment performance of anaerobic, anaerobic-aerobic fixed film system

Type	Type of wastewater	OLR (kg COD/m^3-d)	COD removal (%)	HRT (h or d)	References
AnFBR	TWAS	12	56	4d	Wang et al. 2016
AnFBR	PS	18	62	2.2d	Wang et al. 2016
UASB+AFB[a]	Synthetic textile WW	4.8	80	20 h	Chan et al. 2009
FFB[b]	Slaughter house WW	0.39	92	4.7-7.3d	Chan et al. 2009
EGSB+aerobic biofilm reactor [c]	Palm oil mill effluent	10	95.6	20h	Chan et al. 2009
UBF+MBR[d]	Synthetic WW	7.2	99	1d	Chan et al. 2009
AnFBR	Complex industrial WW	25-30	60-65	3.4-4.3	Chan et al. 2009

[a] Upflow anaerobic sludge bed (UASB) and anaerobic fludized bed (AFB)
[b] Anaerobic–aerobic fixed film bioreactor (FFB)
[c] Expanded granular sludge bed (EGSB)
[d] Anaerobic upflow bed filter (UBF) and membrane bioreactor (MBR)

2.7 Anaerobic Co-digestion of Food Waste

2.7.1 Background

As one of the alternative waste management methods, household food wastes disposers (FWD) have been implemented to divert food wastes from the landfills to wastewater treatment plants. The FWD technology first was introduced in 1924 in USA. Later it has been widely used in Canada, and Australia, with the highest penetration of 50% in some

US jurisdictions while European countries employed it less than other countries (Iacovidou et al., 2012; Battistoni et al., 2007).

Figure 2.6 Schematic diagram of food waste disposer

2.7.2 Advantages and Disadvantages of Food Waste Disposers (FWD)

FWDs are considered to be highly convenient as well require minimum installation and maintenance efforts. Moreover, the technology creates a cleaner and hygienic household, and minimizes the utilization of water for cleaning purposes. Studies reported that FWD can contribute to the diversion of food wastes from the landfill at up to 43% with 75% FWD installation of the households (Iacovidou et al., 2012). Generally, FWDs grind the food waste finely and send it to wastewater treatment plant, where it can be converted to fertilizer or bio-energy.

On the other hand, concerns over the use of FWDs have been mounting due to extra hydro consumption and potential blockage and damage of sewers (Thomas, 2011). Particularly, wastewater treatment plant (WWTP) operators posed their concerns on increased organic loadings to the sewage treatment plants, change of municipal wastewater characteristics, and thereby increase of operational costs. Furthermore, the use of FWD in regions with combined sewers may also increase chances of pollution during wet weather events that cause direct discharge of wastewater into the water bodies (Thomas, 2011).

2.7.3 Impact of Food Waste in Wastewater treatment plant

Different arguments on the FWD impact on WWTP operations persist in the literature. For instance, although COD loading increase by FWD is one of the major concerns, previous investigations did not show consistent results. Thomas (2011) showed elevated pollutant loadings of COD (24%), BOD (28%), and solids (18%) with FWD in UK while Evans et al., (2010) who reviewed 15 years of data from municipal wastewater treatment plants also presented that organic loading did not increase significantly with FWD use. In addition, several studies also addressed the benefit of increased organic strength in terms of enhancing biological nutrient removal and thereby reducing chemical addition (Battistoni et al., 2007; Iacovidou et al., 2012; Bolzonella et al., 2003). The enhanced COD:N:P ratio with FWD arising but nitrogen and phosphorous limited in food waste is beneficial for biological nutrients removal performances (Bolzonella et al., 2003). Moreover, it was reported in the aforementioned study that 78% of the disposed organic wastes reached the wastewater treatment plants

Due to this positive impact, some European local authorities including, UK, Ireland, France, Germany, and Netherland considered permitting FWD in areas with nutrient effluent quality requirements (EPA, 2005). Some studies emphasized that higher concentrations of organics may pass to the aeration tank, which may increase oxygen supply costs (Thomas, 2011). One important aspect to evaluate the impact of FW organics on bioreactors is to assess the settleability of food wastes primary clarifier performance. It is reported that at higher degree of penetration, greater than 40%, the characteristics of the wastewater stream change enough that modifications of municipal wastewater treatment may be required (Delft University, 2004).

Beside the settleability of FW in wastewater treatment plan, metal elements including light metal ions (Na, K, Mg, Ca, Al) and heavy metal ions (Cr, Co, Cu, Zn, Ni) plays an important role in anaerobic co-digestion of FW since enzyme synthesis as well as enzyme activities are maintained by cations (Zhang et al., 2014). However, high concentrations of metal elements can inhibit the digestibility (Appels et al., 2011). It is reported that less than of 400 mg K/L and 350 mg Na/L (Chen et al., 2008), and calcium of 150 mg/L – 300 mg/L (Yu et al., 2001) enhanced the anaerobic digestibility.

2.7.4 Anaerobic Co-digestion of Food Waste

Co-digestion of FW with municipal wastewater treatment biosolids added some beneficial effects like improvement of methane yield as well acceleration of methane production rates (Iacovidou et al., 2012; Koch et al., 2016). However, long term inhibition always occurred when FW is digested alone (Zhang et al., 2014), due to imbalance of nutrients, insufficient amounts of trace elements (Zn, Fe, Mo etc.) and excessive macronutrients (Na, K, etc.)

(Pullammanappallil et al., 2001; Zhang et al., 2011). Moreover, several literature studies reported that C/N ratio is the outside of the optimum ranges of 15-27.2 (Sosnowski et al., 2003; C. Zhang et al., 2013). Moreover, high lipid concentrations in FW may cause inhibition of anaerobic digestion (Zhang, et al., 2013). Therefore, instead of using FW as substrate, several researches have conducted anaerobic co-digestion of FW (Zhang et al., 2013; Mara et al., 2012; El-Mashad & Zhang, 2010; Li et al., 2009). Zhang et al. (2013) have reported that co-digestion of FW with Cattle Manure (CM) improved the maximum acceptable organic loading rate (10 kg VS/m^3-d to 15 kg VS/m^3-d) as well as enhanced the methane yield (55.2%) in semi-continuous digestion whereas Li et al. (2009) achieved a 44% improvement in the methane yield by co-digestion of FW with CM. Co-digestion of FW with CM provided balanced nutrients, and thus provided a more stable environment for promoting methane production in anaerobic digestion (El-Mashad & Zhang, 2010; Li et al., 2009). Moreover, higher methane yields could be obtained through the lipid addition in FW co-digestion, because of the high potential for methane yield of lipids and the higher biodegradation of lipids in co-digestion systems (Zhang et al., 2014). It is also seen that FW with other organic waste improve the biogas production and methane yield as shown in table 2.4.

Various studies on the positive impact of co-digestion in lab-scale and full-scale continuous systems fed with various co-substrates such as FW and Organic Fraction Municipal Solids Waste (OFMSW) are summarized in Table 2.4. A study by Dai et al., (2013) who operated different FW digesters with sewage sludge ranging from 0% to 100% (by w/w VS) showed that VS destruction and methane yield increased from 38% to 86% and 0.24 $LCH_4/gVSS_{added}$ to 0.62 $LCH_4/gVSS_{added}$, respectively at an SRT 30 days with

the similar trends at different SRTs. Sosnowski et al., (2008) also reported that specific methane production ($LCH_4/gVSS_{removed}$/day) increased from 0.32 to 0.44 as organic municipal solids were added to sludge at 25% on a volumetric basis. Similarly, Fitamo et al. (2016) also observed 1.5 times increase in methane yield ($LCH_4/gVSS_{added}$) when the concentration of FW in digestion increased from 0% to 90% (VS basis). Full scale application also shows that co-substrate addition of 94% (w/w VS) of the feed increased biogas production three times compared to mono digestion (Aichinger et al., 2015).

Table 2.4 Continuous-flow FW and wastewater treatment biosolids co-digestion studies

References	System type	Co-substrate source [a]	Temp [b]	SRT	C/N ratio [c]	Biosolids and co-substrate mixing ratio [d]	OLR (kg VS m^3/ d)	VS removal (%) [e]	Methane yield (LCH_4/ gVS_{added}) [f]	SMP (L CH_4/ $gVS_{removed}$) [g]
Dai et al. (2013)	Lab scale (CSTR)	FW	35	8-30	6.7-7.8	100:0	4-13.4	26.8-38.2	0.16-0.24	0.59-0.62
			35	8-30	8.5-9.0	71:29	4.6-15	39.7-51	0.22-0.30	0.54-0.59
			35	8-30	9.6-10.7	47:53	5.1-17.8	52.2-62.2	0.29-0.35	0.54-0.56
			35	8-30	10.2-12.5	29:71	6-18.5	59.2-70	0.3-0.4	0.51-0.57
			35	8-30	11.2-14.8	0:100	6.4-21.8	74.1-86.1	0.38-0.47	0.51-0.54
Sosnowski et al. (2003)	40 m^3 Semi-UASB	KW	56	35	9.3	100:0	0.39	N/A	N/A	N/A
			56	38	14.2	75:25	1.5	N/A	N/A	N/A
	9 m^3 CSTR+ 14 m^3 Semi-UASB		56+36	30	24.5	0:100	2.76	N/A	N/A	N/A
			56+36	62	8.16	100:0	0.67	N/A	0.22	N/A
			56+36	28	14.2	75:25	3.1	N/A	0.18	N/A
Sosnowski et al. (2008)	40 m^3 bioreactor	KW	35	N/A	N/A	0:100	N/A	N/A	N/A	0.23
			35	N/A	N/A	100:0	N/A	N/A	N/A	0.32
			35	N/A	N/A	75:25	N/A	N/A	N/A	0.44
Aichinger et al. (2015)	Full scale1	OFMSW	35	N/A	N/A	100:0	1.17	N/A	N/A	N/A
			35	28.7	N/A	54:46	2.18	N/A	N/A	N/A
	Full scale2		35	N/A	N/A	100:0	1.69	N/A	N/A	N/A
			35	27.7	N/A	85:15	1.98	N/A	N/A	N/A

	Lab scale (CSTR)		35	N/A	N/A	100:0	5.33	53 (52)	N/A	N/A
			35	N/A	N/A	80:20	6.66	55 (57)	N/A	N/A
Gou et al. (2014)	Lab scale (CSTR)	FW	35	4.2-33.3	13	67:23	1-8	48-62	0.23-0.26	N/A
			45	4.2-33.3	13	67:23	1-8	46-68	0.23-0.3	N/A
			55	4.2-33.3	13	67:23	1-8	44-75	0.23-0.4	N/A
Koch et al. (2015)	Full scale	FW	33	40	8.8	54:46:0	N/A	N/A	0.31	N/A
			33	40	17.7	55:35:10	N/A	N/A	0.39	N/A
Kim et al. (2011)	Lab (SBR)	FW	35+35	8	N/A	60:40	3.5	42	0.18	N/A
			55+35	7	N/A	60:40	6.1	45	0.2	N/A
Liu et al. (2012)	Lab scale (CSTR)	FW	35	50	12.9	25:75	2.40	65.6	0.41	0.67
			36	33	12.9	25:75	3.60	62.6	0.38	0.61
			37	25	12.9	25:75	4.8	64.5	0.43	0.67
			38	20	12.9	25:75	6	64.9	0.39	0.62
Cavinato et al. (2013)	Pilot scale	OFMSW	37	22	13	100:0	1.22	N/A	0.09	N/A
			37	24	28	50:50	1.6	N/A	0.21	N/A
			55	22	28	50:50	1.66	N/A	0.30	N/A
Schmit & Ellis (2001)	Lab scale (CSTR)	Synthetic	55+35	15	N/A	100:0/80:20/60:40/40:60/20:80	1.5–3.5	47.5–71.6	0.30–0.42	N/A
		OFMSW	55+35	15	N/A	100:0/80:20/60:40/40:60/20:80	1.5–3.8	39.6–69.3	0.28–0.33	N/A
Fitamo et al. 2016)	Lab scale (CSTR)	OFMSW	55	30	N/A	100:0:0	0.62-0.65	N/A	0.29	N/A
			55	10, 15, 20, 30	N/A	10:67.5:15.7:6.75	2.55, 3.91, 5.04, 7.79	N/A	0.42-0.43	N/A
			55	10, 15, 20, 30	N/A	10:45:31.5:13.5	2.25, 3.74, 4.99, 7.57	N/A	0.32-0.39	N/A

a. Dai et al. (2013) - cafeteria (rice, vegetables, oil and meat) / Sosnowski et al. (2008, 2013) – KW (kitchen waste, potato 55%, fruit and vegetables 28%, bread 5%, paper 2%, rice and pasta 10% wt) / Gou et al. (2014) - university cafeteria/Kim et al. (2011) – cafeteria of academic institute / Liu et al. (2012) - student canteen / Cavinato et al (2013) - a mixture of food waste

from large communities (supermarkets, canteens, restaurants etc.) and separately collected household biowaste / Schmit and Ellis (2001) - 60% paper products+14% FW + 26% Yard waste (dry weight) / Fitamo et al. (2016) - FW (university canteen) + grass and garden waste (garden and recycling centre)

b. Two stages systems for Kim et al. (2011), Sosnowski et al., (2003) and Schmit and Ellis (2001)
c. COD/N for Cavinato et al. (2013)
d. Dai et al. (2013) - dewatered sludge: FW w/w, based on VS
   Sosnowski et al. (2008, 2013) - mixed sludge (PS+TWAS) : OFMSW, based on volume
   Aichinger et al. (2015) - mixture ratio for two full scale tests was estimated using VS loading increase before and after using organic wastes
   Gou et al. (2014) – TWAS : FW (TS basis)
   Koch et al. (2016) – PS : TWAS : FW (TS basis)
   Kim et al. (2011) - sludge : FW (VS basis)
   Liu et al. (2012) - sludge : FW+fruit vegetable waste (TS basis)
   Cavinato et al (2013) – WAS : OFMSW (uncertain basis of mixture)
   Schmit and Ellis (2001) – PS : OFMSW (TS w/w basis)
   Fitamo et al. (2016) – sludge : FW: Grass clipping : Garden waste (VS basis)
e. COD removals are indicated within brackets for Aichinger et al. (2015)
f. L $CH_4/gVSS_{added}$ for Sosnowski et al. (2008). Two full scale tests by Aichinger et al. (2015) show that co-digestion increased specific methane yield maximum 1.59-2.87 times compared to sludge mono-digestion LCH_4/gVSS/day for Sosnowski et al. (2008)

2.8 Synopsis

From the cited literature works, the following knowledge gaps have been identified

- Scum accumulation in AnFBR restricts the digestibility.
- Assessing biodegradability of municipal biosolids through Sotemann (2006) steady-state model is still lacking on microbial activities.
- Impact of anaerobic co-digestion of FW with municipal biosolids
- The distribution of the three main active bacterial groups (methanogenic, acetogenic and acidogenic) during co-digestion of FW

In order to minimize the scum generation, the current study employed ultrasonication based AnFBR which can successfully reduce the scum generation with enhanced methane production.

In order to assess the biodegradability of TWAS, SRT, decay rates, and biomass yield based model was developed and tested with three different influent TWAS concentrations.

Since literature also shows that co-digestion of FW with wastewater treatment biosolids has a significant impact on methane production, this study further explores co-digestion performance emphasizing the specific methanogenic activity (SMA), specific acetogenenic activity (SAtA), and specific acidogenic activity (SAdA) tests that were also conducted to evaluate different microbial behaviors between mono-digestion and co-digestion.

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Chapter 3

Ultrasonically Enhanced Anaerobic Digestion of Thickened Waste Activated Sludge using Fluidized Bed Reactors

3.1 Introduction and Literature Review

The use of wastewater biosolids not only opens a window of opportunities for clean, renewable and CO_2 neutral energy source but also minimizes the use of fossil fuels and lessens global warming. The anaerobic digestion technology is able to treat high-strength, predominantly soluble industrial wastewaters due to its capability of sustaining higher volumetric loadings, low nutrient requirements, low biomass yield, and additional biogas (hydrogen, methane) production (Chan et al., 2009). Additionally, anaerobic digestion can reduce waste volume, and enhance nutrient recovery (Cho et al., 2012). However, a serious issues for the broad implementation of anaerobic digestion for biosolids using conventional technologies is its inability to operate at high organic loading rates, and the long hydraulic retention time (HRT) of 20 to 40 days (Mata-Alvarez et al., 2014; Lee et al., 2011). Additionally, the slow growth rate of the methanogens coupled with the performance fluctuation due to their highly sensitive characteristics remain as major obstacles in anaerobic digestion (Lu et al., 2008; Mata-Alvarez et al., 2000). Anaerobic digestion (AD) of wastewater biosolids is limited by slow biodegradation rates ensuing from slow biomass hydrolysis, and resulting in low solids destruction efficiencies which ultimately necessitate on large footprint and high capital cost.

Recently the anaerobic fluidized bed bioreactor (AnFBR) have been gaining popularity in the wastewater field because of its enhanced mass and heat transfer rates, stability under shock loadings, high treatment efficiency at high organic loading rates, and a uniform distribution within the liquid phase (Wang et al., 2016). Andalib et al., (2012) have investigated the treatability of thin stillage as a by-product of bioethanol production plants using AnFBR and 88% TCOD and 78% TSS removal was achieved at very high OLR of 29 kg COD/m^3-d and solids loading rate of 10.5 kg TSS/m^3-d respectively with HRT of 3.5 days. In AnFBR, wastewater travels through the media, the substrate diffuses to the biofilm where it is digested. The biofilm-coated particles promote the digestion of municipal and industrial biosolids at high loading rates (Andalib et al., 2012). However, one of the major problems of this technology and conventional digestion systems is the generation of scum in the digester. Scum has 6% solids which causes operational and maintenance problems, and also reduces overall efficiency (Wang et al., 2016). Hence one of the main challenges of AD is the minimization of scum generation in the reactor.

To minimize the issues related to anaerobic digestion in conventional digesters, utilization of ultrasonication have gained a lot of success to enhance the reactor performance (Cho et al., 2013; Cho et al., 2012). During the ultrasonication, an acoustic wave propagates in the liquid media, and cavitation bubbles are produced in rarefaction zone (Cho et al., 2012). Ultrasound was first introduced for bacterial cell disruption in order to recover intracellular materials (Harrison, 1991) and later it spread to applications like anaerobic digestion of sewage sludge (Tiehm et al., 1997), low-strength ultrasonication of methanogenic granules (Cho et al., 2013), waste activated sludge disintegration (Cho et al., 2012; Bougrier et al., 2006), and bacterial cell growth (Pitt & Ross, 2003; Liu et al., 2003). Cell

membrane permeability and enzyme activity was increased but it did not cause cell disruption when low strength (US density > 0.1 W/ml) ultrasonication was applied (Pitt & Ross, 2003; Liu et al., 2003). An increase of dehydrogenase activity and adenosine triphosphate content by 257%, and 374%, respectively was found when ultrasonication was used in methanogenic granules to evaluate the performance of UASBr (Cho et al., 2012). Xie et al. (2009) have applied low-intensity ultrasonication at 0.2 W/cm^2 for 10 min in anaerobic sludge and found that the activity of anaerobic sludge was enhanced with 30% increase of organic removal (Xie et al., 2009). However, the aforementioned study investigated an ultrasonic cleaning bath in which anaerobic sludge was taken in a 100 ml serum bottle. The bath had a fixed frequency of 35kHz and variable power from 0 to 80W (Xie et al., 2009). Application of low strength ultrasound in UASBr (1s per min, 0.05 W/ml of US density) can successfully enhanced the CH_4 production from brewery wastewater at an OLR of 2 kg COD/m^3-day by 38% and 19% in ambient and a mesophilic conditions, respectively (Cho et al. 2013). Moreover, the aforementioned study was conducted in a dry digestion system applying ultrasonication (2s per 30s, 0.0025 W/ml of US density) at the same OLR of 2 kg COD/m^3 and found that methane production increased by 40% with deceasing the solids content from 12% to 10% indicating that high solids content reduced the US effect. A different sludge yield was observed in aforementioned study i.e 86.1% and 94.3% of the $COD_{removed}$ were converted to CH_4, while the remaining 13.9% and 5.7% were presumably converted to biomass from the UASBr and the UASBr with the attached ultrasonicator, respectively implying that enhanced CH_4 production was attributed to the increased electron flow toward CH_4 production rather than

biomass synthesis. Table 3.1 summarizes the AnFBR performance and the impact of sonication in different treatment.

Based on the above studies no research so far have been done to minimize solids discharge as well as scum reduction. The current work developed a novel anaerobic fluidized bed digestion incorporating ultrasonication for enhanced biogas production due to break down and reuse of scum. The main concept was to utilize the high scum COD to enhance overall performance. The utilization of ultrasound in the anaerobic fluidized bed reactor successfully reduced the scum production as well as minimized the discharge of stabilized solids, thereby reducing transportation costs, landfill disposal as well as environmental impact.

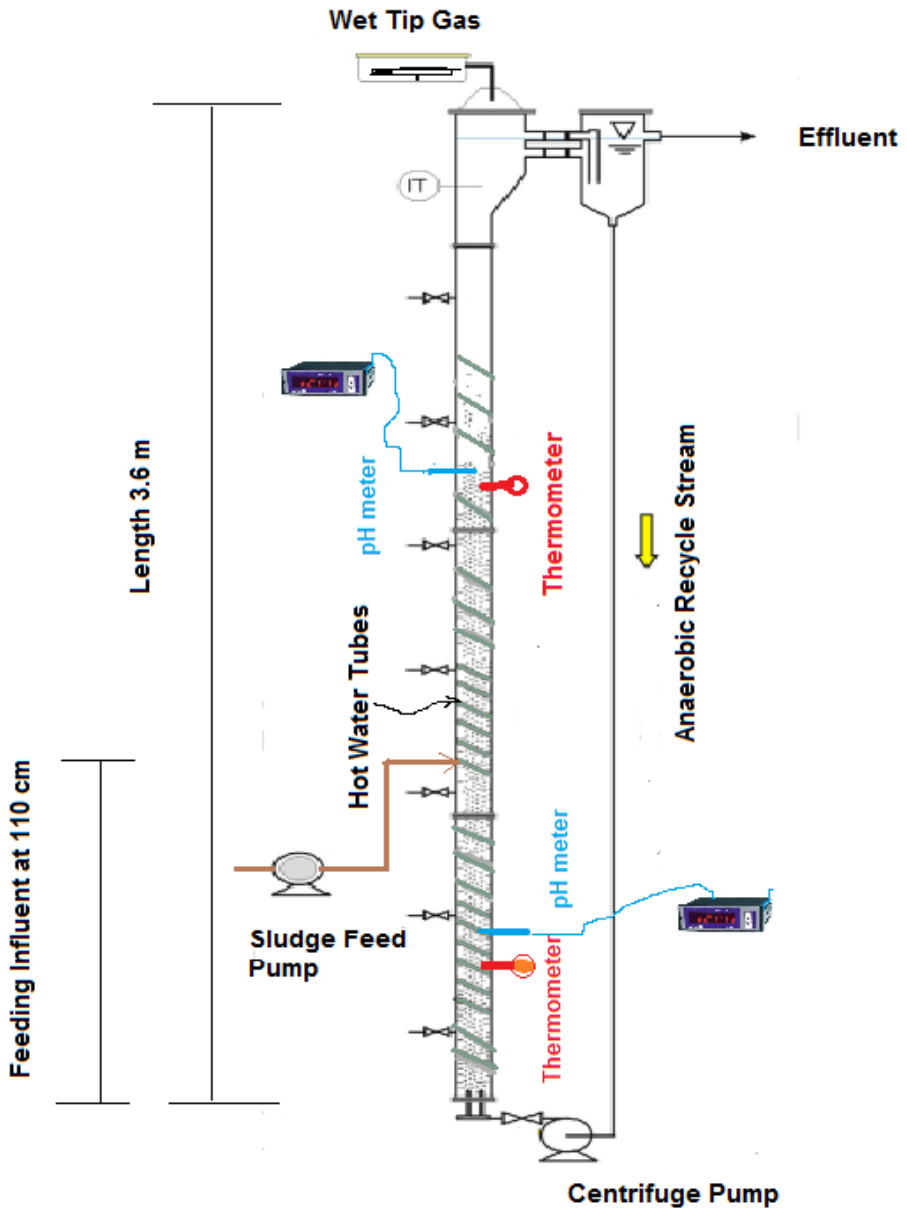
Table 3.1 Studies and applications of ultrasonication and anaerobic fluidized bed reactor

Scale	Reactor or treatment type	US energy* (W/Kg TDS)	US time (min)	US interval	Substrate type	Reactor volume (L)	OLR (kgCOD/m3-d)	HRT (d)	Temperature	CH_4 increased due to US (%)	COD removal (%)	Reference
Lab	Pretreatment	25 kHz	0-250		WAS				37	40		Appels et al., 2008
Lab	Pretreatment	35 kHz	10		Anaerobic sludge				37		92	Xie et al. 2009
Lab	Pretreatment	No			Anaerobic sludge				37		88	Xie et al. 2009
Lab	Batch test	0-0.1 (W/ml)	0-30	1s per min	Brewery WW				37	43		Cho et al. (2012)
Lab	UASBR	237		1s per min	FW	5	2		25	38		Cho et al. (2013)
Lab	UASBR	237		1s per min	FW	5	2		37	19		Cho et al. (2013)
Lab	Dry digester	10		2s per 30s	FW+dewatered sludge cake	60		100	37	40		Cho et al. (2013)
Lab	AnFBR				Starch	50	18	12 hr	37	80		Hickey & Owens (1980)
Lab	AnFBR				Textile	4	3	24 hr	37		82	Şen & Demirer, (2003)
Lab	AnFBR				Thin stillage	16	29	3.5	37		88	Andalib et al. (2012)
Lab	AnFBR				Primary sludge	16	9.5	1.9	37		82	Andalib et al. (2012)
Lab	AnFBR				TWAS	16	12	4	37		56	Wang et al., (2016)
					PS	16	18	2.2	37		62	Wang et al., (2016)

3.2 Materials and Methods

3.2.1 Process Description of Fluidized Bed Reactors

Two identical lab-scale anaerobic fluidized bed reactors (AnFBRs) were used to test the TWAS, as shown in Figure 3.1. TWAS was collected from the Adelaide Wastewater Treatment Plant, London, Ontario. The reactors built with plexiglass consisted of a 16-liters liquid volume main anaerobic column (3.6 m height, 8.9 cm long and 5.1 cm width) and a liquid-solid separator (0.9 m height, 18 cm long, and 8 cm width) from which the digested sludge was separated and circulated to the bottom of the AnFBR for fluidization. An ultrasonic cell disrupter (VCX 500, Sonic and Material Inc., Newtown, USA) was connected with U-AnFBR in upper level of reactor. The ultrasonic vibracell comes with 500 watt (model: CV 33). A wet tip gas meter (Rebel wet-tip gas meter company, Nashville, TN, USA) was connected with each reactor at the top of column for measuring biogas flow rate. Mesophilic temperature (37^0C) was maintained by a water bath (IncuMaxTM WB20C, USA). Feed sludge was continuously pumped (Masterflex I/P, Masterflex AG, Germany) at a specific time interval (3 minutes on in every 4 hours) from a 10 liter container. Each reactor contained around 3kg of High-Density Polyethylene (HDPE) particles (600-850 µm) which occupied 22% volume of the reactor. The HDPE particles had a sphericity of 0.9 and a Brunauer–Emmett–Teller (BET) surface area of 0.86 m^2/g, with bulk density of 810 kg/m^3 and true density of 1554 kg/m^3, respectively. Because of lower energy consumption (Eldyasti et al., 2012), HDPE was preferred over other media in current research.


Wet Tip Gas
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IT
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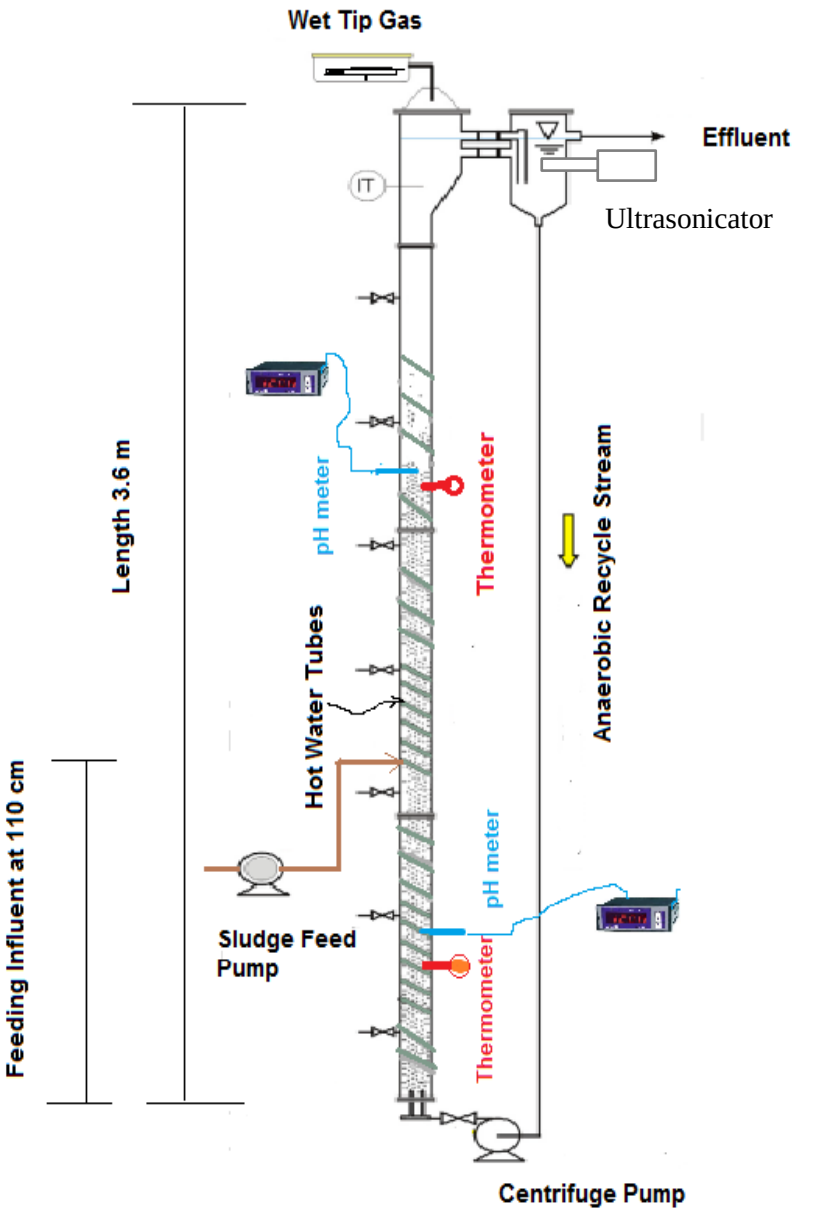
Thermometer
Anaerobic Recycle Stream
Hot Water Tubes
Feeding Influent at 110 cm
Sludge Feed Pump
pH meter
Thermometer
Centrifuge Pump



(b)

Figure 3.1 Schematic diagram of anaerobic fluidized bed reactors (a) AnFBR (b) U-AnFBR

Figure 3.2 HDPE particles used in reactors

Figure 3.3 Sonic vibra cell

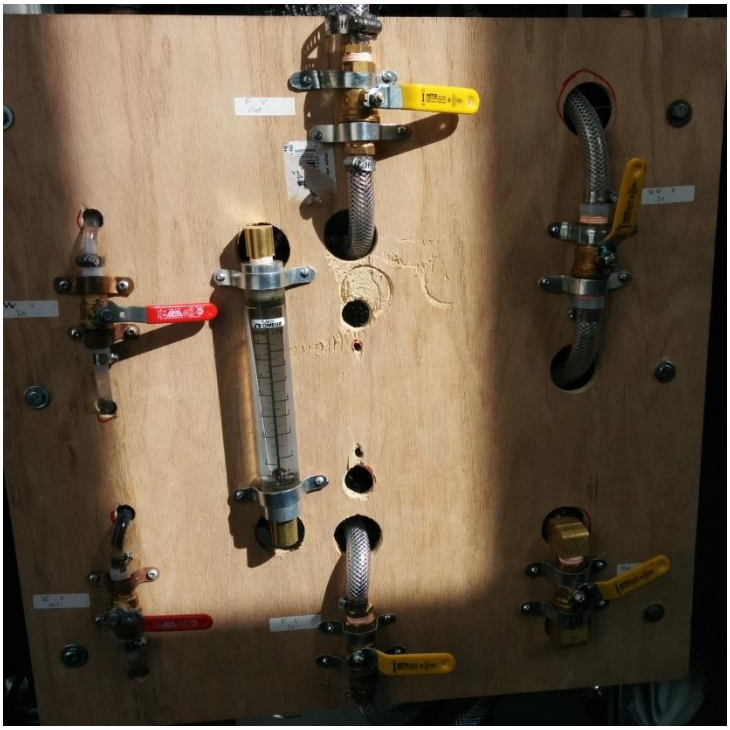


Figure 3.4 Hydraulic control valve

3.2.2 Commissioning and Start-up

As a seed sludge for AnFBRs, anaerobic digested sludge (ADS) was used. ADS was collected from the secondary digester of the St. Mary wastewater treatment plant (St. Mary, ON, Canada). Total suspended solids (TSS) and volatile suspended solids (VSS) for ADS were 32,000 mg/L and 22,000 mg/L. The AnFBRs were filled with around 20 L of ADS (16 L main column, and 4 L of solids liquid separator) after filling the reactor with 2.8 kg media corresponding to a compacted media volume of 3.4L. The reactors were operated in a batch mode at 100% bed expansion for 7 days to induce microbial attachment at an anaerobic conditions provided by initially injecting N_2 gas at the top area. A synthetic acetate based wastewater solution with composition shown in Table 3.2, was fed to both reactors at a flow rate of 1.8 L/d for the first 10 days. The chemical oxygen demand (COD)

for synthetic wastewater solution was close to 10,000 mg/L corresponding to a volumetric OLR of 1.1 kg COD/m^3-d based on the 16 L AnFBR working liquid volume. Even though the pH of synthetic wastewater solution was 4 due to high concentration of acetic acid, pH in the both reactors maintained nearly 7.2.

Table 3.2 Composition of synthetic wastewater (Andalib et al. 2012; Wang et al. 2016)

Feed Comp.	CH_3COOH (mL/L)	NH_4Cl (g/L)	K_2HPO_4 (g/L)	$MgSO_4 \cdot 7H_2O$ (g/L)
Con.	9.5-38	0.93	0.1	0.03
Feed Comp.	$CaCl_2 \cdot 2H_2O$ (g/LF)	Yeast (g/LF)	$NaHCO_3$ (g/LF)	Trace element (mL/LF)
Con.	0.03	0.03	6.2-24.8	1
Trace element	$FeCl_2 \cdot 4H_2O$	$MnCl_2 \cdot 4H_2O$	H_3BO_3	$ZnCl_2$
Con. (mg/L)	2000	500	50	50
Trace element	$CuCl_2$	$AlCl_3$	$CoCl_2 \cdot 6H_2O$	$NiCl_2$
Con. (mg/L)	30	50	50	50

In both reactors, liquid at the top was recycled and pumped back to the bottom of the fluidized bed at a flow rate of 129 L/h to maintain an upflow velocity at 0.8 cm/s. The recycle flow rate was maintained by a control panel. In order to release accumulated gas when necessary, a gas release valve was also installed at the highest point of the pipe line in the control panel. TWAS from the Adelaide wastewater treatment plant (Ontario, Canada) was feed into both reactors after acclimatization. Adelaide Waste Water Treatment Plant (WWTP) is a single-stage nitrifying WWTP operating at an SRT of 3-4 days (Wang et al., 2016).

3.2.3 Analytical Methods

Influent and effluent samples were collected three times a week and analyzed mainly for total suspended solids (TSS), volatile suspended solids (VSS), total chemical oxygen demand (TCOD), and soluble chemical oxygen demand (SCOD). Selected samples were analyzed for total nitrogen (TN), ammonia ($N\text{-}NH_3$), and alkalinity. In addition, gas production and gas composition, pH, temperature were monitored and recorded on a daily basis.

TSS, and VSS were analyzed according to the standard methods (APHA, 1992). Hach methods were followed to analyze TCOD, and SCOD (HACH Odyssey DR/2800) based on the potassium dichromate oxidation and spectrophotometric determination. A 0.45- µm filter paper was used for filtering the sample to analyze soluble parameters. Alkalinity was measured by titration with 0.02 N H2SO4 in accordance with the standard method no. 2320 (APHA, 1992). VFAs were measured by employing gas chromatographs (Model CP-3800, software version 3.2.6.C, CP-1177 injector, VARIAN). The gas pressures were set as 80 psi for helium, 80 psi for nitrogen, 60 psi for air, and 40 psi for hydrogen, respectively. Flowrates of gas were set at 1.5 mL/min. 3.0 mL/min, and 6.0 mL/min for nitrogen, helium, and hydrogen, respectively. Temperatures for the oven and flame ionization detector (FID) were set at 250ºC and 300ºC.

In order to measure the biomass detachment approximately 10 g bioparticles were collected from each reactors and sonicated for 3 hr at 30^0C to detach the biomass from particle using an Aquasonic Sonicator (Model 75HT, ETL Laboratory Investigating Inc., New York).

The VSS content of the detached biomass was measured using standard methods (APHA, 1992) and the sonicated particles were weighted after drying at room temperature for 2 d.

3.2.4 Specific Methanogenic Activity (SMA) Test

The specific methanogenic activity of the suspended and attached biomass was determined by testing the liquid effluent and the reactor media. Serum bottles with a total volume of 155ml (125 ml of working volume and 30 ml of head space) were used for the batch test. The initial substrate-to-biomass (S/X) ratio was set at a constant level of 2.0 g COD/g VSS (Yoon et al., 2014). The same nutrient solution that was fed during the start-up period was added to the batch test. Acetic acid was used as a substrate to test the methane production. For the attached biomass tests, a total bioparticles weight of 37.5 gm was used in each test, the seed VSS of 25.3 g was used for attached and suspended biomass test for the control AnFBR. Similarly in the U-AnFBR, 26.8 g of seed VSS were used for attached and suspended SMA test. A high initial concentration of 5 g/L $NaHCO_3$ in the bottle was required to maintain the pH level throughout the entire test. The volume of the gas produced was measured by releasing the bottles headspace pressure using proper glass syringes (Perfektum; Popper & Sons Inc, NY, USA) until gas production ceased (Andalib et al., 2014). A volume of 0.6 ml of biogas was used to measure the CH_4 content by injecting into a gas chromatograph (Model 310, SRI Instruments, Torrance, CA) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Molesieve 5A, mesh 80/100, 182.88 × 0.3175 cm).

3.3 Results and Discussion

3.3.1 Performance analysis of reactors

The acetic acid-based synthetic wastewater at an OLR of 1.25 kg COD/m^3-d was fed progressively to the both reactor till the TCOD removal reached more than 90%. Since detailed study based on synthetic wastewater was conducted in previous works (Wang et al., 2016), the current study mainly focus on the impact of ultrasonication on TWAS in AnFBR to enhance the reactor performance.

Although, operation of the AnFBR compared to U-AnFBR, was quite smooth during start-up and low OLR (5.2 kg COD/m^3-d), operation and maintenance works were more intense at higher OLR (9.7 kg COD/m^3-d) for the AnFBR because of clogging issues resulting from the accumulation of scum. Thus, the experimental programs for the AnFBR (186 days) was terminated 38 days earlier than the U-AnFBR (224 days). However, both reactors were fed with same municipal wastewater biosolids, ie. TWAS. TCOD and VSS of the raw TWAS was varied from 36240 – 46840 mg/L, and 19200 - 29800 mg/L, respectively. Influent TWAS was initially fed at an OLR of 5 kg COD/m^3-d to the AnFBR for 94 days, and U-AnFBR for 74 days (Table 3.3). At 5 kg COD/m^3-d of OLR, two different ultrasonication energies of 120 kJ and 240 kJ were applied to optimize the maximum reactor performance. Three different ultrasonication energies of 240 kJ/d, 480 kJ/d, and 600 kJ/d were applied to optimize the ultrasonication energy at higher OLR of 10 kg COD/m^3-d.

Table 3.3 Phase details of AnFBR and U-AnFBR

Reactor type	Phase #	Time (d)	Steady state time (d)	OLR (kg COD/ m^3-d)	Input US Energy (kJ/d)	Input US Energy (kJ/g TSS_{feed} -d)	Influent COD (gm)	CH_4 (L/d)	CH_4/ COD -in (%)	CH_4 increase due to US-energy (%)
U-AnFBR	Start-up	30	65-105	1.2	0	0	19±1.4(5)	6.3±1(5)	82	
	Phase Ia	20		5.3	120	2.2	85±2.8(7)	14.1±1(7)	42	14
	Phase Ib	54		5.1	240	4.2	81±1.6(19)	18.2±0.5(19)	56	33
	Phase IIa	81	123-224	9.6	480	4.3	153±3.4(27)	25.6±0.7(27)	42	37
	Phase IIb	14		9.8	600	5.4	156±1.3(4)	21.7±0.8(4)	35	26
	Phase IIc	25		9.6	360	3.1	154±11.4(4)	20.5±0.8(4)	33	22
AnFBR	Start-up	15	62-105	1.3			20±0.3(3)	1.8±0.2(3)	25	
	Phase I	94		5.1			81±5.2(32)	12.2±0.7(32)	38	
	Phase II	77	130-186	9.7			155±6.5(19)	16.1±0.7(19)	26	

Note: Number in the parenthesis are the number of samples

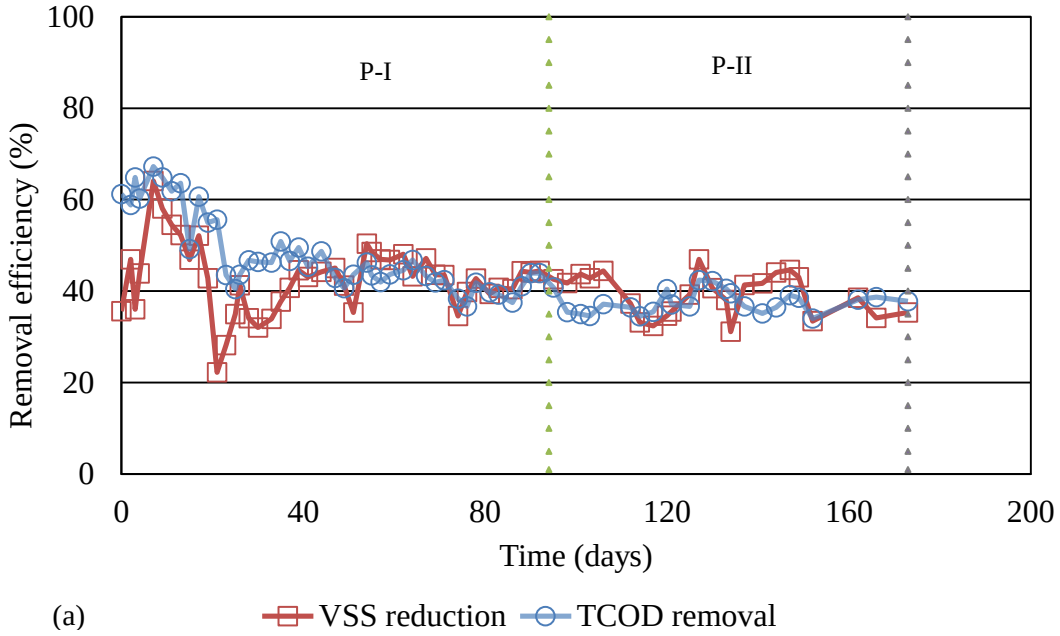
Figure 3.5 shows the temporal variations of VSS destruction and TCOD removal efficiencies in both reactors during the TWAS feeding. Time 0 corresponds to the initial feeding of TWAS. The TCOD removal efficiency and VSS destruction efficiency in any given digester were almost identical due to the relatively low SCOD. On the other hand, fluctuations were observed in the U-AnFBR due to changes of OLR and ultrasonication energy. Biogas production was measured in everyday and COD balance closure was calculated based on the biogas production and COD consumption.

$$Influent\ COD = Effluent\ COD + scum\ COD + biogas\ COD \qquad (3.1)$$

In the AnFBR, a stable performance was observed after 35 days with COD balance closure of more than 93%. The AnFBR was run at 5 kg COD/m^3-d of OLR for 94 days and then OLR increased at 9.7 kg COD/m^3-d. During the low OLR (5.1 kg COD/m^3-d), TCOD removal and VSS destruction efficiencies were recorded as 43% and 41%, respectively. At 9.7 kg COD/m^3-d of OLR, the rate of TCOD removal and VSS destruction slightly

decreased to 38%. A fairly thick scum layer containing wet TSS of 65.5 mg/g and VSS of 48.5 mg/g was observed for AnFBR, floating on the liquid-solid separator with an accumulation rate of 310 g/d (wet) at 4.7 kg COD/m^3-d of OLR and 600 g/d (wet) at 10 kg COD/m^3-d of OLR. Every 2 days, the thick scum was collected to ensure the smooth operation of the reactor. Compressed N_2 was bubbled in each time after collecting the scum.

In the U-AnFBR, 2 different phases (Ia, Ib, IIa, IIb, and IIc) were conducted including start-up time to optimize the US energy. Details of 2 different phases are given in Table 3.3. A steady state condition was achieved after 35 days of TWAS feeding. On the basis of TCOD removal and VSS destruction efficiencies, the optimum US energy of 240 kJ was recorded at the 5 kg COD/m^3-d with TCOD and VSS destruction efficiencies of 65% and 63%, respectively. At the higher OLR of 10 kg COD/m^3-d, US energy was optimized at 480 kJ/d whereas 600 kJ/d of US energy deteriorated the system performance. The reason behind the declining system performance is that specific bacterial activity may have been adversely impacted by ultrasound energy. As a result, excessive use of ultrasound might cause higher effluent concentration as well as lower TCOD removal. Detailed analysis of the sonication impact on reactor performance and energy balance is given in section 3.3.2.



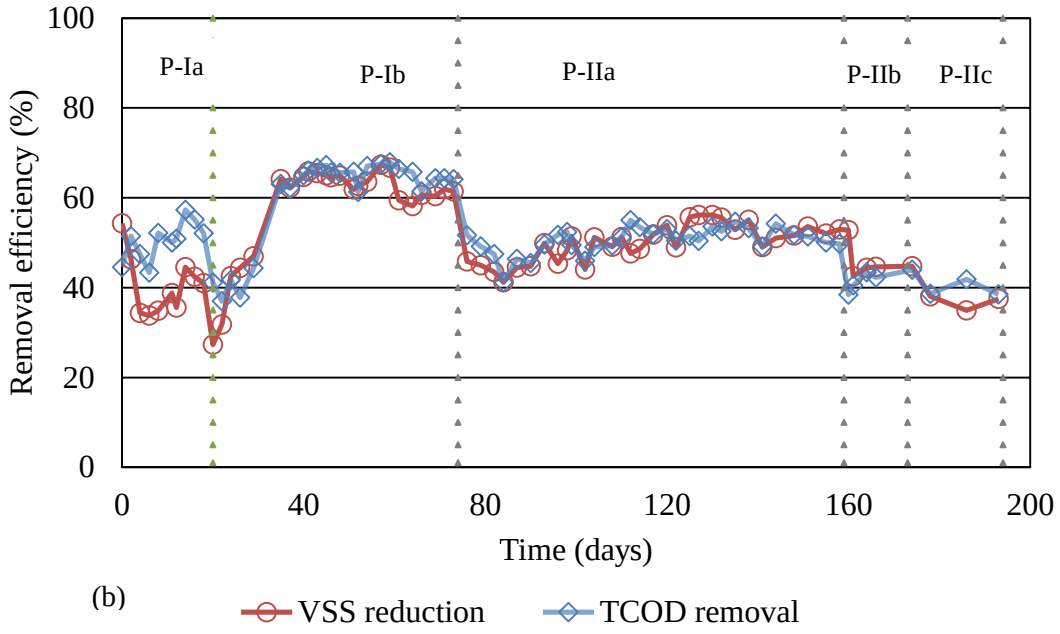


Figure 3.5 Temporal TCOD removal and VSS destruction efficiencies of reactors for treating TWAS (a) AnFBR (b) U-AnFBR

Operational conditions and steady-state data of AnFBR and U-AnFBR are given in Tables 3.4 and Table 3.5, respectively. The influent TCOD and VSS for AnFBR and U-AnFBR were maintained closely at about 40,000 mg/L, and 21,000 mg/L, respectively. The feeding rate of 2 L/d corresponding to an HRT of 8 days and an OLR of 4.5 -5.5 Kg COD/m^3-d (phase I) and ended 4 L/d corresponding to an HRT of 4 days and an OLR of 9.5 -11.5 Kg COD/m^3 d (Phase II). A fairly thick scum layer, containing 69 mg TSS/g and 49 mg VSS/g in AnFBR, 70 mg TSS/g and 42 mg VSS/g in U-AnFBR, was observed floating on the top of the liquid-solid separator. The observed scum accumulation rates were 310 g/d (phase-I), and 600 g/d (phase-II) in AnFBR, and 230 g/d (phase-Ia), 60 g/d (phase Ib), 260 g/d (phase-IIa), 180 g/d (phase-IIb), and 260 g/d (phase-IIc) in U-AnFBR. The VSS in the scum layer were 33% (phase-I) and 30% (phase-II) for AnFBR, and 26% (phase-Ia), 6% (phase-Ib), 11% (phase-IIa), 7% (phase-IIb), and 14% (phase-IIc) for of the influent VSS for the U-AnFBR.

Methane yield, VSS and COD destruction efficiency were calculated as follows:

$$\text{Methane yield } \left(\frac{\text{mL } CH_4}{\text{mg COD}}\right)(\text{STP}) = \frac{V_{CH_4}\left(\frac{mL}{d}\right)\times\frac{273}{273+37}}{S_0\left(\frac{mg}{L}\right)\times Q_{feeding}\left(\frac{L}{d}\right)-S_e\left(\frac{mg}{L}\right)\times Q_{effluent}\left(\frac{L}{d}\right)-S_s\left(\frac{mg}{g}\right)\times Q_s\left(\frac{g}{d}\right)} \quad (3.2)$$

$$VSS\ destruction\ (V_d) = 1 - \frac{VSS_{effluent}\left(\frac{mg}{L}\right)\times Q_{effluent}\left(\frac{L}{d}\right)+VSS_{scum}(mg/g)\times Q_{scum}(g/d)}{VSS_{feeding}(mg/L)\times Q_{feeding}(L/d)} \quad (3.3)$$

$$COD\ destruction\ (C_d) = 1 - \frac{COD_{effluent}\left(\frac{mg}{L}\right)\times Q_{effluent}\left(\frac{L}{d}\right) + COD_{scum}\left(\frac{mg}{g}\right)\times Q_{scum}\left(\frac{g}{d}\right)}{COD_{feeding}\left(\frac{mg}{L}\right)\times Q_{feeding}\left(\frac{L}{d}\right)}$$

Where S_0 denotes the influent TCOD/VSS concentration, S_e denotes the effluent TCOD/VSS concentration, and S_s denotes the TCOD/VSS concentration in the scum layer. All the values involved and the results are illustrated in Tables 3.4 and 3.5. Average TCOD and VSS destruction of AnFBR at an HRT of 8 days and an OLR 5.1 kg COD/m^3-d of were 43% and 41%, respectively. At the shorter HRT of 4 days and higher OLR of 9.7 kg COD/m^3-d, removal rates of both (TCOD and VSS) was 38%. Similarly, the U-AnFBR at an HRT of 4 days and an OLR of 5.1 kg COD/m^3-d achieved maximum TCOD and VSS destruction efficiencies of 65% and 63% (phase-Ib), respectively indicating that 240 kJ/d i.e. 11.1 MJ/Kg-TDS_{scum} of US-energy enhanced the removal efficiency. On the other hand, maximum 51% destruction efficiencies of TCOD and VSS were observed in phase-IIa indicating 480 kJ/d i.e. 11.8 MJ/Kg-TDS_{scum} of US-energy maximized the removal performances during shorter HRT of 4 days in U-AnFBR.

At standard temperature and pressure (STP, 0ºC and 1 atm), the theoretical methane yield is 0.35 mL/mg COD digested (Metcalf & Eddy, 2003), which translates to 0.4 mL/mg COD digested. Temporal methane yields are shown for both reactors in Figure 3.6, with time zero corresponding the start date of TWAS feeding. At an HRT of 8 days, methane yields were 0.35 mL/mg COD digested and 0.36 mL/mg COD digested for AnFBR, and U-AnFBR, respectively. In the AnFBR, the average methane yield was 0.28 mL/mg COD digested in short HRT (4 days), indicating the COD balance was 20% off. The results suggest that the COD concentration in scum layer was underestimated, which also infers that real COD removal and VSS destruction in phase II might have been lower than the 35%. On the other hands, methane yields in the U-AnFBR were in the range of 0.33-0.34 mL/mg COD digested from phase-IIa to phase-IIc.

Table 3.4 Operational conditions and steady-state performance of the control AnFBR fed TWAS at STP

Operating Conditions			
Parameter	Start-up	Phase I	Phase II
Time of operation (d)	1-13	14-105	106-186
Feed flow rate (L/d)	2	2	4
Effluent volume (L/d)	2	1.60±0.08 (32)	3.31±0.45 (19)
OLR based on anaerobic reactor (kg COD/m^3 d)	1-2	4.5-5.5	9.5-11.5
Anaerobic HRT(d)	8	8	4
pH	7.2±0.2 (7)	7.4±0.4 (40)	7.6±0.2 (26)
Total media (kg)	2.8	2.8	2.8
Feed characteristics			
TCOD (mg/L)	9,900~10,120 (7)	39,800±2,430 (48)	
SCOD (mg/L)		4,200±300 (48)	
TSS (mg/L)	-	29,200±2070 (48)	
VSS (mg/L)	-	21,530±2300 (48)	
Effluent characteristics, n = 32(I), 19(II)			
TCOD (mg/L)	-	13800±1820	13430±2060
SCOD (mg/L)		1180±150	1170±160
TSS (mg/L)	-	12000±2230	13120±1810
VSS (mg/L)	-	7000±1060	7870±1200
Scum layer characteristics, n = 32(I), 19(II)			
TCOD (mg/g)	-	78±12	84±5
TSS (mg/g)	-	70±6	68±3
VSS (mg/g)	-	48±6	49±3
Production rate (g/d)	-	310±41	597±59
Removal Efficiencies, n = 32(I), 19(II)			
COD removal eff. (%)	90	43±3	38±2
VSS removal eff. (%)		41±5	38±5
Methane yields, n = 32(I), 19(II)			
Methane yield (LCH_4/gCOD removed) (STP)	-	0.35±0.03	0.28±0.02

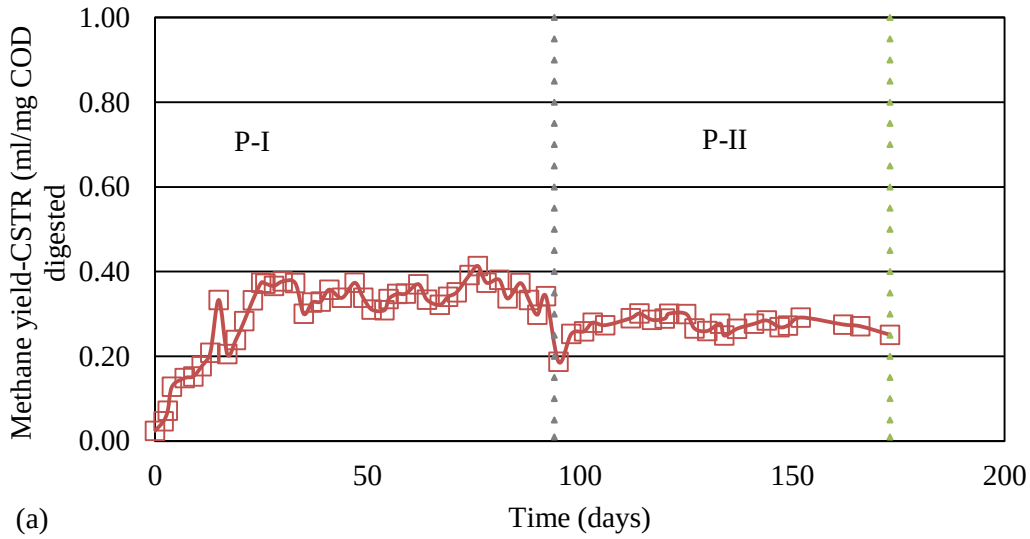
Note: ‘n’ and number within parenthesis denote the samples number

Table 3.5 Operational conditions and steady-state performance of the U-AnFBR fed TWAS at STP

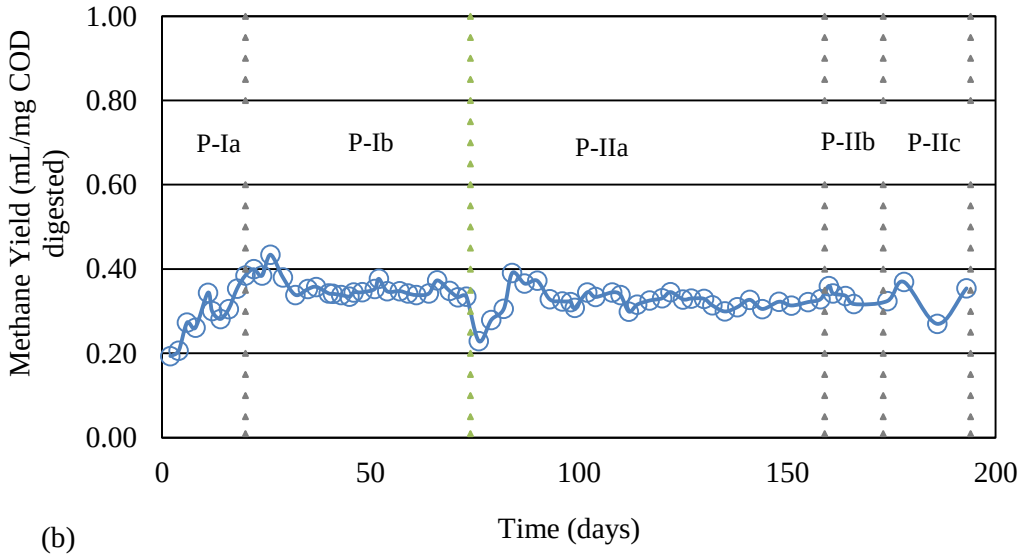
Operating Conditions						
Parameter	Start-up	Phase Ia	Phase Ib	Phase IIa	Phase IIb	Phase IIc
Time of operation (d)	1-30	31-50	51-104	105-189	190-203	204-224
Feed flow rate (L/d)	2	2	2	4	4	4
Effluent Volume (L/d)	2	1.70±0.07 (7)	1.81±0.09 (19)	3.76±0.06 (27)	3.6±0.23 (4)	3.53±0.19 (4)
OLR based on anaerobic reactor (kg COD/m^3 d)	1-1.25	4.5-5.5	4.5-5.5	9.5-11	9.5-11	9.5-11
US-Energy (kJ/d)		120	240	480	600	360
Anaerobic HRT(d)	8	8	8	4	4	4
pH	7.2±0.2 (5)	7.4±0.4 (12)	7.4±0.4 (25)	7.6±0.2 (35)	7.6±0.2 (8)	7.6±0.2 (8)
Total media (kg)	2.8	2.8	2.8	2.8	2.8	2.8
Feed characteristics						
TCOD (mg/L)	10242±1061 (3)	39,800±2,430 (64)				
SCOD (mg/L)		4,200±300 (64)				
TSS (mg/L)	-	29,200±2070 (64)				
VSS (mg/L)	-	21,530±2300 (64)				
Effluent characteristics, n = 7 (Ia), 19 (Ib), 27 (IIa), 4 (IIb), 4(IIc)						
TCOD (mg/L)	535±35 (3)	13580±3840	14550±3380	14610±1330	21840±950	20130±1460
SCOD (mg/L)		1020±130	1340±330	1320±280	2310±250	2480±90
TSS (mg/L)	-	11270±3840	11440±2040	12880±1280	15780±1010	16840±1010
VSS (mg/L)	-	8430±2750	8250±1340	8990±780	11860±1670	11570±1060
Scum layer characteristics, n = 7 (Ia), 19 (Ib), 27 (IIa), 4 (IIb), 4(IIc)						
TCOD (mg/g)	-	79±3.7	67±4.6	77±4.2	74±1.3	78±1.7
TSS (mg/g)	-	70.8±0.41	72±2.3	67±3.5	65±0.6	75±2.4
VSS (mg/g)	-	46±1.8	44±1.1	39±3.8	35±1.4	45±3.7
Production rate (g/d)	-	230±50	62±7.3	259±28.2	183±5	261±65.9
Removal Efficiencies, n = 7 (Ia), 19 (Ib), 27 (IIa), 4 (IIb), 4(IIc)						
COD removal eff. (%)	>95%	51±6%	65±2%	51±3%	41±2%	41±3%
VSS removal eff. (%)	-	38±6%	63±3%	51±4%	46±5%	39±4%
Methane yields, n = 7 (Ia), 19 (Ib), 27 (IIa), 4 (IIb), 4(IIc)						
Methane yield (LCH_4/gCOD removed) (STP)	-	0.33±0.04	0.36±0.02	0.33±0.02	0.34±0.02	0.33±0.04

Note: 'n' and number within parenthesis denote the samples number

Although, methane yields in all phases are slightly lower than theoretical, they are within the typical error accuracy of COD measurement of 10%-15% (Standard Methods) and the 10% accuracy of measuring biogas (Wang et al., 2016). The methane yields for all phases were 0.35 mL/mg COD (phase-I) and 0.28 mL/mg COD (phase-II) in AnFBR, and 0.33 mL/mg COD (phase-Ia), 0.36 mL/mg COD (phase-Ib), 0.33 mL/mg COD (phase-IIa), 0.34 mL/mg COD (Phase-IIb), and 0.33 mL/mg COD (phase-IIc) in U-AnFBR indicating about 6% less than theoretical. Thus, the uncertainty in measured COD removal and VSS destruction data is 6% on average. However, given that typical COD mass balance closures of 80% to 90% in anaerobic reactors are considered satisfactory (Wang et al., 2016), the uncertainty of 6% in this current study is indeed remarkable.



(a)

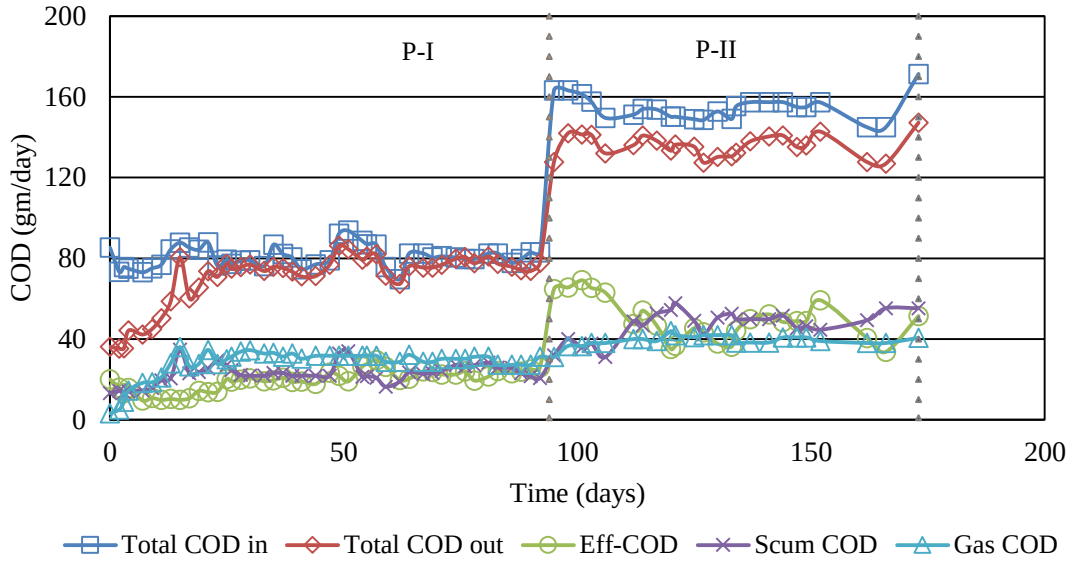


(b)

Figure 3.6 Temporal variation of methane yields for treating TWAS (a) AnFBR (b) U-AnFBR

Figure 3.7 shows the variation of daily mass rate of COD throughout the entire experimental period. Influent COD and total effluent COD comprising liquid, scum, and biogas out were within less than 10% of each other. In the AnFBR, although the average

CH_4 production increased in phase II (16.1 LCH_4/d) compared to phase I (12.2 LCH_4/d), the average ratio of methane as COD vs influent COD dropped from 38% in phase-I to 26% in phase II (Table 3.3).



(a)

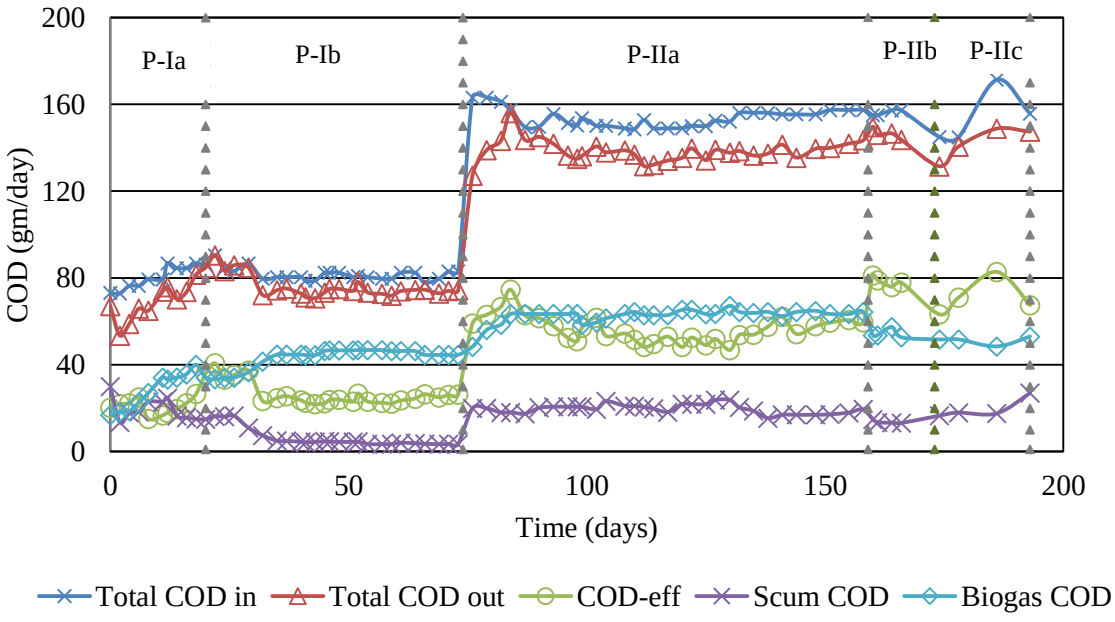


Figure 3.7 Temporal variation of COD as gm (a) AnFBR and (b) U-AnFBR

Digester operational stability is reflected by the VFA/alkalinity ratio, with values less than 0.4 indicating stable performance (Wang et al., 2016). VFA was expressed as concentration of acetic acid which was calculated through converting the summation of individual VFA concentration (as COD) to concentrations of acetic acid. Table 3.6 lists the average and standard deviation of the VFA/alkalinity ratio. During steady-state operations, the ratio of 0.39 for AnFBR (phase-II) and the ratio of 0.3 for U-AnFBR (phase-IIb), indicating stable digestion. The percentage contributions of acetic and propionic acids to the overall VFA on a COD basis in the final effluent are presented in Table 3.6. Acetic acid and propionate acid in U-AnFBR were predominant at 35%-76% and 20%-65% of total VFA, respectively. On the other hand, propionic acid and valeric acid were predominant at 20%-38% and 21%-59% of total VFA, respectively. T-test conducted on the effluent acetic acid, propionic acid, butyric acid, and valeric acid data indicate that difference between the AnFBR and U-AnFBR were not significant for propionic acid, butyric acid, and valeric acid at the 95 percentile confidence level while acetic acid level differences between both reactors were significant at the 95 percentile confidence level.

Table 3.6 VFA composition in final effluent during steady-state operational conditions in phase II for AnFBR (n=3) and phase IIb for U-AnFBR (n=6)

Parameters	**AnFBR**		**U-AnFBR**	
	Concentration (mg COD/L)	Percentage as TCOD	Concentration (mg COD/L)	Percentage as TCOD
Acetic acid (%)	84 ± 60	14 ± 4	252 ± 116	44±1
Propionic acid (%)	146 ± 48	28±10	183 ± 73	31±10
Butyric acid (%)	62 ± 52	10±4	29	5±1
Valeric acid (%)	168 ± 62	34±22	113 ± 89	19±12
VFA to alkalinity ratio	0.39±0.04		0.3±0.06	

Nitrogen mass balances were analyzed to examine the nitrogen generation through VSS destruction as presented in Figure 3.8. Ammonia-nitrogen mass balance closures considering the influent ammonia and ammonia generated from VSS destruction relative to effluent ammonia were 90% (overall) for both reactor (Table 3.7). It must be emphasized that the accuracy of the ammonia nitrogen mas balance closures in both reactors confirm the VSS destruction efficiencies.

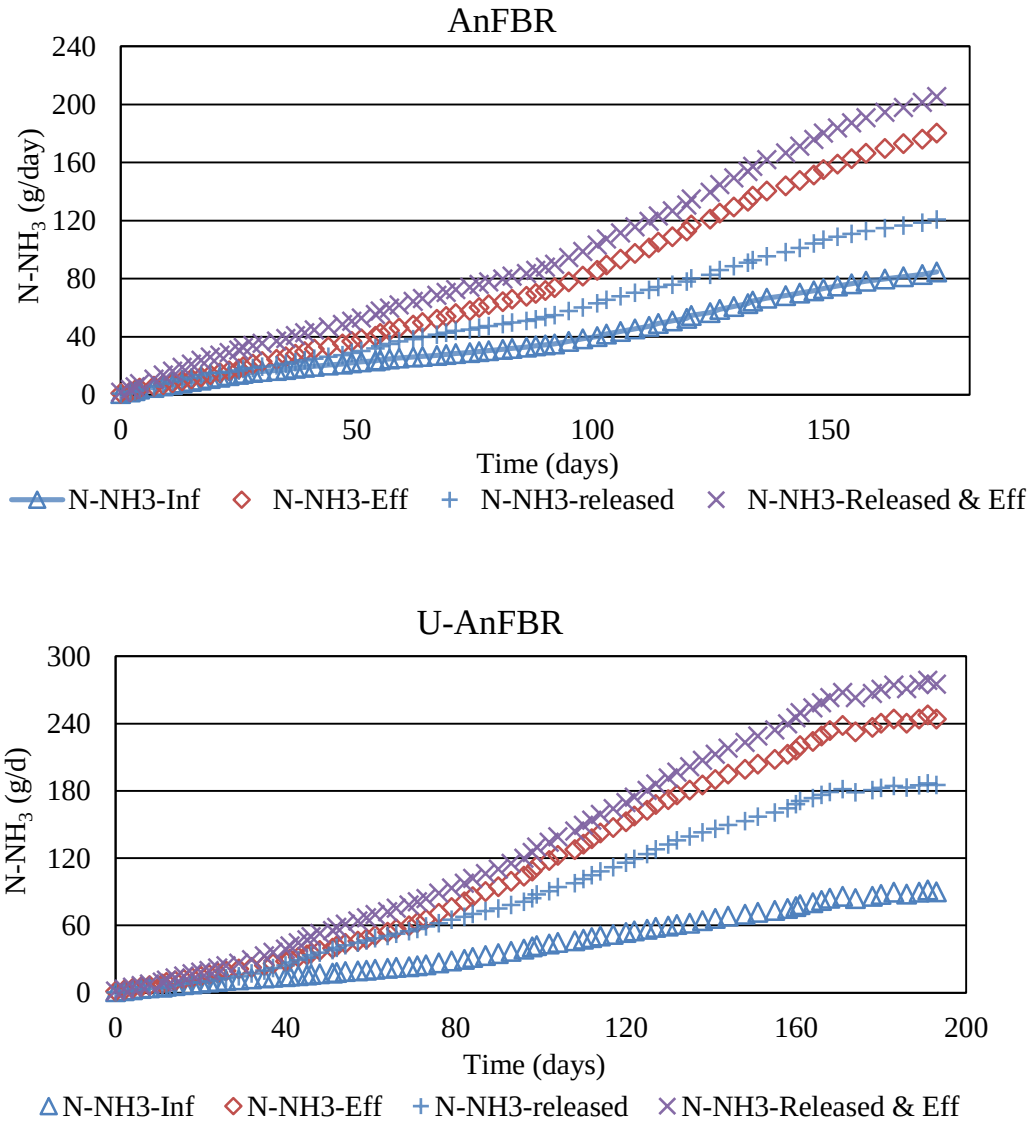


Figure 3.8 Nitrogen balance for treating TWAS

Table 3.7 Nitrogen balance in different phases at steady-state period for AnFBR and U-AnFBR

Phases	AnFBR		U-AnFBR				
	Phase-I n = 32	Phase II n = 19	Phase-Ia n = 7	Phase-Ib n = 19	Phase-IIa n = 27	Phase-IIb n = 4	Phase-IIc n = 4
Steady-state day	38 - 105	130 - 186	41-50	65 - 104	123 - 189	196 - 203	211 - 224
Inf-VSS (g/d)	44.2 ± 4.96	89.9 ± 5.5	40.5 ± 2.7	45.9 ± 2.2	91.1 ± 4.4	90.6 ± 0.01	86.3 ± 4.6
Eff-VSS (g/d)	25.77 ± 3.1	55.5 ± 4.5	25 ± 3.7	16.9 ± 1.2	44.3 ± 2.9	48.8 ± 4.1	52.8 ±5.47
VSS destruction (%)	41	38	38	63	51	46	39
PN/VSS	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Inf-N-NH_3 (gm/d)	0.72 ± 0.09 (10)	1.81 ± 0.17 (6)	0.72 ± 0.05 (3)	0.66 ± 0.13 (5)	1.6 ± 0.25 (10)	1.84 ± 0.08 (2)	1.92 ± 0.07 (2)
Eff-N-NH_3 (gm/d)	1.84 ± 0.18 (10)	3.8 ± 0.3 (6)	1.41 ± 0.03 (3)	2.25 ± 0.47 (5)	4.72 ± 0.3 (10)	4.3 ± 0.46 (2)	3.75 ± 0.05 (2)
Released NH_3 (gm/d)	1.24 ± 0.28	2.35 ± 0.47	1.08 ± 0.22	2.22 ± 0.18	3.57 ± 0.38	2.6 ± 0.51	2.15 ± 0.27
N-balance closures (%)	94	91	78	90	90	92	92

Note: 'n' is number of samples at steady-state conditions except for parameters where the number of samples is specified in parenthesis

3.3.2 Impact of Ultrasonication

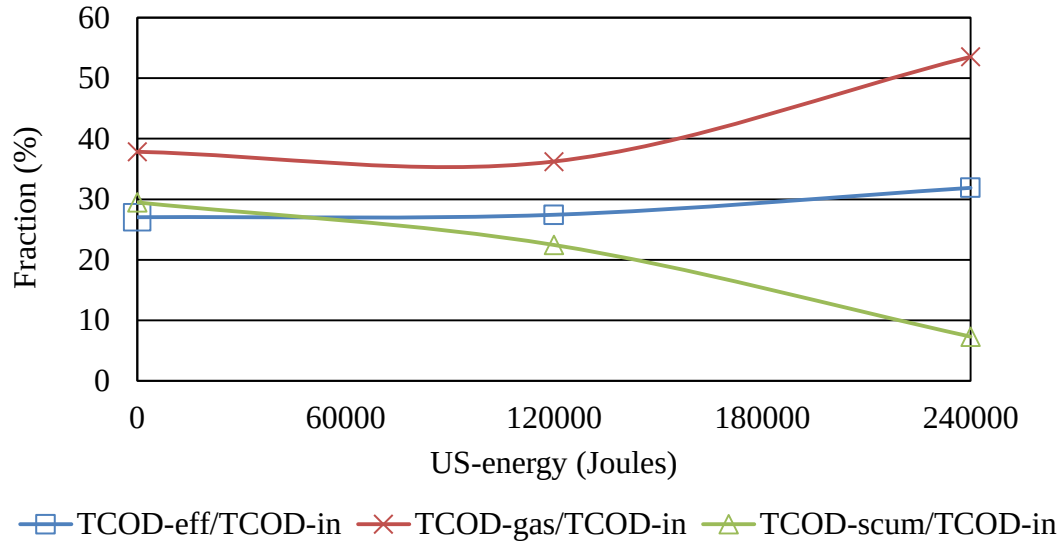
Though the application of ultrasound were investigated in many research fields such as biological cell disruptions (Harrison, 1991), enzyme extraction, pollutant removal, and coal cleaning (Bougrier et al., 2006; Tiehm et al., 2001); research on the impacts of ultrasound in anaerobic digestion is limited. Cho et al. (2012) investigated the impact of low-strength ultrasound in UASBR on the activity of methanogenic granules activity and observed that 5 min of ultrasonication at 237 W/kgTDS was optimal, resulting in the increase of dehydrogenase activity and adenosine triphosphate content by 257%, and 374%, respectively. Cho et al. (2013) also investigated the impact of ultrasound in a dry digestion system treating FW (18% to 22% of TS) and found 40% increase in the production of CH_4 was observed after lowering the total solids content of the reactor from 12% to 10%, implying that a high solid content diminished the ultrasonic stimulation effect. A 2 s per

30 s of ultrasound was applied in aforementioned study to reach the optimum level. Elbeshbishy and Nakhla (2011) have investigated five different mesophilic systems to evaluate the effect of ultrasonication on the anaerobic biodegradability of food waste and found that sonication inside the reactor showed superior results compared to other systems. The aforementioned study reported 67% VSS removal efficiency and a methane production rate of 3.2 $LCH_4/L_{reactor}d$ at an OLR of 45.9 kg COD/m^3-d. To evaluate the efficiency of ultrasonication as a pre-treatment method for hog manure prior to anaerobic digestion, 200 mL of hog manure was sonicated at a range of 0 – 30,000 kJ/kgTS with sonication pulses set to 2 s on and 2 s off (Elbeshbishy et al., 2011). The aforementioned study have reported that methane production was increased by 28% at an input energy of 500 kJ/kgTS and utrasonication was more effective for hog manure with higher TS content than WAS and primary sludges.

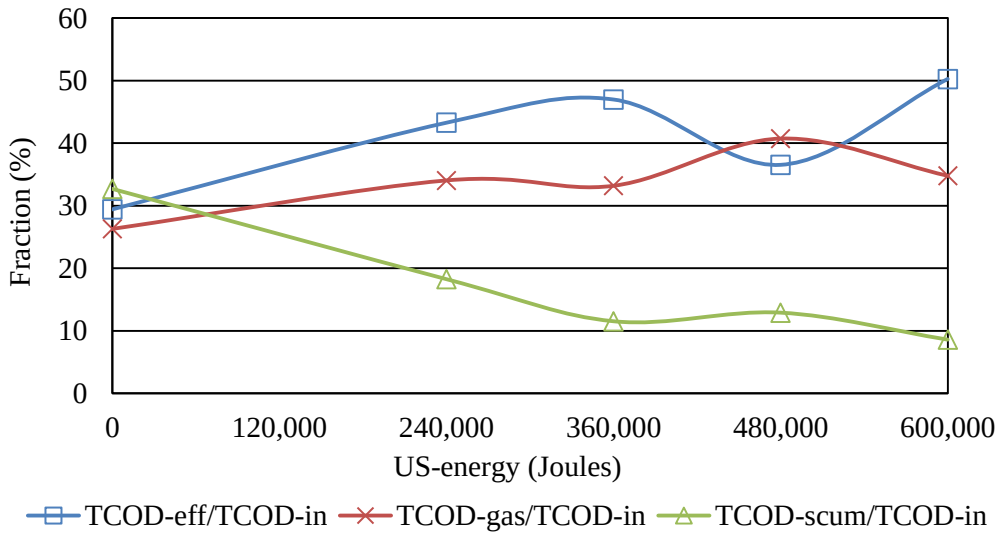
However, our current work applied ultrasound energy at 120-600 kJ/d for 2 s per 30 s corresponding to ultrasonication densities of 5.5 – 14.8 MJ/kgTDS. The volume of sonicated scum ranged from 190 ml to 680 ml while the sonicated solids varied from 21.6 to 40.6 gm TDS/d. In the U-AnFBR, at an OLR of 5.1 Kg COD/m^3-d, the scum accumulation rate varied from 60 - 230 gm/d (wet basis) at US energy in the range of 5.5 – 11.1 MJ/kgTDS. Similarly, at an OLR of 9.7 kg COD/m^3-d, the scum accumulation rate varied from 180 - 260 gm/d (wet basis) at US energy in the range of 8.9 – 14.8 MJ/kgTDS. The best operating conditions with respect to scum minimization occurred at an OLR of 5.1 Kg COD/m^3-d and US energy of 11.1 MJ/kgTDS.

Figure 3.9 shows the fraction of influent total COD at the two different OLR in the liquid effluent, scum, and gas. According to the Figure 3.9, the best operating conditions found

to be 11.1 MJ/kgTDS at an OLR of 5.1 kg COD/m^3-d, and 11.8 MJ/kgTDS at an OLR of 9.7 kg COD/m^3-d. It is noted that maximum biogas was observed at 480 kJ/d of US-energy, with further increases in US energy lowering the biogas production.



(a)



(b)

Figure 3.9 Fraction of TCOD vs US-energy (a) OLR of 5.2 kg COD/m^3-d (b) OLR of 9.7 kg COD/m^3-d

3.3.4 Specific Methanogenic Activity (SMA) Test

Research on anaerobic biofilms' structure is limited and most of the studies focused on the spatial distribution inside the biofilm (Kuba et al., 1990; Bull et al., 1983). Kuba et al., (1990) found that not all of the attached biomass were active methanogens while treating VFAs based synthetic wastewater at an OLR of 4 kg COD/m^3-d in AnFBR using zeolite as support media. Earlier studies (Bull et al., 1983) showed that methanogens mainly grow attached to the carrier surface since acidifiers tend to appear in the suspended phase. However, the SMA test is one of the favoured methods for investigating specific methanogenic activity profile of suspended and attached biomass in anaerobic reactors (Andalib et al. 2014; Sumino et al. 2007; Banik et al. 1997; Ince et al. 1995; Araki & Harada 1994). In anaerobic process, the performance of AnFBR cannot be rationalized on the basis of the widely accepted SRT model based on VSS. Furthermore, SMA test was used to confirm the optimized ultrasonication by the analysis of dehydrogenase activity and adenosine triphosphate content (Cho et al. 2012). Current studies used the SMA test in order to investigate the mechanism of the biofilm reactor and confirm the enhanced performance of AnFBR.

The specific SRT of each bacterial group was determined by the biomass specific growth rate in liquid phase and biofilm phase according to the following equation:

SRT

$$= \frac{Rate\ 1\left(\frac{L}{mgd}\right) * Attachment\ \left(\frac{mg}{g}\right) * W_p(g) + Rate\ 2\left(\frac{L}{mgd}\right) * [VSS_{effluent}\left(\frac{mg}{L}\right) * V_{reactor}(L) + VSS_{scum}\left(\frac{mg}{d}\right)]}{Rate\ 2(\frac{L}{mgd}) * VSS_{effluent}\ (mg/L) * Q_{feeding}(L/d)}$$

…….(3.5)

Rates 1 and 2 reflect the specific biogas production rate of the attached and suspended biomass, respectively. W_p denotes the clean particles in the reactor. The scum layer VSS was also considered in the calculation of effluent VSS concentration. However, in order to determine the maximum specific biogas production rate of R_m (ml g $VSS^{-1}hr^{-1}$), maximum specific cumulative biogas production of P (ml g VSS^{-1}), and lag time of λ (hr); the following Gompertz model has been successfully used.

$$H = P.\exp\left\{-\exp\left[\frac{(R_m \times e)}{P}(\lambda - t) + 1\right]\right\} \quad \ldots\ldots (3.6)$$

Table 3.8 presents the Gompertz parameters. R_m for the attached biomass in U-AnFBR is significantly higher than the control AnFBR whereas the suspended biomass is lower than the control i.e. not only is the attached biomass in the sonicated AnFBR higher but also US-energy affected lower detachment. The ratio of the SRT-to-HRT in the AnFBR and the U-AnFBR were 1.28 and 1.78, respectively. It is noteworthy that Wang et al., (2016) reported an SRT-to-HRT ratio of the 1.25 for the AnFBR. Based on the Liptak equation (Metcalf & Eddy, 2003) using the SRT of table 3.8, the estimated VSS destruction efficiencies in the AnFBR, and U-AnFBR are 41%, and 46%, as compared with the 38%, and 46%, observed experimentally.

Table 3.8 Results of microbial activities tests under steady state conditions at an OLR of 9.7 kg COD/m^3-dfor both reactors

	AnFBR		U-AnFBR	
	Attached	Suspended	Attached	Suspended
P (ml/gmVSS)[a]	1710	480	6170	460
R_m (ml/gmVSS-hr)[a]	19	4.9	32.9	2.9
R_m (ml/gmVSS-hr)[b]	15.7	4.1	26.7	2
λ (hr)[a]	172.5	201.8	201.7	196
OLR (kg/m^3-d)	9.7		9.7	
SRT (days)[a]	5.1		6.7	
SRT (days)[b]	5.1		7.1	
HRT (days)	4		4	

[a] Applying Gompertz model
[b] Maximum specific biogas production rates (R_{max}) are taken at zero order rate under 95 percentile gas/95 percentile time

As shown in Figure 3.10 a-b, specific methanogenic activity was more pronounced in the attached biomass than the suspended phase for both reactors. In fact, activity of methanogenic microbial group for attached biomass in U-AnFBR was more noticeable than the AnFBR (Figure 3.10c) and vice versa is true for suspended biomass. Compared to AnFBR with U-AnFBR, this results justified the enhanced microbial activity for attached biomass than suspended biomass as well as promoted the overall performance of U-AnFBR. Figure 3.11 compares the final pH with the initial pH during the SMA batch test, and shows that although final pHs increased slightly from initial pHs, sodium bicarbonate buffer doses was sufficient to maintain the pH throughout the test within the optimum range of 6.6-7.2 for methanogens.

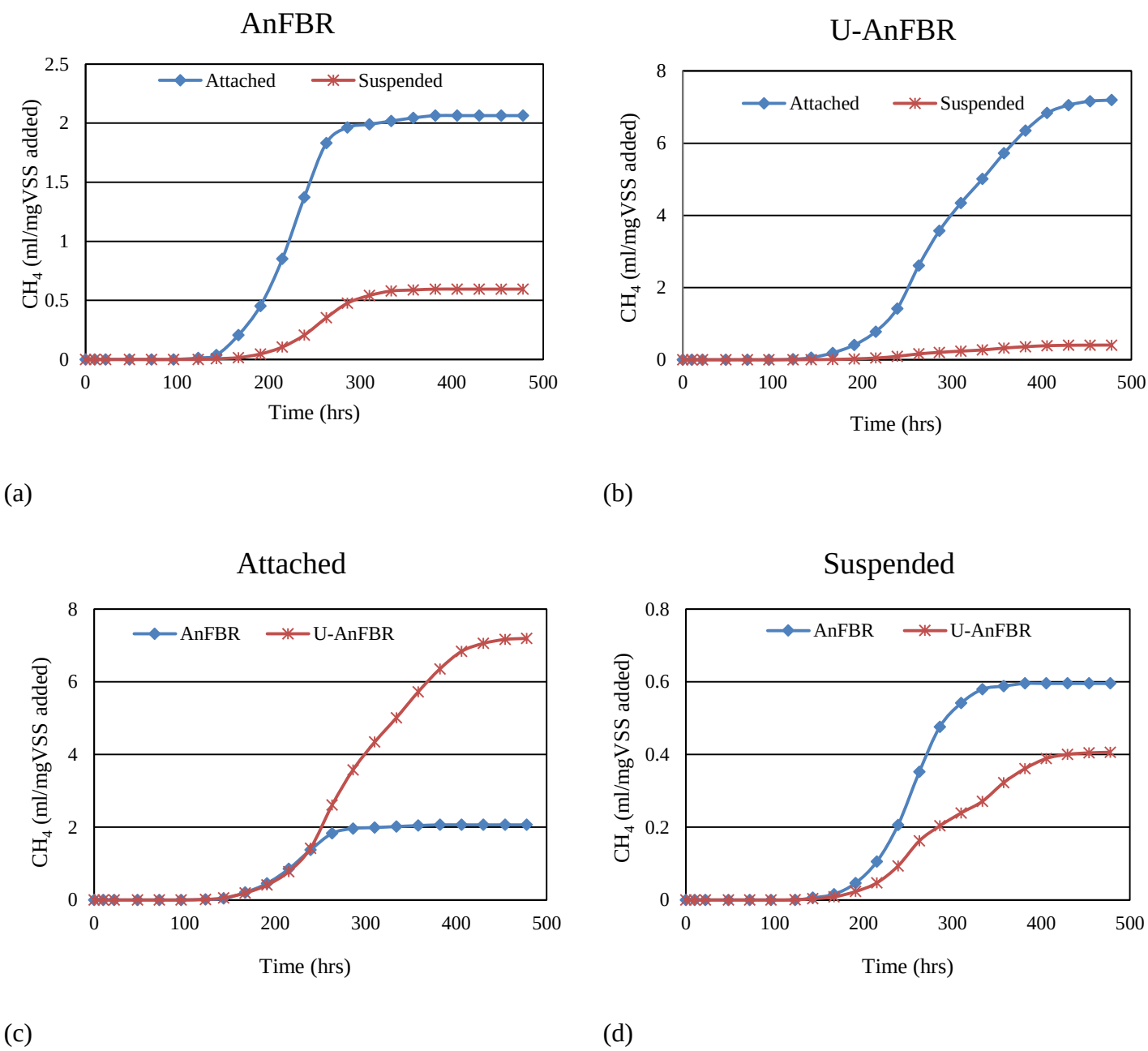


Figure 3.10 SMA test during the steady state period for (a) AnFBR (b) U-AnFBR (c) Attached growth (d) Suspended growth

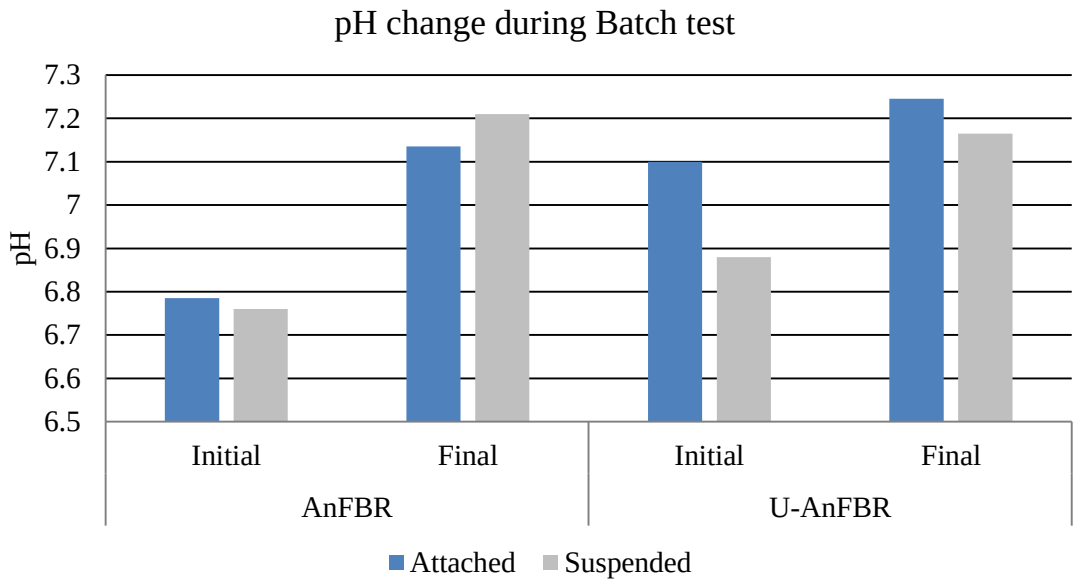


Figure 3.11 Initial and final pH during the SMA batch test

3.4 Conclusions

Mesophilic anaerobic digestion using the U-AnFBR was highly effective for scum control, reducing it from 310 gm/d to 62 gm/d (on wet basis) at an OLR of 5.1 kg COD/m^3-d and from 600 gm/d to 260 gm/d (on wet basis) at an OLR of 5.1 kg COD/m^3-d. The U-AnFBR showed 20% higher TCOD and VSS removal efficiencies (65% COD and 63% VSS) at an OLR of 5.1 kg COD/m^3-d compared to control reactor. Among the four different tested US energies i.e. 120 kJ, 240 kJ, 480 kJ, 600 kJ, the optimum result was observed for a pulse generated at 480 kJ with the sonication cycle of 2 seconds duration for every 30 seconds. However, when the OLR increased to 9.7 kg COD/m^3-d, the removal efficiencies of both TCOD and VSS decreased to 51%. This deterioration necessitates a further investigation to optimize the operational strategy of the pulse in applied US-energy.

In the SMA test, the activity-based sludge retention time was higher for U-AnFBR (7.1 days) compared to AnFBR (5.1 days). Moreover, maximum specific biogas production rates (R_m) were significantly higher for attached biomass in the U-AnFBR (26.7 ml/gmVSS-hr) than in the AnFBR (15.7 ml/gmVSS-hr) The opposite trend was observed for the suspended biomass i.e. 4.1 ml/gmVSS-hr for AnFBR and 2 ml/gmVSS-hr for U-AnFBR indicating higher attached biomass activity and better attachment.

3.5 References

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Chapter 4

Estimation of the Unbiodegradable Fraction of the Thickened Waste Activated Sludge (TWAS)

4.1 Introduction and Literature Review

Management and disposal of the municipal wastewater treatment biosolids is a global challenge and accounts for up to 50% of the operating costs of wastewater treatment plants (Wang et al., 2013; Appels et al., 2008). Compared to conventional technologies, anaerobic digestion (AD) has gained a lot of attraction because of its several advantages such as sludge reduction, biogas production and pathogen destruction (Yu et al., 2016; Appels et al., 2008). Thanks to AD, thickened waste activated sludge (TWAS) can be used as a renewable energy resource because of the biodegradability of organic matter makes up 50%-70% of solids (Appels et al., 2008) enabling total chemical oxygen demand (TCOD) and volatile suspended solids (VSS) removal efficiencies of up to 56% and 50%, respectively (Wang et al., 2016). Research on the optimization of operating conditions in anaerobic digestion has shown that several different parameters like pH, temperature, mixing strategy and intensity, and retention time distinctly impact the rates of biological conversion (Rubia et al., 2006; Bolzonella et al., 2003; Demirel & Yenigün, 2006; Kaparaju et al., 2008).

Therefore several strategies to increase the degradation rate including sonication pre-treatment (Muller et al., 2005; Elliott & Mahmood, 2007), thermophilic digestion (Appels et al., 2008), thermal pre-treatment (Elliott & Mahmood, 2007), enzymatic hydrolysis (Mayhew et al., 2003), and increasing sludge retention time (Appels et al., 2008) have

been investigated with varying degrees of success (Mayhew et al., 2003). Elliott & Mahmood (2007) showed that digester receiving sonicated waste activated sludge (WAS) removed 11%-39% more SCOD than the digester received unsonicated WAS. The aforementioned study also observed that sonication pre-treatment enhanced the destruction rate of volatile solids (VS) by up to 54%. Sonication pre-treatment also improved the gas production by 17% with a 6.2% increase total solids destruction (Muller et al., 2005). Compared to mesophilic digestion, thermophilic digestion has some positive outcomes since biochemical reaction rates are faster, increasing solids and pathogenic reduction, and improved dewatering (Appels et al., 2008). Among with the various pre-treatment technologies, thermal pre-treatment was noted as an effective method since it disrupts the chemical bonds of the cell wall and membrane, thus solubilizing the cell components. Moreover, thermal pre-treatment of WAS showed that soluble COD increased by 25%, 44%, and 60% at 130^0C, 150^0C, and 170^0C, respectively (Elliott et al., 2007). Other pre-treatment technologies like enzymatic hydrolysis can improve biogas by 10% during WAS degradation (Mayhew et al., 2003).

Although significant research has been conducted on optimizing the AD parameters, the control of the mechanism of AD is not still well understood since the process performance is limited with a mean conversion of organic matter from 30% to 50% (Shang et al., 2005). Furthermore, biodegradability of TWAS is more complex because of biological origin and lower availability to anaerobic biomass (Barbusinski & Koscielniak, 1997; Nielsen et al., 2004; Wilén et al., 2008). Ikumi et al. (2014) have noted that unbiodegradable particulate organics originating from the influent wastewater and generated by the activated sludge endogenous process, as determined from response of the activated sludge system, are also

unbiodegradable under anaerobic digestion conditions (Ikumi et al., 2014). On the other hand, due to increasing interest in reducing the mass of biosolids remaining after anaerobic digestion as well as identifying the unbiodegradable fractions of TWAS, the need for a reliable and simple method to assess the anaerobic biodegradability of the organic wastes still persists.

Several studies aimed at identifying the unbiodegradable fractions of municipal biosoilds under anaerobic conditions (Ekama et al., 2007; Ikumi et al., 2014; Mottet et al., 2010; Sötemann et al., 2006; Elsayed et al., 2015). A model was developed by Ekama et al. (2007) to investigate the unbiodegradable particulate fraction ($f_{as'up}$) of activated sludge under anaerobic conditions and noted that $f_{as'up}$ of 0.3 for 2 days retention time in aerated lagoon. Data from a 500 L/d pilot scale study used to determine $f_{as'up}$ in the aforementioned study following the activated sludge (AS) model. It was also mentioned in the aforementioned study that unbiodegradable components in activated sludge remain unbiodegradable under AD conditions. Ikumi et al. (2014) have investigated the biodegradability of wastewater and activated sludge organics in continuous-flow completely mixed anaerobic digesters at HRTs of 10, 18, 25, 40, and 60 days and found that unbiodegradable particulate organics fractions of primary sludge and waste activated sludge calculated from AS models remained essentially unbiodegradable in anaerobic digestion. The unbiodegradable fractions of raw wastewater, primary sludge (PS), and WAS COD in the aforementioned study were estimated as 0.15, 0.31, and 0.51, respectively. On the other hand, Sotemann et al. (2006) have developed a steady state model for anaerobic digestion of sewage sludge and found that primary sludge hydrolysed faster and had a lower unbiodegradable particulate COD fraction ($f_{PS'up}$ = 0.33) than the primary and humus sludge mixture (0.36).

However, the unbiodegradable PCOD fraction of settled wastewater (WW), raw WW, and fraction of COD removed in primary settling tank were required to assess the unbiodegradable PCOD fraction in the aforementioned study. Mottet et al., (2010) have investigated the anaerobic biodegradability indicators for waste activated sludge by biochemical methane potential (BMP) tests and found that the biodegradabilities were 35%, 54% and 66% for volatile solids of 24 gm/L, 29 gm/L, and 43 gm/L, respectively. The BMP tests were carried out with input sludge samples as substrates, under thermophilic conditions for more than 24 days in the aforementioned study. Elbeshbishy et al., (2015) have also carried out BMP test at four substrate to biomass (S/X) ratios for assessing the unbiodegradable fraction of TWAS which ranged from around 12% to 27%. The wide variations of the unbiodegradable fraction of TWAS in AD have significant impacts on methane production rates as well as overall removal performance.

However, the unbiodegradable particulate COD fraction can vary with different anaerobic SRT, decay rates and biomass yields. Jones et al. (2009) have reported that unbiodegradable PCOD were 19%, 23%, and 29% of total PCOD for SRT of 2, 4, and 15 days while investigating AD of WAS using three sequencing batch reactors (SBRs). The aforementioned study also noted that particulates were not retained long enough for hydrolysis to occur at shorter SRT (Jones et al., 2009). Table 4.1 shows the unbiodegradable PCOD fraction for Primary Sludge (PS) and WAS using different methodology. Along with SRT, decay rate and biomass yield also significantly impact biodegrability whereas the Sotemann et al. (2006) steady state model depends on these three parameters.

Table 4.1 Unbiodegradable PCOD fraction for PS and WAS

PS	TWAS	Anaerobic SRT (d)	Decay rate (d^{-1})	Biomass yield	Author
0.31	0.59	60	0.041	0.113	Ikumi et al. (2014)
0.36		7-20	0.041	0.113	Sotemann et al. (2006)[a]
0.33		7-60	0.041	0.113	Sotemann et al. (2006)[b]
0.34		60			Ristow et al. (2005) [c]
	0.34				Jones et al. (2009) [d]
	0.27				Elbeshbishy et al.(2015)[e]

[a] Determined from Izzet et al. (1992) data
[b] Determine from O'Rourke (1967) data
[c] Methanogenic (5-60days), acidogenic (3.33-10 days) and sulfate reducing system was followed
[d] Simulation of the 15-day intensive monitoring period
[e] 90 days batch test

Based on aforementioned literature survey, it is evident that biodegradability of TWAS varied due to SRT, decay rates, and biomass yield. The proposed method can be potentially used to determine the unbiodegradable fraction of TWAS with known SRT, decay rates and biomass yield. Hereafter, the goal of this study was to assess the unibiodegradable particulate fraction ($f_{as,up}$) from which performance limits of AD with respect to volatile solids reduction and methane production rates can be deduced.

4.2 Materials and Methods

4.2.1 Experimental Setup

The anaerobic digester system for the direct treatment of TWAS is shown in Figure 4.1. A 10-liters working volume anaerobic reactor (CSTR: 31cm height and 24 cm inner diameter) was built with plexiglas. TWAS from the Adelaide wastewater treatment plant, London, Ontario) was used as influent. Adelaide WWTP is a single-stage nitrifying wastewater treatment plant with a SRT of 3–4 days. A Masterflex L/S Pump (Cole-parmer, Model

77200-62), with flow rates ranging from 0.0006-3400 mL/min, and pressures up to 125 psi) was used for feeding the influent and discharging the effluent. A wet tip gas meter (Rebel wet-tip gas meter company, Nashville, TN, USA) connected to the top of the reactor was used to measure the biogas flow rate. A mesophilic temperature of 37^0C was uniformly maintained throughout the reactor by a water bath (VWR, Heated Circulating Baths, 89202-950). pH was checked and controlled manually every day. SRT for the experimental works range of 16.7 days to 33.3 days.

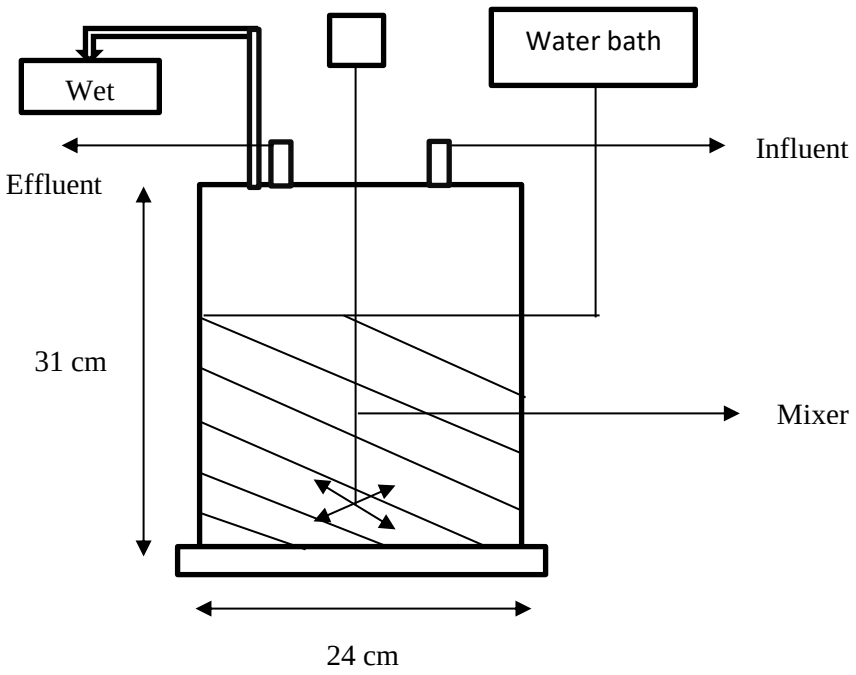

Figure 4.1 Schematic diagram of experimental continuous stirred tank reactor

4.2.2 Commissioning and Operation

Secondary anaerobic digester sludge (ADS), with total suspended solids (TSS) and volatile suspended solids (VSS) of ADS were 32,000 mg/L and 22,000 mg/L) from the secondary digester was collected from St. Mary wastewater treatment plant (Ontario, Canada) and

used as the seed for the CSTR. At the beginning, the reactor was filled with 10 L of ADS and the headspace was spurged with N_2. The reactor were then started by feeding TWAS from next day at a flow rate of 300 ml/day.

4.2.3 Analytical methods

Influent and effluent samples were collected three times a week and analyzed mainly for total suspended solids (TSS), volatile suspended solids (VSS), total chemical oxygen demand (TCOD), and soluble chemical oxygen demand (SCOD). Selected samples were analyzed for total nitrogen (TN), ammonia ($N\text{-}NH_3$), and alkalinity. In addition, gas production and gas composition, pH, temperature were monitored and recorded on a daily basis.

TSS, and VSS were analyzed according to the standard methods (APHA, 1992). Hach methods were followed to analyze TCOD, and SCOD (HACH Odyssey DR/2800) based on the potassium dichromate oxidation and spectrophotometric determination. A 0.45- µm filter paper was used for filtering to analyze soluble parameters. Alkalinity was measured by titration with 0.02 N H2SO4 in accordance with the Standard Method No. 2320 (APHA, 1992). VFAs were measured by employing gas chromatographs (Model CP-3800, software version 3.2.6.C, CP-1177 injector, VARIAN). The gas pressures were set 80 psi for helium, and nitrogen, 60 psi for air, and 40 psi for hydrogen, respectively. Gas flowrates of gas were set at 1.5 mL/min. 3.0 mL/min, and 6.0 mL/min for nitrogen, helium, and hydrogen, respectively. Temperatures for the oven and flame ionization detector (FID) were set at 250ºC and 300 ºC. A wet tip gas meter (Rebel wet-tip gas meter company, Nashville, TN, USA) which was connected to the top of the reactors was used to measure

the rate of biogas. Methane, nitrogen, hydrogen were determined by injecting 0.6 mL of the biogas from the headspace into a gas chromatograph (Model 310, SRI Instruments, Torrance, CA) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Molesieve 5A, mesh 80/100, 182.88 × 0.3175 cm). The temperatures of the column and the TCD detector were 90 and 105oC, respectively. Argon was used as carrier gas at a flow rate of 30 mL/min (Andalib et al. 2012).

4.3 Results and Discussion

4.3.1 Performance Analysis of CSTR

Operational conditions and steady-state data of the CSTR are given in Table 4.2. A total of 4 different phases were conducted during the entire experimental period. TCOD and VSS concentration of TWAS varied in the range of 37000 mg/L - 41000 mg/L and 22000 mg/L - 23200 mg/L, respectively. In order to vary the influent concentration, feed flows in phases I and II were used as is, TWAS was diluted 1:1 with distilled water phase-III, and TWAS was thickened carefully in phase-IV. Therefore, the influent feeding characteristics for CSTR were maintained closely at 40930 mg TCOD/L and 23200 mg VSS/L for phase I, 37680 mg TCOD/L and 22328 mg VSS/L for phase II, 21240 mg TCOD/L and 10780 mg VSS/L for phase III, and 57770 mg TCOD/L and 25150 mg VSS/L for phase IV. The feed rate started at 0.3 L/d corresponding to organic loading rate (OLR) of 1.21 kg COD/m^3-d and ended at 0.6 L/d corresponding to OLR of 3.47 kg COD/m^3-d. At an OLR of 1.2 kg COD/m^3-d (Phases I and III) TCOD removal efficiencies were 37%, and 38%, respectively. On the other hand, TCOD removal efficiencies were around 40% for phase-II and 44% for phase-IV. However, VSS destruction efficiency varied from 39% to 46% for the entire experiment. Figure 4.1 shows the temporal variations of VSS destruction and TCOD

removal efficiencies during the four different phases. Time 0 corresponds to the initial feeding of TWAS. The TCOD removal efficiency and VSS destruction efficiencies were almost identical for both reactors due to the relatively low influent SCOD (accounting for less than 10% of TCOD). Due to changing OLR, fluctuations were observed in reactor. Biogas production was measured in daily and COD balance closure was calculated based on the biogas production and COD consumption, in accordance with equation 4.1. Thus, based on equation 4.1 and 4.2, the ratio of the methane yield (L/g $COD_{removed}$) to the theoretical yield of 0.35 L/g $COD_{removed}$ is a measure of the COD mass balance closure.

$$Influent\ COD = Effluent\ COD + biogas\ COD \qquad (4.1)$$

Methane yield and VSS destruction efficiency were calculated as follows:

$$\text{Methane yield } \left(\frac{\text{mL } CH_4}{\text{mg COD}}\right)\text{(STP)} = \frac{V_{CH_4}\left(\frac{mL}{d}\right)\times\frac{273}{273+37}}{S_0\left(\frac{mg}{L}\right)\times Q_{feeding}\left(\frac{L}{d}\right) - S_e\left(\frac{mg}{L}\right)\times Q_{effluent}\left(\frac{L}{d}\right)} \qquad (4.2)$$

$$VSS\ destruction\ (V_d) = 1 - \frac{VSS_{effluent}\left(\frac{mg}{L}\right)\times Q_{effluent}\left(\frac{L}{d}\right))}{VSS_{feeding}(mg/L)\times Q_{feeding}(L/d)} \qquad (4.3)$$

Where S_0 denotes the influent TCOD concentration, and S_e denotes the effluent TCOD/VSS concentration. It is apparent from Table 4.2 that on average the COD mass balance closed within 10% in phases I-III, and 15% in phase IV.

Table 4.2 Operation conditions and steady-state performance data of CSTR fed TWAS at STP

Operating Conditions				
Parameter	Phase I	Phase II	Phase III	Phase IV
Time of operation (d)	1-53	53-80	80-143	143 -205
Steady-state day	37-53	64-80	111-143	178-205
Feed flow rate (L/d)	0.3	0.6	0.6	0.6
OLR based on anaerobic reactor (kg COD/m^3 d)	1.21±0.02 (8)	2.25±0.12 (8)	1.27±0.06 (13)	3.47±0.06 (10)
Anaerobic SRT(d)	33.33	16.67	16.67	16.67
pH	7.2±0.3 (8)	7.2±0.4 (8)	7.2±0.4 (13)	7.2±0.4 (10)
Feed characteristics				
TCOD (mg/L)	40930±970 (8)	37680±1650 (8)	21240±1130 (13)	57770±1060 (10)
sCOD (mg/L)	3750±230 (4)	3680±90 (4)	2420±160 (4)	5370±70 (3)
TSS (mg/L)	34280±2530 (8)	30030±930 (8)	14430±740 (13)	31980±1310 (10)
VSS (mg/L)	23200±640 (8)	22330±190 (8)	10780±670 (13)	25150±790 (10)
Effluent characteristics				
TCOD (mg/L)	25990±990 (8)	22470±850 (8)	13370±1050 (13)	32170±440 (10)
sCOD (mg/L)	1430±90 (4)	1160±150 (4)	910±70 (4)	4220±1560 (5)
TSS (mg/L)	20000±1350 (8)	18370±1370 (8)	9090±700 (13)	22860±1560 (10)
VSS (mg/L)	14240±440 (8)	11960±510 (8)	6690±740 (13)	14680±780 (10)
Removal Efficiencies				
COD removal eff. (%)	37±1.22 (8)	40±3.05 (8)	38±4.1 (13)	44±0.87 (10)
VSS removal eff. (%)	39±1.32 (8)	46±1.85 (8)	40±2.4 (13)	42±3.04 (10)
Methane yields				
Methane yield (LCH_4/gCOD removed) (STP)	0.41±0.01 (8)	0.37±0.06 (8)	0.33±0.03 (13)	0.30±0.02 (10)

Note: Number within parenthesis denotes the samples number

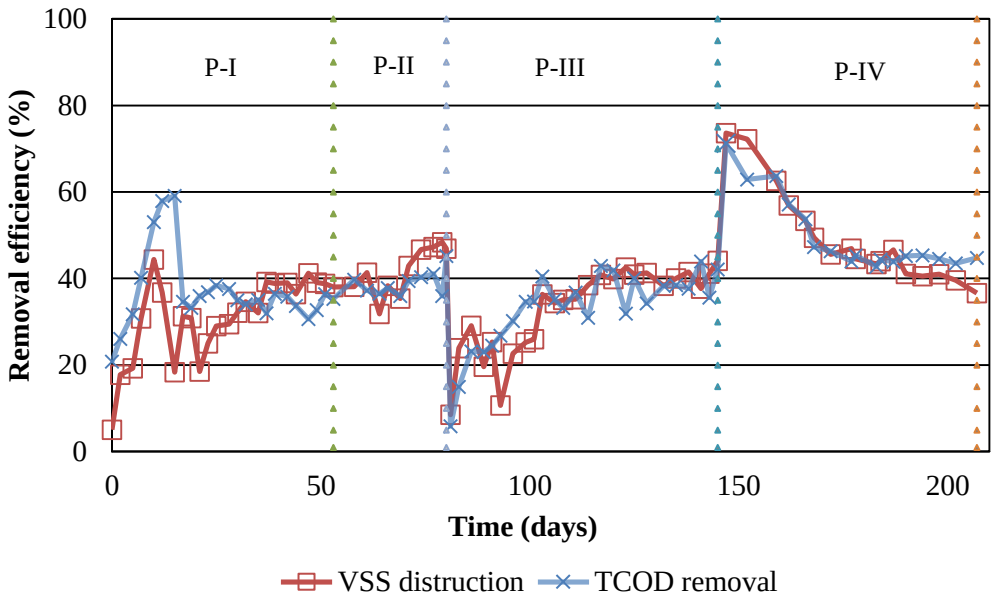

Figure 4.2 Temporal variation of TCOD removal and VSS destruction efficiencies in 4 different phases of CSTR for treating TWAS.

The temporal variation of the methane yields is depicted in Figure 4.3. It is interesting to note that whenever the OLR increases, the methane yield dropped initially but increased later to stabilize at the theoretical value, with the reverse happening when the OLR decreased i.e. from phase II to phase III. This is primarily attributed to the high dilution factor with the long HRTs. In phase I, a methane yield of 0.41 mL/mg $COD_{removed}$ which indicates COD balance close to 100%. On the other hand, nearly 15% of COD balance was off in phase IV in which the observed methane yield was 0.3 mL/mg $COD_{removed}$. However, considering methane yields in all 4 phases, overall methane yields of 0.353 mL/mg COD digested which is close to theoretical. Thus the uncertainty in measured COD removal and VSS destruction data in current works is indeed acceptable. Figure 4.4 shows the temporal

variation of influent and effluent COD. During the steady-state period, the total influent and effluent COD were 12.1gm and 13gm, 22.7gm and 21.9gm, 12.7gm and 12 gm, and 34.7gm and 32.1gm corresponding to COD mass balance closures of107%, 97%, 94%, and 93% for phases I, II, III, and IV, respectively. T-tests conducted on the effluent TCOD, and VSS data indicate that differences between the SRT of 33.3 days and SRT of 16.7 days were significant for both TCOD and VSS concentration at the 95 percentile confidence level.

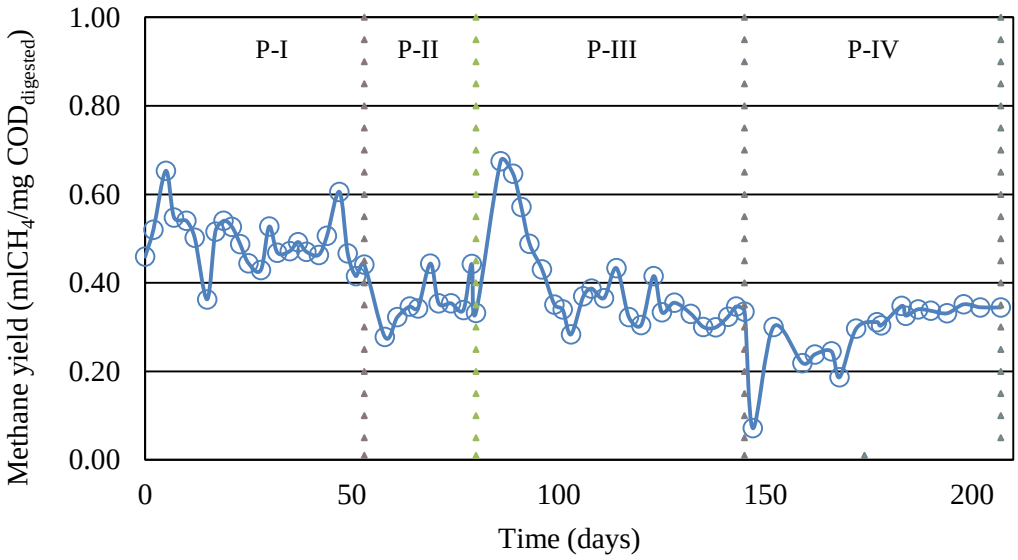

Figure 4.3 Temporal variation of methane yields in CSTR

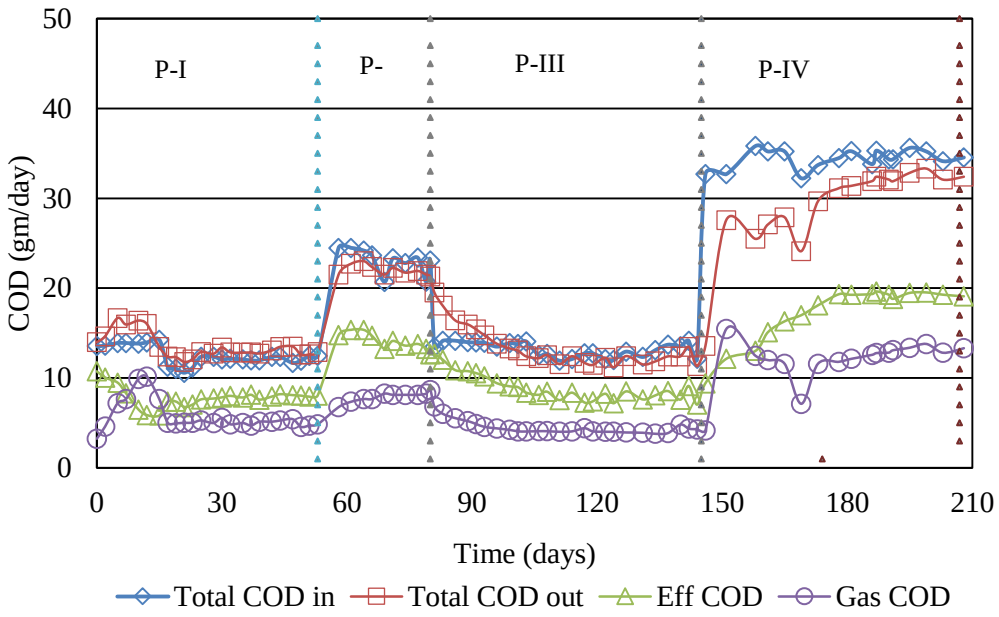

Figure 4.4 Temporal variation of COD as gm/day in CSTR

Digester operational stability is reflected by the VFA/alkalinity ratio, with values less than 0.4 indicating stable performance (Wang et al., 2016). VFA was expressed as concentration of acetic acid, calculated through converting the summation of individual VFA COD. Table 4.3 lists the average and standard deviation of the VFA/alkalinity ratio. During steady-state operation, the ratio of 0.37 for CSTR (phase-II). The percentage contributions of acetic and propionic acids to the overall VFA on a COD basis in the final effluent are presented in Table 4.3. Acetic acid and propionate acid in CSTR were predominant at 14%-67% and 21%-66% of total VFA, respectively.

Table 4.3 VFA composition in final effluent during steady-state operational conditions in phase-IV

Parameters	Concentration (mg COD/L) (n=3)	Percentage of VFA as COD (n=3)
Acetic acid (%)	812 ± 582	67 ± 39
Propionic acid (%)	126 ± 26	8±13
Butyric acid (%)	61 ± 7	5±5
Valeric acid (%)	184 ± 144	21±22
VFA to alkalinity ratio	0.37±0.06	

Note: 'n' is number of samples

Nitrogen mass balances were analyzed to examine the nitrogen generation through VSS destruction as presented in Figure 4.5. Ammonia-nitrogen mass balance closures considering the influent ammonia and ammonia generated from VSS destruction relative to effluent ammonia were 87% (overall) for all phases (Table 4.4).

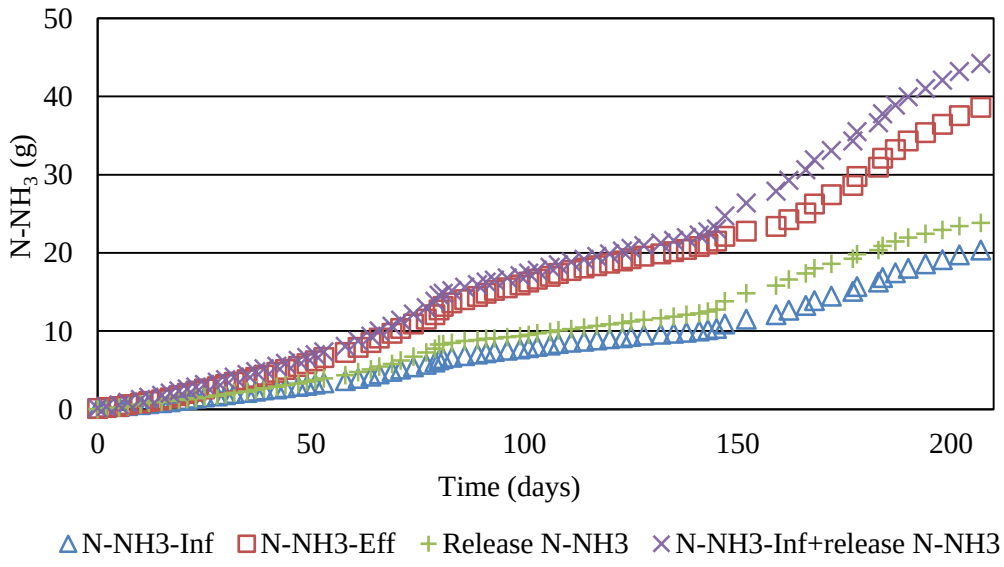

Figure 4.5 Nitrogen balance for treating TWAS in CSTR

Table 4.4 Nitrogen balance in different phases at steady-state period for CSTR

Phases	Phase I	Phase II	Phase III	Phase IV
Steady-state day	37-53	64-80	111-143	178-205
Inf-VSS (g/d)	6.74 ± .2(8)	13.4 ± 0.13(8)	7.2 ± 0.34(13)	15.2 ± 0.6(10)
Eff-VSS (g/d)	4.17 ± 0.16(8)	7.38 ± 0.57(8)	3.78 ± 0.23(13)	8.83 ± 0.44(10)
VSS destruction (%)	39	46	40	42
PN/VSS	0.08	0.08	0.08	0.08
Inf-N-NH_3 (gm/d)	0.15 ± 0.01(3)	0.3 ± 0.01(3)	0.15 ± 0.01(6)	0.59 ± 0.02(4)
Eff-N-NH_3 (gm/d)	0.35 ± 0.04(3)	0.6 ± 0.01(3)	0.32 ± 0.03(6)	1.11 ± 0.04(4)
Released NH_3 (gm)	0.21 ± 0.02(3)	0.44 ± 0.09(3)	0.2 ± 0.02(6)	0.51 ± 0.05(4)
N-balance closures (%)	96	83	89	101

Note: Number within parenthesis denote the samples number

4.3.2 Determination of unbiodegradable particulate COD fraction

Since the unbiodegradable particulate COD fraction ($f_{as'up}$) plays a vital role in anaerobic digestion, Sotemann et al. (2006) have developed a steady-state model for estimating unbiodegradable COD fraction of primary sludge ($f_{ps,up}$) in plant wide in which $f_{ps,up}$ is entirely dependent on the unbiodegradble particulate COD fractions selected for the raw and settled wastewaters and the fraction of COD removed by primary sedimentation. Later, Ikumi et al. (2014) have determined the unbiodegradable COD fractions in two ways: (i) the effluent COD as a fraction of the influent COD for the SRT, which assumes all the biodegradable organics are utilized at SRT, and (ii) applying steady-state anaerobic digestion (AD) model (Sötemann et al. 2006) to measure the influent and effluent COD concentration. The influent unbiodegradable particulate (UPO) COD concentration in the aforementioned study was set to $f_{xU,CODInf} \times COD\ (T,Inf)$, where $f_{xU,CODInf}$ is considered as unbiodegradable COD fraction of the influent sludge. However, the model is simplified using the following equation which is developed by Sotemann et al. (2006).

$$S_{bp} = \frac{S_{ti}\left[f_{AS,up} + E\left(1 - f_{ASup}\right)\right] - S_{te}}{[E-1]} \ldots\ldots (4.4)$$

Here, S_{bp} denotes the biodegradable particulate COD concentration (PCOD), S_{ti} is influent total particulate COD concentration, S_{te} is effluent total PCOD, $f_{AS,up}$ is unbiodegradable PCOD fraction, E is the proportion of the biodegradable COD transformed to biomass, calculated as follows:.

$$E = \frac{Y_{AD}}{1 + b_{AD}R(1 - Y_{AD})} \ldots\ldots (4.5)$$

Where, Y_{AD} is the biomass yield (g COD biomass/g COD organics), R is sludge retention time (d), b_{AD} is decay coefficient (d^{-1}).

Simplifying the equation 4.4

$$S_{bp}(E-1) = S_{ti}f_{AS,up} + S_{ti}E\left(1 - f_{AS,up}\right) - S_{te}$$

$$\frac{St_iE - St_e}{E-1} = f_{AS,up}St_i - Sb_p \ldots\ldots (4.6)$$

Using the constants Y_{AD} as 0.29 gCOD biomass/g COD organics (Elbeshbishy et al., 2015) and, b_{AD} as 0.015 /d (Banik, 1998), and the experimental SRT of 16.67 days in equation 4.3, two linear fitted curves were drawn using equation 4.6 in which 'x' axis corresponds to influent total PCOD (S_{ti}) and 'y' axis corresponds to $\frac{St_iE-St_e}{E-1}$ (Figure 4.6). However, the linear relationships ($R^2 = 0.89$) depicted for COD and VSS, respectively. Figures 4.6(a) and 4.6(b) indicate that the unbiodegradable fractions of PCOD ($f_{AS,up}$) and VSS are 0.28 and 0.26, respectively. Thus, the unbiodegradable particulate COD (S_{bp}) was observed to be in the range of 4500 mg/L to 9000 mg/L (using the equation 4.4) since the influent COD varied in the range of 21000 mg/L to 58000 mg/L.

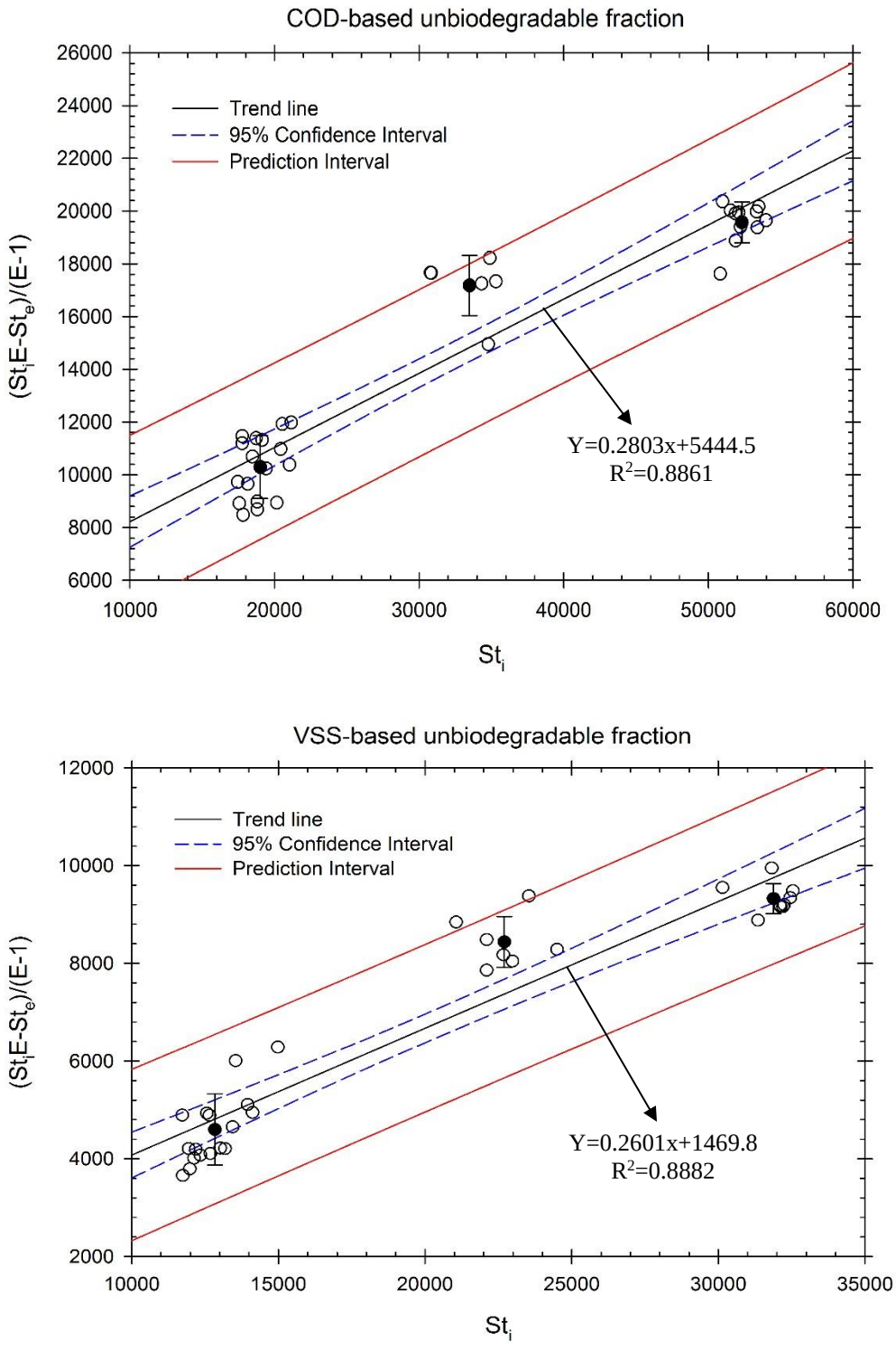

Figure 4.6 Fitted curves for estimating unbiodegradable PCOD fraction

Based on the individual observations, prediction intervals in Figure 4.6 are drawn to estimate the mean variable and it shows that almost all of the future observation will fall under the prediction interval. However, it is also seen from Figure 4.6 that measured data set are not normally distributed and shows minor skewness.

4.4 Conclusion

This study determined the influent unbiodegradable fraction of particulate COD ($f_{AS,up}$) in TWAS. The estimated $f_{AS,up}$ was 0.28, which agreed very well with the unbiodegradable fraction of VSS of 0.26. However, given the testing period of over 200 days, and the three different organic loadings, it is believed that the aforementioned unbiodegradable TWAS fractions are indeed accurate. slight

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Chapter 5

Synergism of Co-digestion of Food Wastes with Municipal Biosolids under Anaerobic Conditions

5.1 Introduction and Literature Review

Anaerobic digestion is the most widely used technology to produce biogas such as methane and hydrogen from the decomposition of organic compounds. The effectiveness of the process depends on the stability of the consecutive reactions i.e. hydrolysis, acidification, acetogenesis, and methanogenesis. The process is widely used in municipal wastewater sludge treatment for stabilization and production of methane gas. Due to the increasing demand on renewable energy, anaerobic digestion has also been used for treating biodegradable wastes; for instance, the organic fraction of municipal solids wastes, wastewater treatment biosolids, and various food and beverage wastes (Iacovidou et al., 2012). Particularly, anaerobic digestion of food wastes (FW) is also considered as one of the effective methods of waste management (Iacovidou et al., 2012). Annual food waste generation in USA is 34.2 million tons. Assuming that potential biogas generation is 367 m^3 per FW (dry tonne) with an energy content of 6.25 kwh/m^3, the annual generated food waste in USA of 34.2 million tons can generate 3.76 ×109 m^3 of biogas with energy value of 23.5×106 MWh, corresponding to 0.12% of the total global electrical energy consumption of 20181 TWh (Curry and Pillay, 2012). However, despite the potential benefit, digestion stability can be hampered when FW is used as single substrate because of potential nutrients imbalance such as insufficient trace metals (Zn, Fe, Mo, etc) and excessive macronutrient (Na, K), high C/N ratio, and lipid content (5 g/L) as well as due

to the high variability of its composition depending on its source (Iacovidou et al., 2012; Zhang et al., 2014). Thus, the use of FW as co-substrate for municipal sludge digestion has emerged to enhance sludge digestibility, and increase energy generation to facilitate the achievability of energy-neutral wastewater treatment.

The positive effect of FW on sludge digestion performance could be related to the increased carbon to nitrogen (C/N) ratio and enhanced kinetics due to the addition of food wastes. In fact, the optimal C/N ratio for anaerobic digestion is 15:1-30:1, much greater than the 6:1 to 9:1 of wastewater sludges (Iacovidou et al., 2012; Koch et al., 2016). The average C/N ratio of nineteen food waste samples reported in different studies was 16:1 (Kim et al., 2015), and thus co-treatment of FW and wastewater sludges could be beneficial to energy production and solids destruction. Moreover, the addition of easily degradable FW helps to overcome the limitation of slow hydrolysis, which is the rate limiting step in sludge digestion. A recent study by Koch et al. (2016) as presented in Table 5.1 reported that methane production in a full scale WWTP digester was enhanced from 0.31 L/kgVS with raw sludge (C/N ratio of 8.8) to 0.39 L/kgVS with addition of FW with a C/N ratio of 17.7 at 10% (w/w), resulting in a C/N ratio of 9.7. Although the 9.7 was still lower than the aforementioned optimal C/N ratio (15:1-30:1), the substantially high hydrolysis rate of food wastes triggered enhancement of acidogenesis and methanogenesis (Koch et al., 2016). More pronounced C/N effect on the continuous co-digestion process can be found in a study by Dai et al. (2013) who reported linear increases in VS destruction (38%-68%) with elevated C/N ratios of feed (7.8- 14.8) although the authors did not elaborate on the relationship.

The accelerated biogas generation from co-digestion compared to mono-digestion was related to the synergistic effect or "priming effect", which can be attributed to promoted microbial enzyme production and metabolic degradation by available readily degradable nutrients, recalcitrant substances are also decomposed (Aichinger et al., 2015; Insam & Markt, 2016; Koch et al., 2016). Despite the increasing interest in the synergistic effects of co-digestion, very few systematic studies are available for providing insight on quantification of synergistic effect and potential factors since the previous studies (Table 5.1) primarily focused on co-digestion performance in relation to of organic loading rate (OLR) (1-21.8 kgVS/m^3/d) (Dai et al. 2013b; Fitamo et al. 2016; Gou et al. 2014), temperature (mesophilic and thermophilic) (Cavinato et al. 2013; Gou et al. 2014; Kim et al. 2011) co-substrate blend ratio (6%-90%) (Dai et al., 2013; Schmit and Ellis, 2001), and SRT (7-62 days) (Dai et al., 2013b; Fitamo et al., 2016; Schmit & Ellis, 2001).

In order to explore synergistic effects, long periods of continuous-flow system operation with steady-state conditions are warranted and are advantageous over short period batch tests which typically run at high inoculum to substrate ratio and do not achieve steady-state conditions with changes of feed (Koch et al., 2016). Additionally, the characterization of the microbial activity of different anaerobic microbes such as acidogenes, acetogenes, and methanogenes which may explain effectiveness of co-digestion is lacking, despite the availability of information on microbial speciation (Kim & Oh, 2011).

Thus, in order to elucidate synergistic effect on co-digestion at steady-state conditions, this study explored different co-digestion performance of five lab-scale semi-CSTR digesters fed with cosubstrates with various FW blend ratio. Moreover, specific methanogenic

activity (SMA), specific acetogenenic activity (SAtA), and specific acidogenic activity (SAdA) tests were also conducted to evaluate different microbial behaviors between mono-digestion and co-digestion.

5.2 Materials and Methods

5.2.1 System Setup and Operation

Five reactors with a total volume of 2.4 L and a working volume of 1.8 L each were used as semi-continuous flow anaerobic digesters. Solids retention time (SRT) was controlled at 20 days through feeding and wasting 90 mL daily. The reactors were completely mixed using a mixer (Stir-Pak, SSM20, Cole-Parmer, Montreal, QC). Feeding and wasting for the system operation was done using a syringe at the same time each day with wastage performed prior to feeding. The reactors were operated for over 100 days i.e. 5 turnovers of the mean SRT, with steady-state data collected over the last three turnovers.

Reactor 1 (R1) was used as a control reactor, fed with a mixture of 50% primary sludge (PS) and 50% thickened waste activated sludge (TWAS) on volumetric basis. PS and TWAS were collected weekly from the Adelaide wastewater treatment plant (WWTP) from day 1 to 51 corresponding to 2.5 turnovers of the mean SRT. However, due to the variations of PS and TWAS between samples, both sludges were taken one time and used throughout the steady-state operation.

Reactor 2 (R2), Reactor 3 (R3), and Reactor 4 (R4) treated a mixture of FW, PS and TWAS at different proportions i.e. R2 with 10% FW, 45% PS and 45% TWAS; R3 with 20% FW, 40% PS and 40% TWAS; R4 with 40% of FW, 30% PS and 30% TWAS on volumetric basis. Reactor 5 (R5) was operated to compare digestion efficiency with R4 without adding

FW at the same ratio of total chemical oxygen demand (TCOD) to total nitrogen (TN) of R4. Feed for R5 was prepared using a mixture of the particulate fractions of PS and TWAS that was obtained after centrifuging each sludge for 15 min at 2500 RPM.

Three different rounds of food wastes collected from the Grind2Energy systems (InSinkErator, Milwaukee, WI) that process food waste from southeast WI supermarkets were regularly characterized prior to use. Three rounds of samples were used for day 1-23, day 24-90, and afterwards. FW samples delivered from InSinkErator were stored in a cold room (4 ºC). To ensure homogeneity, FW was grinded using a blender for 15 min. FW samples after homogenization were analyzed in triplicates. Anaerobically digested sludge from the Guelph WWTP was used as seed to start-up the reactors. Temperature was maintained at 35 ºC using water bath (VWR® Heated Circulating Baths, 89202-950). Biogas production was monitored daily using wet-tip gas meters (Standard Capacity Gas Meter, Wet Tip Gas Meter) with counter meters (Fargo Controls, CH Series totalizing counter) which were connected to digesters.

5.2.2 Analytical Method

Influent and effluent samples were taken for analysis two times a week. Suspended solids, COD and ammonia were measured twice a week while total nitrogen (TN), soluble nitrogen (SN), phosphorus, alkalinity, volatile fatty acids (VFAs) were analyzed once a week during the unsteady operation and twice per week during steady-state operation. All samples were preserved in a cold room at less than 4ºC. The collected samples were analyzed to determine chemical oxygen demand (total COD and soluble COD), total suspended solids (TSS), volatile suspended solids (VSS), total nitrogen (TN), soluble

nitrogen (SN), ammonia-N (NH_4-N), total phosphorus (TP), soluble P (SP), alkalinity, and VFAs concentrations. For all soluble analyses including COD, nitrogen, ammonia, phosphorus, and VFA, sterile 0.45 µm membrane filter papers (VWR International, Canada) were used for filtration of samples. Similarly, 1.2 µm filter papers were used for TSS and VSS analysis in accordance with Standard Methods (APHA, 2005). High range total phosphate (0-100 mg/L of PO43-) vials were used for total and soluble phosphate analysis. On the other hand, high range ammonia (0-50 mg/L), high range total nitrogen (10-150 mg/L), and high range COD (1500 mg/L) vials were used for respective analysis. All of the high range vials were purchased from HACH, Canada.

The concentrations of volatile fatty acids (VFAs) were analyzed after filtering the samples through 0.45 µm filter paper using a gas chromatograph (Varian 8500, Varian Inc., Toronto, Canada) with a flame ionization detector (FID) equipped with a fused silica column (30 m × 0.32 mm). Helium was used as the carrier gas at a flow rate of 2 mL/min. The injector temperature was 200 °C with a split ratio of 5:1. The oven temperature was programmed at 80 °C for 1 min, then a 20 °C / min rate until 130 °C, holding for 2 min, and then a 20 °C / min rate until 165°C holding for 2 min. The detector temperature was 280 °C. The pH of filtered samples was adjusted to less than 2 using phosphoric acid prior to VFA analysis.

Methane content was determined by a gas chromatograph (Model 310, SRI Instruments, Torrance, CA) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Molesieve 5A, mesh 80/100, 6 ft × 1/8 in). The temperatures of the column and the TCD detector were 90 and 105 °C, respectively. Argon was used as the carrier gas at a flow rate of 30 mL/min.

5.2.3 Microbial Activity Tests

In order to investigate the different microbial behaviors of major anaerobic digestion stages i.e. acidogenesis, acetogensis, and methanogenesis, three different batch tests of specific methanogenesis activity (SMA), specific acetogenic activity (SAtA), and specific acidogenesis (SAdA) were conducted on mono-digestion (R1) and co-digestion (R4). The effluents of the anaerobic continuously- stirred tank reactors were used separately as seed in these tests. Initial substrate to biomass (S/X) ratio for all batches was set at 2.0 gCOD/gVSS while seed VSS for reactor R1 and R4 in all batches were maintained close to 2.63 g and 2.93g, respectively. Details of the SMA test can be found in a previous study (Andalib et al., 2014).

The SAtA test used equal COD of ethanol, propionic acid, butyrate acid, and lactic acid as substrates. pH for all batch test was maintained using 5 g/L $NaHCO_3$. It is noted that pH is an important parameter for organic acids production and it was found that butyric acid accumulated at a pH ranging from 5 to 7, while propionate acid tended to accumulate at a pH of 8 (Horiuchi et al., 2002). The initial pH for acetogenic tests was adjusted to 8 to avoid the propionate and butyric acid accumulation. As the process of fermentation progressed, the pH in the bottle was expected to decrease slightly, and thus, would be suitable for propionate fermentation without butyrate accumulation (Wang et al., 2016).

In the SAdA test, glucose was added as the substrate while additional acetic acid was added at a concentration of 5 g/L in order to inhibit further degradation of propionate to acetate as this reaction is considered part of acetogenesis (Wang et al., 2016). The initial pH for acidogenic tests was controlled at 6 by adding 5 N HCl since a pH of 6 is optimal for the

accumulation of butyric acid in acidogenesis. The $NaHCO_3$ concentration was also maintained at 5 g/L in these tests. The seeds for the acidogenic and acetogenic activity tests were preheated at 90 ºC for 30 min to inhibit methanogens. After adding seed and substrate, the headspace was flushed at 5–10 psi for 5 min with nitrogen gas. the sample bottles were then placed in a swirling-action shaker (MaxQ 4000, Incubated and Refrigerated Shaker, Thermo Scientific, CA) and operated at 180 rpm and 37 ºC.

5.2.4 Statistical Analysis

Excel software was used to conduct T-tests and regression analysis. T-tests assessed the significance of statistical difference using the method of Two-Sample Assuming Unequal Variances and the significance was determined with probability (p) values i.e. $p < 0.05$ corresponding to a 95% confidence level. Similarly, regression analysis was done using the LINEST function in Excel.

5.3 Results

5.3.1 FW and Biosoilds Characteristics

The characteristics of the FW are summarized in Table 5.1. The characteristics for the three different rounds were 40-86 g TSS/L, 38-84 gVSS/L, 143-212 gTCOD/L, 4-5 g TN/L, 0.2-2 g TP/L, reflecting the high variability. The last two samples which were used during the steady-state conditions (day 41-101) were comparable in terms of COD, TSS, VSS, and TN concentrations,. The soluble fraction of FW COD in the last two rounds of samples varied from 47% to 51%, while the soluble nitrogen fraction averaged 47.5%. The same trends are confirmed by the TN and SN variations. VFA concentrations in the different food wastes batches ranged from 2 g/L to 3 g/L with acetic acid accounting for

greater than 62% of the total VFA on COD basis. The VFA content accounted for 3%-5% of the SCOD. It should be also noted that ethanol was observed at 6-12 g/L or 7%-15% of SCOD. The sum of VFA and ethanol in food wastes accounted for 10%-19% of SCOD.

The characteristics of the primary sludge used in this study are summarized in Table 5.1. From day 1 to day 51, different batches of primary sludge were used while from day 52 to day 101, one batch was used to minimize the effect of changes of sludge characteristics which varied widely. The concentrations of primary sludge from day 1 to day 51 were 25-30 g TSS/L, 21- 28 g VSS/L, 37-44 g TCOD, 0.8-1.1 g TN/L, and 0.4 g TP/L. Samples used on day 52-101 to achieve steady-state operation yielded ratios of VSS/TSS, PCOD/VSS, and TCOD/TN of 0.92, 1.52, and 42. The soluble fractions of PS COD were identical at 4% for both periods (day 1- day 51 and day 52-day 101) while the soluble nitrogen fraction was 18% for the first period and 10% for the second period.

Similar to PS, different batches of TWAS were used during the first 51 days while one TWAS batch was used for the rest of the study (Table 5.1). The TWAS used during both periods was characterized as 30-32 gTSS/L, 22 gVSS /L, 41-47 g TCOD/L, 2.9-3.0 g TN /L, and 0.7 gTP/L. The characteristics of the fed TWAS after day 51 yield ratios of 0.75 VSS/TSS, 1.95 PCOD/VSS, and 15.6 TCOD/TN. The soluble fraction of TWAS COD were close at 7%-10% for both periods i.e. day 1- day 51 and day 52-day 101 while soluble nitrogen fraction was 18% for the first period and 12% for the second period.

As apparent from Table 5.2, the contribution of FW, PS, and TWAS for suspended solids, COD, and nitrogen in the feed varied in different reactors. During the steady-state operation, FW contribution to total influent TCOD increased from 25% (R2) to 58% (R4).

A similar trend pertains to nitrogen, with FW contribution increasing from 19% (R2) to 50% (R4). It is interesting to note that due to the high soluble fraction of COD in the FW, the relative contribution of FW to influent VSS was much lower than TCOD.

Table 5.1 Characteristics of food waste, primary sludge, and thickened waste activated sludge

(A) Solids, COD, nitrogen, and phosphorous

	Parameter (g/L)	TSS	VSS	TCOD	SCOD	TN	SN	TP	SP
FW	**Day 1- 23**	86±9 (2)	84±9 (2)	212±9 (2)	80±7 (2)	4 (1)	2 (1)	2 (1)	0.4 (1)
	Day 24-90	40±4 (8)	38±4 (8)	152±10 (10)	72±6 (9)	5±1 (10)	3±1 (9)	0.2±0.1 (4)	0.11±0.01 (4)
	Day 90-101	43±3 (4)	40±3 (4)	143±11 (4)	73±6 (3)	4.5±0.5 (4)	1.6±0.5 (4)	0.7±0.2 (2)	0.4±0.1 (2)
PS	**Day 1- 51**	25±6 (4)	21±6 (4)	37±12 (4)	1.6±0.2 (4)	0.8±0.1 (4)	0.1±0.1 (4)	0.4±0.1 (4)	-
	Day 52-101	30±5 (3)	28±3 (3)	44±3 (4)	1.8 (1)	1.1 (1)	0.1 (1)	-	-
TWAS	**Day 1- 51**	32±8 (4)	22±6 (4)	41±8 (4)	4.0±0.4 (4)	2.9±0.2 (4)	0.5±0.1 (4)	0.7±0.2 (4)	-
	Day 52-101	30±1 (4)	22±1 (2)	47±2 (4)	3.5±2 (4)	3.0 (1)	0.4 (1)	-	-

(B) VFA and ethanol (as COD) of FW

Parameter	Acetic acid (g/L)	Propionic acid (g/L)	Ethanol (g/L)	Overall VFA (g/L)	Acetic acid in VFA (%)	VFA in SCOD (%)	Ethanol in SCOD (%)	VFA+ Ethanol in SCOD (%)
Day 1- 23	2±0 (2)	0±0 (2)	12±0 (2)	3±1 (2)	75±13 (2)	4±0 (2)	15±1 (2)	19±0 (2)
Day 24-90	2±1 (7)	1±1 (7)	6±2 (7)	3±1 (7)	62±21 (7)	5±2 (7)	8±3 (7)	12±2 (7)
Day 90-101	2±1 (2)		6±4 (2)	2±1 (2)	100±0 (2)	3±1 (2)	7±5 (2)	10±4 (2)

Note: Number within parenthesis denote the samples number

Table 5.2 Contribution (%) of PS, TWAS, and FW to influent total suspended solids, COD, and nitrogen in the different reactors

	TSS (%)				
	R1 (FW 0%)	**R2 (FW 10%)**	**R3 (FW 20%)**	**R4 (FW 40%)**	**R5 (FW 0%)**
FW	0	12	21	35	0
PS	50	44	40	32	64
TWAS	50	44	40	32	36
	VSS (%)				
	R1 (FW 0%)	**R2 (FW 10%)**	**R3 (FW 20%)**	**R4 (FW 40%)**	**R5 (FW 0%)**
FW	0	13	23	38	0
PS	56	49	43	35	70
TWAS	44	38	34	27	30
	TCOD (%)				
	R1 (FW 0%)	**R2 (FW 10%)**	**R3 (FW 20%)**	**R4 (FW 40%)**	**R5 (FW 0%)**
FW	0	25	40	58	0
PS	48	36	29	21	64
TWAS	52	39	31	22	36
	TN (%)				
	R1 (FW 0%)	**R2 (FW 10%)**	**R3 (FW 20%)**	**R4 (FW 40%)**	**R5 (FW 0%)**
FW	0	19	33	50	0
PS	26	21	17	13	40
TWAS	74	60	50	37	60

5.3.2 Feed and Digested Biosolids Characteristics

The steady-state operation, as reflected by the stability of the digested biosolids was deemed to occur from day 42 to the end of the study (day 101), corresponding to three turnovers of the mean SRT. The steady-state overall average influent (feed) and effluent (digested sludge) characteristics are presented in Table 5.6A while the biogas data is presented in Table 5.6B. Data from a steady-state operation of the five reactors for the last 60 days (3 turnovers of SRT) showed that influent and effluent TSS concentrations were 28-33 g/L and 12-16 g/L with respective VSS/TSS ratios of 0.82-0.9 and 0.70-0.77. Similarly, influent and effluent TCOD of five reactors ranged from 41 g/L to 77g/L and

from 20 g/L to 24 g/L with respective soluble fractions of 5%-31% and 4%-16%. Respective influent and effluent TN levels were 1.5-2.8 g/L and 1.6-2.8 g/L with respective soluble fractions of 11%-41% and 54%-63%.Similarly, respective influent and effluent NH3 concentrations were 0.1-0.4 g/L and 0.7-1.5 g/L. Influent and effluent TP levels were 0.4-0.8 g/L. The stability of operation during the steady-state period is reflected by the low relative standard deviations.

Using regression relationships based on the steady-state data, correlations between the measured parameters were examined (Table 5.3). The VSS to TSS ratio was 0.83 (R1), 0.84 (R2), 0.87 (R3), 0.91 (R4), and 0.85 (R5). PCOD content in VSS for different reactors were 1.63 (R1), 1.78 (R2), 1.87 (R3), 1.84 (R4), and 1.53 (R5). Similarly, nitrogen content of VSS ranged from 0.054 to 0.07 while phosphorus accounted for 2 to 3% of VSS by weight. VSS/TSS ratio and PCOD/VSS ratio increased with the addition of FW while nitrogen/VSS and phosphorus/VSS ratio decreased.

Table 5.3 Relationship between parameters (Steady-state conditions)

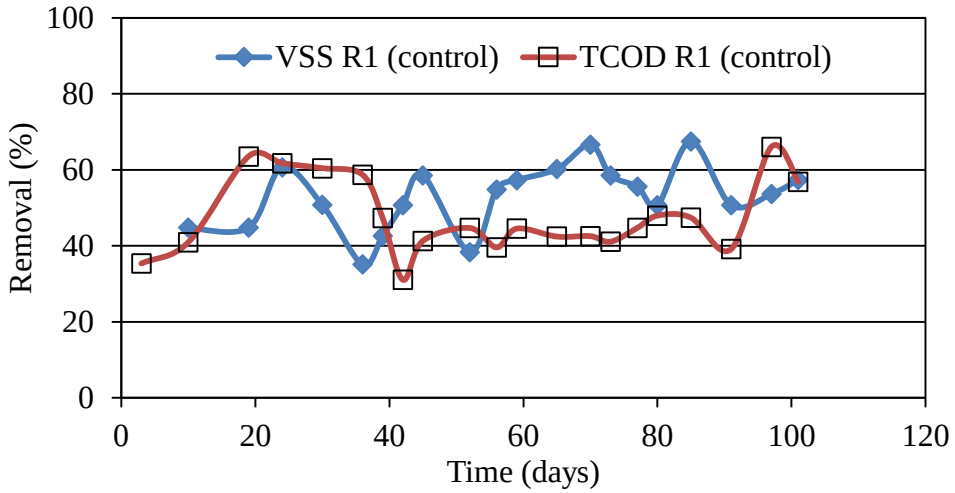
Parameters	**R1 (Control)**	**R2 (FW 10%)**	**R3 (FW 20%)**	**R4 (FW 40%)**	**R5 (FW 0%)**
VSS/TSS ratio	0.83 (R^2= 0.99,)	0.84 (R^2= 0.99)	0.87 (R^2= 0.99)	0.91 (R^2= 0.99)	0.85 (R^2= 0.99)
Particulate COD/VSS ratio	1.63 (R^2=0.99)	1.78 (R^2=0.99)	1.87 (R^2=0.99)	1.84 (R^2=0.99)	1.53 (R^2=0.99)
Particulate N/VSS ratio	0.068 (R^2=0.99)	0.07 (R^2=0.99)	0.063 (R^2=0.99)	0.059 (R^2=0.99)	0.054 (R^2=0.99)
Particulate P/VSS ratio	0.03 (R^2=0.99)	0.03 (R^2=0.99)	0.027 (R^2=0.99)	0.019 (R^2=0.99)	0.025 (R^2=0.99)
TCOD/TN ratio	21 (R^2=0.99)	23 (R^2=0.99)	25 (R^2=0.99)	27 (R^2=0.99)	27 (R^2=0.99)

5.3.3 Systems Performance

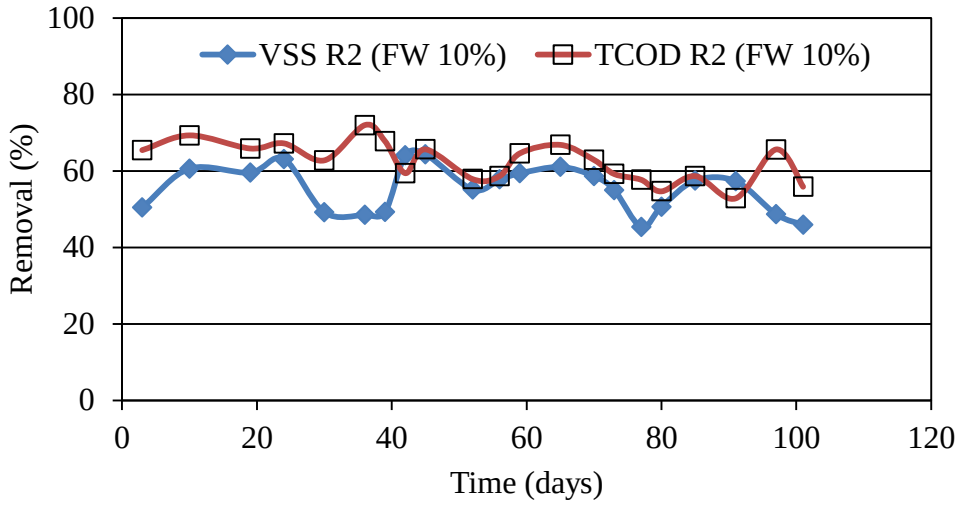
The temporal variations of VSS and COD removal efficiencies in all five reactors are depicted graphically in Figure 5.1. It is evident that significant fluctuations in digesters performance prevailed during the first 40 days of operation or 2 turnovers of the mean SRT of 20 days, after which digester stability improved markedly. The steady-state operation, as reflected by the stability of the digested biosolids was deemed to occur from day 42 to the end of the study (day 101), corresponding to three turnovers of the mean SRT. The steady-state calculated COD removal efficiencies were 45±8% (R1), 60±4% (R2), 65±4% (R3), 69±3% (R4), and 52±8% (R5) with R4 fed with 40% by volume food wastes exhibiting the highest removal efficiency.

The estimated VSS destruction were 56 ±7% (R1), 56±6% (R2), 57±7% (R3), 61±4% (R4), and 64±5% (R5) while the TSS removal efficiencies in R1 to R5 averaged 48% (R1), 49% (R2), 50% (R3), 54% (R4), and 60% (R5) (Table 5.4).

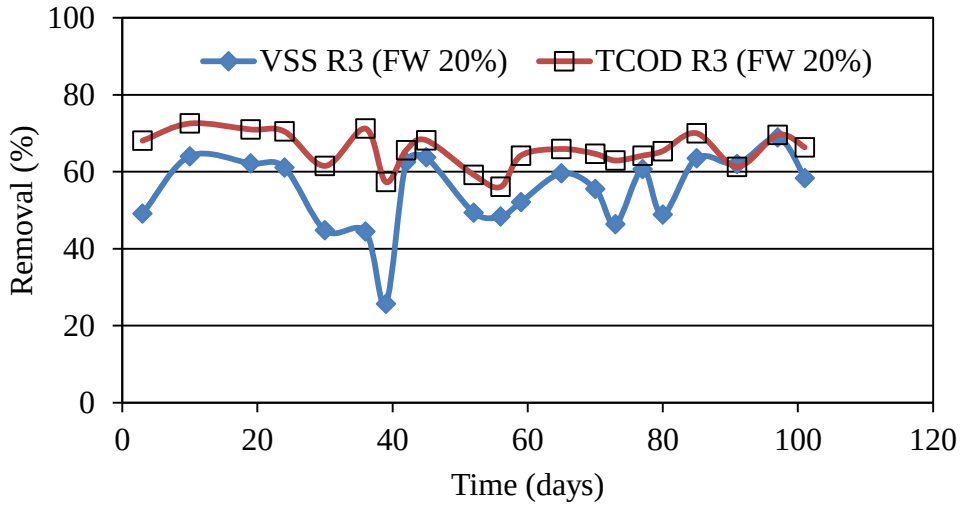
(A)



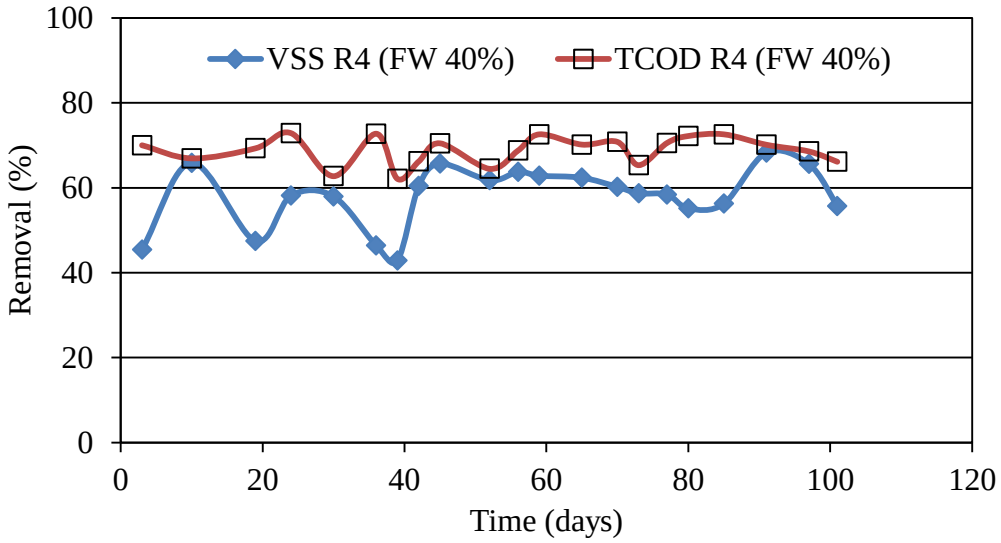
(B)


VSS R2 (FW 10%)
TCOD R2 (FW 10%)
Removal (%)
Time (days)
0
20
40
60
80
100
120

(C)


VSS R3 (FW 20%)
TCOD R3 (FW 20%)
Removal (%)
Time (days)
0
20
40
60
80
100
120

(D)



(E)

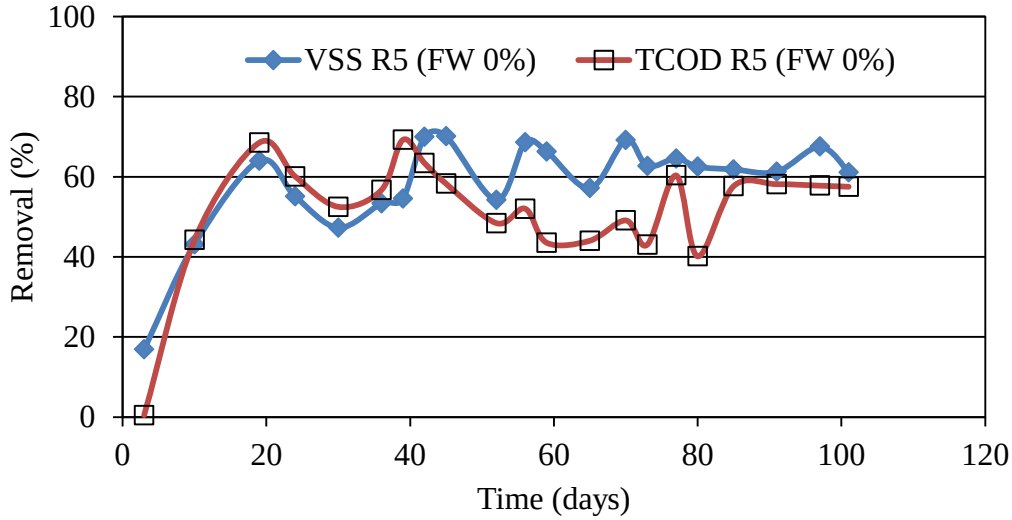


Figure 5.1 VSS and TCOD removal efficiencies under steady-state condition

Our observations are similar to those of Dai et al., (2013). Dai and coauthors operated semi-continuous digesters fed with a mixture of dry sludge and FW at an SRT of 20 days and mass ratios of dry solids-to-FW solids of 2.4:1, 0.9:1 and 0.4:1 (w/w, based on VS), yielding 29%, 53%, and 71% on mass basis FW content in the respective mixtures. The

aforementioned authors observed significant increases in VS destruction efficiencies with addition of FW i.e. 32% (dry sludge only), 46% (29% FW content sample), 58% (53% FW content), and 71% (FW content sample). Based on the correlation between COD and VS, the 53% FW content in Dai's study (0.9:1 on VS basis) closely matches our R4 (58% TCOD basis, Table 5.2), with respective removal efficiencies of 58% versus 69±3% in this study.

The percentage contributions of acetic and propionic acids to the overall VFA on a COD basis in the final effluent are presented in Table 5.5. Acetic acid and propionate acid in R1-R5 were predominant at 15%-40% and 43-69% of total VFA, respectively, with low concentrations of butyric acid (<12%) and valeric acid (3%-9%). Digester operational stability is reflected by the VFA/alkalinity ratio, with values less than 0.4 indicating stable performance (Wang et al., 2016). VFA was expressed as concentration of acetic acid which was calculated through converting the summation of individual VFA concentration (as COD) to concentrations of acetic acid. Table 5.5 lists the average and standard deviation of the VFA/alkalinity ratio. During steady-state operation, the ratio ranged from 0.07 to 0.2 for all reactors, indicating stable digestion.

Table 5.4 Steady-state COD and solids removal efficiencies based on measured influent and effluent values (number of samples is 21)

	R1 (Control)	R2 (FW 10%)	R3 (FW 20%)	R4 (FW 40%)	R5 (FW 0%)
COD removal (%)	45±8	60±4	65±4	69±3	52±8
TSS destruction (%)	48±9	49±5	50±7	54±6	60±6
VSS destruction (%)	56±7	56±6	57±7	61±4	64±5

Table 5.5 VFA composition in final effluent during steady-state operational conditions (number of samples is 10)

Parameters	**R1 (control)**	**R2 (FW 10%)**	**R3 (FW 20%)**	**R4 (FW 40%)**	**R5 (FW 0%)**
Acetic acid (%)	35±27	28±33	40±35	32±41	15±23
Propionic acid (%)	43±46	64±39	53±41	54±41	69±31
Butyric acid (%)	9±22	8±25	12±24	0±0	11±33
Valeric acid (%)	7±21	9±26	4±7	3±8	8±18
VFA to alkalinity ratio	0.11±0.06	0.17±0.15	0.13±0.10	0.07±0.05	0.20±0.16

5.3.4 Methane Production

The steady-state methane production is presented in Table 5.6B. The average pH of the five digesters ranged from 7.2 to 7.4, indicating optimum digestion conditions. Average methane content in biogas was 61% for R1 through R5. Daily methane production for the different reactors averaged at 0.63 L/day (R1), 1.05 L/day (R2), 1.62 L/day (R3), 2.01 L/day (R4), and 0.75 L/day (R5). Thus, the addition of FW at 10%, 20%, and 40% by volume affected 67%, 157%, and 219% increase in methane production, respectively.

Table 5.6 Summary of analysis at steady-state conditions (A) concentration (B) methane production

(A)

Parameters	Influent					Effluent				
	R1 (control)	**R2 (FW 10%)**	**R3 (FW 20%)**	**R4 (FW 40%)**	**R5 (FW 0%)**	**R1 (control)**	**R2 (FW 10%)**	**R3 (FW 20%)**	**R4 (FW40%)**	**R5 (FW 0%)**
TSS (g/L) (n=21)	28±3	29±2	32±3	33±5	29±3	14±2	15±1	16±2	15±1	12±1
VSS (g/L) (n=21)	23±3	25±3	28±3	30±4	25±3	10±1	11±1	12±2	11±1	9±1
TCOD (g/L) (n=21)	44±3	55±4	66±3	77±5	41±3	24±3	22±2	23±3	24±2	20±3
SCOD (g/L) (n=21)	5±1	11±1	16±2	24±4	2±1	1±0	2±0	2±0	2±0	3±1
TN (g/L) (n=10)	2.1±0.1	2.4±0.2	2.6±0.1	2.8±0.2	1.5±0.1	2.0±0.1	2.2±0.4	2.4±0.4	2.8±0.4	1.6±0
SN (g/L) (n=10)	0.5±0.1	0.6±0.1	0.8±0.1	1.2±0.2	0.2±0.0	1.1±0.2	1.3±0.2	1.4±0.2	1.8±0.1	0.9±0.1
NH_3-N (g/L) (n=10)	04±0.1	0.4±0.1	0.4±0.1	0.4±0.1	0.1±0.0	1.0±0.1	1.2±0.1	1.2±0.2	1.5±0.1	0.7±0.1
Alkalinity (g $CaCO_3$/L) (n=10)	2.2±0.5	2.0±0.6	1.6±0.5	0.8±0.4	1.1±0.5	4.3±0.5	4.8±0.6	4.1±1.1	6.0±1.1	2.9±0.2
VFA (g/L as COD) (10)	1.6±1.2	2.1±1.2	1.9±0.9	2.4±1.7	1.1±0.7	0.5±0.3	0.9±0.8	0.7±0.5	0.4±0.3	0.6±0.5
TP (g/L) (4)	0.76±0.06	0.78±0.07	0.81±0.06	0.84±0.11	0.60±0.11	0.56±0.09	0.61±0.06	0.62±0.08	0.59±0.07	0.41±0.09

(B)

	R1 (Control)	**R2 (FW 10%)**	**R3 (FW 20%)**	**R4 (FW 40%)**	**R5 (FW 0%)**
pH (n=60)	7.3±0.2	7.3±0.2	7.3±0.2	7.4±0.1	7.2±0.1

CH_4 content (%)(n=60)	61±4	61±2	61±3	61±3	61±3
CH_4 (L per day)(n=60)	0.63±0.16	1.05±0.19	1.62±0.22	2.01±0.28	0.75±0.18

Note: ‘n’ denotes the number of samples

5.3.5 Mass Balance Analysis

5.3.5.1 COD Mass Balances

Analysis of COD mass balances during the steady-state period (day 42-101) is presented in Table 5.7. Influent and effluent COD were 235 g and 126 g for R1, 301g and 118 g for R2, 350 g and 125 g for R3, 412 g and 126 g for R4, and 221 g and 106 g for R5, respectively. COD removal efficiencies of different reactors were achieved at 47% (R1), 61% (R2), 64% (R3), 69% (R4), and 52% (R5). Overall methane production during the steady-state operation were 37 L (R1), 62 L (R2), 95 L (R3), 119 L (R4), and 44 L (R5). The measured methane generation accounted for 85%-104% of the theoretical methane production estimated from the COD removal. The calculated closures of COD mass balances based on both methane and digested biosolids COD relative to the influent COD were 91%-104%, indicating excellent operation and maintenance of the digesters, as well as data reliability and QA/QC of experimental measurements. Methane production per mass of COD removed ranged from 0.34 L/gCOD-0.43 L/gCOD, close to the theoretical value of 0.4 L/gCOD.

5.3.5.2 VSS Mass Balances

VSS destruction efficiencies based on cumulative mass during the steady-state period are presented in Table 5.7. Influent and effluent VSS were 124 g and 55 g for R1, 132 g and 58 g for R2, 147 g and 62 g for R3, 162 g and 61g for R4, and 131 g and 49 g for R5, yielding 56% to 62% removal efficiencies. It is therefore evident by comparison of the steady-state VSS and COD removal efficiencies, that the primary contributor to the enhancement in COD removal efficiencies in the FW digesters is the high soluble COD.

5.3.5.3 Nitrogen Mass Balances

Nitrogen mass balances were analyzed to examine nitrogen generation through VSS destruction as presented in Table 5.7. The last 60 days of operations at steady-state conditions indicated that respective cumulative influent and effluent ammonia nitrogen were 1.9 g and 5.3 g (R1), 2.0 g and 6.4 g (R2), 2.1 g and 6.9 g (R3), 2.2 g and 8.3 g (R4), and 0.8 g and 4.5 g (R5). VSS destruction for the reactors during the same period ranged from 49 g to 104 g and estimated nitrogen/VSS ratio were 0.054 to 0.07, yielding estimated ammonia nitrogen generation during digestion at 4.5 g to 5.9 g. Ammonia-nitrogen mass balance closures considering the influent ammonia and the ammonia generated from VSS destruction relative to the effluent ammonia were 127% (R1), 112% (R2), 109% (R3), 97% (R4), and 115% (R5). It must be emphasized that the accuracy of the ammonia nitrogen mass balance closures in R2 to R5 of 97% to 115% confirms the VSS destruction efficiencies discussed above. The relatively higher ammonia mass balance closure in R1 of 127% is inconsistent with the VSS removal efficiency in R1. It must be emphasized however that gaseous ammonia emissions were neglected in the nitrogen balance.

Table 5.7 COD, VSS, nitrogen mass balances based on cumulative data during steady-state operation

Mass balance from day 42 to 101	R1 (Control)	R2 (FW 10%)	R3 (FW 20%)	R4 (FW 40%)	R5 (FW 0%)
COD mass balances					
Net influent COD (g)	235.3	300.8	349.5	411.7	220.5
Net effluent COD (g)	125.6	117.6	125.1	126.4	105.6
COD removal (%)	47	61	64	69	52
Actual Methane production (L)	37.3	62.3	95.6	118.6	44.4
Theoretical Methane production (L)	43.9	73.3	89.8	114.1	46.0
Actual methane/theoretical methane production (%)	85	85	106	104	96
COD mass balance closure (%)	93	91	104	103	98
CH4 production (L)/g COD destroyed	0.34	0.34	0.43	0.42	0.39
VSS mass balances					
Influent VSS (g)	124.2	131.7	147.2	161.6	131.4
Effluent VSS (g)	54.5	58.1	62.0	61.0	48.9
VSS destruction (%)	56	56	58	62	63
Nitrogen mass balances					
Influent NH_3-N (g)	1.9	2.0	2.1	2.2	0.8
Effluent NH_3-N (g)	5.3	6.4	6.9	8.3	4.5
VSS destruction (g)	49	63	76	104	69
Influent VSS (g)	124.2	131.7	147.2	161.6	131.4
Effluent VSS (g)	54.5	58.1	62.0	61.0	48.9
VSS destruction (g)	49	63	76	104	69
N/VSS ratio	0.068	0.07	0.063	0.059	0.054
Generated NH_3-N during digestion (g)	4.7	5.2	5.4	5.9	4.5
Closures (%)	127	112	109	97	115

5.3.6 FW Digestion

FW COD removal efficiencies in R2, R3, and R4 were estimated through the calculation of the food waste influent and effluent COD. Food wastes COD in each reactor was calculated by subtracting biosolids COD from the overall COD, where biosolids COD was estimated based on the biosolids COD mass fraction in each reactor and the biosolids COD removal in R1 treating biosolids only. The detailed calculations are presented in Table 5.8.

Influent and effluent FW COD were 86 g and 3 g for R2, 153 g and 20 g for R2, and 244 g and 37 g for R3, respectively resulting in estimated FW COD removal efficiencies of 96% (R2), 87% (R3), and 85% (R4), reflecting the high digestibility of food wastes. Similarly, FW VSS destruction efficiencies were also estimated using the same aforementioned approach for COD (Table 5.8). Influent and effluent FW were 18.6 g and 8.4 g for R2, 44 g and 17 g for R3, and 73 g and 22 g for R4, yielding respective FW destruction efficiencies of 55%, 62% and 70%.

Table 5.8 Estimation of food waste COD and VSS removal in R2, R3, and R4 (steady-state conditions)

	R1 (Control)	**R2 (FW 10%)**	**R3 (FW 20%)**	**R4 (FW 40%)**
Sludge volumetric fraction	1	0.91	0.83	0.71
Food waste volumetric fraction	0	0.09	0.17	0.29
[FW COD removal estimation]				
Influent COD (g)	235	301	350	412
Effluent COD (g)	126	118	125	126
Removal (%)	47	61	64	69
Influent sludge COD (g)		214	196	167
Effluent sludge COD (g)		114	105	89
Influent food waste COD (g)		86	153	244
Effluent food waste COD (g)		3	20	37
Food waste destruction (%)		**96**	**87**	**85**
[FW VSS destruction estimation]				
Influent VSS (g)	124	132	147	162
Effluent VSS (g)	55	58	62	61
VSS destruction (%)	56	56	58	62
Influent sludge VSS (g)		113	103	88
Effluent sludge VSS (g)		50	45	39
Influent food waste VSS (g)		18.6	44	73
Effluent food waste VSS (g)		8.4	17	22
Food waste destruction (%)		**55**	**62**	**70**

Example of COD removal estimation using R2)

Influent sludge COD (g) in R2 = sludge fraction in R2 influent × influent COD (R1)
= 0.91 × 235 g = 214 g
Effluent sludge COD (g) in R2 = Influent sludge COD (g) in R2 × (100-sludge COD removal (%) in R1) / 100
= 214 g × (100-47)/100 = 114 g
Influent food waste COD (g) in R2 = Influent COD in R2 – Influent sludge COD (g) in R2
=301 g – 214 g = 86 g
Effluent food waste COD (g) in R2 = Effluent COD in R2 – Effluent sludge COD (g) in R2
=118 g- 114 g = 3 g
Food waste destruction (%)
= (Influent food waste COD in R2 – Effluent food waste COD in R2) ×100 / (Influent food waste COD in R2)
= (85 g – 3 g) ×100 / 85 g = 96%

5.4 Discussion

5.4.1 Synergies and Effect of COD/N Ratio on COD Removal

The effect of food waste on biosolids digestion was examined to explore the synergistic effects of co-digestion. It is evident that COD removal efficiencies increased with food waste addition, despite a significant increase in volumetric COD loading rate. For example, R4 achieved an overall 69% COD removal efficiency (53% higher than the control—R1), despite operating at an average COD loading of 3.85 kgCOD/m^3-d compared with 2.2 kgCOD/m^3-d in the control.

The first order COD removal kinetic constants based on the completely-mixed reactor model were estimated using steady-state influent and effluent COD as 0.042 d^{-1} (R1), 0.075 d^{-1} (R2), 0.093 d^{-1} (R3), 0.11 d^{-1} (R4), and 0.053 d^{-1} (R5), with linear correlation with the FW COD concentration (Table 5.9 and Figure 5.2a). Particularly, the k value for R4 was more than double the rate of R1 (control), indicating FW content of 40% accelerated significantly the degradation rates. Scrutiny of the data indicated that the extent of kinetic constant increment decreased from 1.8 times (R1 to R2) to 1.25 (R2 to R3), then to 1.18

times (R3 to R4), emphasizing that the enhancement of the degradation rate with FW addition will ultimately level off. Close examination of the linear relationship between the first order COD removal coefficient and additional FW loading depicted in Figure 5.2a indicates that at infinitely low FW loadings, the first-order rate coefficient is 0.0575 d^{-1} while the control without any food waste exhibited a rate of 0.042 d^{-1}. This implies that the addition of food wastes may affect a 37% increase in the first order degradation rate of the primary and WAS solids. This implies that for a typical completely-mixed anaerobic digester, with an SRT of 15 days, the rate enhancement due to co-digestion increases the COD (i.e. VS) removal efficiency from 39% to 53%.

Synergistic effects were also assessed by methane production. At steady-state conditions, methane production per g COD fed was 0.16 L/g COD (R1), 0.21 L/gCOD (R2), 0.27 L/gCOD (R3), 0.29 L/gCOD (R4), and 0.2 L/gCOD (R5), indicating that the values in R2, R3 and R4 were 1.3, 1.7, and 1.8 times higher than R1. Thus, methane generation increased with the proportion of food wastes in the feed. However, the increase also decreased from 1.31 times (R1 to R2), to 1.29 (R2 to R3), and to 1.07 (R3-R4), suggesting that 0.29 L/gCOD could be close to the maximum limit.

Table 5.9 COD removal kinetic constants

Parameters	R1 (Control)	R2 (FW 10%)	R3 (FW 20%)	R4 (FW 40%)	R5 (FW 0%)
Average influent COD (g/L) (day 42-101)	44	55	66	77	41
Average effluent COD (g/L) (day 42-101)	24	22	23	24	20
Kinetic constant (day^{-1})	0.042	0.075	0.093	0.11	0.053

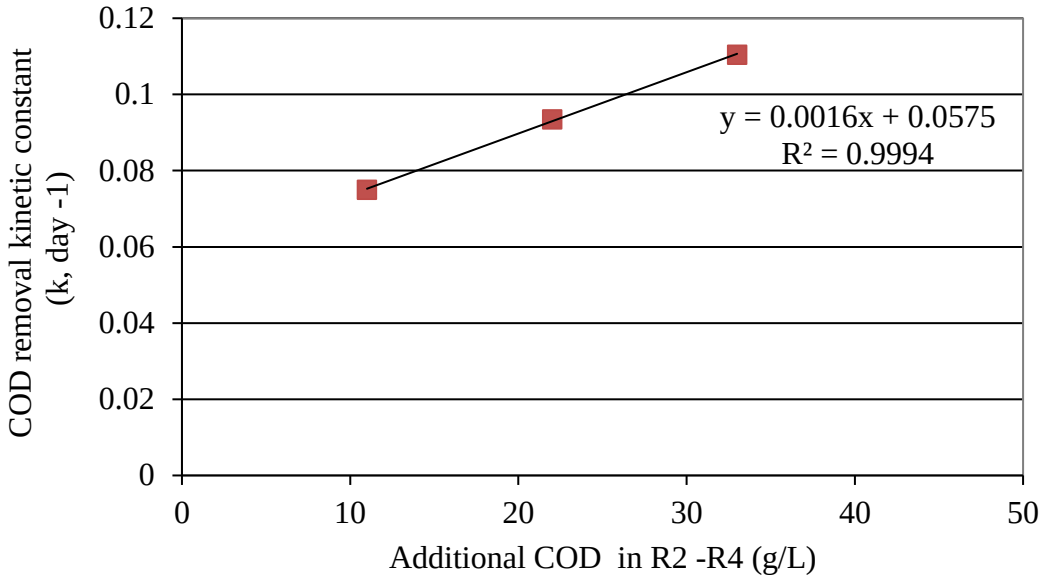
Note) Completely-mixed reactor model: $dC/dt \times V = QCi - QCe - kCV$

where, Q=flowrate (0.09L/d), Ci=influent COD, Ce=effluent COD, k= kinetic constant,

C=Ce, V=reactor volume (1.8L), dC/dt = 0 at steady-state conditions,

k=(44 g/L × 0.09 L/d – 24 g/L × 0.09 L/d) / (24 g/L × 1.8L) = 0.042 day^{-1}

(a)



(b)

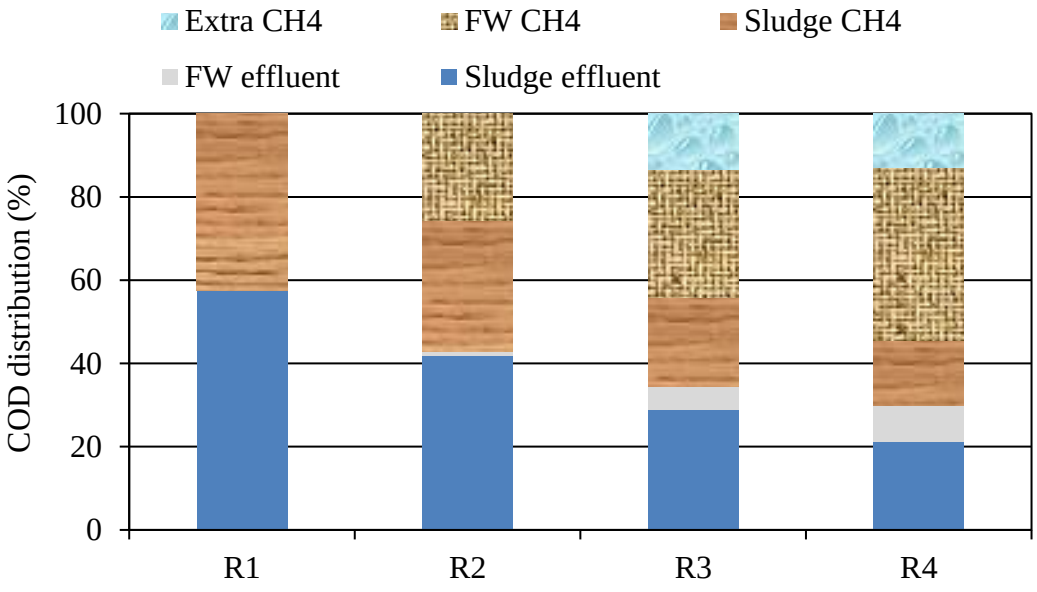


Figure 5.2 (a) First order COD removal coefficient and additional FW loading (b) COD distribution

Synergistic effects were quantified by estimating extra biogas generation in R3 and R4 where 20% and 40% of food waste was added, respectively. Assuming that methane production through PS and TWAS in R2 was similar to R1, methane generation from food waste degradation in R2 was estimated with the assumption of minimal synergism in R2. Accordingly, CH_4 production (as COD) per biosolids COD removal in R1 and CH_4 production (as COD) per FW COD removal in R2 were estimated as 0.86 g COD/g biosolids COD and 0.85 g COD/gFW COD, respectively. These separate methane yields were used to estimate the additional methane generation in R3 and R4 from synergism according the equation below:

Extra methane (COD_{CH4}, g) generated in R3

= A – (B × 0.86 g COD_{CH4}/g biosolids COD) – (C × 0.85 g COD_{CH4}/g FW COD]

= 95.6 L CH_4 / (0.4 LCH_4/gCOD) – (196 g – 105 g) × 0.86 g/g – (153 g – 20 g) × 0.85 g/g

= 48.7 g COD_{CH4}.

where, A: overall methane production (COD_{CH4}, g), B: biosolids influent COD - biosolids effluent COD (g), C: FW influent COD - FW effluent COD (g)

Similarly, additional methane produced in R4 was estimated as 54.7 g. The distribution of effluent COD consisting of effluent biosolids, effluent FW, CH_4 generated from biosolids degradation, CH_4 generated from FW degradation, and CH_4 generated from synergistic degradation is presented in Figure 5.2b. The estimated extra methane production due to

synergistic effects was 13% of the overall COD (COD effluent and COD biogas) in R3 and R4, corresponding to 18%-20% of the overall generated methane gas. The 18%-20% in fact underestimates the impact of synergism since synergistic effects in R2 were ignored.

There is a widely held view that the positive effects of FW are related to the increase of the COD/N ratio. COD removal from five digesters with different influent COD/N ratios were 47% (R1, biosolids only, COD/N ratio of 21), 61% (R2, 10% FW, COD/N ratio of 23), 64% (R3, 20% FW, COD/N ratio of 25), and 69% (R4, 40% FW, COD/N ratio of 27), and 52% (R5, biosolids only, COD/N ratio of 27). Of the two reactors fed with biosolids only (R1and R5), R5 with COD/N ratio of 27 showed a modest 5% higher COD removal than R1 with COD/N ratio of 21, primarily due to higher PS (64% of influent COD in R5 versus 48% in R1) confirming that COD/nitrogen ratio alone is not the major reason for enhanced digestion. However, it should be also noted that compared with R5 performance, co-digestion with food wastes achieved higher COD removal (61%-69%) despite similar or lower COD/N ratio than biosolids alone, which clearly indicates that the biodegradability of food wastes significantly enhanced anaerobic digestion. Our findings correspond well with a study by Koch et al., (2015) who conducted BMP tests on different mixtures of food waste (C/N ratio 17.7) and biosolids (C/N ratio 8.8) ranging from biosolids only to 30% mass based FW addition in increments of 2.5% (C/N ratios 8.4 to 11.7 in increments of 0.3) and reported that the specific methane yield increased from 0.32 L/gVS (biosolids only) to 0.36 L/gVS (12.5% FW addition, C/N ratio 9.4) with no further enhancement at higher mixing ratios.

5.4.2 Mass Microbial activity tests

Different microbial characteristics were explored through three rounds of batch tests. Biogas production patterns for R1 and R4 in the batch tests were presented in Figure 5.3. Average overall biogas production for R1 and R4 samples were 189 and 240 mL for SMA tests, 230 and 300 mL for SAtA, and 102 and 128 mL for SAdA, respectively. The specific microbial activities for different groups of bacteria i.e. methanogenic bacteria, acidogenic bacteria, and acetogenic microbes were assessed by dividing the volume of biogas produced per unit time by the initial weight of VSS in the test bottles. The maximum specific biogas production rate (mL/mgVSS/h) was estimated using the following modified Gompertz model which describes the progression of cumulative biogas production in the batch tests (Wang et al. 2016). The cumulative biogas data from the specific microbial activity tests were fitted with Gompertz equation (eq. 1) using the Newton-Raphson method for non-linear numerical estimation.

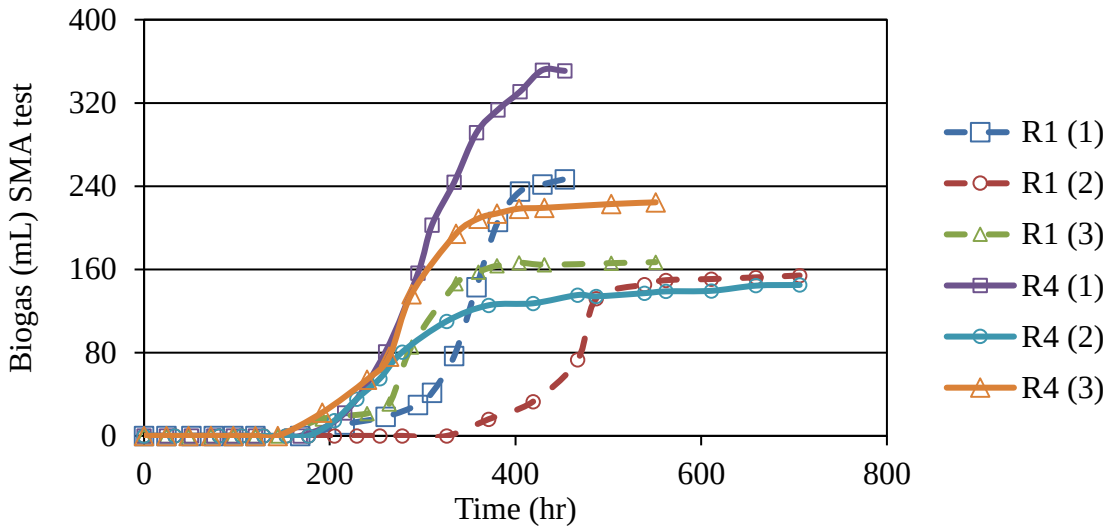
$$H = P \cdot \exp\left\{-\exp\left[\frac{R_m \times e}{P}(\lambda - t) + 1\right]\right\} \quad (1)$$

Where H is the specific cumulative biogas production (mL/mgVSS), R_m is the maximum specific biogas production rate (mL/mgVSS/ h), λ is the lag time (h), t is the incubation time (h), P is the maximum specific cumulative biogas production (mL/mgVSS), and e=2.718.

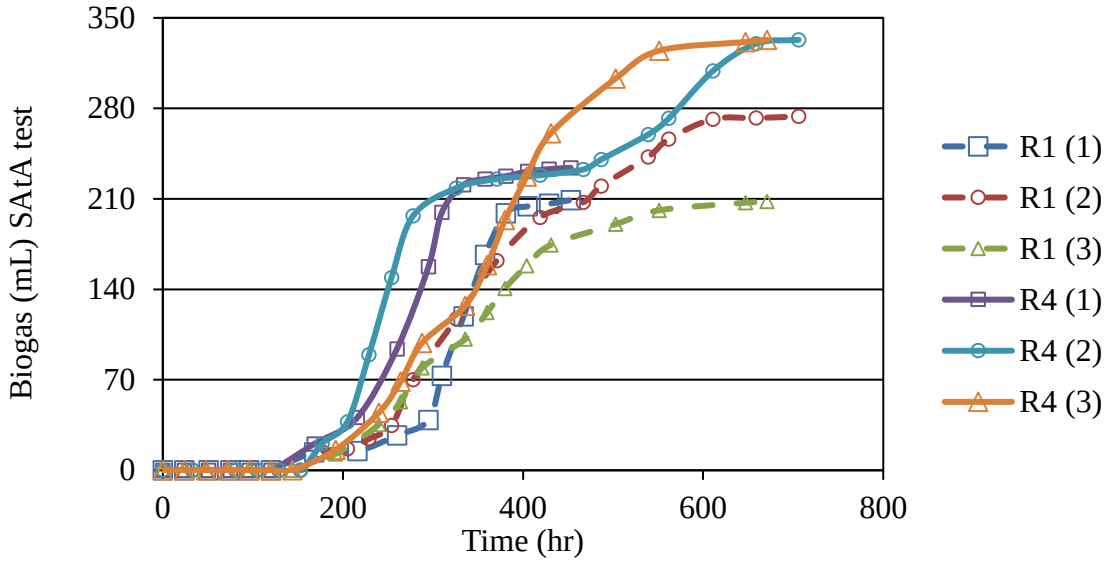
The data comparing R1 and R4 is shown in Figure 5.3. The average maximum specific biogas production (Table 5.10) of R1 and R4, obtained from lag phase were 7.3 and 5.9 mL/gVSS/hr for SMA, 5.2 and 5.3 mL/gVSS/hr for SAtA, and 8.8 and 9.1 mL/gVSS/hr for SAdA, indicating that SAtA and SAdA were higher for R4 than R1 with an opposite trend for SMA. It should be also emphasized that SAtA values are 64%-93% lower than

SMA values. SAtA test used mixed substrates to produce acetic acid and then biogas while SMA used acetic acid as single substrate to generate biogas. The relatively lower biogas production rate of SAtA than SMA may show that acetogenesis is rate limiting in producing biogas in SAtA, indicating the validity of the batch tests used in this study to characterize acetogens and methanogens behavior separately.

(A)



(B)



(C)

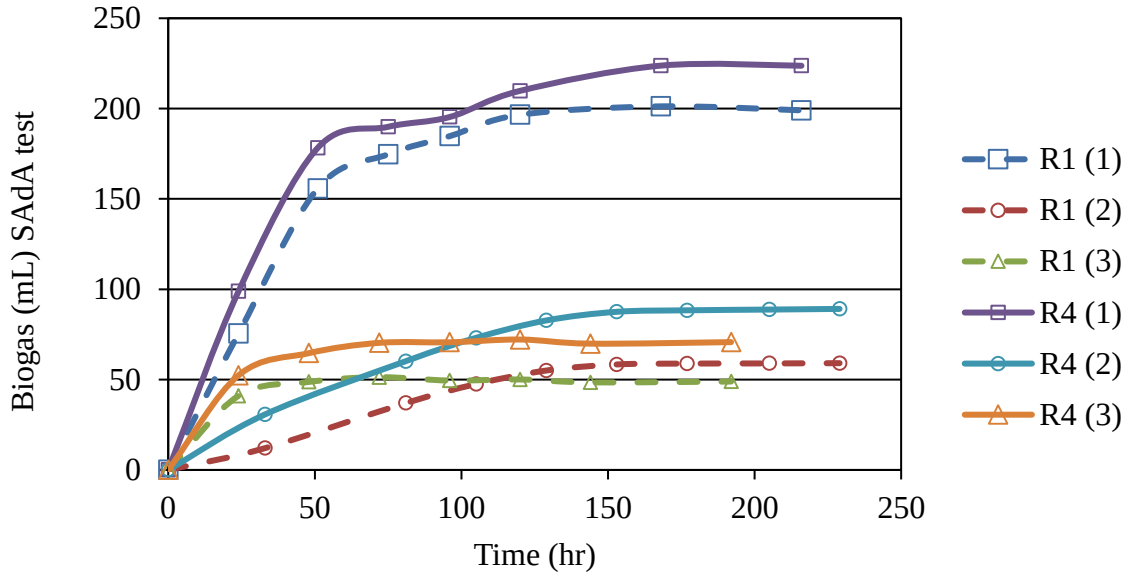


Figure 5.3 Biogas production during three batch tests (A) SMA (B) SAtA (C) SAdA

Scrutiny of data, however, also shows that when zero order rates were used to account for the lag phases, the average maximum specific biogas production of the three different microbial groups were 1.22-1.3 times higher in R4 than R1, indicating enhanced activity of methanogens, acetogens, and acidogens in co-digesters. Considering that the digested sludge VSS concentration in R4 was 10% higher than R1 (Table 5.6A), the active biomass in R4 is about 25% higher than R1. Furthermore by comparing the COD removal rates in R1 and R4, it is evident that the 25% higher concentration of active biomass readily affected more than double the COD removal rate. Thus, considering that the average COD removal rate in R4 was 2.65 times that of R1 (Table 5.6), the biomass-specific COD removal rate in R4 was approximately twice that of R1.

Table 5.10 Maximum specific biogas production rate (R_{max}) (n=3)

R_{max}	SMA		SAtA		SAdA	
(ml/gVSS-hr)	R1	R4	R1	R4	R1	R4
Gompertz Model	7.3 ± 2.1	5.9 ± 2.6	5.2 ± 3.1	5.3 ± 1.4	8.8 ± 6.0	9.1 ± 5.8
Zero order rate*	1.6 ± 0.6	2.0 ± 0.8	1.7 ± 0.3	2.2 ± 0.1	4.2 ± 2.9	5.1 ±3.3

*Maximum specific biogas production rates (R_{max}) are taken at zero order rate under 95 percentile gas/95 percentile time

5.4.3 Effect of Metals on Co-digestion

To explore the effect of metals on co-digestion, total and dissolved Cd, Cu, Fe, K, Mg, Na, Ni, and Zn in the digestate were analyzed in three samples of the digested sludge (Table 5.11). Total K and Na of FW were 9.7 and 3.5 times higher than those of biosolids. On the contrary, the content of Cu, Fe, Ni, Zn of FW was 9%-33% lower than that of biosolids, indicating FW addition did not supplement these metals for co-digestion. Metals of FW reported in previous studies ranged from 1.2-3.1 mg Cu/L, 3-23 mg Fe/L, 546-1123 mg K/L, 63-88 mg Mg/L, 1004-1529 mg Na/L, 0.2-2.7 mg Ni/L, and 3-10 mg Zn/L, which were maximum 98 times higher than this study (Zhang et al., 2011; Facchin et al., 2013). It should be noted that the aforementioned studies used restaurant and kitchen wastes while the FW in this study was from a supermarket. Additionally, FW metal concentrations in this study were one or three orders magnitude lower than the reported threshold levels for inhibition methanogens such as 36-3400 mg Cd/L, 12.5-350 mg Cu/L, 400-28934 mg K/L, 3500-8000 mg Na/L, 35-1600 mg Ni/L, and 7.5-1500 mg Zn/L (Romero-Güiza et al., 2016). The most abundant soluble metals in FW were K (75.5 mg/L) and Na (12.5 mg/L) with the levels of other metals ranging from 0.006 mg/L (Cu) to 1.1 mg/L (Fe). All soluble

metal concentrations of FW were higher than those of biosolids i.e. 2-7 times for Cd, Cu, Fe, Mg, Na, and Ni, 15 times for K and 35 times for Zn.

Table 5.11 Metal concentrations in digesters (n=1-3)

Parameters (mg/L)		**FW**	**R1 (control)**	**R2 (FW 10%)**	**R3 (FW 20%)**	**R4 (FW 40%)**
Cd	**Total**	0.021±0.01	0.005±0.001	0.017±0.002	0.009±0.002	0.01±0.002
	Soluble	0.007±0.004	0.003±0.003	0.005±0.003	0.004±0.002	0.004±0.001
Cu	**Total**	0.076±0.05	0.8±0.3	1.2±0.4	1.3±0.4	1.4±0.5
	Soluble	0.006±0.007	0.003±0.001	0.009±0.011	0.003±0.003	0.005±0.004
Fe	**Total**	3.6±3.9	38.8±12	43.6±18	37.8±12	31.1±12
	Soluble	1.1±0.96	0.2±0.12	0.1±0.04	0.1±0.02	0.1±0.07
K	**Total**	71.8±18.2	7.4±2.3	14.6±2.2	17.0±1.6	28.9±3.2
	Soluble	75.5±7.1	5.1±2.2	14.1±5.6	15.6±7.0	17.4±0.5
Mg	**Total**	6.4±0.4	4.6±1.4	4.6±1.5	4.2±1.2	5.2±1.9
	Soluble	4.4±0.1	2.5±0.3	3.0±0.1	3.0±0.0	3.2±0.7
Na	**Total**	27.1±4.5	7.8±1.8	9.8±2.6	10.5±2.1	14.8±3.6
	Soluble	12.5±7.2	2.6±0.8	3.8±0.9	4.2±1.1	6.5±1.3
Ni	**Total**	0.01±0.00	0.03±0.0	0.02±0.0	0.03±0.0	0.03±0.0
	Soluble	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
Zn	**Total**	0.23±0.04	0.8±0.3	1.3±0.4	1.4±0.4	1.7±0.6
	Soluble	0.24±0.13	0.01±0.01	0.004±0.00	0.02±0.02	0.01±0.01

Metals play an important role in digestion and insufficient concentrations of certain metals deteriorates FW digestion while proper supplement of metals recover performance of digesters. For instance, addition of 100 mg Fe/L, 10 mg Ni/L, 5 mg Mo/L, and 2 mg Co/L to the poorly performing digester fed with FW only increased methane production and decreased high VFA concentrations (Choong et al., 2016). It can be expected that the synergistic effects of co-digestion can be induced through addition of biodegradable substrates and metals, both of which are essential for successful performance. Interestingly, in this study, Fe concentration was reversely related to digestion performance. Fe content in digestate were 64% (R1), 58% (R2), 52% (R3), and 37% (R4), evidently decreasing with the extent of FW addition. However, the content was inversely related to COD degradation

kinetics (d^{-1}) of 0.042 (R1), 0.075 (R2), 0.093 (R3), and 0.11 (R4) with regression model R^2=0.86, contradicting that higher Fe concentration promotes digestion (Choong et al., 2016). A study by Takashima et al. (2011) who tested minimum requirements for trace metals for methane fermentation using glucose reported that Fe is required at 0.2 mg Fe/gCODremoved. In this study, the ratio of influent Fe/gCODremoved in the five reactors ranged from 0.5 to 1.9 mg Fe/gCOD, indicating that Fe was not deficient in the reactors. It is thus evident that the effect of metals on the observed synergism in this study may not be pronounced i.e. synergistic effects were more related to biodegradability.

5.4.4 Effect of Co-digestion on Solids and Nitrogen Generation

Scrutiny of the data presented in Table 5.2a indicates that while influent TSS concentrations to the co-digestion reactors were generally 7%-26% higher than the control, the effluent TSS were within 10% of the control. T-tests conducted on the effluent TSS data indicate that differences between the control (R1) and co-digestion reactors (R2-R4) were not significant at the 95 percentile confidence level while VSS level differences between R1 and two co-digested reactors (R3 and R4) were significant at a 95 percentile confidence level (Table 5.12). This indicates that solids loading increase due to FW addition did not adversely affect residual solids.

Nitrogen loading increase with co-digestion could be a drawback because the rejected water increases nitrogen loading in the liquid stream, thus increasing aeration energy in spite of its potential offset by the additional biogas (Koch et al., 2015). Soluble nitrogen concentrations of digestates increased from 1.1 g/L (R1) to 1.2 g/L (R2), 1.4 g/L (R3),

Table 5.12 Statistical analysis results (T-tests, Two-Sample Assuming Unequal Variances)

TSS (average effluent)	**R1 (14.5 g/L)**	**R2 (14.9 g/L)**	**R3 (15.7 g/L)**	**R4 (15.0 g/L)**
R2 (14.5 g/L)	p>0.05			
R3 (15.7 g/L)	p>0.05	p>0.05		
R4 (15.0 g/L)	p>0.05	p>0.05	p>0.05	
R5 (11.7 g/L)	p<0.05	p<0.05	p<0.05	p<0.05
VSS (average effluent)	R1 (10.1 g/L)	R2 (10.9 g/L)	R3 (11.8 g/L)	R4 (11.5 g/L)
R2 (10.9 g/L)	p>0.05			
R3 (11.8 g/L)	p<0.05	p>0.05		
R4 (11.5 g/L)	p<0.05	p>0.05	p>0.05	
R5 (8.9 g/L)	p<0.05	p<0.05	p<0.05	p<0.05
TCOD (average effluent)	R1 (24.0 g/L)	R2 (21.9 g/L)	R3 (23.3 g/L)	R4 (23.8 g/L)
R2 (21.9 g/L)	p<0.05			
R3 (23.3 g/L)	p>0.05	p>0.05		
R4 (23.8 g/L)	p>0.05	p<0.05	p>0.05	
R5 (19.6 g/L)	p<0.05	p<0.05	p<0.05	p<0.05
VSS/TSS (average effluent)	R1 (0.70)	R2 (0.73)	R3 (0.75)	R4 (0.77)
R2 (0.73)	p>0.05			
R3 (0.75)	p<0.05	p>0.05		
R4 (0.77)	p<0.05	p>0.05	p>0.05	
R5 (0.77)	p<0.05	p>0.05	p>0.05	p>0.05

and 1.8 g/L (R4) with ammonia content of 88%-90%, indicating that co-digestion yielded 1.23 to 1.64 times higher soluble nitrogen than the control ($p<0.05$). The difference in SN concentrations between R4 compared to R2 and R3 were also statistically significant. Moreover, average SCOD/SN ratios were 1.02 (R1), 1.21 (R2), 1.31 (R3), and 1.31 (R4) although statistical differences between ratios were not significant ($p>0.05$). Thus, the rejected nitrogen-loaded water may increase aeration cost for nitrification and necessitate the use of additional external carbon for denitrification.

5.5 Practical Implications

The most significant finding of this study i.e. that the increased loadings from addition of FW do not deteriorate digester performance but on the contrary improve the digestibility of primary and WAS solids clearly dispels a common myth in the industry that the addition of FW does in fact decrease the available capacity for biosolids. To illustrate the impact of FW co-digestion on digesters, the following example for a 100,000 population equivalent plant is presented. The following information pertains (Tchobanoglous et al., 2003):

- Per capita TSS and BOD_5 generation rates of 85 and 95 g/d,
- TSS and BOD removal efficiencies in primary clarification of 65% and 35% respectively, with biomass yield of 0.85 $gTSS/gBOD_5$.
- Volatile fraction of primary and biological solids based on the data of this study of 93%, and 73% resulting in an overall volatile fraction of 84% in the combined biosolids.
- Inorganic SS concentrations in PS+WAS and Food wastes based on this study are 5 and 4 g/L, respectively.
- The ratio of PCOD/VSS in the primary and WAS suspended solids based on the data of this study is 1.85 g PCOD/gVSS, with the combined biosolids at 2.7% dry solids.
- Digester SRT is 20 days i.e. the calculated digester volume is 8055 m^3
- Co-digestion affects a 30% increase in first order COD removal of primary and WAS solids, increasing it from 0.042 d^{-1} to 0.055 d^{-1}.
- Average FW COD is 150 g/L.

- The TCOD/TN ratios for PS, TWAS, and FW from this study based on the steady-state characteristics are 40, 15, and 30 respectively.
- Aeration energy is based on 1 kg O_2/KwH

Figure 5.3 and Table 5.13 illustrate the results of three scenarios: control (PS and WAS), FW at 15% of the combined flow rate of PS and WAS, and FW at 30% of the combined flow rate of PS and WAS. It is evident from the tabulated data that addition of 15% by volume FW corresponding to a 56% increase in volumetric COD loading increased the digested volatile SS by a mere 2.9% and the inorganic SS by 10% while increasing biogas production by more than 100%. Furthermore, the energy required for nitrification of the additional soluble nitrogen emanating from the co-digestion of food wastes is negligible compared to the methane energy.

Table 5.13 Impact of food wastes on full-scale digesters

Parameter (units)	100% (PS+WAS)	15% FW + 85% (PS+TWAS)	30% FW + 70% (PS+TWAS)
Influent PS+WAS (kgCOD/d)	16110	16110	16110
SRT (days)	20	17.4	15.4
COD Destruction Efficiency (%)	45	49	45
Digested PS+WAS (kgCOD/d)	8860	8216	8860
Influent FW (kgCOD/d)	0	9000	18000
FW COD Destruction Efficiency (%)	0	90	85
Effluent FW COD (kg/d)	0	900	2700
Total Effluent COD (kg/d)	8860	9116	11560
Effluent inorganic TSS (kg/d)	2015	2255	2495
Effluent VSS (kg/d)[a]	4922	5064	6422
Methane (m^3/d)	2538	5600	7890
Incremental CH_4 energy (GJ/d)	-	113	192
N-produced (kg N/d)	350	650	860
Incremental energy for nitrification (GJ/d)		5	85

[a] It was estimated using PCOD/VSS ratio of 1.8 observed in this study

5.6 Conclusions

Synergism of co-digestion affected an estimated 37% increase in biosolids degradation rate, and a minimum of 18% increase in methane production rates. The aforementioned rate enhancement increases the COD and VS removal efficiencies in a 15-d SRT digester from 39% to 53%. Higher acidogenic, acetogenic, and methanogenic activity was observed in the co-digesters than in the control. For a 100,000 p.e. plant, the addition of FW at 15% by volume to PS & TWAS while affecting a 56% increase in volumetric COD loading and increasing methane production by over 100% caused a mere 2.9% increase in digested volatile solids.

5.7 References

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Chapter 6

Conclusions and Recommendations

6.1 Conclusions

This study demonstrated the effectiveness of using a control fluidized bed reactor and ultrasonicated fluidize-bed reactor (U-AnFBR), with HDPE (600 μm~850 μm) as biomass support media for TWAS digestion. In the U-AnFBR system (4 s active per min, 480 kJ), ultrasonication enhanced the CH_4 production by 33% at an OLR of 5.1 kg COD/m^3-day and 37% at an OLR of 9.7 kg COD/m^3-day, achieving TCOD removal and VSS destruction efficiencies of 65% and 63%, respectively, at an OLR of 5.1 kg COD/m^3-d, and 51% and 50.8% oat an OLR of 9.7 kg COD/m^3-d, about 20% higher than the control AnFBR. The specific methanogenic activity (SMA) test, and the rates of maximum specific biogas production (R_m) showed enhanced attached microbial activity due to application of US-energy.

This work estimated the unbiodegradable fraction of PCOD in TWAS using Sötemann et al. (2006) steady-state model for CSTR systems at 28% i.e. 72% of the PCOD was biodegradable. The results provide experimental evidence that the biodegradability of the particulate TWAS is justified since the non-biodegradable fraction of TWAS in batch tests (Elbeshbishy et al. 2015) ranged from around 12% to 27% by previous co-workers.

In this study, five semi-continuous flow anaerobic digesters were also operated to investigate co-digestion performance treating a mixture of food waste (FW) and municipal biosolids (primary sludge and thickened wasted activated sludge) at an SRT of 20 days and

different blend ratios i.e. 0, 10%, 20%, 40% by volume with the fifth digester treating only biosolids at the same COD/N ratio as the 40% FW digester. Over 100 days of operation including sixty days at steady-state at organic loading rates of 2.2 kgCOD/m^3/d to 3.85 kgCOD/m^3/d showed that COD removals were higher for the three co-digesters than for the two municipal biosolids digesters i.e. 61%-69% versus 47%-52%. The estimated extra methane production due to synergistic effects was 13% of the overall COD (COD effluent and COD biogas) in R3 and R4, corresponding to 18%-20% of the overall generated methane gas.

6.2 Limitations of the Current Study

This research mainly focused on the impact of ultrasonication in an AnFBR at 2 different organic loading rates but did not optimize the US-energy as well as reduced scum generation in reactor. Moreover, detailed microbial activities of the various population bacteria such as specific acetogenic and acedogenic activity were not assessed in this study. The relationship between bioparticle size, liquid upflow velocity, bed height, and other hydrodynamic elements were not studied.

In the CSTR system, the unbiodegradable fraction of PCOD estimation was limited to a single SRT or HRT. In the FW co-digestion study, digestion of FW alone could not be investigated due to inherent reactor instability arising from the very high ammonia.

6.3 Recommendations and Future Works

Since the impact of ultrasonication in AnFBR was limited to TWAS only, and scum layer disintegration was not optimized at higher OLR, the following works merit further investigation

- Performance evaluation of other municipal biosolids like primary sludge for U-AnFBR reactor
- Optimization of sonication energy to reduce scum volume at higher OLR
- Optimization of mass of HDPE to maximize the process performance
- To develop a model for anaerobic digestion in the fluidized bed reactor, further investigations are needed including the uses of different size of HDPE
- FW digestion in fluidize bed reactor

Curriculum Vitae

Of ***Mohammad Monirul Islam Chowdhury***

Being an Academic in a world class university where honesty, integrity and hard work are the key to success

Key Skills

- Extensive experience in the university sector spanning various roles: research assistantship, lab analysis, advanced oxidation process (AOP), waste management, waste minimization, soil quality analysis, water quality measurement, wastewater treatment, risk assesment study for disinfection by-products (DBPs) in drinking water, rejection of micropollutants as pharmaceutical active compounds (PhACs) in drinking water.
- Rigorous experiences on laboratory equipments: Gas chromatograph (GC), Ion chromatograph (anion & cation), high performance liquid chromatograph (HPLC), TOC analyzer, UV-vis spectroscopy, turbidimeter, and surface charge analyzer.
- Wide experience in geotechnical investigation, planning & desingning of high-rise building structure, and supervision of civil Engineering projects.
- Highly developed interpersonal skills including team-leadership and conflict resolution.
- Highly developed analytical, strategic and problem solving skills.
- Clear and effective communication across the full range of professional and academic contexts.
- Commitment to higher education and research demonstrated in professional, academic and representative roles.

Scholastic attainments

- Master of Science (Research based) in Engineering from the department of Civil Engineering, King Fahd University Of Petroleum & Minerals
- Bachelor of Science (Honors) in Civil and Environmental Engineering from Shah Jalal University of Science and Technology (SUST), Bangladesh.

Published Journal

- Mingu Kim, Mohammad M Chowdhury, George Nakhla, PhD; Michael Keleman; Synergism of co-digestion of food wastes with municipal wastewater treatment biosolids, Waste management, 2016.
- Mohammad M I Chowdhury, Mingu Kim, Basem Mikhaeil Haroun, George Nakhla, Michael Keleman, Flocculent Settling of Food Wastes, Water Environment Research, 2015.
- Mingu Kim; Mohammad M I Chowdhury; George Nakhla; Michael Keleman, Characterization of Typical Household Food Wastes from Disposers: Fractionation of Constituents and Implications for Resource Recovery at Wastewater Treatment, Bioresource Technology, May 2015.
- Chowdhury, M.M.I. and Vohra, M.S., Photocatalytic Degradation of some charged aqueous phase pollutants Using nafion and silica Modified TiO_2, *Sains Malaysiana*, August 2015.
- Saleh M., Essa M.H., Chowdhury M.M.I., Rahman M., Bashir, Vohra M.S., Application of solar photocatalysis and solar photo-fenton processes for the removal of some critical charged pollutants: mineralization trends and formation of reaction intermediates, Arabian Journal for Science and Engineering, 2015.
- G. M. Munna, M.M.I. Chowdhury, A.A. Masrur Ahmed*, Sadia Chowdhury and M. M. Alom; March 2013; A Canadian Water Quality Guideline-Water Quality Index (CCME-WQI) based assessment study of water quality in Surma River; Journal of Civil Engineering and Construction Technology Vol. 4(3), pp. 81-89.
- M.M.I. Chowdhury*, S. M. Rahman, R. A. Khan, G. M. Munna, Jan 11-13, 2013; The Effects of Climate Change on Food System and Security in Bangladesh and

	Relevant Adaptation Initiatives, International Conference on Engineering Research, Innovation and Education, ICERIE 2013, ICERIE -268. • M.M. I. Chowdhury*, A.A. Masrur Ahmed and G.M. Munna; July 2013; Prioritizing e-waste management needs for mitigating hazardous pollutants in north-east zone, Bangladesh; J. of applied tech. in environmental sanitation, Volume 3 (1), 61-70.
Refereed International Conference Paper	• Md. Monirul Islam, Md Rafiul Hassan, Md. Imtiaz Hossain; "Hybrid Computational IntelligenceTechniques for Accurate Surface Temperature Analysis and Prediction"; Proceeding of the 6th International Conference on Environmental Science and Technology; ICEST, June 25-29, 2012; Environmental Conference Program, American Academy of Sciences; Abstract accepted. • M.S. Vohra*, M.M. I. Chowdhury, and M.S. Al-Suwaiyan; Nov 2-6, 2013; Use of solar radiation energized photocatalytic degradation process for aqueous phase ammonia removal: a case study for Saudi Arabia, 9th Int conference & exhibition on ChemIndix, Bahrain.
Work Experiences	
Position Held	Research Engineer Duration: 16 Jun, 2010 – till date
Job description	Heavy equipment operation and maintenance, lab analysis, conducting water & wastewater engineering lab, conducting project work's experiments.
Name & address of employer	Civil Engineering Department, King Fahd University of Petroleum & Minerals, Dhahran, Saudi Arabia.
Position held	Research Assistant Duration: 13 Feb, 2008 – 15 Jun 2010
Job description	Water Quality measurement (BOD, COD, Alkalinity, TSS, SS, Total hardness, pH, Ca, Mg, Sedimentation, Adsorption, Filtration,) Grading of undergraduate student
Name & address of employer	Civil Engineering Department, King Fahd University of Petroleum & Minerals, Dhahran, Saudi Arabia.
Position held	Research Engineer (Part Time) Duration: 14 Nov 2008 – 15 Jun 2010
Project Title	• National Inventory of Anthropogenic Emissions by Sources and Removal by Sinks of Greenhouse Gases (GHG) not Controlled by the Montreal Protocol – Year 2000. • Environmental Monitoring for Seismic and Exploratory Drilling Operations in LUKSAR Contract Area, North Rub Al-Khali Desert, Saudi Arabia. • Environmental Monitoring for Exploratory Drilling Operations in SSG Contract Area, North Rub Al-Khali Desert, Saudi Arabia
Job description	Data analysis of green house gases, Water quality monitoring such as BOD, COD, TOC, NH_4 test, Chemical Exchange Capacity (CEC) analysis.
Name & address of employer	Centre for Environment & Water, Research Institute, King Fahd University of Petroleum & Minerals, Dhahran, Saudi Arabia.
Position held	Assistant Engineer Duration: Dec 2005 - Feb 2008
Job description	Geotechnical Investigation, Planning & designing of high-rise building structure and Supervision of Civil Engineering projects
Name & address of employer	Engineering Planning Consultancy Team (EPCT), 27/3 Jalalabad Abashik Elaka, Sylhet-3100, Bangladesh
Position held	Field Investigator Duration: Jun 2005 - Nov 2005

Project Title	Delineation of slum boundary in six-city corporation of Bangladesh
Job description	Mapping slum settlement and collecting information from slum dwellers
Name & address of employer	**University of North Carolina at Chapel Hill, USA & Center for Urban Studies**, **Bangladesh,** House # 59/B, Road # 16 (new), Dhanmondi RA, Dhaka -1209.

Position held	Junior Engineer (Part Time) Duration: March 2002 - March 2004
Project Title	Community Participated Rural Piped Water Supply
Job description	Conducting the feasibility study of the project, ensuring community participation and design the components of water supply system.
Name & address of employer	**Mothers' Society NGo(financed by World Bank)**, Shah Jalal Uposhahar, Sylhet - 3100, Bangladesh.

Position held	Part Time Faculty Duration: March 2005 - Feb 2008
Job description	Taking Basic English language and IELTS classes
Name & address	Mentors' Education, Zindabazar, Sylhet -3100, Bangladesh.

Professional Experiences	Training for Lab-in-charge Duration: April 2010 – Jun 2010 (Environmental Engineering lab)
Job description	• Preparation of experiments (CE 370) including reagent preparation and experimental setup. • Analysis and uses of Ion Chromatograph, Total Organic Carbon, Atomic Absorption Spectrophotometer, UV Spectrophotometer, High Performance Liquid Chromatograph, pH meter, DO meter, Oven, Furnace, GC chromatograph, surface charge analysis, centrifuge.

Linguistic skills	English, Bangla, and Hindi.

Computer skills	Programming Languages: C Operating Systems: Windows, Linux Simulation Software: Neural Network Office Software: MS word, MS Visio, MS Excel, MS PowerPoint and MS Access Aplication Packages: Adobe PhotoShop, AutoCAD, Arc View (GIS), ArcGIS, Mathmatica, and Excel.

Organizational achievements	Member of IEB, Bangladesh; Tourist Club, SUST, Sylhet, Bangladesh.

Extra-curricular activities	• English Debating • Playing Cricket, Chess, Badminton, Table tennis, and Football.