

ABSTRACT

WANG, LING. Anaerobic Co-digestion of Thickened Waste Activated Sludge with Grease Interceptor Waste. (Under the direction of Dr. Francis Lajara de los Reyes III and Dr. Tarek Aziz.)

Municipal water and wastewater treatment systems are extremely energy-intensive. As a result, efforts have been undertaken to facilitate energy independence at wastewater treatment facilities (WWTFs). Achieving this goal calls for not only improvement of efficiency in existing treatment processes, but also onsite energy production from alternative energy supplies. Anaerobic digestion produces biogas that consists of methane, carbon dioxide, and other constituents. This renewable source of bioenergy presents a great potential for cost-effective biogas utilization at WWTFs. However, most of the WWTFs operating digesters flare excess biogas instead of employing utilization technologies. Biogas production enhancement thus plays an essential role in improving the economic feasibility of anaerobic biotechnology at WWTFs.

In this study, substantial enhancement in biogas production was achieved by anaerobic co-digestion of thickened waste activated sludge (TWAS) with grease interceptor waste (GIW) from a food service establishment (FSE). The experiments were conducted to co-process TWAS and the entire content of the grease interceptor (GI) including fat, oil, and grease (FOG), food residuals, and associated wastewater in lab scale semi-continuous digesters. Stepwise increase of GIW addition was employed to identify the maximum methane production and the corresponding threshold input of GIW that leads to methanogenic inhibition. The highest GIW loading rate achieved without digester failure was 20 vol%, or 65.5 % volatile solid (VS). This GIW input concentration enhanced the methane yield from 0.180 to 0.752 L-CH₄/g-VS added, biogas production from 2.2 to 13.7 L/d, and

methane content from 60.2% to 70.1%. The 0.752 L-CH₄/g-VS added methane yield was the highest value reported for co-digestion of lipid-rich substrates. The substantial enhancement in methane yield was likely due to the stepwise increase of co-substrate addition as it provided longer time for microbial acclimation and reduced the GIW inhibitory effect. The threshold limit for GIW addition leading to an inhibited digestion process was identified in range of 20 to 40 vol% or 65.5 to 83.5 %VS. Further investigation on the exact nature and inhibition mechanism of GIW is needed for a successful full scale application of GIW co-digestion at WWTFs.

Aside from contributing to a significant enhancement of biogas production, stepwise increase of GIW addition also produced more robust methanogenic communities in surviving higher GIW loading rate. Microbial communities fed with increasing GIW loading rate were able to survive 20 vol% GIW input; while in microbial communities that have never experienced a GIW addition, a sudden input of 20 vol% GIW resulted in a quick failure in the digestion process. Moreover, with a onetime GIW loading "shock" that led to a digester failure with 40 vol% GIW addition, the recovered microbial communities were able to reach the same biogas production as before when the GIW loading rate was again increased to 20 vol%. These demonstrate the robustness of the "trained" methanogenic communities through stepwise increase of GIW addition. The recovered microbial communities are expected to sustain a higher GIW loading rate above 40 vol% after the onetime 40 vol% GIW loading that caused digester failure. Furthermore, in this study the alkalinity to total volatile acid ratio was identified as an unsuitable parameter for GIW inhibition monitoring. Instead, monitoring results of methane yield successfully identified the digester failure in earlier stage compared to all the other parameters by showing an apparent drop in concentration.

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Anaerobic Co-digestion of Thickened Waste Activated Sludge with Grease Interceptor Waste

by
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DEDICATION

This thesis is dedicated to God my heavenly Father, for His mercy and grace that have given me new birth through the resurrection of His son, Jesus Christ; for His blessing and deliverance that restore my soul and spirit; for His comfort and guidance that lead me in paths of righteousness; for He is my rock, my shield, my strength, my hope, and the source of all my joys; for He listens my prayers and sees my affliction and distress; for He is my shepherd and all I ever need.

BIOGRAPHY

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Chapter 1 Introduction

1.1 Overview

Municipal water and wastewater treatment systems are extremely energy-intensive, accounting for up to 3% of the total U.S. electricity consumption. This energy requirement represents approximately \$4 billion in costs, 56 billion KWh of energy consumption, and 45 million tons of greenhouse gases emission annually (EPRI, 2002; U.S. EPA, 2009; U.S. EPA, 2010a). As climate change and rising energy demand focus attention on energy management at critical infrastructure, efforts have been undertaken to facilitate energy independence at wastewater treatment facilities (WWTFs) (U.S. EPA, 2008; U.S. EPA, 2009). Achieving zero-net energy at WWTFs often calls for not only improvement of efficiency in existing treatment processes by upgrading or implementing new devices, but also onsite energy production from alternative energy supplies (U.S. EPA, 2010b).

Anaerobic biotechnology has been applied to solids management as one of the most essential steps in wastewater treatment processes. Anaerobic digestion provides many advantages such as reduction of waste biosolids disposal costs, elimination of aeration energy requirements, and most importantly, additional bioenergy production (Speece, 1996; van Staikenburg, 1997). Anaerobic biotransformation of organic matter in the absence of oxygen produces bioenergy in the form of biogas that consists of methane, carbon dioxide, and other constituents. This renewable source of bioenergy generated during the anaerobic digestion process presents the WWTFs a feasible option for sustainable energy development. Anaerobic digestion produces 0.35 m^3 of methane in theory, approximately 12,000 BTU, per

kg of chemical oxygen demand (COD) destroyed at 0 °C and 760 mm-Hg pressure (STP). This amount of energy is a great potential for cost-effective biogas utilization as a source of renewable fuel for WWTFs to generate thermal energy and electricity for onsite use or export of surplus electricity back to the grid.

However, of the 544 operational WWTFs with influent flow rates greater than 5 million gallon per day (MGD) employing anaerobic digestion in the United States, only about 19% utilize biogas to offset onsite energy demand and/or generate electricity as identified by the 2004 Clean Watersheds Needs Survey (CWNS) (U.S. EPA, 2007a). A more recent update by the 2008 CWNS stated that of the 1,351 WWTFs with influent flow rates greater than 1 MGD operating anaerobic digesters, only 15% employ biogas utilization technologies, excluding those installed with combined heat and power for concurrent production of useful thermal and electrical energy (U.S. EPA, 2011). Moreover, it was found that smaller WWTFs have less tendency to operate anaerobic digesters for solids management, let alone implement biogas utilization. At some smaller facilities between 1 and 5 MGD with anaerobic digesters, biogas utilization is still viable with adequate biosolids loadings. Some WWTFs blend the biogas with purchased natural gas when the volume of biogas is not sufficient to offset all or most of the onsite energy requirements for digester or space heating. However in many cases, smaller facilities operating anaerobic digesters often find it less economically or operationally appealing than flaring excess biogas (U.S. EPA, 2007a; U.S. EPA, 2011). As a result, biogas production enhancement plays an essential role in improving the economic feasibility of anaerobic biotechnology for sustainable energy independence at WWTFs, especially for those treating smaller influent flow rates.

Substantial enhancement in biogas production can be achieved by anaerobic co-digestion of biosolids with substrates of high bioenergy potential such as FOG waste streams. The term FOG generally refers to a layer of lipid-rich material generated during cooking and food processing. More specifically, FOG wastes can be divided into (a) mineral oils including petroleum and hydrocarbon (petroleum-based, non-polar FOG) and (b) materials of vegetable and animal origin (animal- and vegetable-based, polar FOG) (U.S. EPA, 2004a). Petroleum-based FOG (gasoline, kerosene, fuel oil, and motor oil, for example) consists of a mixture of hydrocarbons such as paraffins, cycloparaffins, and aromatic compounds (e.g. benzene and toluene) generated from industries using oil and grease such as automotive-related facilities (Groenewold et al., 1982; Pawlak et al., 2008). Moreover, petroleum-based FOG is generally not compatible with either aerobic or anaerobic treatments as it tends to coat the organisms and cause reduction in process effectiveness (U.S. EPA, 2004a). Animal- and vegetable-based FOG, on the other hand, is readily biodegradable and mostly produced from restaurants, residences, food processors, and food-based industries (U.S. EPA, 2004a). Animal- and vegetable-based FOG is formed from the combination of glycerol and several different fatty acids such as stearic acid, oleic acid, and palmitoleic acid. Individual fatty acids differ in terms of carbon chain length and number of unsaturated bonds, and thus result in various physical and chemical properties of the corresponding animal- and vegetable-based FOG (Groenewold et al., 1982; Lam et al., 2010).

In addition to waste animal fats from food-based industries and waste industrial FOG from food processors, other major sources of animal- and vegetable-based FOG are food service establishments (FSEs) such as restaurants and fast food outlets. FSEs refer to those

places or locations, whether permanent, temporary, seasonal or itinerant, primarily engaged in activities of preparing, serving, or otherwise making available for consumption foodstuffs. One or more of the following preparation activities might involve during these processes: cooking by frying, baking, grilling, sautéing, rotisserie cooking, broiling, boiling, blanching, roasting, toasting, or poaching, infrared heating, searing, barbecuing, and any other food preparation or serving activity. These activities produce a consumable food product in or on a receptacle requiring washing to be reused (City of Rocky Mountain, 2009; Tennessee Department of Health, 2005). As a comparison, food processing establishments refer to those commercial establishments (aside from FSEs, retail food stores, and commissary operations) where food is processed, manufactured or packaged for human consumption and is not provided directly to the consumer (Tennessee Department of Health, 2005; New York City, 2004).

Although animal- and vegetable-based FOG is relatively biodegradable compared to petroleum-based FOG, its discharge can still lead to damages to collection systems, pumping stations, and all the way through the treatment facilities as it accumulates and congeals on pipe walls, forming hardened deposits through a chemical reaction or a physical aggregation process (He et al., 2011; NCDENR, 1999; U.S. EPA, 2004a). Polar FOG of animal and vegetable origin results in the greatest threat of obstruction in sewer lines, responsible for up to 47% of the reported blockages and 50 to 75% of sanitary sewer overflows as it tends to solidify, reduce conveyance capacity, and eventually block flow in sewer line system (Keener et al., 2008; Southerland, 2002; U.S. EPA, 2003; U.S. EPA, 2004a; U.S. EPA, 2004b). Since 2002, Kansas City has experienced approximately 1,294 illegal sewer

overflows and has agreed to pay over \$2.5 billion over 25 years for extensive improvements to its sewer systems, and an additional civil penalty of \$600,000 to the United States due to its violation of the federal Clean Water Act and the terms of the city's National Pollution Discharge Elimination System permits (U.S. Department of Justice, 2010). In 2000, the City of Raleigh, NC budgeted \$100,000 for program set-up and \$50,000 annually for implementation of FOG education program (U.S. EPA, 2004b). The San Francisco Public Utility Commission also estimated \$3.5 million annual costs for service calls for grease-related problems in the city (Missouri Department of Natural Resources, 2011; Greer, 2010).

At FSEs, grease abatement devices such as grease interceptors (GIs) and grease traps (GTs) are commonly installed to prevent polar FOG discharge from entering the collection systems. The entire contents of a GI or GT comprising FOG, food particles, and associated wastewater produced at FSEs are collectively referred to as grease interceptor waste (GIW) or grease trap waste (GTW). A GT is a small reservoir installed inside the FSEs with a typical volume of around 50 gallons, while a GI is a vault built outside of the building with a larger volume ranging from 1,000 to 2,000 gallons (Long et al., 2011). These grease abatement devices retain and accumulate FOG and food particles before they enter the collection systems. Adequate residence time is provided to separate them through gravity sedimentation, allowing the grease to congeal and float to the top of the basin, the solids to settle to the bottom of the basin, and the remaining wastewater to continuously flow down to the collection systems (Aziz et al., 2011; Chapin, 2008; Gallimore et al., 2011). As a result, GIW/GTW can be roughly divided into three layers: top layer of FOG, middle layer of associated wastewater, and bottom layer of settled food particles.

1.2 GIW/GTW disposal

GIW/GTW disposal has always been an issue due to its ubiquity and problematic characteristics; hence, illegal discharges of grease from FSEs are not rare cases. Authorities have enforced measures such as fines for violations, rewards for illegal discharge reports, and monthly surcharges added to restaurant sewer bills ranging from \$100 to \$700 and more (U.S. EPA, 2007b). For instance, beginning on Oct. 1, 2010, the City of Raleigh offered a \$1,000 reward for identification of large illegal grease discharges (City of Raleigh, 2010). New York City also instituted a \$1,000-per-day fine for violation after identifying a 73% noncompliance rate with its restaurants ordinance (U.S. EPA, 2007b).

To prevent violations of regulations, it is in the best interest of the food service business owners to perform periodic cleaning and maintenance for their GIs or GTs to reduce the likelihood of back-ups or blockages within their building sewer line (Colorado Department of Public Health and Environment, 2002). In most cases, professional pumping service contractors/grease waste haulers provide GIW/GTW removal service by transporting them to (a) rendering/tallow plants for recycling into beneficial products such as animal feed additives, soaps, cosmetics, and composts, (b) municipal solid waste treatment plants for landfilling, land application, composting, or incineration, or (c) WWTFs for biodiesel production through chemical conversion and purification or for biogas production through anaerobic co-digestion process (Colorado Department of Public Health and Environment, 2002; Missouri Department of Natural Resources, 2011; NCDENR, 1999; U.S. EPA, 2007b).

Though GIW/GTW can be treated and recycled in a variety of applications, only biodiesel and digester biogas provide sustainable production of bioenergy from GIW/GTW.

Nevertheless, the current commercial homogeneous base-catalyzed transesterification process for biodiesel production utilizes refined vegetable oils derived from agricultural crops as the main feedstock (Lam et al., 2010; Montefrio et al., 2010). The homogeneous base catalysts such as sodium hydroxide (Leung and Guo, 2006) and potassium hydroxide (Demirbas, 2009) are not applicable to waste cooking oil from FSEs as its high water and free fatty acids contents can negatively affect the process performance (Lam et al., 2010). Other attempts proposed for biodiesel production from waste cooking oil include homogeneous acid catalysts such as sulfuric acid (Wang et al., 2006), two-step homogeneous acid and base catalysts (Patil et al., 2010; Wang et al., 2006), heterogeneous base catalysts such as calcium oxide (Kouzu et al., 2008) and calcium and zinc mixed oxide (Dehkordi and Ghasemi, 2012), heterogeneous acid-catalysts such as zirconium oxide (Park et al., 2008) and aluminium hydrogen sulfate (Ramachandran et al., 2011). Nevertheless, obstacles including additional pretreatment that adds on the expense on chemicals, undesirable soap formation, generation of large amount of wastewater, corrosion on equipment from using corrosive chemicals, long reaction time, and intensive energy requirement still remain (Lam et al., 2010). As these obstacles limit the biodiesel production from waste cooking oil collected directly from FSEs, biodiesel conversion of FOG from GI installed at FSEs also has its barriers. Due to the presence of high moisture content and food residuals in GIs, more free fatty acids in FOG portion are formed through hydrolysis. The hydrolysis of FOG is catalyzed by the lipase activity which is mostly associated with the presence of food residuals, either as endogenous lipase in the food particles or via microbial reaction (Montefrio et al.,

2010). The presence of food residuals in GI results in a more complex and expensive biodiesel conversion process for FOG from GIs.

In addition, since only the FOG component of GI/GT is refinable for biodiesel production, converting FOG waste into biodiesel leaves the remaining waste disposal of food residuals and wastewater a challenge. A BlackGold Biofuels' 250k FOG-toFuel® system was proposed for a FOG-to-biodiesel installation at Missouri Bissell Point WWTF (Missouri Department of Natural Resources, 2011). The system includes (a) a dewatering process to remove water portion from inlet FOG waste streams (GIW/GTW, grease from dissolved air flotation unit at WWTFs, and scum from internal WWTF processes), and (b) a chemical conversion and purification process to convert the processed dry FOG into biodiesel. It is clear that processing FOG wastes into dry grease and eventually biodiesel is feasible with proper pretreatment and chemical reaction process, but often comes with the generation of liquid and solid waste byproducts as new waste streams.

Anaerobic co-digestion on the other hand, is able to address the entire content of GIs/GTs and utilizes locally available digesters. In most cases, several advantages can be obtained by performing anaerobic co-digestion, such as increased biogas production enhancement due to additional sources of biodegradable materials, degradation improvement of substrates due to positive synergisms, moisture or nutrients supplies from co-substrates, treatment of toxic compounds by co-metabolism, and financial benefits when treatment for co-substrates is eliminated (Bond et al., 2012; Long et al., 2011; Mata-Alvarez et al., 2000). Co-processing sewage sludge with an extensive range of organic wastes has been demonstrated by previous studies, including organic fraction of municipal solid waste (Del

Borghetti et al., 1999; Sosnowski et al., 2003; Stroot et al., 2001), manure (Rao and Baral, 2011), confectionery waste (Lafitte-Trouqué and Forster, 2000), brewery sludge (Babel et al., 2009), food waste (Habiba et al., 2009; Heo et al., 2003; Kim et al., 2011; Murto et al., 2004), and coffee waste (Neves et al., 2006). Past experiences have also demonstrated the feasibility of GIW/GTW co-digestion in lab scale (Kabouris et al., 2009a; Kabouris et al., 2009b; Luostarinen et al., 2009; Wan et al., 2011), pilot scale (Davidsson et al., 2008), and full scale implementations at Millbrae WWTF, Ca. (1.6 MGD), Essex Junction Wastewater Treatment Facility, Vt. (3.3 MGD), Johnson County Wastewater, Kan. (14.5 MGD), and Pinellas County South-Cross Bayou Water Reclamation Facility, Fla. (33 MGD) (FOG Energy Corporation, 2010; Johnson et al., 2011).

During the wastewater treatment process, emissions of carbon dioxide (CO₂), the ultimate product from the transformation of organic materials, along with the onsite energy consumption, are inevitable tradeoffs. Nevertheless, for WWTFs operating anaerobic digesters, instead of wasting produced methane by flaring it into the atmosphere, enhancement of the existing biogas generation through anaerobically co-digesting GIW/GTW can translate into energy savings when proper biogas utilization technologies are employed, and also serve as a sustainable GIW/GTW disposal alternative. The increase in amount of biogas produced from co-digestion ranges from 9% to 197% depending on the GIW/GTW loading rates (Davidsson et al., 2008; Kabouris et al., 2009a; Luostarinen et al., 2009; Wan et al., 2011). If this amount of bioenergy is properly utilized at WWTFs to offset the onsite energy requirement, reduction of global warming potential of CO₂ emissions from WWTFs due to additional electricity supply can be achieved. The integration of biosolids

management, GIW/GTW biodegradation treatment, and sustainable bioenergy production presents a great financial incentive to promote energy independence at WWTFs as an environmentally and economically beneficial biotechnology.

1.3 Annual production and energy potential of GIW/GTW

To compare the annual production and energy potential of FOG waste streams, data obtained from five previous studies are displayed in Table 1-1. According to the technology evaluation report conducted for the City of San José (City of San José, 2007), restaurants and food processors in the San José/Santa Clara Water Pollution Control Plant service area, California, produced approximately 10,000 tons of FOG annually. This was an estimate of only FOG portion, excluding food solids and wastewater in the GIW/GTW, but including FOG from food processing facilities. Based on the FOG density of 7.3 lb/gal (City of San José, 2007), a person living in the area generated approximately 3.10 gallons of FOG annually. On the other hand, a waste grease resource assessment conducted within 30 metropolitan areas in the U.S. determined a FOG production of 13.37 lb/person/yr or 1.60 gal/person/yr (Wiltsee, 1998). This estimate included FOG portion from restaurants and additional trapped FOG collected at the WWTFs. A more recent research conducted for the Wake County, North Carolina metropolitan area estimated a GIW/GTW production (the complete content of GIs/GTs) of 16.16 million gal/yr or 18.65 gal/person/yr (Austic, 2010). The amount of FOG (3.10 gal/person/yr) generated from the City of San José (2007) was much higher than that from Wiltsee (1998) (1.6 gal/person/yr) due to its additional waste stream from food processing facilities. Based on these estimates, up to 5.4 billion gallons or

45 billion pounds of GIW/GTW (in average of 18.65 gal-GIW/GTW/person/yr and 16.0 gal-FOG/person/yr, assuming a 10 vol% FOG content within GIW/GTW) are generated annually in the U.S., with a population of 314 million (U.S. Census Bureau, 2012). This estimate of U.S. annual GIW/GTW production is consistent with the data obtained from FOG Energy Corporation (2012a) that stated 4.5 billion pounds of FOG, or 45 billion pounds of GIW/GTW (assuming 10 vol% FOG contained in GIW/GTW), are produced annually.

GIW/GTW is an energy dense material with energy content ranging from 7,000 to 10,000 BTU/lb when dewatered, solidified, and burned as a substitute for wood (6,500 BTU/lb) or coal (12,000 BTU/lb) (Dayton, 2010). For FOG portion in GIW/GTW, up to 90,664 BTU per gallon or 10,845 BTU per pound of FOG processed can be extracted, compared to methanol (65,000 BTU/gal), ethanol (85,000 BTU/gal), and biodiesel (126,000 BTU/gal) as refined liquid fuels (FOG Energy Corporation, 2012b) (Table 1-1). Based on an average 9,282 BTU/lb of energy content for dewatered GIW/GTW, an assumption of 50 vol% water portion within GIW/GTW, and a national production of 45 billion pounds of GIW/GTW per year, this energy potential equates to approximately 211 trillion BTU or 62 billion kWh/yr in the U.S., with an economic value of \$0.92 billion at a natural gas cost of 4.37\$/MMBTU (U.S. EIA, 2010a) and \$5.5 billion at an electricity cost of 0.089 \$/kWh (U.S. EIA, 2010b).

Table 1-1 Annual production and energy potential of FOG waste streams.

Reference	City of San José, 2007	Wiltsee, 1998	Austic, 2010	Dayton, 2010	FOG Energy Corporation, 2012a,b	
Area	San José/Santa Clara Water Pollution Control Plant service area, Ca	30 metropolitan areas	Wake County, NC metropolitan area	-	U.S.	
FOG stream	FOG portion from restaurants and food processing facilities	FOG portion from restaurants and trapped grease at WWTFs	GIW/GTW	Dewatered GIW/GTW	FOG portion from restaurants	
Population	973,672 ^a	-	866,410	-	-	
FOG or GIW/GTW production	(ton/yr) (lb/yr) (gal/yr) (lb/person/yr) (gal/person/yr)	10,000 22,050,000 ^b 3,020,548 ^b 22.6 ^c 3.10 ^c	- - - 13.37 1.60 ^d	- - 16,156,800 - 18.65	- - - - -	- - - - -
Estimated U.S. GIW/GTW production	(gal/yr) (lb/yr)	- -	5,434,794,890 ^e 45,434,885,279 ^d	- -	- 4,500,000,000	
Energy content	(BTU/lb) (BTU/gal)	16,800 -	- -	- -	7,000-10,000 10,845 ^d 90,664	
Density (lb/gal)		7.3	-	-	-	

^a California Energy Commission, 2007.

^b Estimated based on FOG density 7.3 lb/gal.

^c Estimated based on the population.

^d Estimated based on the assumption of 8.36 lb/gal density.

^e Estimated based on the average GIW/GTW production of Wiltsee, 1998 (assuming 10 vol% FOG contained in GIW/GTW) and Austic, 2010, and U.S. population of 313,761,664 in 2012 (City of San José, 2007 was excluded due to its excess amount of FOG from food processing facilities).

Anaerobic digestion itself theoretically produces 350 L of methane, approximately 12,000 BTU, per kg of COD destroyed at STP. Co-digestion of the highly digestible waste and/or wastewater provides the anaerobic digestion process additional biodegradable materials with high bioenergy potential, and therefore enables substantial biogas production improvement. Labatut et al. (2011) investigated the biomethane potential of 175 organic substrates using biochemical methane potential assay. The specific methane yield defined as the total volume of methane produced per amount of volatile solid (VS) in substrate initially added (i.e. L-CH₄/g VS added) was applied to quantify the biomethane potential for each sample. It was found that substrates rich in lipids such as suspended and settled FOG were able to yield up to 0.402-0.413 L-CH₄/g VS added, higher than most of the substrates studied. Lab scale experiments also confirmed the feasibility of achieving higher methane yield ranging from 0.295 to 0.473 L-CH₄/g VS added or 9% to 197% increase in methane production by co-digesting sewage sludge with FOG waste streams (Davidsson et al., 2008; Kabouris et al., 2009a; Luostarinen et al., 2009; Wan et al., 2011).

For a 7 MGD WWTF that operates four anaerobic digesters treating a total 1.5 million gallons of sewage sludge monthly, the total biogas production is approximately 4.6 million ft³ on a monthly basis (data based on South Durham Water Reclamation Facility Sludge Report, July 2011). Assuming an average 103% increase in methane production, 66% methane content, and a methane energy content of 35.8 KJ per liter of methane through anaerobic co-digestion of GIW/GTW, this will yield an annual methane production of 2.2 billion liters, equivalent to 78 billion KJ, or 22 million kWh of energy produced per year. From an economic and environmental perspective, this energy produced equates to

approximately \$0.3 million at a natural gas cost of 4.37\$/MMBTU (U.S. EIA, 2010a), 1.9 million at an electricity cost of 0.089 \$/kWh (U.S. EIA, 2010b), and 15,000 tons of greenhouse gases emission that can be avoided annually at 0.676 tons/MWh CO₂ emission factor of electricity (U.S. EIA, 2007). This improvement presents a great potential for the WWTFs to reach sustainable energy management with the enhanced bioenergy generation through the beneficial reuse of GIW/GTW.

1.4 Characteristics of GIW/GTW streams

Waste pumping service contractors typically collect GIW/GTW along with wastes from sand traps and septic tanks in the same truck, resulting in commingled wastes with various characteristics (Colorado Department of Public Health and Environment, 2002). Furthermore, rather than a single lipid compound, GIW/GTW generated at FSEs is a complex mixture of animal- and vegetable-based oils, lipids, greases, food solids, and possibly soaps and detergents, along with many other materials with various origins and concentrations. The characteristics of this complex mixture vary with the type of FSE, and configuration and maintenance frequency of GIs/GTs, which also change with time (de los Reyes and He, 2009; Lesikar et al., 2006).

Aside from its diverse properties, characterization of GIW/GTW has also been challenging because of the inconsistency in previous studies when describing their FOG waste streams obtained for analysis. Table 1-2 shows the interpretations of FOG waste sources from previous literatures. In this study, FOG wastes are roughly classified into three categories: (a) GIW/GTW from FSEs including FOG, food solids, and wastewater portions,

(b) waste animal fats from food-based industries, and (c) waste industrial FOG from food processors. In Table 1-2, sources of FOG wastes in previous studies are interrelated based on the following assumptions: (a) unless the authors stated otherwise, the "FOG" from GTs/GIs would include the food particles and wastewater portions, (b) if the co-substrates were dewatered then a portion of the wastewater would have been removed, and (c) grease wastes obtained from a FOG receiving/handling facility would potentially contain all sources of FOG wastes collected from FSEs, food processors, and food-based industries.

Table 1-3 tabulates the characteristics of various FOG waste sources. Comparing the three main categories, FOG waste from food-based industries had the highest total solid (TS) concentrations (Battimelli et al., 2010), followed by that from food processing facilities (Luostarinen et al., 2009) and GIW/GTW from FSEs (Davidsson et al., 2008). The FOG portion within GIW/GTW also contained high amounts of total solids (TS) and VS due to its high lipid content (Li et al., 2011). Wan et al. (2011) best described the properties of a mixed waste from all sources, as the combined water portion might have diluted the overall solid content. GIW/GTW from FSEs contained 17.3% TS of which 98% is volatile (Davidsson et al., 2008); these results correspond to a typical GIW/GTW TS range of 1% to 20% of which 88% to 99% is volatile (Johnson et al., 2011). Dewatered co-substrates typically consist of 30% to 50% of which 85% to 98% is volatile (Johnson et al., 2011), which is in concord with the analytical results of Kabouris et al. (2009a). Trapped grease waste from WWTFs (Bouchy et al., 2012; Martín-González et al.; Silvestre et al., 2011) tend to have lower solid concentrations due to dilution effect as it is difficult to separate this kind of influent FOG from wastewater (Austic, 2010). There is no significant difference in pH observed among

samples, generally ranging from pH 4 to 5. Others such as COD, alkalinity, total volatile acids (TVAs), carbohydrate, protein, total nitrogen, ammonia, and total phosphorous are not common parameters for analysis.

Table 1-2 Interpretations of FOG waste sources.

Reference	Co-substrates	
	Source stated in literature	Interpretation
Wan et al., 2011	FOG obtained from a FOG receiving facility	All sources of FOG wastes: GIW/GTW (from FSEs, entire content), waste industrial FOG (from food processors), and waste animal fats (from food-based industries)
Kabouris et al., 2009a	FOG from a FOG dewatering facility handling FOG from GTs	Dewatered GIW/GTW from FSEs (with FOG and food particles, without wastewater portion)
Luostarinen et al., 2009	Grease trap sludge obtained from a meat processing plant	Waste industrial FOG from food processors (not GIW/GTW from FSEs)
Davidsson et al., 2008	Sludge from grease traps	GIW/GTW (from FSEs, including FOG, food particles, wastewater)
Battimelli et al., 2010	FOG from slaughterhouses	Waste animal fats from food-based industries
Li et al., 2011	FOG from a garbage oil receptacle of a restaurant (a mixture of waste frying oil, bacon grease, and animal fat)	FOG portion from a FSE
	Synthetic kitchen waste of a mixture of potato, strawberry, orange, tomato, chicken breast, apple, green peas, cabbages, pork, and distilled water	Food particles portion from a FSE

Table 1-3 Characteristics of various FOG waste sources.

Reference	Total solids (TS, %)	Volatile solids (VS, %)	VS/TS (%)	Density (g/mL)	COD (g/L)	pH	Alkalinity (mg-CaCO ₃ /L)	Total volatile acids (TVAs, mg-acetic acid/L)	Carbohydrate (g/L)	Protein (g/L)	FOG content (g/L)	Total nitrogen (g-N/L)	Total kjeldahl nitrogen (TKN, g-N/L)	Ammonia (mg/L)	Organic nitrogen (mg-N/L)	Total phosphorus (mg-P/L)
Wan et al., 2011	3.2	3.0	93.9%	nd ^c	nd	4.2	nd	857.4	nd	nd	nd	nd	nd	nd	nd	nd
Kabouris et al., 2009a	42.4	40.9	96.5%	nd	1211	4.03	nd	3473 mg-COD/L	60.1	29.7	319.3	5.4	5.1	356	0.01 mg-N/ng-VS	670
Luostarinen et al., 2009	25.4	25.2	99%	nd	nd	5.10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Davidsson et al., 2008	17.3	17.0	98%	nd	nd	4.38	nd	nd	nd	nd	nd	nd	nd	136	nd	nd
Battimelli et al., 2010	15.5-93.5	13.8-93.4	89%-100%	nd	410-1680	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Li et al., 2011	94.7	94.2	99.6%	1.01	nd	4.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	12.4	11.4	92.1%	1.11	nd	4.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Silvestre et al., 2011 ^a	7.5-16.0	6.3-14.3	80%-89%	nd	177-321	nd	nd	nd	nd	nd	15-100	nd	3.166-4.287	348-659	nd	nd
Martín-González et al., 2011 ^b	11.66	10.39	89%	nd	nd	nd	nd	nd	nd	nd	50%	nd	2.4%	nd	nd	nd
Bouchy et al., 2012 ^a	7.5-16.0	6.3-14.3	nd	nd	177-335	nd	nd	nd	nd	nd	15-100	nd	nd	nd	nd	nd

^a FOG source: trapped grease waste from the dissolved air flotation unit of a WWTF.

^b FOG source: FOG waste from a WWTF.

^c nd: not determined.

1.5 Anaerobic co-digestion performance

Lab and pilot scale experiments have demonstrated the feasibility of co-digesting sewage sludge with FOG-rich wastes. Table 1-4 presents a comprehensive comparison of anaerobic co-digestion feedstock and performance. Parameters for performance comparison such as co-substrate concentration, organic loading rate (OLR), methane yield, biogas production, percent increase in methane yield, CH₄ and CO₂ contents, and VS and COD reduction rates are described as follow.

- (a) Co-substrate concentration: the amount of VS concentration in co-substrate per amount of VS concentration in the entire substrate (feedstock and co-substrate) added, i.e. %VS.
- (b) OLR: the amount of VS added per volume of the digester sludge treated per day, i.e. kg-VS/m³ day.
- (c) Methane yield: the total volume of methane produced per amount of co-substrate added, i.e. L-CH₄/g-VS added. Since the digester volumes, solid retention time (SRT), and OLR employed for anaerobic co-digestion vary in previous literatures, methane yield can better help quantify and compare the methane production compared to biogas production.
- (d) Biogas production: the amount of biogas containing methane, carbon dioxide, and other constituents produced from a treated reactor per day, i.e. L/d.
- (e) Percent increase in methane yield: percent increase in methane yield between treated (with co-substrate) and control (single substrate) reactors. Note that comparing the methane production of treated reactor to that of the control reactor

might be "unfair" due to the inconsistency in OLRs applied for the control reactors in previous studies.

- (f) CH₄ or CO₂ contents: the volume of CH₄ or CO₂ contained in produced biogas in percentage.
- (g) VS and COD reduction rates: the amount of VS or COD destroyed per amount of VS or COD added.

Anaerobic co-digestion of various FOG wastes produced methane ranging from 0.295 to 0.598 L-CH₄/g VS added, 61% to 70 % methane content, 31% to 67% VS reduction rate, and 29% to 58% COD reduction rate (Davidsson et al., 2008; Kabouris et al., 2009a; Luostarinen et al., 2009; Wan et al., 2011). Comparing the highest co-substrate loading rates in previous studies, results of 47.6 % VS (Kabouris et al., 2009a) and 46 % VS (Luostarinen et al., 2009) co-substrate additions yielded the similar methane productions of 0.473 L-CH₄/g-VS added and 0.463 L-CH₄/g-VS added, respectively, even with different sources of co-substrates (one was dewatered GIW/GTW from FSEs, and the other was waste industrial FOG from food processing facilities, respectively). Their % increases in methane yields, however, varied because of the differences in baselines from the control reactors.

Wan et al. (2011) conducted the anaerobic co-digestion experiment with high co-substrate addition (64 % VS). The employed OLR (2.34 kg-VS/m³ day) was relatively low, however, due to its low VS concentration in co-substrate (VS of 3.0%). The high co-substrate concentration resulted in the highest methane yield reported (0.5984 L-CH₄/g-VS added). The co-substrate used in Wan et al. (2011) was FOG waste from a FOG receiving facility, which is interpreted as a combined waste consisting of GIW/GTW from FSEs, waste

industrial FOG, and waste animal fats in this study. In addition to the differences in co-substrate source and concentration from other studies, Wan et al. (2011) conducted the experiment with thickened waste activated sludge (TWAS) while the rest operated mostly with sewage sludge (SS) that includes TWAS and primary sludge (PS).

Table 1-4 Comparison of feedstock and performance in previous anaerobic co-digestion studies.

Ref.	Feedstock	Co-substrates (VS basis)	OLR (kg-VS/m ³ day)	Methane yield (L-CH ₄ /g-VS added)	Biogas Production (L/d)	% increase in methane yield	CH ₄ content (vol%)	CO ₂ content (vol%)	VS reduction %	COD reduction %	
[1] ^a	TWAS ^d	R1 ^b	-	2.34	0.2524	1.849	-	65.1%	28.1%	40.0%	nd
		R2	FOG (64% VS)	2.34	0.5984	4.1736	137%	66.8%	29.5%	57.1%	
		R3	FOG (75% VS) (failed)	3.40	nd ^c	0.04	nd	21.3%	51.9%	29.4%	
[2]	PS ^d (40.5 % VS), TWAS (59.5 % VS)	R1	-	2.45	0.159	1.274	-	66.1%	nd	25.2%	25.3%
	PS (21.1 % VS), TWAS (31.3 % VS)	R2	FOG (47.6 % VS)	4.35	0.473	6.775	197%	65.8%		45.1%	50.2%
[3]	PS (21.1 % VS), TWAS (31.3 % VS)	R3	FOG (47.6 % VS)	4.35	0.449	nd	182%	nd	nd	45.0%	47.8%
[4]	PS (50 vol%), WAS ^d (50 vol%)	R1	-	2.50	0.271	nd	-	65%	nd	45%	nd
		R2	GS (10 % VS)	2.50	0.295-0.308		9-14%	66%		54%-55%	
		R3	GS (30 % VS)	2.40	0.344		26.9%	69%		58%	
	-	R4	GS (100 % VS) (failed)	0-2.3	nd	nd	nd	nd			
[5]	SS ^d	R1	-	1.56-2.09	0.278	-	-	63%	nd	52%	nd
		R2	GS (5 % VS)	1.67-2.23	0.374	3.48	34.5%	66%		59%	
		R3	GS (20 % VS)	1.93-2.45	0.441	4.59	58.6%	64%		58%	
			GS (28 % VS)	2.8	0.444	6.83	59.7%	61%		52%	
			GS (38 % VS)	3.13	0.447	6.09	60.8%	65%		64%	
			GS (46 % VS)	3.46	0.463	7.47	66.5%	62%		67%	
			GS (55 % VS) (failed)	4.01	0.318	6.10	14.4%	63%		72%	
			GS (71 % VS) (failed)	4.41	0.315	6.75	13.3%	58%		70%	

Table 1-4 Continued.

Ref.	Feedstock	Co-substrates (VS basis)		OLR (kg-VS/m ³ day)	Methane yield (L-CH ₄ /g-VS added)	Biogas Production (L/d)	% increase in methane yield	CH ₄ content (vol%)	CO ₂ content (vol%)	VS reduction %	COD reduction %
[6]	PS (70%), WAS (30%)	R1	-	1.5	nd	nd	-	72%	nd	36%	35%
			GW (4% VS)	1.2			138%	70%		46%	40%
			GW (23% VS)	1.6			nd	70%		52%	55%
			GW (37% VS)	1.7				68%		56%	44%

^a

[1] Wan et al., 2011.

[2] Kabouris et al., 2009a.

[3] Kabouris et al., 2009b.

[4] Davidsson et al., 2008.

[5] Luostarinen et al., 2009.

[6] Silvestre et al., 2011.

^b R1 refers to reactor 1. If three reactors (e.g. R1, R2, R3) are displayed, the research was conducted in three parallel reactors with various co-substrate concentrations. If multiple concentrations of co-substrates are displayed for only one reactor, the experiment was carried out in stepwise increase of co-substrate concentration in a single reactor.

^c nd: not determined.

^d TWAS (thickened waste activated sludge); WAS (waste activated sludge); PS (primary sludge); SS (sewage sludge).

1.6 Inhibition and threshold input of FOG wastes

Despite the proven feasibility of conducting anaerobic co-digestion of GIW/GTW to enhance methane production, a successful full scale application at WWTFs is unlikely without more in-depth research and evaluation on various related issues. It is most beneficial for the utility to address the entire content within the GIS/GTs, reducing the troubles associated with the remaining high COD, lipid-rich wastewater and food residuals. However, dewatering might be more cost-effective in terms of lowering the costs in transporting GIW/GTW to the WWTFs and maximizing the VS concentrations in digester feed (Johnson et al., 2011). In previous lab scale experiments, dewatering was only carried out by Kabouris et al. (2009a), resulting in higher TS and VS concentrations in co-substrate compared to other studies. The WWTF size also plays an important role in GIW/GTW collection. For smaller WWTFs treating less influent flow rate in their digesters, the amount of GIW/GTW produced from FSEs in the associated WWTF service area that can be processed by anaerobic treatment might be limited. Evaluating the necessity of GIW/GTW pretreatment and determining proper biogas utilization technology for onsite energy generation based on site-specific conditions at local WWTFs are also needed. Most importantly, a comprehensive evaluation on the economic feasibility of full scale GIW/GTW co-digestion cannot be achieved without identifying the maximum amount of GIW/GTW that can be co-processed without digester failure.

Though presenting a desirable nature for bioenergy generation, lipid-rich materials can still be problematic if excessive amounts are introduced into the system (Hanaki et al., 1981; Hwu et al., 1998; Kim et al., 2004; Rinzema et al., 1994). The digester upset or even failure

is primarily associated with the inhibitory effect of FOG wastes on methanogenesis because of their presence in substantial concentrations. Earlier studies (McCarty and Smith, 1986; Parkin and Owen, 1986; Speece, 1996) have demonstrated the methanogenic series metabolism, in which the conversion of complex mixtures of substrates into biogas involves many classes of bacteria and multiple intermediate reactions. Complex organic polymers such as carbohydrates, proteins, and lipids are first hydrolyzed by hydrolytic fermentative bacteria into monomers such as sugars, amino acids, and peptides. These monomers then are fermented into long chain fatty acids (LCFAs) and then subsequently into short chain fatty acids such as propionate and butyrate by fermentative bacteria. The fatty acids longer than two carbons are further transformed by syntrophic H_2 -producing acetogens to produce acetate and H_2 (acetogenesis). Finally, the acetate and H_2 are converted into CH_4 by acetoclastic methanogens and H_2 -utilizing methanogens, respectively (methanogenesis). Within this complex biodegradation process, propionate, as a common intermediate that accounts for up to 30% of the electron flows associated with the methane production, plays an deciding role in a well functioning anaerobic digestion process (Speece, 1996). Propionate conversion to acetate and H_2 is thermodynamically possible only at H_2 partial pressure below 10^{-4} atm or 100 ppm; while conversion of H_2 to methane is thermodynamically possible only at H_2 partial pressure above 10^{-6} atm or 1 ppm. As a result, the proper functioning of an anaerobic digestion process calls for a close syntrophic cooperation between H_2 -producing acetogens and H_2 -utilizing methanogens to produce H_2 concentration higher than 10^{-6} atm for the conversion of H_2 to methane by H_2 -utilizing methanogens which simultaneously

reduces/maintains the H₂ concentration lower than 10⁻⁴ atm to convert propionate into acetate and H₂.

According to the methanogenic series metabolism, one of the possible hypotheses for methanogenic inhibition mechanism is GIW/GTW overdose. When the microorganisms are confronted with excessive input of co-substrates such as FOG wastes, the enhanced loads of biodegradable solids and lipid-rich materials may result in an prompt increase in propionate concentration and generate excessive amount of H₂ and acetate. The increase in acetate concentration along with the introduction of low pH FOG wastes may also lower the pH in the digester sludge, resulting in an unfavorable environment for efficient methanogenesis. As the amount of H₂ generated from propionate overwhelms the capacity of H₂-utilizing methanogens to process H₂ and eventually exceeds 10⁻⁴ atm, an inhibited conversion of propionate to acetate and H₂ is likely to occur, followed by a drop in methane production. Finally, as the accumulation of propionate and all the other fatty acids that cannot be further metabolized decreases the pH, it will further stress the methanogens and interfere with the conversion of acetate into methane. The accumulation of acetate along with other undigested fatty acids will further reduce the pH in the digester sludge, compounding the problem, and eventually result in process failure.

Inhibitory substances, or inhibitors, refer to those materials that cause an adverse shift in the microbial population or inhibition/toxicity on bacterial growth. In addition to co-substrate overdose, the inhibitors commonly present in anaerobic digesters include ammonia, sulfide, light metal ions, heavy metals, and organics (Chen et al., 2007). GIW/GTW is a very strong waste containing FOG and readily biodegradable food residuals in substantial concentrations.

The degradation of protein and lipid rich in GIW/GTW during anaerobic digestion could result in accumulation of ammonia and LCFAs (Salminen and Rintala, 1999). Ammonia in the form of free ammonia (NH_3) was proposed to be inhibitory to anaerobic digestion as it tends to diffuse into the bacterial cell and cause proton imbalance, potassium deficiency, and/or activity lost in microorganisms, due to its membrane-permeability and hydrophobic property (Chen et al., 2007; de Baere et al., 1984; Gallert et al., 1998; Kroeker et al., 1979; Koster and Lettinga, 1988; Sprott and Patel, 1986).

Animal waste such as swine manure consists of high ammonia concentration due to the presence of protein and urea (Hansen et al., 1999; Krylova et al., 1997; Zeeman et al., 1985). However, for GIW/GTW the concentrations of ammonia, and/or lipid and protein that can be biodegraded into ammonia, not only can vary greatly from one GI/GT to another but also could have been diluted by the presence of food residuals and wastewater in GIs/GTs. Previous studies have reported wide ranges for ammonia concentration from 136 to 659 mg/L, FOG content from 15 to 319 g/L, and protein concentration from 9.2 to 29.7 g/L in various FOG wastes (Davidsson et al., 2008; de los Reyes and He, 2009; Kabouris et al., 2009a; Silvestre et al., 2011). Inhibiting concentration of ammonia from 1.7 to 14 g/L was suggested to cause reduction in methane production (Chen et al., 2007). As the quantity of ammonia released from the biodegradation of protein and lipid can be estimated through stoichiometric calculation, obtaining detailed information on waste composition is necessary for successful evaluation on the degree of ammonia inhibition. For diluted GIW/GTW with low protein and lipid concentrations, inhibitory effect of ammonia to GIW/GTW co-digestion may not be the leading cause of digester inhibition or failure. Additionally, as lower pH may

shift the free ammonia into non-toxic ammonium ion (NH_4^+) and decrease its inhibitory concentration (Chen et al., 2007), the introduction of low pH FOG wastes into the anaerobic digestion process may also alleviate the level of ammonia inhibition.

In addition to ammonia, LCFAs present and/or degraded during anaerobic digestion process in GIW/GTW are also inhibitory substances. LCFAs may exert detrimental effects on the microbial communities such as (a) substrate and product transport limitation due to LCFA adsorption onto the microbial cell wall/membrane (Hanaki et al., 1981; Long et al., 2011; Pereira et al., 2003; Pereira et al., 2004; Rinzema et al., 1994), (b) surfactant effect that reduces the membrane surface tension, interferences with proton transport and energy flow, and even leads to cell damage (Coles and Lichstein, 1963; Galbraith and Miller, 1973a; Galbraith and Miller, 1973b; Kabara et al., 1977; Kodicek and Worden, 1945; Long et al., 2011; Rinzema et al., 1994; Zeikus, 1977), (c) lag phase in methanogen activity due to transport limitation (Coles and Lichstein, 1963; Galbraith and Miller, 1973b; Hanaki et al., 1981; Koster and Cramer, 1987; Pereira et al., 2004; Rinzema et al., 1994), and (d) sludge flotation and washout due to LCFA adsorption onto biomass (Hawkes et al., 1995; Hwu et al., 1998; Jeganathan et al., 2006; Pereira et al., 2004; Rinzema et al., 1989; Rinzema et al., 1993).

It should be noted that the exact nature and inhibition mechanism of single LCFAs on methanogenesis is not yet well understood, let alone that of GIW/GTW comprising various LCFAs from mixed animal- and vegetable-based oils and greases, food residuals, wastewater, and possibly other substances derived from FSEs. This complex and commingled FOG waste may present multiple inhibition mechanisms simultaneously during anaerobic treatment, as

the presence of mixed LCFAs complicate and accentuate the inhibitory effect (Koster and Cramer, 1987; Long et al., 2011; Pereira et al., 2003). A comprehensive investigation on the mechanism of GIW/GTW inhibition is necessary for a successful full scale GIW/GTW co-digestion application.

Besides identifying the potential inhibitors and mechanism, the exact threshold concentration for FOG-rich wastes that inhibits methanogenesis along with the corresponding biogas production enhancement in response to co-digestion with sewage sludge has been of great interest. Davidsson et al. (2008) performed the anaerobic co-digestion experiment with GIW/GTW from FSEs, including FOG, food particles, wastewater portions. The threshold limit for GIW/GTW addition was identified in the range of 30 %VS to 100 %VS in which the microbial communities to survive the FOG inhibition (Table 1-4). For anaerobic co-digestion of other FOG-rich waste streams, a rough range between 64 %VS to 71 %VS was also recognized (Davidsson et al., 2008; Kabouris et al., 2009a; Luostarinen et al., 2009; Wan et al., 2011). However, up to the present time an in-depth evaluation on the threshold limit that can be adapted by GIW/GTW from various sources for full scale application of anaerobic co-digestion of GIW/GTW is still lacking.

Furthermore, previous studies were mostly conducted in high rate anaerobic digesters such as up-flow anaerobic sludge blanket reactors (UASB) and expanded granular sludge bed (EGSB) for LCFA inhibition analysis (Alves et al., 2001; Angelidaki et al., 1992; Koster and Cramer, 1987; Long et al., 2011; Rinzema et al, 1994; Pereira et al., 2003). However, anaerobic digesters at municipal WWTFs are mostly performed as continuously stirred tank reactors (CSTRs). Better lipid degradation performance was observed in CSTR digesters

compared to UASB reactor, likely due to more intensive mixing strategy in CSTR digesters (Elefsiniotis and Oldham, 1994). Inadequate mixing in digesters co-digesting FOG could cause the formation of a scum layer and reduction in process performance (Joyce and Donaldson, 2005). To prevent unnecessary expenses on energy, a further investigation is needed to evaluate the optimal mixing intensity in CSTR digesters for co-digesting GIW/GTW.

1.7 Research objectives

The goal of this study is to explore the limits of anaerobic co-digestion to co-process TWAS and the entire content of GIW including FOG, food residuals, and associated wastewater in lab scale digesters through stepwise increase of GIW fraction. The specific objectives include:

- [1] Determine the maximum methane production of GIW co-digestions.
- [2] Identify the corresponding threshold input of GIW that leads to methanogenic inhibition.
- [3] Study the impacts on effluent pH, alkalinity, total volatile acids (TVA), and VS and COD reduction rates in response to GIW loading variations.

Chapter 2 Methodology

2.1 Experimental setup

The anaerobic co-digestion experiments were conducted using two identical anaerobic reactor systems operating in parallel. Each configuration consists of a reactor system, a feeding and decanting system, and a biogas system. Figure 2-1 shows the configuration of the experimental set-up. Specific designs and functions of the setup are described in the following sections.

2.1.1 Reactor system

Experiments were carried out in two identical laboratory scale reactors (digester A and B) constructed by Precision Machine Research lab (Broughton Hall 1136, North Carolina State University) with the help of Darwin D. (Jake) Rhoads (Department of Civil, Construction, and Environmental Engineering, North Carolina State University). Each anaerobic reactor system includes a reactor chamber, cover, flange, and mixing device. Figure 2-2 provides the reactor chamber design details. Figure 2-3 shows a cross section of the proposed parts. Table 2-1 lists the experimental equipment and catalog numbers used in this research.

Reactor cover

The reactor cover was made of 1/2-inch thick Plexiglas with a 9-inch diameter. Equally-spaced bolt holes around the circumference of the cover to fit 12 bolts ensuring air- and water-tight seal. Four 3/4-inch nylon single-barbed tube fitting adapters were screwed into the Plexiglas cover. Thread seal tape was applied to all the threaded joints to help tighten the screw connections. One of the four adapters (Cat. No. 5463K261, McMaster-Carr, Atlanta, GA) has a larger opening to connect with 1/2-inch ID tygon lab tubing (Cat. No. EW-06408-74, Cole-Parmer Instrument Company, Vernon Hills, IL), while the rest (Cat. No. 5463K259, McMaster-Carr, Atlanta, GA) were inserted to attach tygon lab tubing with a 3/8-inch ID (Cat. No. EW-06408-12, Cole-Parmer Instrument Company, Vernon Hills, IL). To ensure air- and water-tight seal, all adapters were clamped tight with the tubing using double grip hose clamps (Size C, Speedy Products, Jacksonville, TX).

For the reactor system to adapt future research design, some of these openings not used in the present study were connected with tubing of corresponding size and sealed with screw tubing clamps (Cat. No. EW-06833-10, Cole-Parmer Instrument Company, Vernon Hills, IL). The three 3/8-inch ID openings served as a feeding port, a biogas/foam outlet, and a liquid recycle line, respectively; while the 1/2-inch ID opening was sealed. The feeding port was fitted with the 3/8-inch ID tygon lab tubing which was clamped shut using a screw tubing clamp while not in use.

Reactor flange

The reactor chamber is attached with a 9-inch diameter flange made of 1/2-inch thick Plexiglas. Circular, equally-spaced bolt circles were drilled in the flange for clamping the chamber flange and cover in place with 12 fitting bolts. A 7.5-inch diameter O-ring was placed in the O-ring groove and pressed between reactor cover and flange, with one flat cover and one grooved flange. Properly lubricated O-ring, cover, and flange with vacuum grease provide leakage prevention for the reactor system.

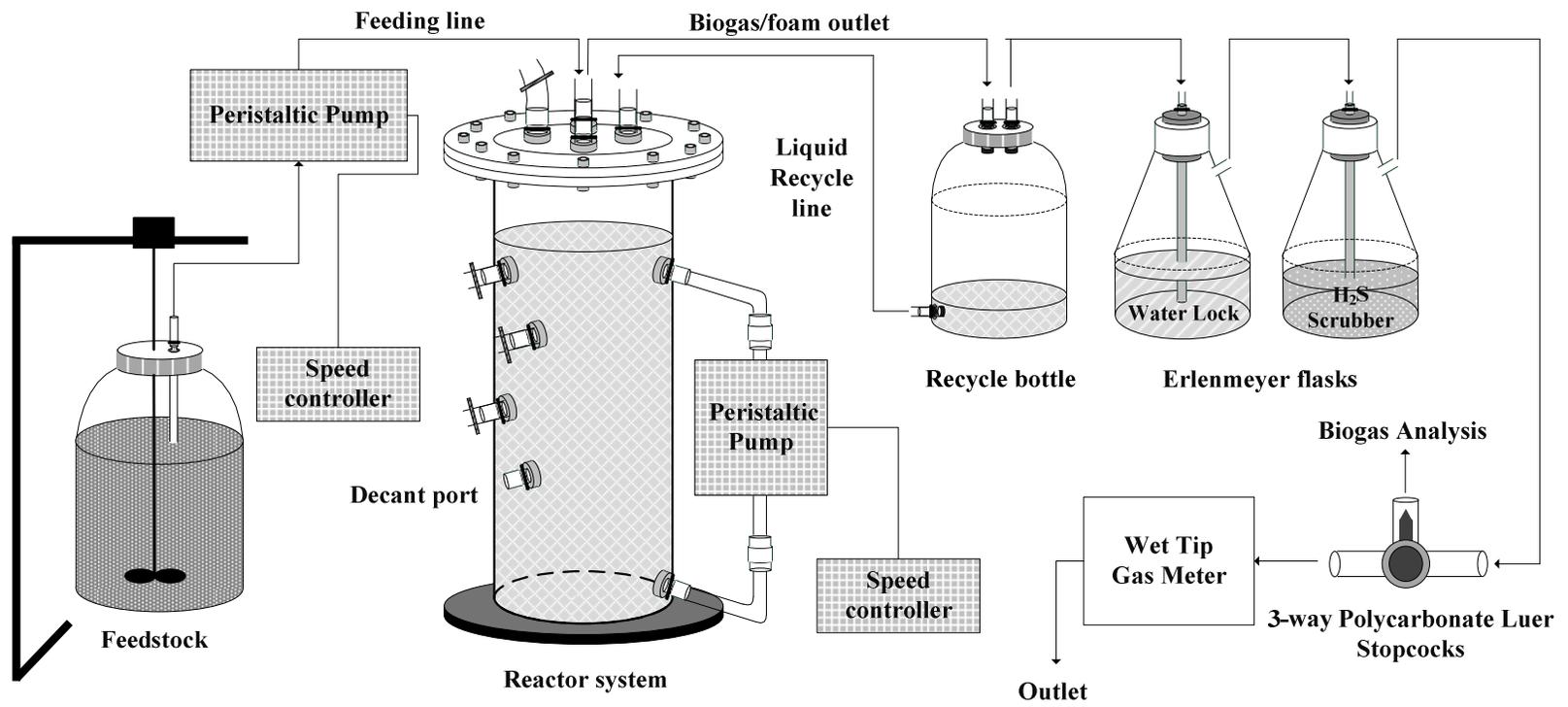


Figure 2-1 Experimental set-up.

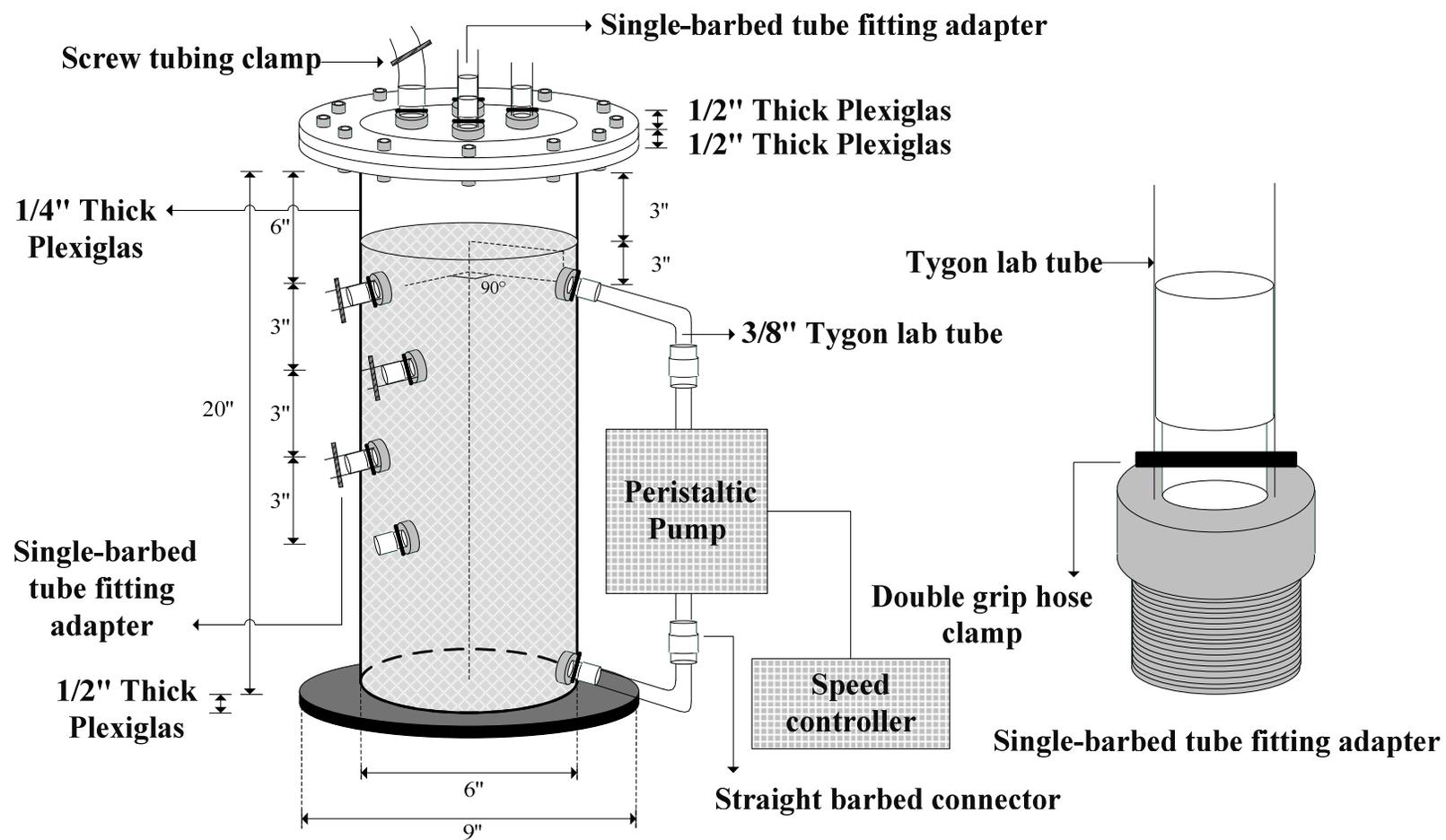


Figure 2-2 Reactor system.

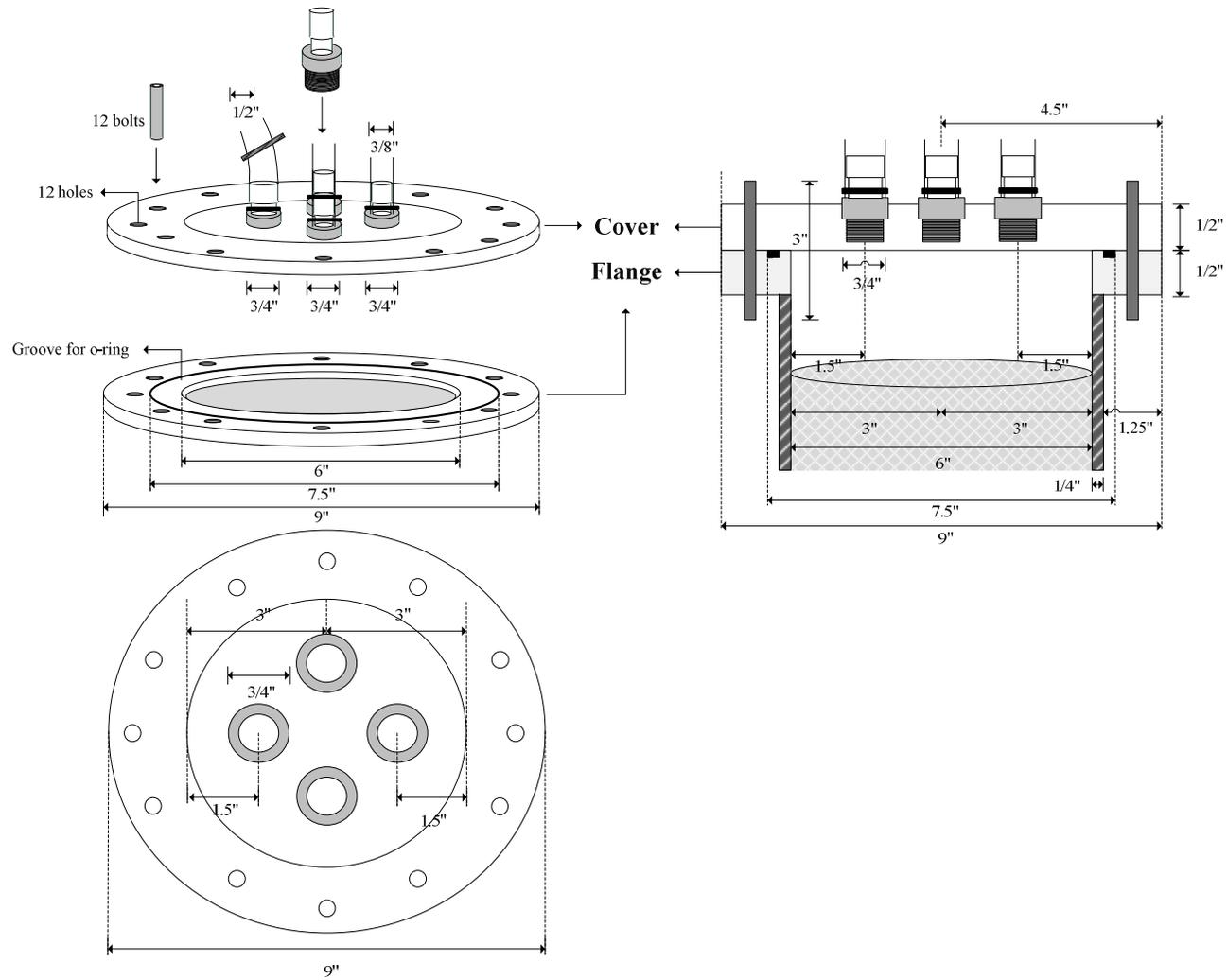


Figure 2-3 Dimensional and sectional views of the reactor system.

Table 2-1 Equipment used to construct the reactor system.

Device	Cat. No.	Company
Durable nylon single-barbed tube fitting adapter, for 1/2" Tubing ID	5463K261	McMaster-Carr
Durable nylon single-barbed tube fitting adapter, for 3/8" Tubing ID	5463K259	McMaster-Carr
Tygon lab tubing, 1/2" ID	EW-06408-74	Cole-Parmer
Tygon lab tubing, 3/8" ID	EW-06408-12	Cole-Parmer
Tygon lab tubing, 1/4" ID	EW-06408-50	Cole-Parmer
Double grip hose clamp	Size A~F	Speedy Products
Screw tubing clamp	EW-06833-10	Cole-Parmer
Masterflex L/S variable-speed modular drive	EW-07553-70	Cole-Parmer
Masterflex L/S variable-speed modular drive	EW-07553-80	Cole-Parmer
Masterflex L/S easy-load II pump head	EW-77200-52	Cole-Parmer
Masterflex high-performance precision tygon fuel & lubricant tubing, 3/8" ID (L/S 36)	S-06401-36	Cole-Parmer
Masterflex high-performance precision tygon lab (R-3603) tubing, 3/8" ID (L/S 36)	S-06409-36	Cole-Parmer
Nylon straight barbed connector, for 3/8" tubing ID	S-30621-90	Cole-Parmer
Extended-life sealless plastic centrifugal (magnetic-drive) pump	9930K37	McMaster-Carr
ChronTrol timer	XT-4F	Chrontrol Corporation
Polypropylene, heavy-walled jars, 1 gallon	71168	US Plastic Corporation
Polypropylene, heavy-walled jars, 1/8 gallon	71165	US Plastic Corporation
Stir-Pak dual-shaft mixer	EW-04554-10	Cole-Parmer
PVDF barbed reducing connector, for 3/8" x 1/4" ID tubing	EW-30703-54	Cole-Parmer
Thermo Scientific Nalgene barbed bulkhead fitting kits	EW-06259-10	Cole-Parmer
3-way polycarbonate Luer stopcock	EW-30600-07	Cole-Parmer
Wet tip gas meter	-	Wet Tip Gas Meter.com

Reactor chamber

The reactor chamber was made of 1/4-inch thick Plexiglas with an ID of 6 inches, a height of 20 inches, and a total volume of 8L. The working volume was 6L, resulting in a headspace of 2L (approximately 3 inches of fraction above the designed digester sludge surface). Along the side of the reactor chamber, four staggered 3/4-inch nylon single-barbed tube fitting adapters (Cat. No. 5463K259, McMaster-Carr, Atlanta, GA) were fitted at 3-inch intervals, and clamped tight with 3/8-inch ID tygon lab tubing (Cat. No. EW-06408-12, Cole-Parmer Instrument Company, Vernon Hills, IL) using double grip hose clamps. Thread seal tape was used to help seal connections of the threaded pipes. The first three openings from the top were sealed, while the bottom opening served as a decant port. The reactor column base plate was made of 1/2-inch thick Plexiglas with a 9-inch diameter.

Mixing device

Perpendicular to the four staggered 3/4-inch nylon single-barbed tube fitting adapters, two other identical adapters were inserted along the side of the chamber to connect with the mixing tubing. The mixing intensity for each reactor was controlled by a Masterflex peristaltic pump, introducing a circular mixing into the reactors. The peristaltic pump system comprises two major components: a Masterflex L/S variable-speed modular drive (Cat. No. EW-07553-70, Cole-Parmer Instrument Company, Vernon Hills, IL) with a speed controller, and a Masterflex L/S easy-load II pump head (Cat. No. EW-77200-52, Cole-Parmer Instrument Company, Vernon Hills, IL). Masterflex high-performance precision tygon fuel &

lubricant tubing (Cat. No. S-06401-36, Cole-Parmer Instrument Company, Vernon Hills, IL) with a 3/8-inch ID (L/S 36) was loaded for both mixing systems.

In this research, specific pumping requirements include: compatibility with viscous fluids (e.g. sludge with various concentrations of lipid-rich materials), larger tubing ID to reduce potential occlusion, and thicker tubing wall to lower the frequency of maintenance. Therefore, the L/S easy-load II pump head was selected for accepting high-performance precision tubing with several different tubing sizes. High-performance precision pump tubing features a thicker wall compared to regular pump tubing. The largest tubing size (L/S 36) was used, along with the tygon fuel & lubricant tubing that is designed specifically for handling fluids such as gasoline, kerosene, heating oils, cutting fluids, and glycol-based coolants.

The two 3/4-inch nylon single-barbed tube fitting adapters inserted into the reactor chamber were connected to the 3/8-inch ID Masterflex tygon fuel & lubricant tubing and further attached to two 3/8-inch ID nylon straight barbed connectors (Cat. No. S-30621-90, Cole-Parmer Instrument Company, Vernon Hills, IL) for convenient maintenance of the mixing tubing. As peristaltic pump tubing wears out over time, constant pumping of the mixing system required periodic tubing replacements. The L/S easy-load II pump head allowed quick tubing changes by simply clamping both sides of the tubing connected to the adapters, unclamping the other sides attached to the straight barbed connectors, and replacing the tubing.

The peristaltic mixing systems were controlled using a ChronTrol timer (Cat. No. XT-4F, Chronrol Corporation, San Diego, CA), that allowed setting of the mixing duration. Mixing intensity is defined as input power of the pump times the mixing duration. The initial

mixing intensity for start-up period was 1/100 horse power times 2 minute per hour, providing a 1241 mL/min jet flow rate. The mixing duration followed a stepwise increase pattern in the later research phases.

2.1.2 Feeding and decanting system

Two one gallon polypropylene, heavy-walled jars (Cat. No. 71168, United States Plastic Corporation, Lima, OH) served as feeding containers for two reactor systems. Constant mixing during feeding was provided by a Stir-Pak dual-shaft mixer (Cat. No. EW-04554-10, Cole-Parmer Instrument Company, Vernon Hills, IL) with a speed controller. Periodic feeding and decanting were performed simultaneously by unclamping the decant port for effluent collection, and pumping in the feedstock from the feeding container using a Masterflex L/S variable-speed modular drive (Cat. No. EW-07553-80, Cole-Parmer Instrument Company, Vernon Hills, IL) with a speed controller. The pump drive was mounted with two Masterflex L/S easy-load II pump heads (Cat. No. EW-77200-52, Cole-Parmer Instrument Company, Vernon Hills, IL). Masterflex 3/8-inch ID high-performance precision tygon lab (R-3603) tubing (Cat. No. S-06409-36, Cole-Parmer Instrument Company, Vernon Hills, IL) and tygon fuel & lubricant tubing (Cat. No. S-06401-36, Cole-Parmer Instrument Company, Vernon Hills, IL) were used for feeding sludge and with lipid-rich material, respectively.

2.1.3 Biogas system

Openings from the 3/4-inch nylon single-barbed tube fitting adapters, that is, the biogas/foam outlet and liquid recycle line, were connected to the biogas system. The biogas/foam outlet was connected to the lid of the recycle bottle, a 1/8 gallon polypropylene jar (Cat. No. 71165, United States Plastic Corporation, Lima, OH), with 3/8-inch and 1/4-inch ID tygon lab tubing (Cat. No. EW-06408-50, Cole-Parmer Instrument Company, Vernon Hills, IL). The size of the 3/8-inch ID tubing was reduced to 1/4 inch by a PVDF barbed reducing connector (Cat. No. EW-30703-54, Cole-Parmer Instrument Company, Vernon Hills, IL). The liquid recycle line was connected to the recycle bottle at the bottom also with a 3/8-inch and 1/4-inch ID tygon lab tubing and a PVDF barbed reducing connector.

The recycle bottle had three openings; one was connected to the biogas/foam outlet, one to the liquid recycle line, and one to the following water lock bottle. Biogas produced from the reactor was led to the recycle bottle, along with the excessive foam that was directed back to the reactor through the recycle line. The openings were made by drilling holes on the polypropylene jar and applying the Thermo Scientific Nalgene barbed bulkhead fitting kits (Cat. No. EW-06259-10, Cole-Parmer Instrument Company, Vernon Hills, IL). The fitting kits include a polypropylene fitting, an acetal nut, and a silicone gasket. Air- and water tight seal was achieved by screwing the polypropylene fitting into the jar wall using the acetal nut with the silicone gasket. The silicone gasket filled the space between the fitting and jar wall to prevent leakage. Figure 2-4 presents the design details of the recycle bottle.

Two Erlenmeyer flasks with side tabulation served as water lock and hydrogen sulfide (H_2S) scrubber bottles. Each flask was sealed by fitting a rubber stopper at the top. A 3-inch

plastic tube was inserted into the stopper to connect with 1/4-inch ID tygon lab tubing. Through the plastic tube the biogas effluent from the recycle bottle was directed below the level of water (approximately 100 ml of water), serving not only to prevent biogas back flow but also to give warning of potential gas leakage through the change of water level.

The water lock bottle was connected to the H₂S scrubber bottle by 1/4-inch ID tygon lab tubing. Steel wool in the bottle removed H₂S from the biogas through a reaction with iron. All joint connections were made air- and water-tight using double grip hose clamps and Parafilm sealing film. The gas effluent from the scrubber then passed through a 3-way polycarbonate Luer stopcock (Cat. No. EW-30600-07, Cole-Parmer Instrument Company, Vernon Hills, IL) where biogas sampling took place using a needle, and finally entered a wet tip gas meter (Wet Tip Gas Meter.com, Nashville, TN) for biogas production measurement.

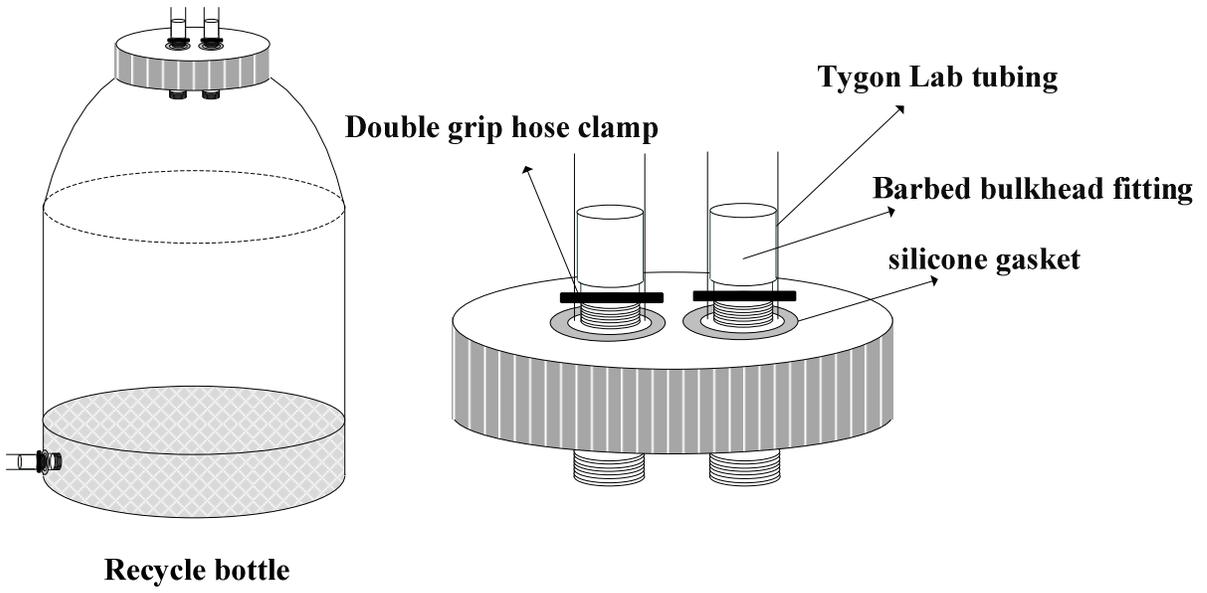


Figure 2-4 Recycle bottle.

2.2 Experimental procedures

2.2.1 Substrates and inoculum

Anaerobic sludge obtained from South Durham Water Reclamation Facility in North Carolina was used as inoculum, and TWAS obtained from North Cary Water Reclamation Facility in North Carolina was used as feedstock. GIW provided by a food service establishment in Cary, North Carolina was used as co-substrate. FOG, food particles, and wastewater portions within GIW were separated at the food service establishment with the help of professional grease waste haulers. The substrates and inoculum were stored in 4 °C upon delivery.

2.2.2 Loading schedule

The present research was conducted in two 8 L Plexiglas reactors in a temperature-controlled room to maintain mesophilic conditions (37 °C) with a working volume of 6 L. The reactors were fed every other day in a draw-and-fill semi-continuous mode with a solids retention time (SRT) of 20 days. Each feed time, 600 mL effluent is removed through the decant port while 600 mL feedstock was pumped into the digester through the feeding line.

Eleven research phases were initially designed to explore the limit of anaerobic co-digestion of sewage sludge with GIW by stepwise increase of GIW loading rate from 10 vol%, 20 vol%, 40 vol%, 80 vol%, and finally 100 vol%, followed by dewatering and FPs removal, as shown in Figure 2-5. During phase 1 (start-up), both reactors were fed with only TWAS. A mixing intensity experiment was implemented in phase 2 for digester B. After the optimal mixing intensity was obtained, mixing duration of digester A (control) was adjusted

to this desired value. In phase 3 to 7, GIW addition was designed to be increased from 10 to 100% to evaluate the optimal GIW fraction for maximum biogas production. However, the anaerobic digesters were operated for roughly 9 months with the GIW inhibition observed during phase 5, and therefore the subsequent phases were not performed in the present research.

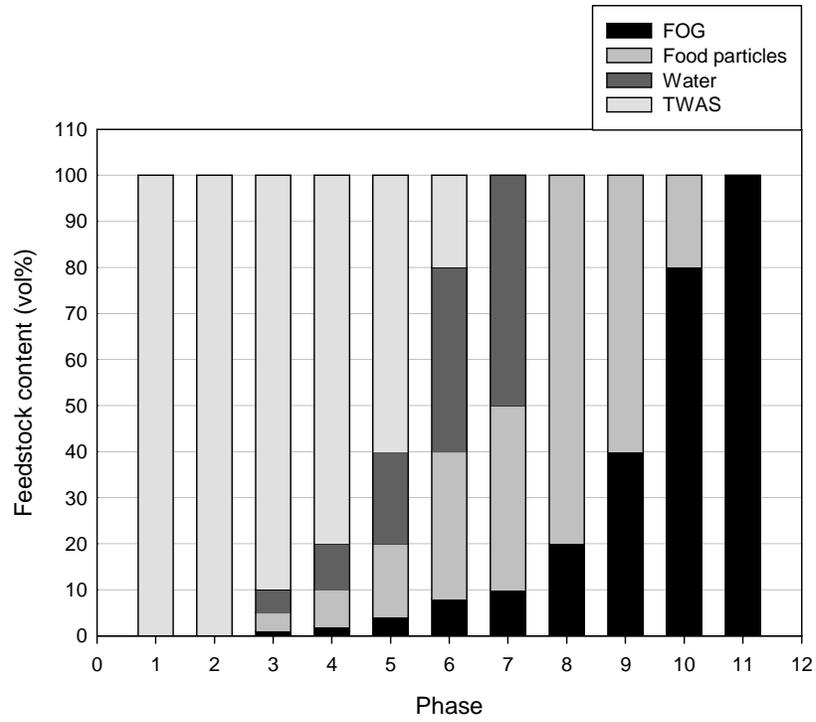


Figure 2-5 Loading schedule.

2.3 Analytical methods

Biogas production in liters per day was recorded on a daily basis by the wet tip gas meter. Daily biogas production was measured based on the principle of liquid displacement. Each discrete incremental volume of 100 ml biogas produced will initiate a flip-flop or "tip" in the gas meter and recorded by the internal magnetic actuated counter. Total daily volume of biogas can be calculated by multiplying the total counts recorded by 100 ml. The measured biogas production were further corrected by STP condition based on the daily local climatological data obtained from National Climatic Data Center of U.S. Department of Commerce (NCDC, 2012).

Biogas samples were taken daily for methane and carbon dioxide content analysis by a gas chromatography (SRI 8610C) equipped with a thermal conductivity detector (TCD). TS, VS, alkalinity, pH, and TVAs concentration were determined according to the Standard Methods (APHA, 2005). COD was analyzed using test kits of COD high plus range, 15,000 mg/L (HACH Company, Loveland, CO). The accuracy check for COD analysis was conducted by preparing a 10,000 mg/L standard solution and applying 0.2 mL (200 μ L) of this std solution for COD analysis (the expected result is 10,000 mg/L, or read "1000" on the colorimeter). The standard solution was prepared by adding 8.500g of dried (120 °C, overnight) potassium acid phthalate (KHP) into 1 L deionized water.

Chapter 3 Results and discussion

3.1 Feedstock characterization

The characteristics of TWAS and GIW are shown in Table 3-1. The GIW is defined as the entire contents of a GI and therefore comprise FOG, food particles, and associated wastewater. The GIW was mixed with 10 vol% of FOG, 40 vol% of food particles, and 50 vol% of wastewater, based on the average depths of the three portions measured in a previous analysis conducted within two GIs in North Carolina by Dr. de los Reyes research group in 2011. As the FOG portion tends to affect the accuracy of the analysis, standard deviations of GIW for TS, VS, and TCOD are higher than the others. The TS and VS concentrations of GIW in the present study are 19% and 18.9%, respectively, which corresponds to the TS and VS concentrations (17.3% and 17%, respectively) of sludge from grease traps (interpreted as GTW) obtained in Davidsson et al. (2008). The characteristics of feedstock prepared for feeding in phases 4-6 are presented in Table 3-2.

3.2 Continuous experiment

This research was performed for approximately 270 days, and divided into six experimental phases corresponding to various feeding strategies and OLRs. Figures 3-1 to 3-3 present the monitoring parameters including biogas production, methane yield, methane content in biogas, effluent pH, alkalinity, and TVA concentration, and VS and COD effluent concentrations and reduction rates. To better illustrate and compare changes between

parameters in response to various OLRs as the experiment proceeded, all variables from monitoring data are displayed as functions of time during all six phases.

Table 3-1 Raw feedstock characterization.

	TWAS			GIW									
				Wastewater			Food particles			FOG	GIW ^b		
TS (g/kg-wet sample)	31.5	±	1.81 ^a	2.72	±	0.05	75.0	±	3.25	nd	190	±	14.7
VS (g/kg-wet sample)	24.9	±	1.48	2.15	±	0.09	73.8	±	3.21	nd	189	±	14.7
VS/TS (%)	78.9			79.0			98.4			nd	99.5		
TCOD (g/L)	37.9	±	3.33	10.9	±	0.52	195	±	28.3	> 1500 ^c	86.9	±	32.9

^a Mean ± standard deviation (n ≥ 3).

^b GIW composition: FOG (10 vol%), food particles (40 vol%), and water (50 vol%).

^c Value above the highest standard range.

nd: not determined.

Table 3-2 Prepared feedstock characterization.

	10 vol% GIW 90 vol% TWAS			20 vol% GIW 80 vol% TWAS			40 vol% GIW 60 vol% TWAS		
	TS (g/kg-wet sample)	37.9	±	0.77	49.1	±	0.75	75.8	±
VS (g/kg-wet sample)	31.5	±	0.79	43.3	±	0.76	70.9	±	5.21
VS/TS (%)	83.2			88.1			93.5		
%VS	45.8			65.5			83.5		
TCOD (g/L)	55.6	±	3.86	66.2	±	13.2	114	±	27.2

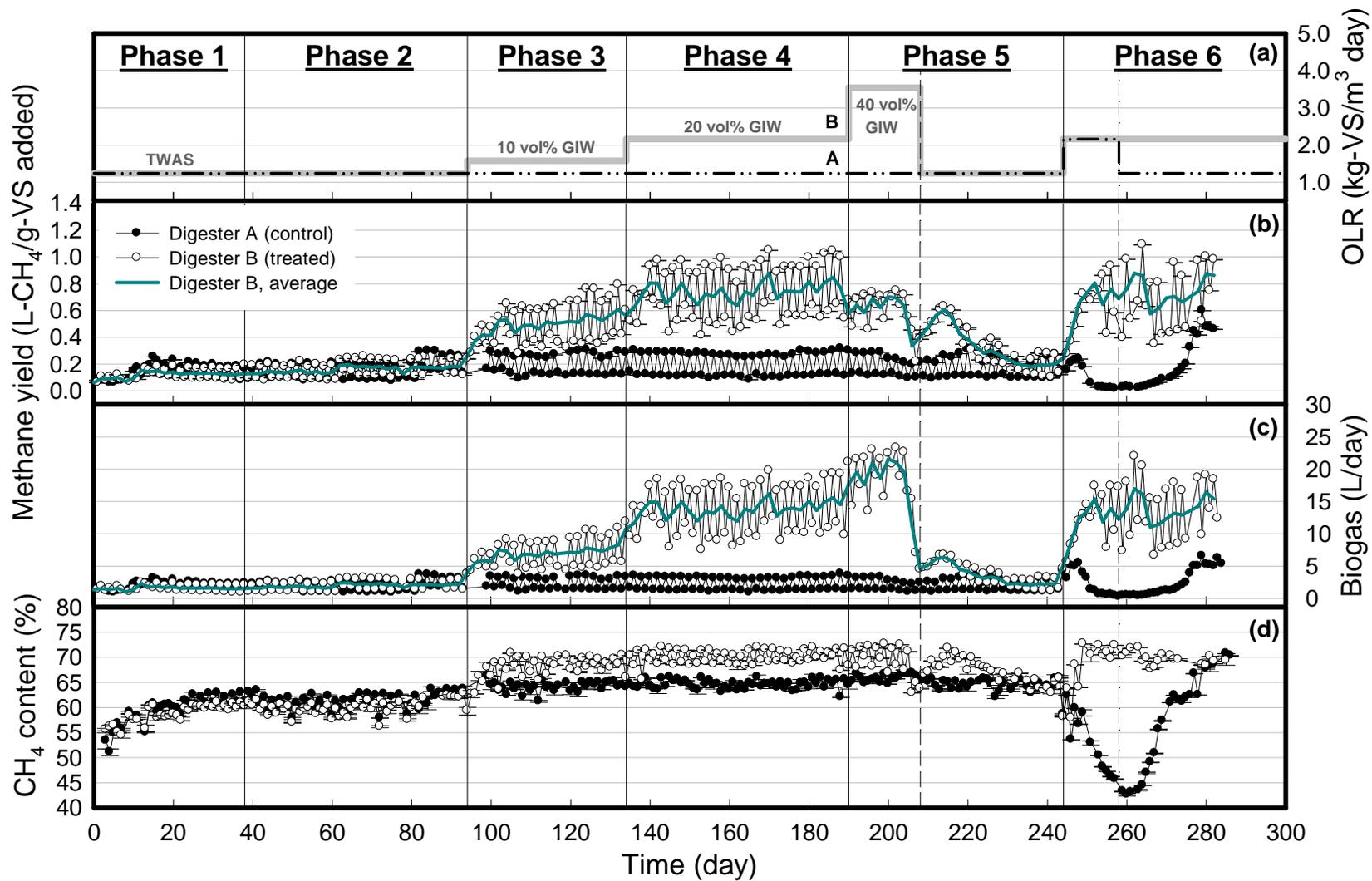


Figure 3-1 Biogas production, methane yield, and methane content of control and treated digesters.

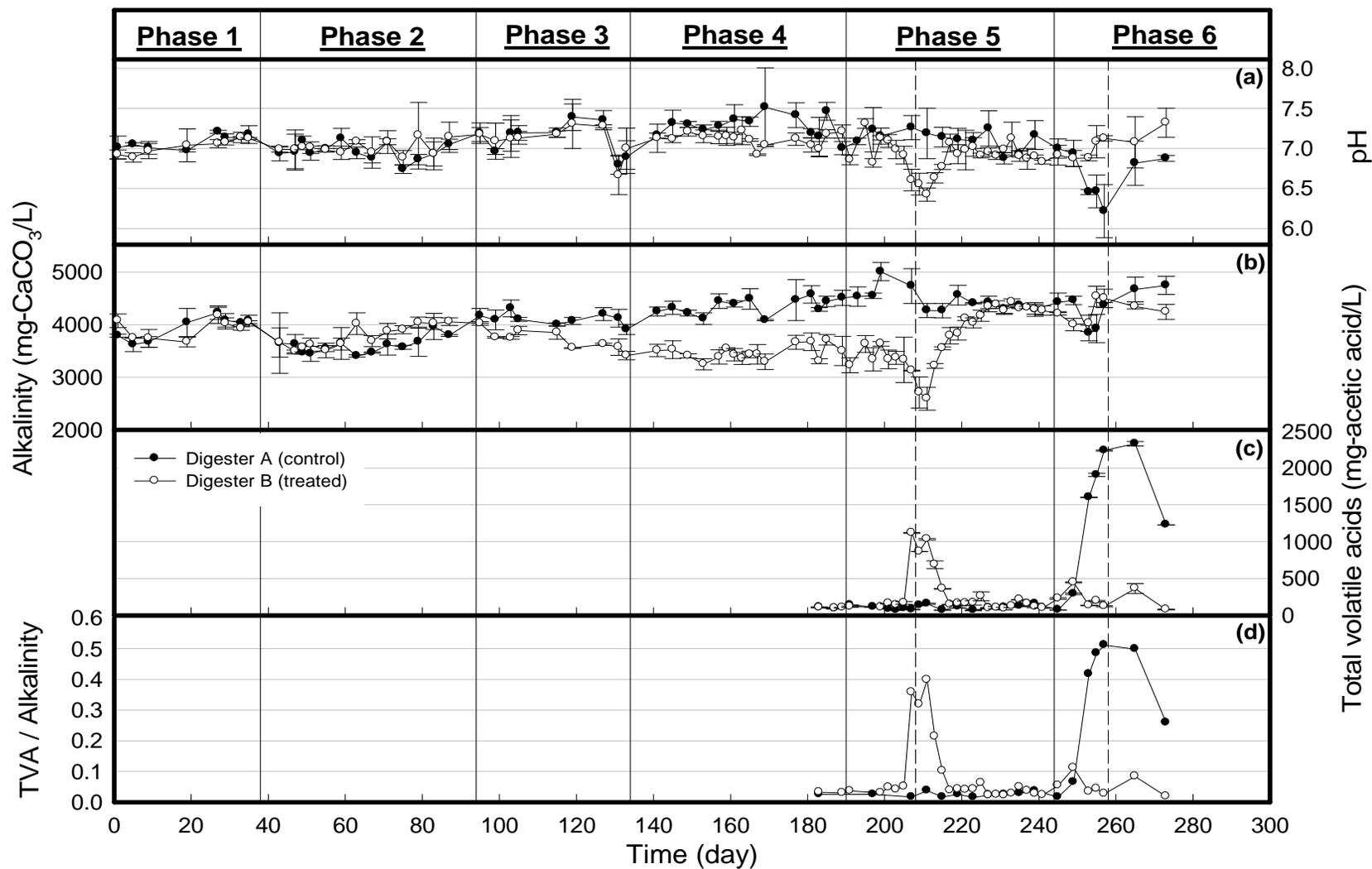


Figure 3-2 Effluent pH, alkalinity, and total volatile acids concentrations of control and treated digesters.

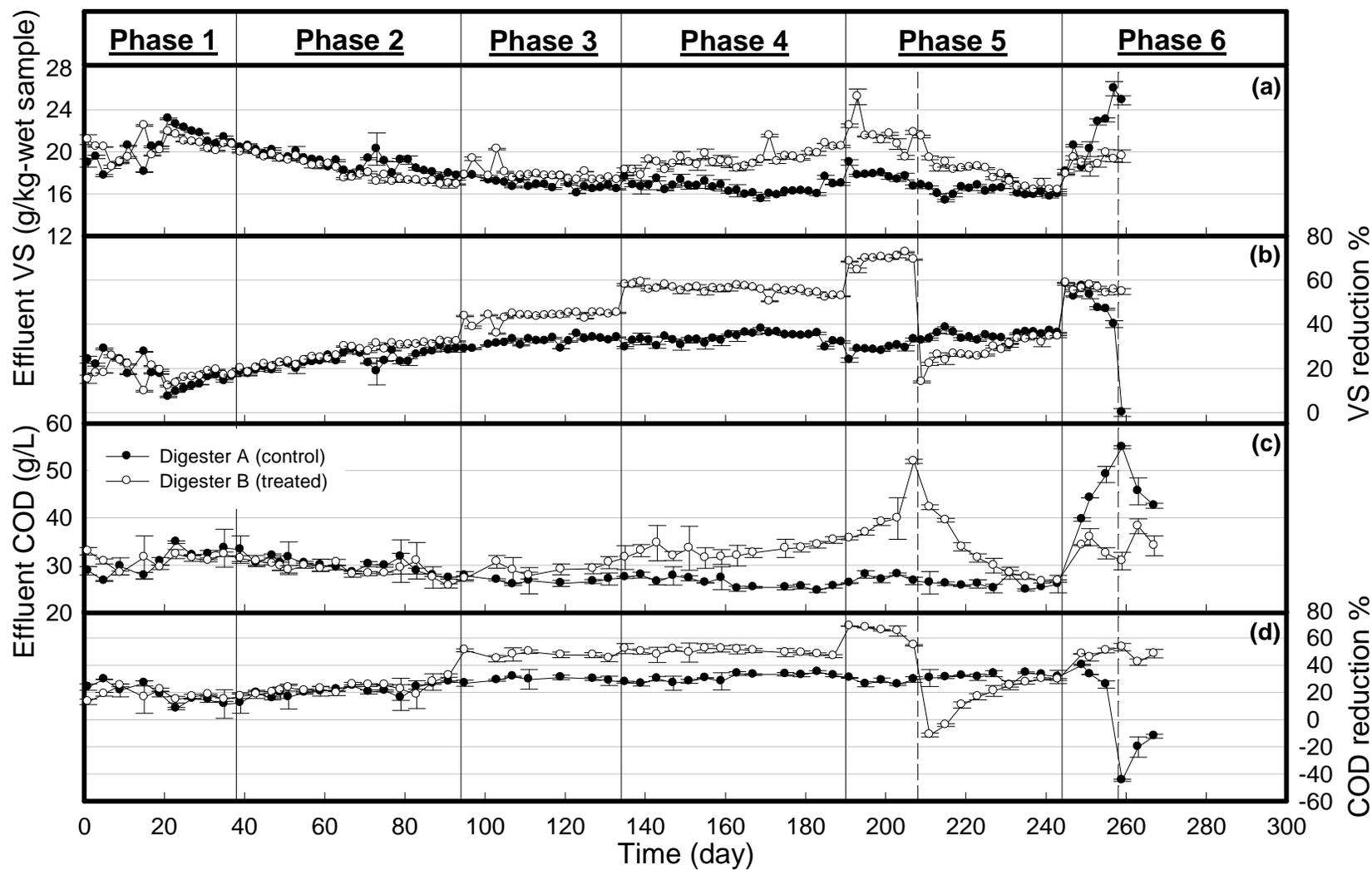


Figure 3-3 VS and COD effluent concentrations and reduction rates of control and treated digesters.

3.2.1 Phase 1 and 2

Phase 1 (day 0-38) was conducted as a start-up period when both of the digesters were fed with only TWAS and mixed for 2 minute per hour (Figure 3-1). The OLR for both digesters during phase 1 and 2 was $1.24 \text{ kg-VS/m}^3 \text{ day}$. Digester A and B were stable both in biogas production and methane content starting at day 24 and 18, respectively. The fluctuation of biogas production and methane content were related to the semi-continuous feeding mode; that is, on the first day after feeding, the biogas production and methane content were generally higher due to sufficient amount of feedstock, while on the second day the partly-consumed feedstock led to a reduction in both parameters, leading to a lower methane yield as well. Average biogas production and associated methane yield are marked by the thick grey line with no symbols in Figures 3-1 (a) and (b).

After 20 days, phase 2 (day 38-94) started with an increase in mixing duration from 2 to 4 minute per hour for digester B which lasted for 24 days. The mixing duration was again increased to 30 minute per hour on day 62. As shown in Table 3-4, the average biogas productions of digester B resulted from 2, 4, and 30 minute per hour of mixing were 1.6, 1.7, and 2.2 L/d, respectively. An increase of 36% (1.6 to 2.2 L/d) in biogas production was observed due to the increase in mixing duration time. Similarly, an increase of 40% was observed in the average methane yield of digester B (from 0.129 to 0.180 L-CH₄/g-VS added). The average methane contents of digester B due to 2, 4, and 30 minute per hour mixing durations were 59.6%, 59.7%, and 60.2%, respectively.

Table 3-3 Digester A average biogas production, methane yield, and methane content.

	Phase	Day	OLR (kg-VS/m³ day)	Average methane yield (L-CH₄/g-VS added)	Average biogas production (L/d)	Average methane content (%)
1	2 min/hr	24-82		0.144	1.8	61.2
2-5	4 min/hr	-	1.24	-	-	-
	30 min/hr	82-244		0.192	2.2	64.4

Table 3-4 Digester B average biogas production, methane yield, and methane content.

	Phase	Day	OLR (kg-VS/m³ day)	Average methane yield (L-CH₄/g-VS added)	Average biogas production (L/d)	Average methane content (%)
1	2 min/hr	18-38		0.129	1.6	59.6
2	4 min/hr	38-62	1.24	0.138	1.7	59.7
	30 min/hr	62-94		0.180	2.2	60.2
3	10% GIW	94-134	1.58	0.502	6.8	68.2
4	20% GIW	134-190	2.16	0.752	13.7	70.1
5	40 % GIW	190-205	3.54	0.630	19.6	68.7

Table 3-5 Digester A average effluent pH and alkalinity.

	Phase	Day	OLR (kg-VS/m³ day)	pH	Alkalinity (mg-CaCO₃/L)
1	2 min/hr	24-82		7.01	3692
2-5	4 min/hr	-	1.24	-	-
	30 min/hr	82-244		7.17	4278

Table 3-6 Digester B average effluent pH and alkalinity.

	Phase	Day	OLR (kg-VS/m³ day)	pH	Alkalinity (mg-CaCO₃/L)
1	2 min/hr	18-38		7.09	3969
2	4 min/hr	38-62	1.24	6.99	3579
	30 min/hr	62-94		7.03	3937
3	10% GIW	94-134	1.58	7.10	3715
4	20% GIW	134-190	2.16	7.14	3428
5	40 % GIW	190-205	3.54	6.96	3376

Table 3-7 Digester A average effluent VS and COD concentrations and reduction rates.

Phase	Day	OLR (kg-VS/m³ day)	Effluent VS (g/kg-wet sample)	VS reduction rate (%)	Effluent COD (g/kg-wet sample)	COD reduction rate (%)	
1	2 min/hr	24-82		19.8	20.5	31.1	18.0
2-5	4 min/hr	-	1.24	-	-	-	-
	30 min/hr	82-244		16.8	32.5	26.7	29.6

Table 3-8 Digester B average effluent VS and COD concentrations and reduction rates.

Phase	Day	OLR (kg-VS/m³ day)	Effluent VS (g/kg-wet sample)	VS reduction rate (%)	Effluent COD (g/kg-wet sample)	COD reduction rate (%)	
1	2 min/hr	18-38		20.8	16.4	31.4	17.3
2	4 min/hr	38-62	1.24	19.4	22.1	30.1	20.6
	30 min/hr	62-94		17.4	30.1	28.6	24.6
3	10% GIW	94-134	1.58	17.9	43.4	29.6	46.8
4	20% GIW	134-190	2.16	19.3	55.5	33.1	50.0
5	40 % GIW	190-205	3.54	21.7	69.4	40.7	64.4

At the end of phase 2, digester B had reached the typical anaerobic digestion methane content of 60% and also exhibited to a constant and stable biogas production with a mixing duration of 30 minute per hour; therefore, the mixing duration of 30 minute per hour was maintained throughout the rest of the experimental phases for digester B. For digester A, the mixing duration was increased from 2 to 30 minute per hour on day 82 and served as the control reactor with only TWAS feeding until day 244 (phase 6). Table 3-3 shows the monitoring results of digester A. The average biogas production rates of digester A at 2 and 30 minute per hour mixing durations were 1.8 and 2.2 L/d, respectively. The average methane yields of digester A at 2 and 30 minute per hour mixing durations were 0.144 and 0.192 L-CH₄/g-VS added, respectively. The average methane contents of digester A at 2 and 30 minute per hour mixing duration were 61.2% and 64.4%, respectively.

During phases 1 and 2, pH and alkalinity of the digester effluent were monitored periodically for anaerobic process stability (Figure 3-2). As shown in Tables 3-5 and 3-6, effluent decanted from digester A were at pH 7.01 and 7.17 with average alkalinity of 3692 and 4278 mg-CaCO₃/L for 2 and 30 minute per hour mixing duration, respectively; that of digester B were maintained at pH 7.09, 6.99 and 7.03 with average alkalinity of 3969, 3579 and 3937 mg-CaCO₃/L for 2, 4, and 30 minute per hour mixing duration, respectively. It was suggested that the safe ranges for a healthy anaerobic digestion process are 6.5–8.2 (Speece, 1996) and 2,500–5,000 mg-CaCO₃/L (McCarty, 1964) for pH and alkalinity, respectively. Monitoring results indicated maintenance of a stable and active anaerobic process during phases 1 and 2.

Effluent VS and COD concentrations and reduction rates for both digesters are presented in Figure 3-3. Fluctuation in effluent VS concentrations of both digesters had ceased since day 21 during phase 1. Since then effluent VS concentrations had gradually decreased to 17.7 and 16.9 g/kg-wet sample for digester A and B, respectively, at the end of phase 2 (day 94).

The average effluent VS concentrations of digester A and B mixed for 2 minutes per hour were 19.8 (20.5% reduction rate) and 20.8 (16.4% reduction rate) g/kg-wet sample, respectively. The average effluent VS concentrations of digester A and B mixed for 30 minutes per hour were 16.8 (32.5% reduction rate) and 17.4 (30.1% reduction rate) g/kg-wet sample, respectively. The VS reduction rate of digester A stabilized at around 38% with an average value of 32.5% as the experiment proceeded. The monitoring results of the present research were in accordance with the typical 20-30% VS reduction rate reported in the literature pertaining to WAS digestion with 20 days of SRT (Long et al., 2012; Parkin and Owen, 1986).

Effluent COD concentrations of digester A in Figure 3-3 also illustrated the similar climbing phenomenon as the experiment proceeded. The average effluent COD concentrations of digester A and B operated with 30 minute per hour mixing duration were 26.7 g/L (29.6% reduction rate) and 28.6 (24.6% reduction rate) g/L, respectively, in agreement with the typical COD reduction rate of 20-30% with 100% WAS feeding (Parkin and Owen, 1986).

3.2.2 Phase 3

In phase 3 (day 94-134), 10 vol% GIW (45.8 % VS) addition was applied for digester B, increasing the OLR from 1.24 to 1.58 kg-VS/m³/day; while digester A remained at 1.24 kg-VS/m³/day (Figure 3-1 (a)). The biogas production gradually increased and stabilized on day 106 for digester B. During the first few days after phase 3 was carried out, approximately 1 cm of residual feedstock with food particles and FOG was observed to float to the top of the digester sludge. During the phase transition, the addition of GIW seemed to have built up in the system and resulted in the increasing biogas production during the first 12 days (day 94-106) without obvious fluctuation. After the microorganisms accommodated to the new environment with higher OLR, the biogas production stabilized at an average biogas production of 6.8 L/d and methane yield of 0.502 L-CH₄/g-VS added. Anaerobic digestion with 10 vol% GIW addition then contributed to a 208% increase in biogas production and 178% increase in methane yield compared to that with that without GIW addition (2.2 L/d and 0.180 L-CH₄/g-VS added). Moreover, the 10 vol% GIW addition also boosted the methane content from 60.2% to 68.2%.

Effluent alkalinity and pH decreased after 10 vol% GIW addition with average values of 7.10 and 3715 mg-CaCO₃/L during phase 3. No significant reduction on both parameters was observed, indicating a stable anaerobic digestion process. The effluent VS concentration and reduction rate of digester B stabilized at 17.9 g/kg-wet sample and 43.4%, respectively. Compared to the previous 17.4 g/kg-wet sample and 30.1% VS reduction rate in phase 2, a 44% increase in VS reduction rate was observed due to the 10 vol% GIW addition. Effluent COD concentration and reduction rate were 29.6 g/L and 46.8% on average, leading to a 91%

increase in COD reduction rate compared to the previous 24.6% COD reduction rate during phase 2.

3.2.3 Phase 4

In phase 4 (day 134-190), 20 vol% GIW (65.5 % VS) was added to digester B, further increasing the OLR to 2.16 kg-VS/m³/day (Figure 3-1 (a)). Around 10 days (day 134-144) of adapting the increased OLR were required for the stabilization of biogas production. The average biogas production, methane yield, and methane content were 13.7 L/d, 0.752 L-CH₄/g-VS added, and 70.1%, respectively, for digester B during phase 4. The 20 vol% GIW input enhanced the biogas production and methane yield by 522% and 317%, respectively, compared to monitoring data in phase 2.

Effluent pH and alkalinity were successfully maintained at 7.14 and 3428 mg-CaCO₃/L. Compared to the alkalinity of 3937 and 3715 mg-CaCO₃/L during phases 2 and 3, respectively, alkalinity in phase 4 dropped but was still within the 2,500–5,000 mg-CaCO₃/L range. To confirm the anaerobic process stability and explore the possibility of further pushing the GIW input concentration, TVA concentrations were analyzed for digester B effluent. As indicated in Figure 3-2 (c) and (d), TVA concentrations of digester B effluent for the last few days (day 183, 187, and 189) ranged from 95.7 to 104.5 mg-acetic acid/L with TVA/alkalinity ratios below 0.05, indicating the potential for further increases in the GIW loading rate.

The average effluent VS concentration and reduction rate of digester B was enhanced to 19.3 g/kg-wet sample and 55.2%, respectively, resulting in an 84% increase in VS reduction

rate compared to 30.1% during phase 2. Effluent COD concentration and reduction rate were 33.1 g/L and 50.0% in average, leading to a 104% increase in COD reduction rate compared to the 24.6% during phase 2.

3.2.4 Phase 5

In day 190, GIW input concentrations of digester B was elevated to 40 vol% (83.5 % VS) with a 3.54 kg-VS/m³/day OLR (Figure 3-1 (a)). The biogas production climbed from the average 13.7 L/d during phase 4 to 19.6 L/d (from day 190 to 205), achieving a 43% increase due to the GIW addition (Figure 3-1 (c)). However, in comparison with the 102% biogas production increase from 6.8 L/d (phase 3, 10 vol% GIW) to 13.7 L/d (phase 4, 20 vol% GIW), the 40 vol% GIW input did not seem to have reached its full potential. Methane yield in Figure 3-1 (b) was calculated by multiplying the biogas production and methane content from the monitoring data and divided by the amount of VS input, providing a better way to quantify the biogas/methane production as a normalized unit. As shown in Figure 3-1 (b), the digester B methane yield from day 190 to 205 had a 16% decrease (from 0.752 to 0.630 L-CH₄/g-VS added, in average) compared to that in phase 4. Also, the average methane content decreased from 70.1% (phase 4) to 68.7% (from day 190 to 205) with a more obvious data fluctuation. These observations along with a layer of foam that slowly built up in digester B indicated an impending methanogenic inhibition.

On day 206 the biogas production started to drop from 22.6 L/d (day 204) to 4.5 L/d (day 208), accompanied by a methane content decrease from 71.1% (day 205), 62.9% (day 206), 65.9% (day 207), to 63.8% (day 208). The occurrence of methanogenic inhibition was

also verified by the monitoring feedback of effluent pH, alkalinity, and TVA concentrations, and VS and COD effluent concentrations. Since day 199, both effluent pH and alkalinity of digester B had been decreasing, from pH 7.13 and 3636 mg-CaCO₃/L (day 199) to pH 6.60 and 3126 mg-CaCO₃/L (day 207) with the TVA concentrations increasing from 167.7 (day 205) to 1119 (day 207) mg-acetic acid/L. The TVA/Alk ratio was higher than the 0.1 limit for a well-functioning anaerobic digestion process.

The process inhibition was also reflected in the effluent VS and COD concentrations. As more and more foam layer (likely composed of residual GIW) was observed in digester B, the effluent VS and COD concentrations were affected. Average effluent VS concentration increased from 19.3 (phase 4) to 21.7 g/kg-wet sample (from day 190 to 205). Average effluent COD concentration was 33.1 g/L during phase 4; however, it had been climbing to 51.9 g/L in day 207.

The feeding strategy was switched from 40 vol% GIW to TWAS in day 208 (Figure 3-1 (a)) to alleviate the toxicity effect of GIW. Digester B was operated for approximately 15 days (day 190-205) at 40 vol% GIW loading, and it took 24 days (day 208-232) for it to recover. From day 208 to 232, the methane production first increased without the typical fluctuation, because the residual GIW that had not yet been completely digested. The methane production decreased to the same level as digester A after the GIW inhibition was lessened. Digester B was then operated for another 12 days (day 232-244) to ensure a good recovery.

Monitoring data of methane content, effluent pH, alkalinity, and TVA concentrations, along with the effluent VS and COD concentrations also suggested successful recuperation of

the anaerobic process. The methane content was again brought back to around 65.0% (day 242); the effluent pH and alkalinity climbed back to 6.90 and 4293 mg-CaCO₃/L (day 239); the effluent TVA concentration dropped back to 115.7 mg-acetic acid/L (day 239). Data of the effluent VS and COD concentrations also confirmed the recovery of digester B.

3.2.5 Phase 6

On day 244, the GIW input concentrations were increased to 20 vol% (OLR 2.16 kg-VS/m³/day) for both digesters. The objectives of this experiment were to evaluate if digester B was recovered from the inhibition in phase 5 and the influence of stepwise increase in GIW addition on methanogenesis. Digester A which had been fed with only TWAS since day 1 seemed to fail in surviving the sudden increase of OLR. The biogas production increased from 3.1 L/d (day 242) to 5.5 L/d (day 248) and dropped to 0.7 L/d (day 253) due to GIW overloading (Figure 3-1 (c)). The methane content in digester A decreasing from 65.7% (day 242) to 50.4% (day 253) also revealed the occurrence of methanogenic inhibition (Figure 3-1 (d)). As shown in Figure 3-2 (a) and (b), effluent pH and alkalinity in digester A also decreased from pH 7.17 and 4291 mg-CaCO₃/L (day 239) to pH 6.45 and 3843mg-CaCO₃/L (day 253), respectively. As the TVA concentration increased from 156.9 mg-acetic acid/L (day 239) to 1602 mg-acetic acid/L (day 253), the TVA/Alk ratio also exceeded 0.1 in day 253. Effluent VS and COD concentrations both increased from 15.9 g/kg-wet sample and 26.1 g/L (day 243) to 23.0 g/kg-wet sample and 49.1 g/L (day 255), respectively due to GIW inhibition.

On day 258, the feeding strategy for digester A was switched from 20 vol% GIW to TWAS in response to the inhibition. The biogas production and methane content increased from 0.4 L/d and 45.7% (day 257) to 6.5 L/d and 68.4% (day 279), respectively, due to residual GIW, and had been dropping with decreasing residual GIW since day 279. Effluent pH, alkalinity, and TVA concentration, along with VS and COD concentrations showed a gradual recovery from GIW toxicity in digester A.

On the other hand, digester B which had experienced inhibition at 40 vol% GIW addition during phase 5 (and recovered) survived this 20 vol% GIW input concentration. The biogas production increased from 1.6 L/d (day 243) to 15.9 L/d (day 254) and then stabilized with typical fluctuation. The digester B average biogas production methane yield, and methane content were 13.7 L/d (day 254-283), 0.727 L-CH₄/g-VS added (day 254-282) and 70.2% (day 249-285), respectively, consistent with the monitoring data during phase 4. Effluent pH, alkalinity, and TVA concentration, along with VS and COD concentrations all were the same levels as in phase 4.

3.3 Discussion

3.3.1 Methane production enhancement

In this study, substantial enhancement in methane production was achieved by anaerobic co-digestion of TWAS with GIW. Stepwise increase of GIW addition was employed to identify the maximum methane production and the corresponding threshold input of GIW that leads to methanogenic inhibition. Table 3-9 compares the methane yield enhancement with previous studies.

The TWAS used in the present research had a lower VS concentration (24.9 g/kg-wet sample, or 2.49%); while TWAS in the work done by Wan et al. (2011) had a VS concentration of 4.94%. This resulted in its higher OLR of 2.34 kg-VS/m³ day compared to 1.24 kg-VS/m³ day of the present research without the presence of co-substrates. The higher OLR also led to a higher methane yield of 0.252 L-CH₄/g-VS added and methane content of 65.1% compared to 0.180 L-CH₄/g-VS added and 60.2% methane content of the present study. When co-digesting TWAS with similar co-substrate loading rates (65.5 %VS of the present study and 64 %VS of Wan et al., 2011) and OLR (2.16 of the present study and 2.34 kg-VS/m³ day of Wan et al. (2011)), 20 vol% GIW co-digestion contributed to a higher methane yield of 0.752 L-CH₄/g-VS added and higher methane content of 70.1% (compared to a methane yield of 0.598 L-CH₄/g-VS added and 66.8% methane content in Wan et al. (2011)).

In addition, comparing the experimental results of the present research with investigations done by Kabouris et al. (2009a) and Luostarinen et al. (2009), it was found that co-digesting TWAS seemed to present better performance than co-digesting TWAS and PS

under similar co-substrate input concentrations (45.8 %VS of the present study, 47.6 %VS of Kabouris et al. (2009a), and 46% VS of Luostarinen et al. (2009)). A methane yield of 0.502 L-CH₄/g-VS added and methane content of 68.2% were achieved with 10 vol% GIW addition (45.8 %VS) in the present research, higher than that of 0.473 L-CH₄/g-VS added with 65.8% methane content (Kabouris et al., 2009a), and 0.463 L-CH₄/g-VS added with 62% methane content (Luostarinen et al., 2009).

Table 3-9 Comparison of maximum methane yield enhancement.

Reference	Feedstock	Co-substrates (VS basis)	OLR (kg-VS/m ³ day)	Methane yield (L-CH ₄ /g-VS added)	% increase in Methane yield	Biogas Production (L/d)	Methane content (vol%)
Present research	TWAS	-	1.24	0.180	-	2.2	60.2%
		GIW (10 vol%, 45.8% VS)	1.58	0.502	178%	6.8	68.2%
		GIW (20 vol%, 65.5% VS)	2.16	0.752	317%	13.7	70.1%
Wan et al., 2011	TWAS	-	2.34	0.252	-	1.8	65.1%
		FOG ^a (64% VS)	2.34	0.598	137%	4.2	66.8%
Kabouris et al., 2009a	PS (40.5 % VS), TWAS (59.5 % VS)	-	2.45	0.159	-	1.3	66.1%
	PS (21.1 % VS), TWAS (31.3 % VS)	FOG ^b (47.6 % VS)	4.35	0.473	197%	6.8	65.8%
Davidsson et al., 2008	PS (50 vol%), WAS(50 vol%)	-	2.50	0.271	-	-	65%
		GS (30 % VS)	2.40	0.344	26.9%	-	69%
Luostarinen et al., 2009	Sewage sludge	-	1.56-2.09	0.278	-	2.8	63%
		GS ^c (46 % VS)	3.46	0.463	66.5%	7.5	62%

^a FOG obtained from a FOG receiving facility.

^b FOG obtained from a FOG dewatering facility receiving FOG from grease traps.

^c GS: grease trap sludge obtained from a meat processing plant.

3.3.2 Stepwise increase of GIW addition

In this study, the highest GIW loading rate achieved without digester failure was 20 vol%, or 65.5 %VS. This GIW input concentration enhanced the methane yield from 0.180 to 0.752 L-CH₄/g-VS added, biogas production from 2.2 to 13.7 L/d, and methane content from 60.2% to 70.1%. These values contributed to a 317% increase in methane yield (Figure 3-4 and 3-5). The 0.752 L-CH₄/g-VS added methane yield was the highest value reported for co-digestion of lipid-rich substrates.

The stepwise increase in GIW loading rates in the present study very likely played an essential role in the significant enhancement of methane production. Wan et al. (2011) used parallel reactors with various co-substrate additions, while the present experiment was carried out with a stepwise increase of co-substrate concentration in a single reactor. Silvestre et al. (2011) demonstrated the biomass acclimation to FOG waste due to a stepwise increase in co-substrates dose. It was suggested that a slow increase in co-substrates input may allow a gradual increase in the acetogenic community to process LCFAs and reduce the LCFA inhibition due to increased LCFAs degradation (Silvestre et al. ,2011). The substantial enhancement in methane yield was likely due to the stepwise increase of co-substrate addition as it provided longer time for microbial acclimation and reduced the GIW inhibitory effect.

Stepwise increase in co-substrate addition was also employed in research done by Luostarinen et al. (2009). However, comparing the methane production of Luostarinen et al. (2009) at 46 %VS co-substrate input (0.463 L-CH₄/g-VS added, with stepwise addition) to that of Kabouris et al. (2009a) at 47.6 %VS (0.473 L-CH₄/g-VS added, without stepwise

addition), no significant contribution of stepwise input strategy was observed. The co-substrate used in Luostarinen et al. (2009) was FOG waste from a meat processing plant, whose characteristics are essentially different from that of GIW used in the present study. Although not shown in their feedstock characterization, waste industrial FOG from food processors might contain higher amount of fats, lipids, and other substances that may potentially present greater inhibition on the anaerobic co-digestion.

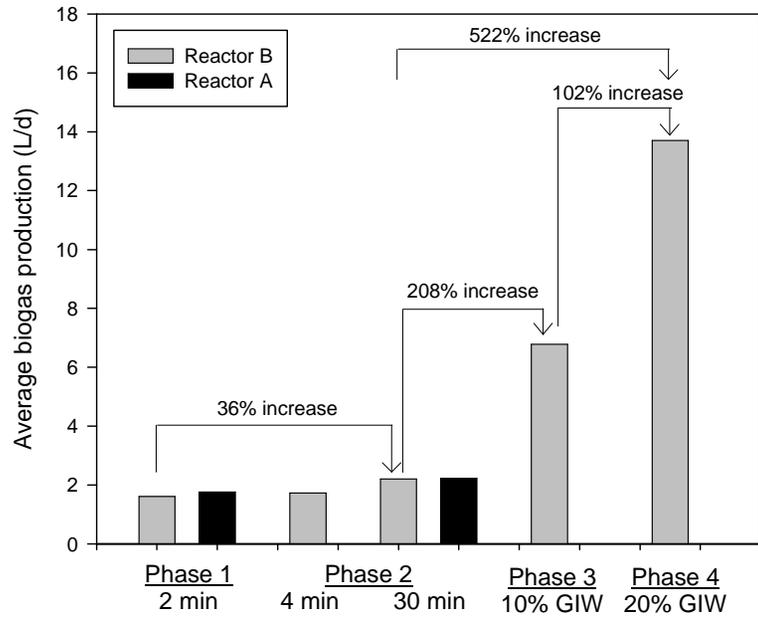


Figure 3-4 Average biogas production enhancement.

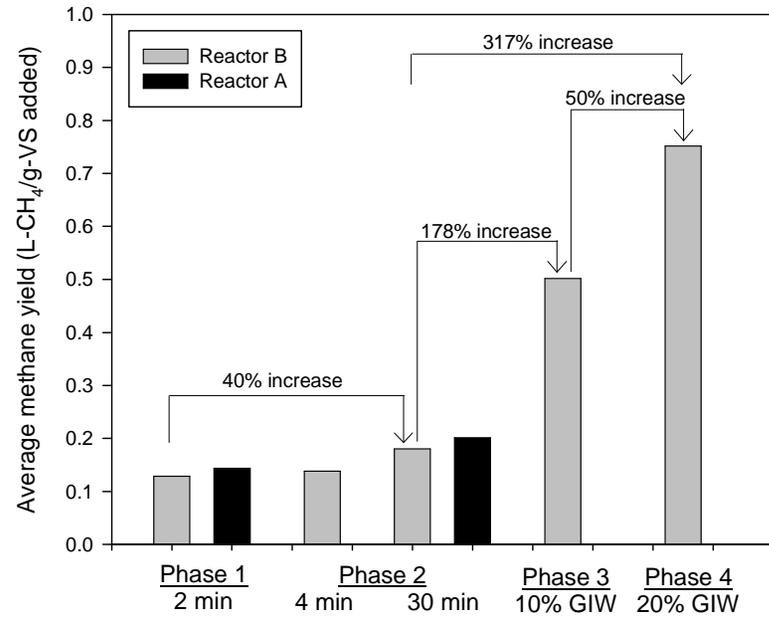


Figure 3-5 Average methane yield enhancement.

3.3.3 Threshold GIW input concentration

The threshold input of GIW was identified in phase 5 by a drastic decrease in methane yield and dramatic changes in monitoring parameters including effluent pH, alkalinity, and VS and COD reduction rates in response to the FOG inhibition. Identification of threshold GIW input is essential as it serves to evaluate the financial feasibility of GIW co-digestion for WWTFs. The threshold limit for GIW addition leading to an inhibited digestion process was identified in range of 20 to 40 vol% or 65.5 to 83.5 %VS as the inhibition occurred during phase 6. Past experience has demonstrated the approximate range of lipid-rich co-substrate maximum allowable inputs, shown in Table 3-10. Combining the present experimental results of 65.5-83.5 %VS with that of 64-75 %VS (Wan et al., 2011), 30-100 %VS (Davidsson et al., 2008), and 46-71 %VS (Luostarinen et al., 2009), a narrower but rough range from 65.5 to 71 %VS can be identified, if ignoring the fact that different combinations of feedstock and co-substrates with their various characteristics could exert different influences on the anaerobic process performance.

The stepwise increase of GIW addition from 10 vol%, 20 vol%, to 40 vol% contributed to the highest GIW loading rate reported, 65.5 %VS, without digester failure. However, stepwise GIW increase in narrower interval such as 5 vol% and longer SRT for the adaptation of microbial communities may lead to a higher GIW input threshold and corresponding methane yield. Biomass acclimation by proper stepwise GIW input strategy is expected to reduce the LCFA inhibition and subsequently increase the GIW threshold input limit. Additionally, the threshold input of GIW affected by the degree of LCFA inhibition may be associated with the characteristics of co-substrate. Since GIW from different sources

contain LCFAs and constituents that can be biodegraded into LCFAs in various concentrations and mixtures, the degree of inhibitory effect is expected to vary with the GIW characteristics. In this study, besides the GIW overdose, the interaction among LCFA inhibition, GIW characteristics, and microbial acclimation due to stepwise GIW loading rate collectively might have played an essential role in determining the threshold GIW input and digester failure. Further investigation on the exact nature and mechanism of GIW inhibition is needed for a successful full scale application of GIW co-digestion at WWTFs.

Table 3-10 Comparison of co-substrate threshold input concentration.

Reference	Feedstock	Co-substrates (VS basis)	OLR (kg-VS/m ³ day)	Methane yield (L-CH ₄ /g-VS added)	% increase in methane yield	Biogas Production (L/d)	Methane content (vol%)	
Present research	TWAS	R1 ^a	-	1.24	0.180	-	2.2	60.2%
			GIW (10 vol%, 45.8 % VS)	1.58	0.502	178%	6.8	68.2%
			GIW (20 vol%, 65.5 % VS)	2.16	0.752	317%	13.7	71.1%
			GIW (40 vol%, 83.5 % VS) (failed)	3.54	-	-	19.6	-
Wan et al., 2011	TWAS	R1	-	2.34	0.252	-	1.849	65.1%
		R2	FOG ^b (64% VS)	2.34	0.598	137%	4.1736	66.8%
		R3	FOG (75% VS) (failed)	3.40	-	-	0.04	21.3%
Davidsson et al., 2008	PS (50 vol%), WAS(50 vol%)	R1	-	2.50	0.271	-	-	65%
		R2	GS (10 % VS)	2.50	0.295~0.308	9-14%	-	66%
	-	R3	GS (30 % VS)	2.40	0.344	26.9%	-	69%
		R4	GS (100 % VS) (failed)	0-2.3	-	-	-	-
Luostarinen et al., 2009	SS	R1	-	1.56-2.09	0.278	-	2.82	63%
		R2	GS ^c (5 % VS)	1.67-2.23	0.374	34.5%	3.48	66%
			GS (20 % VS)	1.93-2.45	0.441	58.6%	4.59	64%
		R3	GS (28 % VS)	2.80	0.444	59.7%	6.83	61%
			GS (38 % VS)	3.13	0.447	60.8%	6.09	65%
			GS (46 % VS)	3.46	0.463	66.5%	7.47	62%
			GS (55 % VS) (failing)	4.01	0.318	14.4%	6.10	63%
			GS (71 % VS) (failing)	4.41	0.315	13.3%	6.75	58%

^a R1 refers to reactor 1. If three reactors (e.g. R1, R2, R3) are displayed, the research was conducted in three parallel reactors with different amounts of co-substrate added. If multiple concentrations of co-substrates are displayed for only one reactor, the experiment was carried out in stepwise increase of co-substrate concentration for a single reactor (e.g. R1 in the present research and R3 in the Luostarinen et al., 2009).

^b FOG obtained from a FOG receiving facility.

^c GS: grease trap sludge.

3.3.4 Digester foaming

During phase transition when higher GIW doses were added, digester foaming was observed, and resulted in blockage in the mixing system. Digester foaming/scum is one of the concerns associated with anaerobic co-digestion of GIW/GTW due to its FOG content (Ganidi et al., 2009; Kabouris et al., 2008; Jeganathan et al., 2006). Since adequate mixing intensity is essential for digester treating FOG waste to avoid the formation of a scum layer (Joyce and Donaldson, 2005), an increase in mixing duration from 30 min per hour to a higher level in the present study might resolve the foaming issue and potentially contribute to a better performance in maximum methane yield, GIW threshold limit, and the degree of GIW inhibition.

3.3.5 GIW inhibition warning signal

In digester operation, inhibition or digester failure is often indicated by a decrease of methane yield, methane content, and/or increase in alkalinity to TVA ratio (Speece, 1996). Alkalinity to TVA ratio is a common monitoring parameter for inhibition; however, it might not be a suitable option to monitor digesters co-processing GIW, as it failed to give early warning signal during phase 5 (Figure 3-1 and 3-2). On the other hand, monitoring results of methane yield successfully identified the digester failure in earlier stage of phase 5 compared to all the other parameters by showing an apparent drop in concentration.

3.3.6 Influence of loading shock and stepwise increase of GIW addition

As verified by Silvestre et al. (2011), a slow increase in co-substrates dose benefits the biomass acclimation to lipid-rich co-substrate, leading to better degradation performance and reduction of inhibition. Aside from contributing to a significant enhancement of biogas production, stepwise increase of GIW addition also produced more robust methanogenic communities in surviving higher GIW loading rate. Microbial communities fed with increasing GIW loading rate (digester B) were able to survive 20 vol% GIW input (phase 4); while in microbial communities that have never experienced a GIW addition (digester A), a sudden input of 20 vol% GIW resulted in a quick failure in the digestion process (phase 6). Moreover, with a onetime GIW loading "shock" that led to a digester failure with 40 vol% GIW addition (phase 5), the recovered microbial communities were able to reach the same biogas production as before when the GIW loading rate was again increased to 20 vol% (phase 6). These demonstrate the robustness of the "trained" methanogenic communities through stepwise increase of GIW addition. The recovered microbial communities are expected to sustain a higher GIW loading rate above 40 vol% after the onetime 40 vol% GIW loading in phase 5 that caused digester failure.

Chapter 4 Conclusions

Municipal water and wastewater treatment systems are extremely energy-intensive. As a result, efforts have been undertaken to facilitate energy independence at WWTFs. Achieving this goal calls for not only improvement of efficiency in existing treatment processes, but also onsite energy production from alternative energy supplies. Anaerobic digestion produces biogas that consists of methane, carbon dioxide, and other constituents. This renewable source of bioenergy presents a great potential for cost-effective biogas utilization at WWTFs to generate thermal energy and electricity for onsite use or export of surplus electricity back to the grid. However, most of the WWTFs operating digesters flare excess biogas instead of employing utilization technologies. Biogas production enhancement thus plays an essential role in improving the economic feasibility of anaerobic biotechnology at WWTFs.

Substantial enhancement in biogas production can be achieved by anaerobic co-digestion of biosolids with substrates of high bioenergy potential such as GIW/GTW from FSEs. Up to 45 billion pounds of GIW/GTW are generated annually. With an average 9,282 BTU/lb of energy content in GIW/GTW, approximately 211 trillion BTU or 62 billion kWh/yr of energy potential, with an economic value of \$0.92 billion at a natural gas cost of 4.37\$/MMBTU and \$5.5 billion at an electricity cost of 0.089 \$/kWh. This amount of energy can translate into energy savings when proper biogas utilization technologies are employed, and also serve as a sustainable GIW/GTW disposal alternative. Furthermore, anaerobic co-digestion is able to address the entire content of GIs/GTs, reducing the trouble associated with the remaining high COD, lipid-rich wastewater and food residuals. Consequently, the

integration of biosolids management, GIW/GTW biodegradation treatment, and sustainable bioenergy production presents a great financial incentive to promote energy independence at WWTFs as an environmentally and economically beneficial biotechnology.

In this study, substantial enhancement in biogas production was achieved by anaerobic co-digestion of TWAS with GIW. The experiments were conducted to co-process TWAS and the entire content of the GI including FOG, food residuals, and associated wastewater in lab scale semi-continuous digesters. Stepwise increase of GIW addition was employed to identify the maximum methane production and the corresponding threshold input of GIW that leads to methanogenic inhibition. The highest GIW loading rate achieved without digester failure was 20 vol%, or 65.5 %VS. This GIW input concentration enhanced the methane yield from 0.180 to 0.752 L-CH₄/g-VS added, biogas production from 2.2 to 13.7 L/d, and methane content from 60.2% to 70.1%. These values contributed to a 317% increase in methane yield. The 0.752 L-CH₄/g-VS added methane yield was the highest value reported for co-digestion of lipid-rich substrates. The substantial enhancement in methane yield was likely due to the stepwise increase of co-substrate addition as it provided longer time for microbial acclimation and reduced the GIW inhibitory effect. The threshold limit for GIW addition leading to an inhibited digestion process was identified in range of 20 to 40 vol% or 65.5 to 83.5 %VS.

The stepwise increase of GIW addition from 10 vol%, 20 vol%, to 40 vol% contributed to the highest GIW loading rate reported, 65.5 %VS, without digester failure. However, stepwise GIW increase in narrower interval such as 5 vol% and longer SRT for the adaptation of microbial communities may lead to a higher GIW input threshold and

corresponding methane yield. Biomass acclimation by proper stepwise GIW input strategy is expected to reduce the LCFA inhibition and subsequently increase the GIW threshold input limit. Additionally, the threshold input of GIW affected by the degree of LCFA inhibition may be associated with the characteristics of co-substrate. Since GIW from different sources contain LCFAs and constituents that can be biodegraded into LCFAs in various concentrations and mixtures, the degree of inhibitory effect is expected to vary with the GIW characteristics. In this study, in addition to the GIW overdose, the interaction among LCFA inhibition, GIW characteristics, and microbial acclimation due to stepwise GIW loading rate collectively might have played an essential role in determining the threshold GIW input and digester failure. Further investigation on the exact nature and mechanism of GIW inhibition is needed for a successful full scale application of GIW co-digestion at WWTFs.

Aside from contributing to a significant enhancement of biogas production, stepwise increase of GIW addition also produced more robust methanogenic communities in surviving higher GIW loading rate. Microbial communities fed with increasing GIW loading rate were able to survive 20 vol% GIW input; while in microbial communities that have never experienced a GIW addition, a sudden input of 20 vol% GIW resulted in a quick failure in the digestion process. Moreover, with a onetime GIW loading "shock" that led to a digester failure with 40 vol% GIW addition, the recovered microbial communities were able to reach the same biogas production as before when the GIW loading rate was again increased to 20 vol%. These demonstrate the robustness of the "trained" methanogenic communities through stepwise increase of GIW addition. The recovered microbial communities are expected to

sustain a higher GIW loading rate above 40 vol% after the onetime 40 vol% GIW loading that caused digester failure.

Furthermore, in this study the alkalinity to total volatile acid ratio was identified as an unsuitable parameter for GIW inhibition monitoring. Instead, monitoring results of methane yield successfully identified the digester failure in earlier stage compared to all the other parameters by showing an apparent drop in concentration.

Chapter 5 Future work

Despite the proven feasibility of conducting anaerobic co-digestion of GIW to enhance methane production, a successful full scale application at WWTFs is unlikely without more in-depth research and evaluation on various related issues in the future. These issues include

- (a) necessity of GIW pretreatment such as dewatering,
- (b) selection of proper biogas utilization technology for onsite energy generation based on site-specific conditions at local WWTFs,
- (c) distribution/transportation of GIW produced in the associated WWTF service area,
- (d) GIW characterization for inhibition evaluation,
- (e) evaluation of the exact nature and inhibition mechanism of GIW, and
- (f) influence of food particles and wastewater on co-digestion process.

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APPENDICES

Appendix A – Total solids (TS) and volatile solids (VS) measurement procedure

1. Remove the six evaporating dishes with “dried” samples (not “wet” anymore) from last time from the 105°C drying oven into a desiccator (use a cart for transportation every time).
2. Preheat a muffle furnace (the two in the back, make sure you have reservations every week) at 550°C.
3. After the six evaporating dishes with dried samples from last time cool down to room temperature, weigh them using an analytical balance, and record the numbers (in Dish + dried sample column, be alert to change in weight due to air exposure).
4. Put the six clean evaporating dishes (bottom) and six evaporating dishes with dried samples from last time (top) in an furnace (be aware of the heat) at 550°C for 2 hours.
5. Turn off the furnace and open the furnace door to cool down for 1.5 - 2 hours.
6. Remove them into a desiccator (move them while the dishes are still hot, not completely cool down) (keep the dishes from contacting the desiccator surface as they might melt it)
7. Weigh the cool-down six evaporating dishes with “ignited” (not “dried” anymore) samples using an analytical balance, and record the numbers (in Dish + ignited sample column, be alert to change in weight due to air exposure).
8. Weigh the cool-down six clean evaporating dishes, and record the numbers (in Dish column) (compare with the numbers last time, they should be close).

9. Add different sample portions of effluents (stored in the cold room, add immediately after taking them out) into the six clean evaporating dishes, and record the numbers (in Dish + wet sample column, be alert to change in weight due to CO₂ escaping).
10. Put the six evaporating dishes with wet samples into the 105°C drying oven for evaporation to dryness for 2 days.
11. Discard the remaining effluents, and clean the six evaporating dishes with ignited samples and the effluents bottles.

Appendix B – Total volatile acids (TVAs) recovery factor (f) measurement

Base: NaOH
Normality of base (eq): 0.1
Equivalent weight of HAc: 60000
Sample Specific gravity: 1.0289

Final recovery factor (f)

Mean **SD**
 0.982 0.015

Date	Distillation (to 200 ml)				Titration						
	C Sample (mg/L)	V sample (ml)	Dilut times	C Dilu (mg/L)	V sample (ml)	pH start	pH end	V NaOH (ml)	TVA (mg/L)	Final TVA (mg/L)	f
2/29	500	50	4	125	50	2.91	-	1.36	163.2	652.8	1.306
	500	50	4	125	50	2.91	-	1.32	158.4	633.6	1.267
3/2	500	100	2	250	50	3.64	8.61	2.09	250.8	501.6	1.003
	500	100	2	250	50	3.58	8.73	2.02	242.4	484.8	0.970
	500	100	2	250	50	3.59	8.44	2.02	242.4	484.8	0.970
3/5	500	100	2	250	80	3.24	8.58	3.23	242.25	484.5	0.969
	500	100	2	250	75	3.25	8.59	3	240	480.0	0.960
3/24	500	100	2	250	70	3.7	8.55	2.96	253.71	507.42857	1.015
	500	100	2	250	65	3.58	8.58	2.68	247.38	494.76923	0.990

Appendix C – Individual volatile fatty acids analysis standard curve

Prepared by David Black, CCEE, NCSU, Raleigh, NC

Date Analyzed: 6/14/12

Direct Injection on Shimadzu 2014 with packed inlet.

Preliminary data for general info only. New stock to be generated.

Standard Curve Information: 4/17/12 w/9/19/11 stock on Shimadzu GC by direct injection by David C. Black

Equation: $y \text{ (ug/L)} = (10^{ax^2 + bx + c}) * \text{(Dilution Factor)}$ where $x = \log(\text{area})$

0.5 uL splitless injection in packed inlet

	Acetic	Propionic	i-Butyric	n-Butyric	i-Valeric	Valeric	i-Caproic	Hexanoic
a	0.8166	0.9262	1.0170	0.9769	1.0750	0.9509	0.9413	0.8334
b	-1.8309	-2.7661	-3.4763	-3.2206	-3.8774	-3.1291	-3.0966	-2.4548
R ²	0.995	0.9985	0.9991	0.9997	0.9959	0.999	0.9991	0.9929
low std area	4964	21199	5411	38652	9547	3559	961	994
hi std area	10,512,459	17,267,433	2,197,207	21,963,175	2,693,761	2,591,823	2,619,784	2,705,360
Lo/Hi Quant. Limit mg/L	20 10,000	20 10,000	2 1,000	20 10,000	2 1,000	2 1,000	2 1,000	2 1,000
QC Information:								
	Acetic	Propionic	i-Butyric	n-Butyric	i-Valeric	Valeric	i-Caproic	Hexanoic
QC value	1073	1043	92	968	89	101	96	105
Std 4	915,568 1090.0	1485344 891.8	200414 82.4	1805657 778.9	242014 81.3	188187 77.0	165409 65.4	114156 57.6
	101.6%	85.5%	89.5%	80.5%	91.4%	76.2%	68.1%	54.8%