ANAEROBIC CO-DIGESTION OF MULTIPLE FEEDSTOCKS FOR BIOMETHANE RECOVERY- THE IMPACT OF LIPIDS:PROTEINS:CARBOHYDRATES RATIO

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ANAEROBIC CO-DIGESTION OF MULTIPLE FEEDSTOCKS FOR BIOMETHANE RECOVERY- THE IMPACT OF LIPIDS:PROTEINS:CARBOHYDRATES RATIO

Anahita Rabii, Doctor of Philosophy, 2020
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ABSTRACT

Municipalities are facing increasing challenges regarding management and disposal of solid waste. Anaerobic digestion (AD) of municipal biowaste enables waste reduction and biogas production that can be utilized as a renewable source of energy for heat and power generation. Anaerobic co-digestion (AnCoD) enhances the performance of conventional mono-digestion. The mixing ratio of the feedstocks is an important criterion in AnCoD design which is typically determined based on the optimum carbon to nitrogen (C:N) ratio within the range of 25-30 or COD:N ratio in the range of 50-140. However, literature has shown contradictory results for the optimum C:N and COD:N ratios. Therefore, the main objective of this study was to primarily investigate the influence of the mixing ratio of the feedstocks including thickened waste activated sludge (TWAS), manure and source separated organics (SSO) on improving biomethane production and introducing a new methodology for optimizing the mixing ratio in AnCoD based on the lipids, proteins, and carbohydrates contents as the three main compounds existing in biowaste. The hydrolysis/acidification performance in AnCoD of manure, TWAS and SSO individually and in different combinations was also investigated. This study has introduced an empirical model to explain the relationship between the biomethane production and lipids: proteins: carbohydrates ratio of the feedstocks in anaerobic co-digestion of TWAS, manure and SSO. Among the binary ad ternary combinations, the ternary co-digestion of TWAS/manure/ SSO at the mixing ratio of 2:4:4 and lipids: proteins: carbohydrates ratio of 1:3:12 resulted in the maximum ultimate methane production. The maximum methane yield of 363 ml CH_4/g COD added corresponded to co-digestion of manure/SSO at the mixing ratio of 7:3. The maximum hydrolysis rate corresponded to the co-digestion of TWAS/manure at the ratio of 9:1. Overall, the best performance in both hydrolysis and methanogenesis was achieved by the co-digestion of TWAS with SSO at the ratio of 3:7 as well as TWAS/manure/SSO at the ratio of 2:4:4 compared to other feedstock mixes. It was observed that the proposed second order polynomial model could describe the relationship between biomethane production and lipids, proteins, and carbohydrates content of the feedstock.

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Dedication

To the light of my eyes

To my heavenly gift

And to all of my

inspiration

Armin

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List of Nomenclature

AD	Anaerobic digestion
AnCoD	Anaerobic co-digestion
TWAS	Thickened waste activated sludge
SSO	Source separated organics
F:M	Food to microorganism ratio
COD	Chemical Oxygen Demand
TCOD	Total Chemical Oxygen Demand
SCOD	Soluble Chemical Oxygen Demand
VSS	Volatile suspended solids
TSS	Total suspended solids
VS	Volatile solids
TS	Total solids
VFAs	Volatile fatty aids
GC	Gas chromatograph
S Proteins	Soluble proteins
S Lipids	Soluble lipids
S Carbohydrates	Soluble carbohydrates
LCFAs	Long chain fatty acids
TN	Total nitrogen
TSN	Total soluble nitrogen
C:N	Carbon to nitrogen ratio
COD:N	COD to nitrogen ratio

Chapter 1
Introduction

1. INTRODUCTION

Waste materials including biowaste are constantly being generated due to inevitable human activities. Different techniques have been employed to manage and reduce the growing amount of biowaste. However, such technologies result in secondary environmental impact. Landfilling can lead to soil and groundwater contamination imposing further actions and cost to remediate the secondary contamination. If not well managed and maintained, incineration will cause air pollution and subsequent environmental and health impact (Elbeshbishy et al., 2017; Feng et al., 2018). Mitigation of climate change and fossil fuel consumption demands a shift to alternative, renewable energy sources (Cornelissen et al., 2012; Kwietniewska and Tys, 2014). As reported by International Energy Outlook, 2011, total world energy consumption will increases from 505 quadrillion British thermal units (Btu) in 2008 to an estimated number of 619 quadrillion Btu in 2020, and it is expected to rise to 770 quadrillion Btu in 2035 which is equivalent to 53% increase in spam of 27 years (OECD/EIA, 2011). Energy obtained from biomass is regarded as an important future renewable source, as it is capable of providing a continuous power generation and it is also an essential part of the current CO_2-mitigation policy (Appels et al., 2011; Kwietniewska and Tys, 2014).

Production of biofuel from biomass, has received increasing attention during recent years. Several treatment processes and technologies have been established to obtain sustainable and affordable biofuel which includes syngas (SNG). SNG is a synthetic gas produced by gasification of a carbon containing fuel that has some energy value. However, production of SNG is narrowly practiced due to its cost (Guo et al., 2015; Schuetzle et al., 2015). Anaerobic digestion (AD) which is widely used for the treatment of wet residual biomass is considered to be one of the most favorable processes for biofuel production from biomass.

In Canada, more than 27 million tons of food waste are disposed yearly. The produced organic waste can lead to serious health and environmental issues. Municipalities have taken different management actions to manage the organic fraction of municipal solid waste (OFMSW). Biological processes for the treatment and/or conversion of OFMSW to value-added products has aroused significant attention due to its financial benefits and less environmental impacts compared to the other waste disposal methods such as landfilling, incineration, gasification, etc. (Luk and Bekmuradov, 2014; Naroznova et al., 2016; Razavi et al., 2019). Organic matters in waste includes food scraps, yard trimmings, wood waste, paper and cardboard products which normally make up around 33% by weight of the municipal solid waste. Source Separated Organics (SSO) refers to the organic waste which is segregated from other waste materials at the source for separate collection. SSO comprises mostly of food waste which is separated from the residential waste (Kelleher Robins, 2013).The study by Kelleher Environmental have indicated that almost 23% of waste from non-residential industrial, commercial and institutional (IC&I) sector is food waste which is generated by institutions and businesses in communities across Canada. The majority of food waste from this sector is created by restaurants, hotels, food processing facilities and hospitals. A considerable amount of biogas through anaerobic digestion process can be produced by food wastes from all of these sources (Government of Canada, 2013; Kelleher Robins, 2013).

In areas with vast numbers of large-scale livestock farms, the development of a treatment process for manure is necessary to properly handle the high amount of produced waste. Animal manure provides adequate nutrients for anaerobic digestion to produce biogas as a source of renewable energy. High degradability of manure when flushed and fibrous materials are separated makes its anaerobic digestion a good treatment option to minimize waste above and beyond bioenergy recovery. However, in some farms manure is collected in lagoons or in the open areas which can result in air and water pollution. Manure stockpiling can lead to serious health environmental issues as a result of emissions such as methane, nitrous oxide, ammonia, hydrogen sulfide, volatile organic compounds and particulate matter (Lin et al., 2018; Peter Wright et al., 2013). Co-digestion of manure with additional substrates provided that appropriate mixture ratios are applied can improve digestion process and increase biogas production.

In addition, in Canada more than 660,000 metric tons of dry stabilized biosolids is produced each year. Biosolids are produced during the treatment of wastewater due to the removal of the solids content (sludge) from the liquid effluent. Biosolids management is responsible for almost 50% of the total operating annual cost of wastewater management. According to Canadian Council of Ministers of the Environment (CCME), a Canada-wide approach for the management of wastewater biosolids was approved on October 11, 2012 which encourages the favorable and thorough management of biosolids. Biosolids are the nutrient-rich, organic materials which makes them a useful resource, containing necessary plant nutrients, and organic matter. They can be recycled as a fertilizer and soil improvement for agricultural utilization. Annually, around 195,000 tons of biosolids is processed by the Ashbridges Bay and Highland Creek wastewater treatment plants in City of Toronto. The City's biosoilds management can be classified as the following options (City of Toronto, 2019):

- Land application for agricultural and other purposes
- Pelletization for fertilizer production
- Alkaline stabilization for producing fertilizer, landfill cover, or for the pH adjustment of acidic soil
- Landfilling
- Incineration

There are some pros and cons associated with any of the above options. However, Anaerobic Digestion (AD) for producing bioenergy from organic waste has shown to be an energy-efficient technology while causing less environmental footprint compared to other technologies such as composting that requires large land and can release uncontrolled odorous volatile organic compounds and pathogens (Bordeleau and Droste, 2011; Lin et al., 2018; Razavi et al., 2019). Nevertheless, in conventional anaerobic digestion of single feedstock some problems such as nutrient imbalance, low biodegradability, toxic substances, etc. can hinder the process causing low production of biogas. Such problems can be modified by simultaneous digestion of two or more feedstocks referred to as anaerobic co-digestion (AnCoD).

1.2. Research objective

Anaerobic digestion involves sequential phases comprising hydrolysis, acidogenesis, acetogenesis and methanogenesis. Anaerobic digestion of multi feedstocks has been advantageous compared to conventional anaerobic digestion of single feedstock. Literature has showed contradictory values for the optimum range of the C:N or COD:N ratio for optimizing the mixing ratio of the feedstocks in anaerobic co-digestion systems. In addition, hydrolysis of particulate organic matter which is the rate-limiting stage, remains as the least well-defined phase of the process. Therefore, this research focused on the three main following objectives:

1- Study biomethane potential in AnCoD of TWAS, Manure, and SSO. This part of the study included the following purposes:

- Evaluate the influence of the mixing ratio on improving biomethane production in anaerobic co-digestion of TWAS, manure, and SSO.
- Investigate the impact of lipids: proteins: carbohydrates on improving biomethane production in anaerobic co-digestion of TWAS, manure, and SSO.
- Compare the impacts of COD:N ratio, lipids:proteins, lipids:carbohydrates, and proteins:carbohydrates on biomethane production in anaerobic co-digestion of TWAS, manure, and SSO.

2- Study the hydrolysis/acidification in AnCoD of TWAS, Manure, and SSO. This part of the present research comprised the following areas:

- Monitor the dynamic changes of soluble and particulate COD, lipids, proteins, and carbohydrate content in co-digestion of TWAS, manure, and SSO.
- Evaluate the influence of the mixing ratio on hydrolysis/acidification performance in co-digestion of TWAS, manure, and SSO.
- Study the hydrolysis kinetics of lipids, proteins, carbohydrates and COD and calculate their hydrolysis rate coefficients for co-digestion of TWAS, manure, and SSO.

3- Investigate the relationship between biomethane production and the lipids, proteins, and carbohydrates contents of the feedstocks aiming to:

- Propose an empirical model that explains the relationship between biomethane yield and concentration of lipids, proteins, and carbohydrates at different mixing ratios in anaerobic digestion of manure, TWAS and SSO individually and in their co-digestion.

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Chapter 2

Literature review

2. LITERATURE REVIEW

2.1. Anaerobic digestion

Biological treatment is an integral part of any wastewater treatment plant that treats wastewater from either municipality or industry which contains soluble organic contaminants or a mix of both source types. There are clear economic advantages of biological systems in terms of capital investment and operating costs when compared to other methods such as chemical oxidation, thermal oxidation, etc. In addition, mostly biological treatment converts toxic contaminants to end products that are less harmful or non-harmful with the help of microorganisms (Mittal, 2011). The two types of biological treatment: aerobic and anaerobic processes are directly related to first the type of microorganisms involved in the degradation of organic compounds in a particular wastewater and second the operating conditions of the bioreactor. Aerobic treatment processes occur in the presence of air and consume those microorganisms also called aerobes, which use molecular/free oxygen to assimilate organic contaminant. They convert them in to carbon dioxide, water and biomass (Eddy, 2003). The anaerobic processes, on other side occur in the absence of air (molecular/free oxygen) by those microorganisms also called anaerobes which do not require air or molecular/free oxygen to assimilate organic contaminants. The end products of organic assimilation in anaerobic treatment contains methane (CH_4) and carbon dioxide (CO_2) and biomass.

Anaerobic digestion of municipal wastewater sludge has been widely developed since the early 1900s and is the most applied sludge treatment method. Throughout the process about 40% to 60% of the organic solids is converted to methane and carbon dioxide. The chemical composition of the produced gas contains 60-65% methane, 30-35% carbon dioxide, and small quantities of H_2, N_2, H_2S, and H_2O. Among these, methane has the most value since it is a hydrocarbon fuel producing 36.5 MJ/m^3 in combustion (Lema and Suarez, 2017). The residual organic matter is chemically stable, nearly odorless, and contains considerably reduced levels of pathogens.

Hydrogen production by the use of anaerobic microbial communities also referred to as dark fermentation from organic waste has raised attention due to its ability to produce an environmentally benign energy source, while it stabilizes waste material simultaneously (Sung et al., 2003). Albert Lea facility in Minnesota with 12 million gallons/day of sewage and 4.5 million gallons/day sludge produces 75,000 ft^3/day of biogas. Their facility which consists of 4 Capstone microturbines, each of them 30 kW, is producing 2,500 kWh per day electricity at peak production in addition to 28,000 Btu/day of heat.

According to Canadian Biogas Study conducted by Canadian Biogas Association in 2013, biogas production has significant potential for extension and development. All biogas sources excluding energy crops, could meet nearly 3% of Canada’s natural gas demand as biogas contribution is 2,420 Mm^3 /year of renewable natural gas (RNG) or 1.3% of its electricity demand where biogas contribution is 810 MW. Anaerobic digestion (AD) is a multi-step process during which organic material is converted to biogas and digestate in the absence of oxygen and presence of anaerobic microorganisms. The AD process occurs in four stages including hydrolysis/liquefaction, acidogenesis, acetogenesis, and methanogenesis. The pathways in anaerobic degradation is shown in figure 1.

The first stage of the process is hydrolysis/liquefaction. This stage is very important in AD process since polymers cannot be directly consumed by the fermentative microorganisms. Therefore, hydrolysis makes the substrate available for the following conversion steps. In hydrolysis stage, insoluble complex organic materials are decomposed into their constituents. This allows them transport through microbial cell membrane (Madigan, 2014). Hydrolysis is accomplished due to the function of hydrolytic enzymes. In the first step of hydrolysis, or liquefaction, fermentative bacteria convert the insoluble complex organic compounds like cellulose, into soluble molecules such as sugars, fatty acids, and amino acids.

Hydrolysis/Liquefaction reactions

$$\text{Lipids} \rightarrow \text{Fatty Acids}$$

$$\text{Polysaccharides} \rightarrow \text{Monosaccharides}$$

$$\text{Protein} \rightarrow \text{Amino Acids}$$

$$\text{Nucleic Acids} \rightarrow \text{Purines and Pyrimidines}$$

The next stage is acidogenesis/fermentation through which facultative and anaerobic bacteria convert sugars, amino acids and fatty acids to hydrogen, acetate, carbon dioxide, VFAs such as propionic, butyric and acetic acid, ketones, alcohols and lactic acid. Although a simple substrate like glucose can be fermented, various products are created by the diverse bacterial community.

Conversion of glucose to acetate, ethanol and propionate are shown in the reactions 1, 2 and 3 below respectively (Kangle et al., 2012).

$$C_6H_{12}O_6 + 2\ H_2O \rightarrow 2\ CH_3COOH + 2\ CO_2 + 4\ H_2 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2\ CO_2 \quad (2)$$

$$C_6H_{12}O_6 + 2\ H_2 \rightarrow 2CH_3CH_2COOH + 2\ H_2O \quad (3)$$

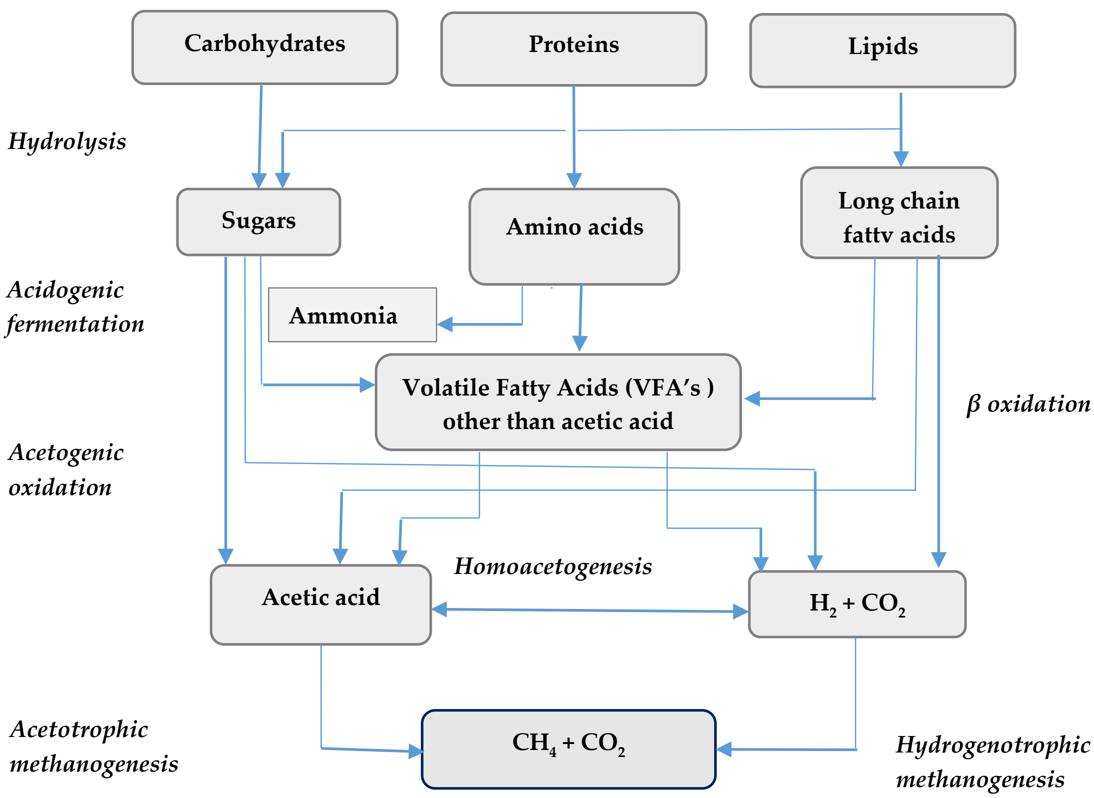


Figure 2.1. Pathways of anaerobic digestion (Salminen and Rintala, 2002)

In equilibrium condition, most of the organic material is converted into substrates such as acetate, hydrogen and carbon dioxide that are readily available for methanogenic microbes. However, a significant portion of about 30% is converted to short chain fatty acids or alcohols. Degradable organic material is eliminated in this step (Angelidaki et al., 2009a; Kangle et al., 2012). The byproduct of amino acids fermentation, ammonia and hydrogen sulfide are released. These compounds can be inhibitory for AD process (Kangle et al., 2012; Salminen and Rintala, 2002).

During the third stage, acetogenesis, certain fermentation products including VFAs with more than two atoms of carbon, alcohols and aromatic fatty acids are converted into acetate and hydrogen these conversions take place via obligate hydrogen producing bacteria (Boe, 2006, Kangle et al., 2012). In this step, the products of the first phase are converted to simple organic acids, carbon dioxide and hydrogen by acetogenic bacteria, also called acid formers. The main acids produced include acetic acid (CH_3COOH), propionic acid (CH_3CH_2COOH), butyric acid ($CH_3CH_2CH_2COOH$), and ethanol (C_2H_5OH). The activities of different microorganisms cause the formation of the products that are created during acetogenesis. These microorganisms include syntrophobacter wolinii, a propionate decomposer and sytrophomonos wolfei, a butyrate decomposer. Also other acid formers include clostridium spp., peptococcus anerobus, lactobacillus, and actinomyces (Kangle et al., 2012; Themelis, 2002). Hydrogen-producing acetogenic bacteria yield acetate, H_2 and CO_2 from volatile fatty acids and alcohol, whereas, homoacetogenic bacteria produce acetate from CO_2 and H_2 (Sterling et al., 2001). However, most of the acetate is formed via hydrogen-producing acetogenic bacteria (Angelidaki et al., 2009b). A reaction that occurs during acetogenesis is presented below:

$$C_6H1_2O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

The final stage of AD process is methanogenesis. Various methane-forming bacteria are required in an AD system. That is because a single species is not able to degrade all the existing substrates. The methanogenic bacteria comprise methanobacterium, methanobacillus, methanococcus, and methanosarcina. Methanogenesis can be classified into two groups. These two groups involves acetate and H_2/CO_2 consumers. *Methanosarcina spp*. and *methanothrix spp*. Also known as *methanosaeta* are important in AD process both as H_2/CO_2 and acetate consumers. Almost 70% of the methane is produced from acetate. The remaining 30% is produced due to the reduction of carbon dioxide via hydrogen and other electron donors (Smith and Mah, 1966; Varel et al., 1980). With regard to the type of the substrate consumed by the methanogens, methanogenesis can be classified into two main types (Bitton, 2010; Kangle et al., 2012):

- Hydrogenotrophic methanogenesis

In this type of methanogenesis, hydrogen and carbon dioxide are converted into methane through the following reaction:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

- Acetotrophic or aceticlastic methanogenesis

This type of methanogenesis involves the formation of methane from the conversion of acetate by the following reaction:

$$CH_3COOH \rightarrow CH_4 + CO_2$$

Any substrate that can be converted to methane by anaerobic bacteria is referred to as feedstock. The main components of organic wastes (feedstock) are carbon, oxygen, nitrogen, hydrogen and phosphorus, and microbial cell material of those elements is approximately 50, 20, 12, 8 and 2 % respectively (Gerardi, 2003). Sulfur is also required to synthesize vital proteins in metabolic and anabolic pathways (Madigan, 2014). Feedstocks can be a range of different waste materials from easily degradable wastewater to complex high-solid waste.

AD technologies have shown sufficient adaptability to different feedstocks (Bordoloi et al., 2014). Although AD is a commercial reality for a range of wastes, anaerobic digestion of single waste may be associated with certain drawbacks such as unbalanced nutrients, rapid acidogenesis, poor buffering capacity, high ammonia nitrogen concentration, inhibition of long chain fatty acids which can inhibit methanogenesis and lead to severe instability and process disruption (Bayr et al., 2014; Silvestre et al., 2014).

The growth rate of anaerobic microorganisms and subsequent biogas production depends highly on the composition of the organic matter in feedstock. The constituents of the feedstock added to the digester are consumed selectively by a range of different microbial consortia. In addition, the existence of nitrogen in the feedstock is necessary for the synthesis of amino acids, proteins and nucleic acids. It is also required for ammonia formation to neutralize VFAs produced during the fermentation process and to maintain neutral pH condition for cell growth. However, an excess of nitrogen in the feedstocks can result in toxic effects to bacteria by extreme ammonia formation. Therefore, a suitable amount of nitrogen is required to provide sufficient nutrient while avoiding ammonia toxicity (Hagos et al., 2017; P. et al., 2014).

During anaerobic digestion, a series of complex biological degradation pathways are involved which are influenced by numerous factors. Therefore, a profound understanding of the biochemical activities of anaerobic microorganisms in the AD system is required to support an effective control of the governing factors in anaerobic digestion process (Viotti et al., 2004).

2.2. Process parameters of anaerobic digestion

A number of parameters affect the rate of the different steps of the digestion within the anaerobic environment. Overall, two groups of parameters can affect the anaerobic digestion performance including environmental and operational parameters. Environmental factors comprise temperature, pH, alkalinity, waste characteristics such as the amounts of volatile solids (VS) carbon to nitrogen (C:N) ratio, total solids (TS), nutrients, organic loading rate (OLR), and volatile fatty acids (VFAs). The performance of anaerobic digesters can be reduced by various environmental factors including low pH, accumulation of ammonia and volatile fatty acids (VFAs) which inhibit the activity of methanogenic microorganisms (Heo et al., 2004; Towey, 2013). VS consists of both biodegradable volatile solids (BVS) and the refractory volatile solids (RVS) fractions. BVS fraction of substrate is helpful in better biodegradability of the waste, organic loading rate, C:N ratio, and biogas production. Apart from the environmental factors, a number of operational factors including solid retention time (SRT), hydraulic retention time (HRT), digestion mode in terms of single or multistage approaches, digester design being batch or continuous types, and digester mixing also affect the AD performance. These parameters are individually discussed in the following sections.

2.2.1. Waste composition and volatile solids

The wastes to be treated by AD process can be comprised of a biodegradable organic fraction, a combustible and an inert fraction. Waste materials containing high VS and low non- biodegradable material, or refractory volatile solids, are the most suited to AD treatment. Kitchen waste, food waste, and garden waste are biodegradable organic fraction of the waste. The combustible fraction can be slowly degrading lignocellulosic organic compounds such as coarser wood, paper, and cardboard. The lignocellulosic organic content does not readily degrade under anaerobic conditions. Therefore, they are more suitable for waste-to-energy plants. The inert fraction of the waste may contain stones, glass, sand, metal, etc. that can ideally be removed, recycled or used at land fill. It is important to remove the inert fraction before digestion, otherwise it causes the increase of digester volume and wear of equipment. The Volatile Solids (VS) of organic wastes are measured as total solids excluding the ash content which is obtained by complete combustion of the feed wastes. The VS contains the Biodegradable Volatile Solids (BVS) fraction and the Refractory Volatile Solids (RVS). Knowing the BVS fraction of substrate can be helpful in better estimation of the biodegradability of the waste, of biogas production, organic loading rate and C:N ratio. Lignin is a complex organic compound which is not easily degraded by anaerobic bacteria and forms refractory volatile solids (RVS) in organic matter. Waste materials containing high VS and low non-biodegradable material, or RVS, are the most suited to AD treatment (Elbeshbishy et al., 2017; Kangle et al., 2012).

2.2.2. Carbon to Nitrogen Ratio (C:N)

The amount of carbon and nitrogen present in feedstock or C:N ratio is a very important parameter of the AD process. A high C:N ratio indicates rapid consumption of nitrogen by methanogens and leads to lower gas production. On the contrary, a lower C:N ratio results in accumulation of ammonia and exceeding pH values which is toxic to methanogens. Low C:N ratios occur when too much nitrogen is present. On the other side, a high C:N ratio leads to deficiency in AD system (Mata-Alvarez et al., 2000a; Poliafico and Murphy, 2007). According to a study (Gerardi, 2003), the optimal gas production can be achieved by feedstock with C:N ratio of 25:1. Other studies reported that the optimum range falls within 25-30 and can be achieved by the co-digestion of different waste streams (Gonzalez-Avila et al., 2011; Monnet, 2003). Some other studies even though reported that the optimum C:N ratios in anaerobic digesters are between 20 and 30 while some other studies even reported lower values than 20. Although, a very low C:N ratios occurs when too much nitrogen is present and leads ammonia (NH_3) to be accumulated which leads to either high pH values or methanogenic inhibition (Salminen et al., 2002, Kangle et al, 2012). On the contrary, the high C:N ratio indicates that nitrogen is rapidly depleted by methanogens and leads to lower gas production. A lower C:N ratio results in accumulation of ammonia and exceeding pH values to over 8.5, which is toxic to methanogens. Mixing materials of high and low C:N ratios, such as organic solid waste mixed with animal manure or sewage can help achieve optimum C:N ratios (Poliafico, 2007, Kangle et al, 2012).

2.2.3. Nutrients

Some nutrient elements are needed for the growth of methane-forming bacteria. Particular metals comprising nickel, iron, cobalt, and molybdenum are essential for optimal growth and methane production. Trace metals stimulate methanogenic activity. Some metals including selenium, molybdenum, manganese, aluminum, and boron have been suggested as additional components in media. Addition of metal ions solutions to anaerobic digesters can improve the performance of the AD system. The amounts of 0.002, 0.004, 0.003, 0.02 mg/g are suggested for iron, cobalt, nickel, and zinc respectively. The requirement for nickel is quite unusual for biological systems and can exclusively characterize methanogenic bacteria. Addition of metal ions solutions to anaerobic digesters can improve the performance of the AD system (Azbar and Speece, 2001; Kangle et al., 2012; Mata-Alvarez et al., 2000a).

2.2.4. Total solids content (TS)/Organic loading rate (OLR)

The increase of the total solids (TS) fraction leads a corresponding decrease in the reactor volume. High solids (HS) AD system contain 22% to 40% TS, medium solids (MS) system about 15 to 20% and low solids (LS) AD systems contain less than 10 % of TS. The organic loading rate (OLR) is defined as the organic matter flowing into the digester per time which is expressed as mass of organic matter over digester volume over time (Appels et al., 2008; Kangle et al., 2012). OLR is also defined as measure of the biological conversion capacity of the AD system. Typical values of OLR ranges between 0.5 and 3 kg VS/m^3/d. OLR is particularly important parameter in continuous systems. System failures as a result of overloading have been reported by numerous plants (Kangle et al., 2012; Poliafico and Murphy, 2007).

When feeding the system above its sustainable OLR, low biogas yield is obtained. This is caused by the accumulation of the inhibiting substances such as fatty acids in the digester slurry. Any substrate that can be converted to methane by anaerobic bacteria is referred to as feedstock. The main components of feedstock are carbon, oxygen, nitrogen, hydrogen and phosphorus, and microbial cell material of those elements is reported to be approximately 50, 20, 12, 8 and 2 % respectively. Feedstocks can be a range of different waste materials from easily degradable wastewater to complex high-solid waste (Gerardi, 2003).

2.2.5. pH, alkalinity and volatile acids/alkalinity ratio

There is a different optimum pH range for each group of micro-organisms. Methanogenic bacteria are very sensitive to pH. The optimum range for them is between 6.5 and 7.2. The fermentative microorganisms are relatively less sensitive and can tolerate a wider range of pH between 4.0 and 8.5. At low pH, mainly acetic and butyric acids are produced, while at a pH of around 8.0, acetic and propionic acids are mainly produced. The VFAs produced during AD process result in a pH reduction. This reduction is normally adjusted by methanogenic bacteria, which produce alkalinity in the form of ammonia, carbon dioxide, and bicarbonate (Appels et al., 2008).

The pH of the system is controlled by the CO_2 in the gas phase and the HCO_3-alkalinity of the liquid phase. If the concentration of CO_2 remains constant, the addition of HCO_3-alkalinity can increase the pH of digester. In order to maintain a stable and well-buffered digestion process a buffering capacity of 70 meq $CaCO_3$/L or a molar ratio of at least 1.4:1 of bicarbonate/VFA is required. However, previous studies have shown that particularly the stability of the ratio is very significant and not so much its level.

Except for ammonia, other factors such as sulfide, sodium and potassium, heavy metals, volatile fatty acids, long-chain fatty acids, and hydrogen can also affect the activity of methanogens. Molecular hydrogen is formed throughout different stages of anaerobic digestion. Inhibition can occur due to the lack of balance between the rates of hydrolysis and methanogenesis. A suitable balance between those rates is essential for higher methane production. Rapid methanogenesis is required to prevent accumulation of organic acid lowering pH to an extent that inhibits methanogenesis (Appels et al., 2008; Pouget et al., 2012).

2.2.6. Temperature

The temperature has an important effect on the physicochemical properties of the substrate. Moreover, it is effective on the growth rate and metabolism of micro-organisms and the population dynamics in the reactor. A stable operating temperature is very important to be maintained in the digester, since fluctuations in temperature affect the bacteria particularly the methanogens. Acetotrophic methanogens are one of the most sensitive groups of bacteria to increasing temperatures. The degradation of propionate and butyrate is also sensitive to temperatures above 70 ˚C. (The propionate or propanoate ion is $C_2H_5COO^-$ the conjugate base of propionic acid- A propionic or propanoic compound is a small salt or ester of propionic acid. Butyric acid, also known under the systematic name butanoic acid, abbreviated BTA, is a carboxylic acid with the structural formula $CH_3CH_2CH_2$-COOH. Salts and esters of butyric acid are known as butyrates or butanoates) (Appels et al., 2008; Turovskiy and Mathai, 2005).

The temperature has also a substantial effect on the partial pressure of H_2 in digesters and therefore, it affects the kinetics of the syntrophic metabolism. Thermodynamic studies show that endergonic reactions under standard conditions, for example the decomposition of propionate into acetate, CO_2, and H_2, would be more favorable energetically at higher temperature, whereas the exergonic reactions such as hydrogenotrophic methanogenesis are less favored at higher temperatures. Increasing temperature can be favorable for several reasons. It can increase solubility of the organic compounds, enhance chemical and biological reaction rates, and an increase the rate of pathogens' death in thermophilic conditions. Although, the application of high temperatures (thermophilic) has some adverse effects as there will be an increase of the fraction of free ammonia which can be an inhibiting factor for the microorganisms. The increasing pKa of the VFA will make the process more susceptible to inhibition. Therefore, temperature control is a very important concern for thermophilic digestion in comparison with mesophilic digestion. A stable operating temperature is very important to be maintained in the digester, since fluctuations in temperature affect the bacteria particularly the methanogens. Process failure can occur at changes in temperature over 1℃/day and changes of more than 0.6℃/day in temperature should be avoided Process failure can occur at changes in temperature over 1℃/day, and changes of more than 0.6℃/day in temperature should be avoided (Appels et al., 2008; Turovskiy and Mathai, 2005).

2.2.7. Solids and hydraulic retention time

The average time that the solids spend in a digester is referred to as solids retention time (SRT), and the average time that the liquid sludge is held in the digester is referred to as hydraulic retention time (HRT). SRT and HRT are an important design and operating parameter for all anaerobic processes. Reduction of SRT decreases the extent of the reactions and vice versa. A fraction of the bacterial population is removed each time when the sludge is withdrawn. Therefore, the cell growth must at least compensate the cell removal to maintain steady state and to prevent from process failure.

The ratio between the solid content of the reactor by the solid flow rate indicates the average SRT. HRT specifies the average time that waste and wastewater are exposed to microorganisms for degradation. In a conventional mixed reactor, the SRT and HRT are equal while in a retained biomass reactor the SRT is usually higher than HRT. Higher SRT can be achieved by increasing the digester volume, increasing the solid content or using a retained biomass reactor. Equation (1.1) shows the calculation for HRT and equation (1.2) shows the calculation for SRT in continues stirred tank reactor (CSTR).

HRT= Volume of the reactor (V)/ Influent flow rate= L/L/d= d Eq. 2.1

SRT= Mass of the biomass in reactor/ mass of the biomass leaving

$= (V \times VSS)/(Q_{out} \times VSS) = V/Q_{out} = HRT\ d$ Eq. 2.2

The effect of the retention time on the gas production is mostly studied on laboratory scale. Studies indicated that in a semi-CSTR system retention times shorter than 5 days are insufficient for a stable digestion. VFA concentrations are increasing due to washout of methanogenic bacteria. VFA concentrations are relatively high for 5–8 days SRT. There is an incomplete breakdown of compounds, especially of the lipids for this SRT. It has also been indicated that stable digestion is obtained after 8–10 days. There is low VFA concentrations and the breakdown of lipids starts. According to the studies, the breakdown curve stabilizes at SRT>10 days as all sludge compounds are significantly reduced. Figure 2 shows a schematic relationship between SRT and degree of digestion (Appels et al., 2008).

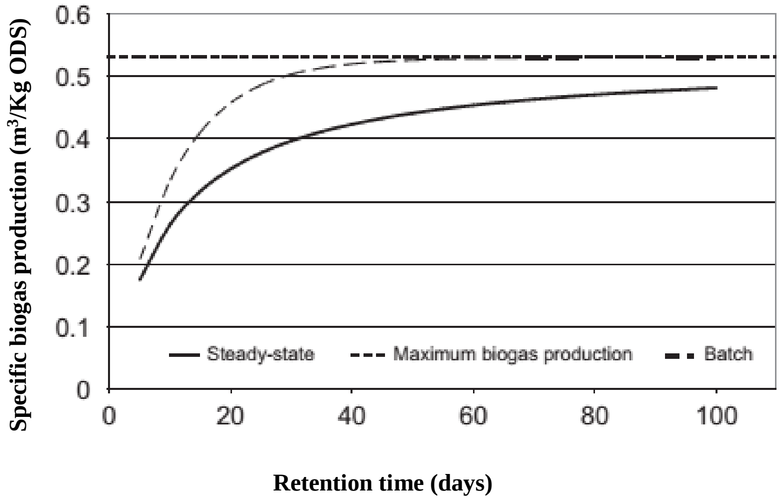


Figure 2.2. Schematic relationship between SRT and degree of digestion (Appels et al., 2008)

2.2.8. Digester mixing

To attain an optimum performance for an AD system, it is essential to maintain a proper mixing. Mixing causes near contact between the feed sludge and active biomass, yielding uniformity of several parameters including temperature, substrate concentration, other chemical, physical and biological aspects all through the digester. Mixing also prevents from the formation of surface scum layers and the sludge deposition on the bottom of the tank (Appels et al., 2008). The rise of gas bubbles and the thermal convection currents caused by the addition of heated sludge, results in some degree of natural mixing in the digestion tank. However, this is not adequate for an optimum performance and auxiliary mixing is required. There are some methods of auxiliary mixing including external pumped recirculation, internal mechanical mixing and internal gas mixing as it is shown in Fig. 3.

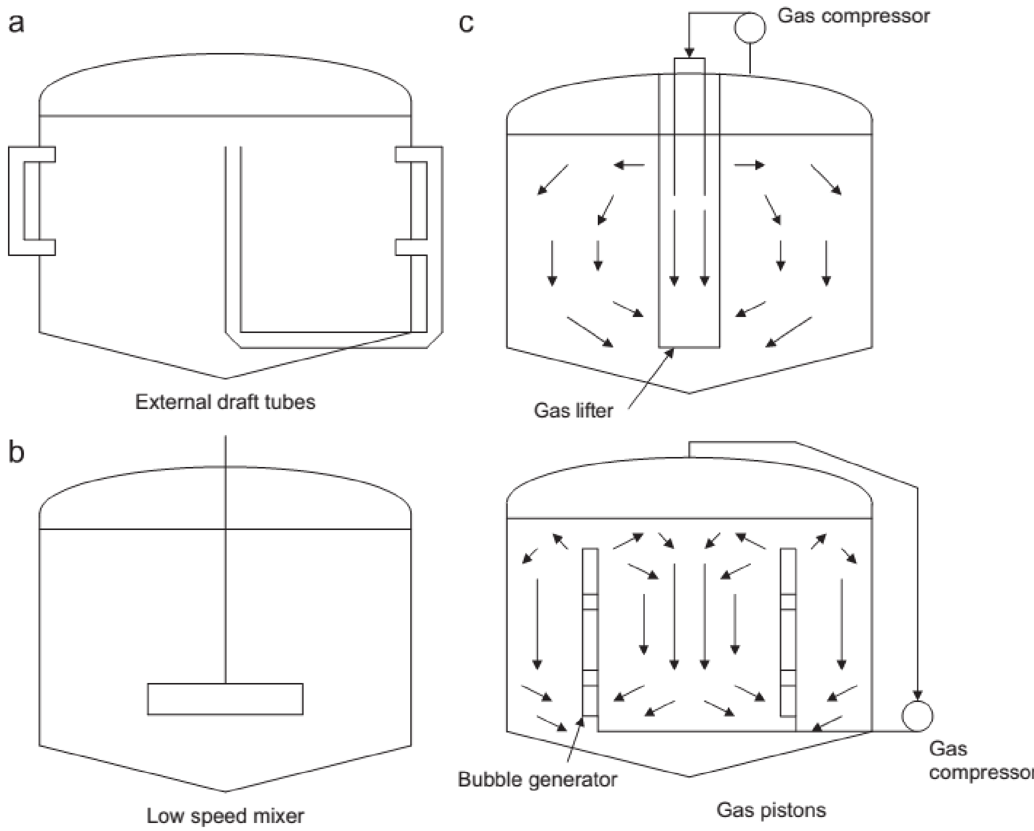


Figure 2.3. Types of mixing methods for digesters (a) external, pumped recirculation mixing, (b) internal mechanical mixing, and (c) external gas recirculation mixing (Appels et al., 2008).

2.3. Anaerobic Co-Digestion (AnCoD)

Anaerobic Co-digestion (AnCoD) involves the simultaneous anaerobic digestion of two or more organic waste feedstocks. The anaerobic co-digestion can be referred to as the simultaneous treatment of two or more organic biodegradable waste streams by anaerobic digestion. It provides a proper method of disposal for the organic fraction of solid waste which comes from source or from separate collection systems. This method of treatment, makes it possible to use the existing anaerobic reactors in wastewater treatment plants, with minor modifications and some additional requirements. By combining the treatments of two problematic wastes including for instance organic part of municipal solid waste and paper pulp sludge, higher yield in the production of biogas can be attained. Conventionally, anaerobic digestion was a single substrate and single purpose treatment. Recently, it has been indicated that when an increased variety of substrates applied at the same time, more stable AD is achieved. The most common state is when a major amount of a main basic substrate for example manure or sewage sludge is mixed and digested accompanied by minor amounts of a single, or a variety of additional substrate. The usage of co-substrates usually improves the biogas yields from anaerobic digester due to positive interaction established in the digestion medium and the supply of missing nutrients by the co-substrates (Alvarez et al., 2008, Kangle et al, 2012). Anaerobic co-digestion offers several benefits including: improved nutrient balance and digestion, possible gate fees for waste treatment, additional biogas collection, and additional fertilizer i.e. soil conditioner (Elbeshbishy and Nakhla, 2012; Viotti et al., 2004).

Substrates with high C:N ratio have the poor buffering capacity and produce excessive amounts of VFAs during the fermentation. In contrast, substrates characterized by low C:N ratio have high buffer capacity and the increased concentration of ammonia in the fermentation process leads to microbial growth inhibition. Anaerobic co-digestion (AnCoD), which entails the simultaneous digestion of two or more feedstocks has shown to be beneficial for its economic viability and increasing methane yields (Gelegenis et al., 2007; Karagiannidis and Perkoulidis, 2009; Kwietniewska and Tys, 2014). These problems have made AnCoD of multi feedstock to become a hot research area in the enhancement of conventional AD technology. The publications on AnCoD significantly increased within the last fifteen years indicating its capability for improving biogas production (Hagos et al., 2017). The main goal of anaerobic co-digestion is to increase biogas mainly biomethane for heat and electricity. As shown in Figure 2, a range of feedstocks can be co-digested at suitable blend ratio to maintain optimum condition required for metabolic activity and improved biogas production for thermal energy and power generation. Anaerobic co-digestion has shown to be a viable option to alleviate the drawbacks of mono-digestion while enhancing economic feasibility of the existing AD plants by increasing methane yields (Bayr et al., 2014; Mata-Alvarez et al., 2014, 2000b; Shah et al., 2015),(Mata-Alvarez et al., 2000a).

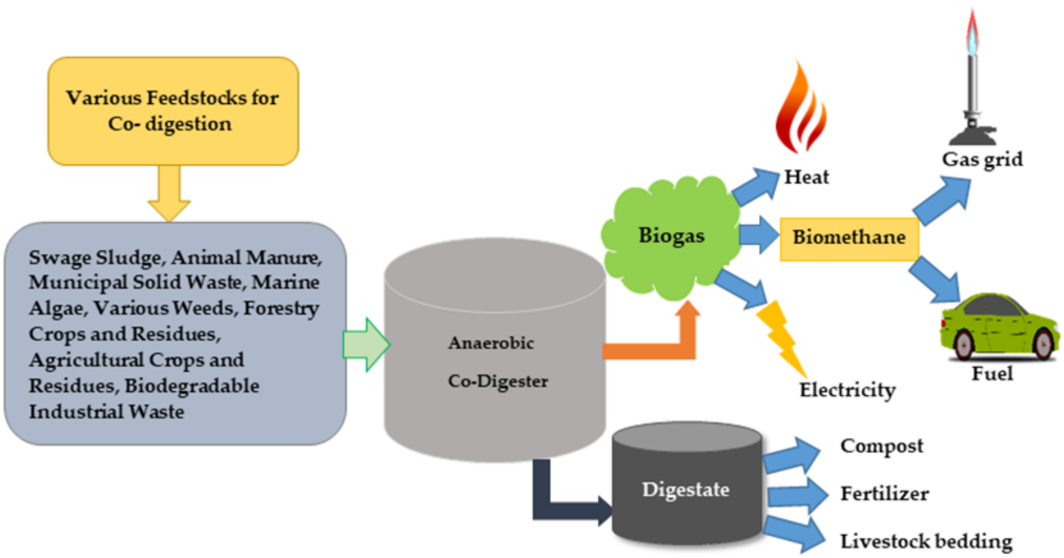


Figure 2.4. Co- digestion of multi feedstocks for waste reduction and energy recovery

Co-digestion can increase biogas production from 25% to 400% compared to the mono-digestion of the same substrates. Feedstocks characterized by higher C:N ratio (>50) such as rice and wheat straws, corn stalks, seaweed and algae can be co-digested by the feedstocks of lower C:N ratio for instance pig manure, poultry manure, food and kitchen wastes to achieve nutrient balance and to avoid the inhibitions which leads to system instability and reduced biogas production as a result of unsuited C:N ratio (Hagos et al., 2017; R et al., 2017; Sosnowski et al., 2003). Table 2.1 shows the possible feedstocks for co-digestion with regard to C:N ratio.

Various advantages of AnCoD systems are presented in Figure 3. Multiple aspects are considered when applying AnCoD. Cost of transporting the co substrate from the generation point to the AD plant seems to be the primary consideration, the selection of the best co-substrate and blend ratio in order to enhance synergism, dilute disruptive compounds, optimization of methane production and digestate quality, are also important consideration that treatment plants need to evaluate when using the AnCoD (Divya et al., 2015; Mata-Alvarez et al., 2014). Operational and environmental factors as mentioned previously are factors that influence methane yield in anaerobic co-digestion of multi feedstocks (Long et al., 2012).

Table 2.1. Potential feedstocks for co-digestion to balance nutrient with regard to C/N ratio [(Hagos et al., 2017),(R et al., 2017)–(Sosnowski et al., 2003)]

Feedstocks with max C/N ratio <20	C/N ratio	Feedstocks with max C/N ratio ≤40	C/N ratio	Feedstocks with C/N ratio around or >50	C/N ratio
TWAS[1]	6-9	OFMSW[3]	24	Potatoes	35-60
CSW[2]	11	Cow dung	16-25	Oat straw	48-50
Poultry manure	5-15	Horse manure	20-25	Corn stalks/straw	50-56
Pig manure	6-14	Kitchen Waste	25-29	Fallen leaves	50-53
Goat manure	10-17	Peanut shoots/ hulls	20-31	Rice straw	51-67
Grass/Grass trimmings	12-16	Slaughterhouse waste	22-37	Seaweed	70-79
Alfalfa	12-17	Mixed food waste	15-32	Algae	75-100
Food Waste	3-17	Waste cereal	16-40	Sugar cane/ bagasse	140-150
		Sugar beet/ Sugar foliage	35-40	Sawdust	200-500
		Waste cereals	16-40		

[1] Thickened Waste Activated Sludge,

[2] Caned Seafood Waste,

[3] Organic Fraction of Municipal Solid Wastes

However, for the implementation of AnCoD, other than aforementioned factors that govern overall AD process, additional factors including the selection of co-substrates and their mixing ratio should be taken into consideration. For instance, mixing materials of high and low C:N ratios, such as organic solid waste mixed with animal manure or sewage can help achieve optimum C:N ratios (Akyol et al., 2016). In order to attain an improved co-digestion process, some precautions and suitable procedures are necessary. There may be requirements for supplementary digester equipment depending on the size of the operation, quality of waste, and characteristics of the wastes to be co-digested. Mainly, precautions or supplementary equipment would be required for: homogenization and mixing of co-substrates, delivery of the waste, prevention of excessive foaming and scum layer formation, and removal of sediments from the digester.

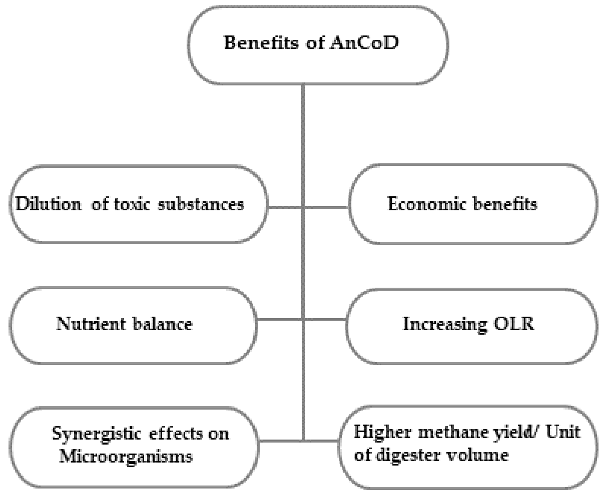


Figure 2.5. Advantages of anaerobic co-digestion systems

Furthermore, proper monitoring parameters should be determined to control and regulate the AnCoD digesters to help maintain an efficient performance when it is under operation. Applying suitable monitoring and control procedures when running the AnCoD process, allows for utilizing the full capacity of the system without overload risks. Monitoring can be performed by measuring indirectly the activity of different groups of organisms for example by measuring the rate of gas production, or the accumulation of intermediates of anaerobic degradation which reflect the existing metabolic status of the active organisms in the system (Gujer and Zehnder, 1983).

Several recommendations in the literature has been proposed specifying what control parameters should be chosen to be measured for this purpose. Some of the more common ones include pH, alkalinity, volatile fatty acids (VFA), gas production rate and the amounts of hydrogen, methane and carbon dioxide in the gas (Ahring et al., 1995; Moletta et al., 1994).

Partial alkalinity (PA) has been considered as a reliable monitoring parameter (Jenkins et al., 1991; Rozzi et al., 1994; Supaphol et al., 2011; Wilcox et al., 1995). The applicability of pH as a process indicator was reported to be intensely dependent on the buffering capacity making it an unreliable monitoring parameter (Ahring et al., 1995). It is expected that the selection and applicability of a specific parameter could not be generalized depending on the individual process configuration and the waste characteristics. For a full-scale municipal system, co-digested excess sludge from the municipal wastewater treatment plant with carbohydrate-rich food processing waste, different parameters were assessed for monitoring and control of the system performance (Björnsson et al., 2000). Those parameters included the volume of gas produced, pH, VFA, and alkalinity. In addition, gas composition and the degradation of organic matter were also measured at steady state and during process changes. Both full-scale and lab-scale experiments were carried out by that research to evaluate the suitability of those parameters. The digester was run below maximum capacity in order to avoid overload. The only operational limit set for the plant, was that the pH should not have been below 6.8. So, the pH was compared with alkalinity, VFA concentration, gas production rate, and the gas composition. Alkalinity was measured as partial alkalinity (PA). OLR changes were monitored both in the full-scale digester and in the lab-scale models (Björnsson et al., 2000).

As indicated by the results of Björnsson et al's study, the load's fluctuations were reflected in the pH, PA, and VFAs concentration. At overload condition, all the three parameters clearly demonstrated the process imbalance. The VFA concentrations proved to be a better indicator for an overload of the microbial system, although alkalinity and pH showed to be good monitoring parameters as well. The results indicated that gas-phase parameters demonstrated a slow response to load changes. The response of gas production and gas composition was behind and significant change occurred only after severe overload. Prior studies had observed that change in the gas phase parameters only takes place after well-developed imbalance. For that reason, the gas-phase concentration would not always reflect the actual concentration in the liquid, caused by limitations in liquid-to-gas mass transfer (Frigon and Guiot, 1995; Pauss et al., 1990).

At higher OLR, the process showed to be more sensitive to system disturbances. The changes in VFA concentration were not accurately reflected in pH. The increased amounts of VFA were demonstrated in a lower pH, because of the low buffering capacity of the process. Nevertheless, the pH was not presented as a reliable means of process monitoring, because of possible variation in buffering capacity as a result of variations in substrate composition. Therefore, a process imbalance causing significant accumulation of VFAs, could be unseen by this buffering effect. Therefore, relying on pH measurements for the process monitoring was not advised and the usage of pH measurements together with measurements of the PA or VFA was suggested by authors (Björnsson et al., 2000).

Different studies have been carried out to evaluate the impact of various effective factors on AnCoD processes. The main aim of the studies was to assess the influence of those parameters on biogas yield, biogas composition including biomethane or biohydrogen content. However, no comprehensive guidelines have been compiled so far to standardize the AnCoD systems. This would be firstly due to the complexity of the process caused by the variety of co-substrates and wastes composition, and secondly because it has not been broadly implemented in full scales.

A study for IEA Bioenergy has presented most important control parameters which regulate anaerobic co-digesters. The control parameters included the overall daily substrate flow (m^3/d or tones/d) and the amount of biogas produced daily (m^3/d). Based on the results obtained by this study, for appropriate control of the process, the determination of the CH_4 concentration was highly advised. In addition, a periodic calculation of the biogas yield referred to as daily biogas amount divided by daily substrate flow was recommended since it provides the efficiency of the digestion process. The analysis of the ammonia and the volatile fatty acids concentration were also suggested for large-scale operations. In addition, identifying the influences of co-substrates on the digester behavior was recommended. Particularly, detecting the formation of scum layers and bottom sediments was specified. It was suggested to maintain the record of the type and amount of separated contaminations in co-digestion. In the cases that sterilization is also involved, monitoring the type and the amount of waste streams and the treatment conditions such as time and temperature were also considered to be of necessary control parameters. The sampling frequency and methods for analysis required for quality assurance of the end product digestate or compost were also comprehensively defined in that study (Kim et al., 2006).

Although co-digestion of feedstocks such as poultry manure and kitchen waste with low C:N ratio with those of higher C:N ratio such as agricultural waste including rice and wheat straw is a solution to adjust its ratio at the optimum level, the existence of lignocellulosic material in the agricultural waste cause limitation during AnCoD as a result of long retention time and low biodegradability (Saha et al., 2011a).

Such problems still may demand for pretreatment techniques in order to speed up the hydrolysis which is the rate-limiting step in the anaerobic digestion process. The main purpose of the pre-treatment is to increase the solublization by the breakdown of the complex substrates such as lignin in lingocellulosic feedstocks or tough cell wall in seaweed biomass, in order to accelerate the hydrolysis rate (Elbeshbishy et al., 2011; Giordano, 2012; Noike et al., 1985).

C. Rodriguez et al, 2017 studied the effect of using co-substrate on methane production in co-digestion of waste paper (WP) with microalgal biomass (MA). Their study (Rodriguez et al., 2017) was carried out in batch mode and was intended to investigate the influence of the feedstocks mixing ratio (WP:MA) as well as feedstock to inoculum (F:I) ratio. They achieved the highest methane yield of 608 ml CH_4/ gVS at the F/I and WP:MA ratios of 0.2 and 50:50 respectively. At this mixing ratio of the feedstocks, the obtained methane yield was more than that of the feedstock's mono-digestion. The maximum 49.58% increase of the methane yield occurred at the same co-digestion ratio of 50:50 and F:I ratio of 0.4. Their study verified the synergetic effect at the feedstocks mixing ratio of 50:50 and all F:I ratios of 0.2, 0.3, and 0.4.

Pretreatment has proved successful at increasing the methane yield of numerous strains of microalgae in the digestion process. Most species of microalgae reduce the digestion rate due to their tough cell wall consisting of slowly biodegradable material (Uggetti et al., 2017).

With the increasing attention to anaerobic co-digestion, several researches have been allotted for co-digestion of various feedstocks and pre-treatment techniques from mechanical particle size reduction, thermal, chemical and ultrasonic treatment to enzymatic degradation and so on. For instance, mechanical pretreatment with Hollander beater in co-digestion of seaweed biomass with digester sludge increased biogas production by 20% at the ratio of 2:3 of algal pulp to sludge per reactor for 10 min beating time (Tedesco et al., 2013).

2.3.1. Microbial Diversity and Synergy in AnCoD

The selection of sludge inocula plays an important role in the effectiveness of biological anaerobic treatment of organic wastes. The analysis of microbial community dynamics has revealed that various waste streams and environmental factors can affect microbial community dynamics in an anaerobic co-digestion processes (Lin et al., 2012; Supaphol et al., 2011). Reportedly, mesophilic anaerobic co-digestion of mixed wastes allows for a better variety of substrates which in turn supports a wider diversity of bacteria and archaea. More diverse resource input results in more diverse communities and greater metabolic activity (Ike et al., 2010; Supaphol et al., 2011). However, there is limited awareness about the microbial consortia in anaerobic co-digestion process due to the lack of metabolic data on the microorganisms involved in the process. A comprehensive understanding of the microbial community is hindered by limitations of conventional molecular technology approaches that are restricted in terms of detecting sophisticated microbial diversity in the environment. Attempts for the analysis of the 16S rRNA gene sequencing have been carried out as an alternative to conventional culture techniques. This method is used to identify and compare microorganisms present within a given sample and it is a well-established method for studying complex microbial community or environments that are difficult or impossible to study. The method of 16S rRNA gene-based fingerprints could provide less biased and higher coverage information and can support many unknown details about the mechanism of microbial response to the digester enhancement. An improved understanding of the function and the metabolic role of microorganisms in the anaerobic co-digestion of various pollutants can be obtained b by the molecular inventories (Rivière et al., 2009; Zhang et al., 2011). Some of the results of these studies are presented in Table 2.2.

Organic matter in AD process, is decomposed synergistically by a bacterial consortium producing biogas including biomethane (Chandra et al., 2012; Liew et al., 2012). The process involves at least three functional groups of microorganisms that mainly regulate the mutual metabolic interactions under anaerobic conditions. The first microbial community hydrolyzes complex polymeric substances such as lipids, cellulose and protein to fundamental structural building blocks like glucose and amino acids. Subsequently, fermentation of these products to fatty acids, acetate and hydrogen is proceeded by the second community.

Among degradation processes involved in anaerobic digestion, the acidogenesis process has been shown to be the most important step. The third community develops methanogenesis process through which acetate and hydrogen are converted to methane and carbon dioxide. Therefore, microbial communities are vital to a stable and efficient transformation of organic matter to biogas (Zhang et al., 2011). General metabolism of microbial consortia involves extracellular polymeric substances (EPS) of sludge aggregates. It has been indicated that EPS is partly the result of the microbial metabolism that is affected by the microbial community structure and its activity. Growth conditions control the quantity of EPS which in turn affect the anaerobic digestibility and biogas production. It is not yet clear that how different microorganisms contribute to EPS secretion. A comparative study on the pathways of substrates degradation and the by-products of EPS sub-fractions could provide supplementary data on long-term impacts of microbial activity on anaerobic co-digestion reactors (Sheng et al., 2010; Yu et al., 2012). Monitoring qualitative and quantitative changes in a bacterial community structure, allows for the evaluation of the influence of the co-substrate on bacteria contributing to the biogas production.

However, there is not enough literature on this topic. Some attempts have been made to study microbial community structure and its influence on anaerobic co-digestion processes. Such studies as presented in Table 2.2 have been aimed to increase methane production by co-digestion of different organic-rich waste streams and they have been mostly developed with a view to the influencing parameters such as mixing ratio, organic loading rate (OLR), and carbon to nitrogen (C:N) ratio on the population of methanogenic archaea species (Benn and Zitomer, 2018; Gaby et al., 2017; A. J. Li et al., 2011).

It is reported that an even distribution of hydrogenotrophic and acetotrophic methanogen populations in a reactor is indicative of a stable operating condition (Demirel and Scherer, 2008; Kim et al., 2014; Yang et al., 2016). Yang et al. studied the performance of a co-digester for the treatment of sewage sludge with fat, oil and grease (FOG) using a mesophilic semi-continuous reactor and compared it to that of a mono-digester receiving only sewage sludge. It was indicated that the secretion of EPS increased by 40% in comparison with the mono-digester and that the improvement in co-digestion performance was stimulated due to the release of EPS. The analysis of microbial 16S rRNA gene showed the dynamic change of microbial community through the process. Both bacterial and archaeal community went through a progress with FOG addition, and a large amount of consortia such as Methanosaeta and N09 were involved in the process. As compared to sewer sludge mono-digestion, biogas production and TS removal efficiencies increased up to 35% and 26%, respectively. It was shown that FOG addition resulted in nutrition balance and regulating microbial composition. Also, metabolic activities were stimulated, and more EPS were obtained with the progressive addition of FOG.

Jihen et al. investigated microbial community's structures in anaerobic co-digestion of dairy wastewater and cattle manure. A maximum volatile solids (VS) reduction of 88.6% and biogas production of 0.87 L/g VS removed were obtained through their research corresponding to the C:N ratio of 24.7 at HRT of 20 days. The bacterial profile analysis showed a large quantity of Uncultured Bacteroidetes, Firmicutes and Synergistetes bacterium.

The Syntrophomonas strains associated with H_2-using bacteria comprising *Methanospirillum* sp., *Methanosphaera sp*., and *Methanobacterium formicicum* were observed as well. These syntrophic associations are necessary in anaerobic digestion reactors allowing for maintaining a low hydrogen partial pressure. On the other hand, high concentrations of volatile fatty acids (VFAs) resulted from dairy wastes acidogenesis allowed the growth of Methanosarcina species. It was indicated that high concentrations of VFAs would result preferentially in the growth of the acetoclastic Methanosarcina species (Jihen et al., 2015).

Table 2.2. Microbial consortia diversity in various AnCoD systems for biogas and methane improvement

Feedstocks	Microbial Consortia	Digester mode	HRT	Methane / Biogas increase%
Fruit vegetable waste +Food waste (1:1)	Methanoculleus, Methanosaeta, Methanosarcina	CSTR (mesophilic)	NA[1]	0.49 m^3 CH_4/kg VS
Food, fruit, vegetable + night soil waste	Methanosaeta (predominant methanogen) + hydrogenotrophs	Full scale wet fed-batch	18-20 d C:N 8.6	NA
Cow manure + grass silage	Clostridia, unclassified Bacteria, Bacteroidets	CSTR mesophilic	20 d	NA
Cow manure + oat straw	Clostridia, unclassified Bacteria, Bacteroidets, Deltaproteobacteria	CSTR (mesophilic)	20 d	NA
Cow manure + sugar beet tops	unclassified Bacteria,Clostridia, Bacteroidets, Bacilli	CSTR (mesophilic)	20 d	NA
STP-OGW + SC-OFMSW (1:6)	Methanobacterium, Methanoculleus, Methanothermobacter uncultured archaea	Batch (thermophilic)	14.4 d	52% biogas and 36% methane increase
biodiesel waste glycerin + municipal waste sludge (1.35:0.65)	Dominated by:Methanosaeta and Methanomicrobium	Two-stage CSTR (mesophilic)	20 d	100% biogas and 120 % methane increase
Sewage sludge +FOG	Dominantly Methanosaeta, and N09	Semi-continuous (mesophilic)	15 d	35% biogas increase
Dairy wastewater + Cattle manure	Uncultured Bacteroidetes, Firmicutes, Synergistetes, Syntrophomonas strains Methanosarcina species	ASBR (mesophilic)	20 d (C:N 24.7)	biogas produced: 0.87 L/g VS removed
Food wastewater +WAS (3:1)	Dominated by: Methanothermobacter and Methanosarcina	CSTR (thermophilic)	20 d	Max biogas: 316.11 mL CH_4/g COD removed

[1] Not available

Former studies verify that the AnCoD of cellulosic materials such as grass silage, oat straw, and sugar beet tops to methane to be mediated by bacteria and methanogenic archaea. The polymers which are hydrolyzed into soluble compounds under fermentative condition, are converted to acetate and one-carbon constituents by acidogens and acetogens and these intermediates in turn can be transformed directly by methanogenic archaea into methane and carbon dioxide (Nopharatana et al., 1998; Veeken and Hamelers, 1999).

Anaerobic digestion process of cellulosic material including grass silage, oat straw and sugar beet tops, is a multistep process mediated by Bacteria and methanogenic Archaea to produce methane. The process involves the hydrolysis of polymers into soluble compounds under fermentative condition. Subsequently, Acidogenic and acetogenic bacteria convert these soluble intermediates to acetate and mono-carbon compounds. These compounds subsequently can be converted to methane and carbon dioxide by methanogenic Archaea. It was found that in anaerobic digestion of cellulytic feedstocks, significant cellulolytic competences occurs due to the existence of species belonging to the order *Clostridiales* (Lynd et al., 2002).

Martín-González et al. found that thermophilic conditions was superior to mesophilic conditions for the enhancement of AnCoD along with the use of sewage treatment plant fat, oil and grease wastes (STP-FOGW) as co-substrates in co-digestion with organic fraction of municipal waste (OFMSW). Monitoring of microbial structure demonstrated that the bacterial profiles clustered in two separate groups, before and after the extended contact with STP-FOGW, whereas, archaeal community structure remained relatively constant throughout the operation. Bacterial population structure showed a dynamic change determined to be due to introducing FOG residues to the reactor.

2.3.2. Effect of Digester Mode on AnCoD

In general, anaerobic digesters can be configured as one-stage, two-stage, or multi-stage reactors in which the hydrolysis/acidogenesis and acetogenesis/methanogenesis steps occur in either the same or separated digesters (Figure 4). Separating the digesters makes the process easier to control, and makes it possible to separately optimize the operational and environmental conditions for hydrolysis/acidogenesis and methanogenesis processes in order to enhance the overall reaction rate and biogas yield (Lalman and Bagley, 2002; Nathao et al., 2013).

Fluctuations in organic loading rate, heterogeneity of wastes, and or the presence of excessive inhibitors can lead to instability of the process, and multi-stage systems have shown to be more stable as compared to single-stage ones. Two or multi-stage systems allow for the selection and enrichment of different types of microorganisms in each digester which results in extending the possibility of processing different biomass constituents, improving substrate conversion, enhancing the chemical oxygen demand (COD) reduction, and increasing energy recovery.

Although, multi-stage digesters are associated with greater construction and maintenance costs, multi-stage digesters provide higher performances as compared to single-stage systems (Azbar and Speece, 2001; Cuetos et al., 2007). Using two-stage digestion, controlled acidogenesis in the first digester, helps maintaining a high soluble feed to the second stage which subsequently enhances the biogas production (Dareioti and Kornaros, 2015).

In the two-stage anaerobic digestion systems, acid fermentation and methanogenesis are separated in two reactors in order to optimize reactor conditions for the different groups of microorganisms. The acidogenic stage is typically operated at a low HRT in the range of 2 to 3 days and a pH between 5 and 6. While the second stage, methanogenesis, is typically operated with a HRT of 20 to 30 days and a pH between 6 and 8 facilitating the development of slow-growing methanogenic archaea.

Moreover, acidogenesis phase allows for long chain fatty acids (LCFAs) saturation and degradation (Kangle et al., 2012; Kim et al., 2004). Owing to the bent molecular structure of unsaturated LCFAs because of the existence of double bonds, they have a greater cover area of cell wall per molecule as compared to saturated LCFAs. As a result, unsaturated LCFAs have demonstrated stronger inhibitory effects in comparison with saturated LCFAs (Demeyer and Henderickx, 1967; Thies et al., 1994). As such transformation of unsaturated to saturated LCFAs is beneficial. In addition, LCFA saturation would be necessary for the oxidative breakdown of fatty acid molecules and formation of acetic acid (Hanaki et al., 1981; Lalman and Bagley, 2002). The outcome of a study using a two-stage AD system treating a synthetic fat-containing wastewater comprised of glucose and LCFAs mixture revealed that, 19% of LCFAs were degraded and 12% of unsaturated LCFAs were saturated in the acidogenic phase (Kim et al., 2004). Acidogenic phase also can convert the unsaturated LCFAs to palmitic acid which reduces the lipid inhibition of methanogenesis in the second stage (Beccari et al., 1998). Food waste, which composes a large portion of OFMSW, contains a substantial amount of organic soluble compounds which can be simply converted to VFA.

Therefore, it can be an ideal substrate for biogas production. Nevertheless, formation of excessive amount of VFA at initial digestion stages can result in a remarkable pH reduction and subsequently methanogenesis inhibition. Utilization of two-stage anaerobic digestion systems for food waste has shown to be an effective solution for the pH inhibition of one stage systems (Dinsdale et al., 2000; Kinnunen et al., 2015; Klocke et al., 2008; Y. Li et al., 2011; Shen et al., 2013; Shin et al., 2010). Most of the studies on anaerobic co-digestion process have aimed to evaluate the digesters performance and optimal operating conditions for a particular type of waste.

Lafitte-Trouque et al. found two-stage thermophilic/mesophilic AnCoD systems to be effective for the co-digestion of sewage sludge and confectionery waste. The system with the second digester operating at a HRT of 12 days provided the best performance in terms of stability, VS reductions, and specific methane yield corresponding to an average 82% methane in the gas composition. However, a HRT of 8 days in the second stage digester was not able to assimilate high concentrations of volatile acid and low pH from the first digester. This was related to the insufficient retention time for maintaining a substantial methanogenic population. In a single-stage digester, a HRT of less than 20 days may cause methanogens to be washed out of the digester. Therefore, HRT is one of the important design parameters for the single-phase operations (Lafitte-Trouqué and Forster, 2000).

The study by Ratanatamskul et al. was conducted in pilot-scale on the development of an energy recovery system using a novel prototype single-stage anaerobic digester. Their system (Ratanatamskul et al., 2015) co-digested food waste with sewage sludge from a high-rise building for on-site biogas production.

The food waste to sewage sludge mixing ratio of 10:1 by weight was selected according to the result of lab experiments. Different HRTs of 27, 22, 19 days corresponding to 7.9, 10.8 and 14.0 kgCOD/m^3 d OLRs were applied and the optimal methane yield of 76.8% was achieved by their proposed single-stage reactor when the digester was operated at an HRT of 27 days. Although maximum biogas production occurred at the shortest HRT of 19 days. This indicated that the improvement of methane content of biogas could be attained by adequate operating HRT.

Although, the advantages of two-stage over single-stage digestion systems are addressed in the literature (Bertin et al., 2013; Shen et al., 2013; Yu et al., 2002) there exists a lack of adequate research available in terms of comparing the performances of single, two and or multi-phase co-digestion systems. Kim et al. developed a two-stage system comprised of a continuously stirred tank reactor for acidogenesis and a methanogenic up-flow bed reactor for the treatment of a high lipid wastewater from a milk and ice cream factory co-digested with slaughterhouse wastewater. They obtained 1.2 times increase in the COD removal, 1.9 times increase in lipids removal, and 1.4 times increase in the methane production compared to the single phase system (Kim and Shin, 2010).

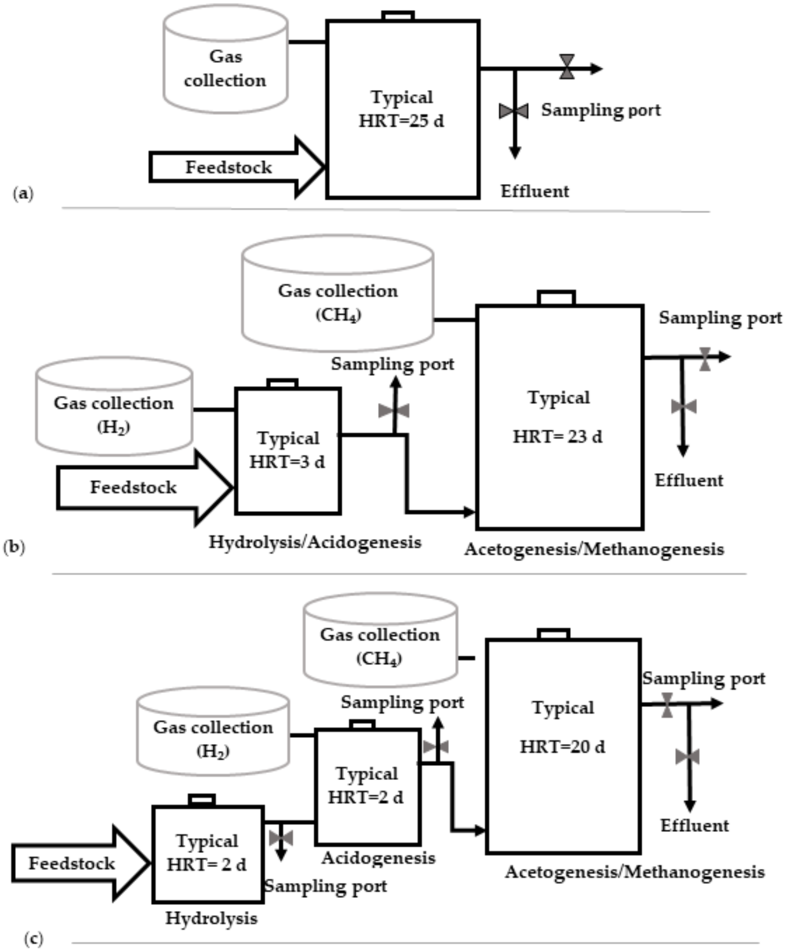


Figure 2.6. Types of digester configuration: (a) Single- stage; (b) Two- stage; and (c) Three- stage digester

Contrary to the result of that research (Kim and Shin, 2010), no significant increase in the overall energy recovery was attained by other study (Schievano et al., 2012) using a two-stage digester co-digesting swine manure and market biowaste in comparison with a single-stage one. Volumetric biogas productions were found to be 0.1 and 0.079 m^3 /L reactor for the single-stage and the two-stage systems, respectively. Even though, the average biogas methane content of the two-stage system showed 25% increase over that of the single-stage digester. The accumulation of un-degraded intermediate metabolites such as volatile fatty acids, ketones, amines, amino acids, and phenols was believed to be responsible for the reduced efficiency of the two-stage digester though. It was concluded that although the two-stage system could be capable of a higher bioenergy production, certain incompetent fermentative pathways may lead to formation of recalcitrant and toxic metabolites.

Hidalgo et al. compared a single-phase with a two-phase reactor for the co-digestion of residues from the used vegetable oil processing industry and pig manure. The maximum methane production of 1.06 m^3 /kg VS removed in the single-stage digestion corresponding to a methane production of 0.69 m^3 CH_4/kg VS removed (65 % CH_4) was obtained at the end of first 50-day operational period. The average biogas productions of 0.46 and 0.33 m^3 /kgVS $_{removed}$ were observed for the second and third operational period with methane productions of 0.30 (65.5 % CH_4) and 0.22 (66 % CH_4) m^3 CH_4/ kg VS $_{removed}$, respectively (Hidalgo et al., 2014).

The two-phase anaerobic digestion improved VS removal efficiencies and process stability in comparison with the single-phase reactor. Although the single-stage system produced more biogas, a higher methane content in produced biogas was obtained by the two-phase system and the latter was deemed to be more beneficial. Table 2.3 summarizes the results achieved by a number of studies on single and two-stage AnCoD systems.

The results obtained by applying a novel compact three-stage anaerobic digester in co-digestion of food waste and horse manure verified the advantages of the three-phase digester over single and two-stage ones as controls. By using three compartments in the three-stage anaerobic digester, three separated functional zones hydrolysis, acidogenesis and methanogenesis were created. This configuration significantly accelerated the solublization of solid organic matters and the formation of volatile fatty acids leading to an increase of 11 and 23% in methane yield in the two-stage and three-stage digesters in comparison with the single-stage one respectively. The analysis of 16 S rDNA showed that different microbial communities comprising hydrolyzing bacteria, acidogenic bacteria and methanogenic archaea were selectively enriched in the three separate reactors of the three-stage digester. It was also indicated that the abundance of the methanogenic archaea was increased by 0.8 and 1.28 times in the two-stage and the three-stage digesters compared to the single stage one correspondingly (Zhang et al., 2017b).

Despite its benefits, AnCoD implementation can be limited owing to long retention time and low biodegradability (Saha et al., 2011b). Therefore, certain techniques are required in order to overcome these obstacles. With the increasing interest in anaerobic co-digestion, a number of researches have been conducted during recent years with the purpose of improving co-digestion of various substrates. Part of those studies has been allotted for pre-treatment techniques from mechanical particle size reduction, thermal and ultrasonic treatment to enzymatic degradation and so on. The main purpose of the pre-treatment methods is to increase the solublization of the complex substrates by the breakdown of the complex substrates such as lignin in lingocellulosic feedstocks or tough cell wall in seaweed biomass, in order to accelerate the hydrolysis rate as hydrolysis is the limiting step in the anaerobic digestion process (Eastman and Ferguson, 1981; Esposito et al., 2012; Noike et al., 1985).

Table 2.3. Comparison of single-stage and two-stage digestion in AnCoD systems

Digester mode	Feedstocks	Mixing ratio	HRT	Biogas / Methane content
Single- stage (CSTR, mesophilic)	Sewage sludge + confectionery waste	NA[1]	20 d	Methane yield: 0.36-0.28 m3/kg VS applied (76- 82% methane)[2]
Two- stage (CSTR, thermophilic/mesophilic)			12 d	Methane yield: 0.3-0.34 m3/kg VS applied (66- 76% methane)[2]
Single stage (plug flow)	Food waste +sewage sludge	10:1 (weight)	27 d	Biogas production: 1045 ± 52.81 L/d
			19 d	1662.58 ± 37.32 L/d
Single- stage (UASB)	Slaughter house + milk wastewater	NA	2.14 d	40% Methane increase by two-stage reactor
Two- stage (CSTR/ UASB)			2.9 d	
Single- stage (CSTR, thermophilic)	Market biowaste + swine manure	1:4 (weight)	25 d	0.55 dm3/L digester d
Two- stage (CSTR, thermophilic)			3/22 d	0.54 dm3/L digester d
Single- stage (Batch)	Oil processing wastewater + pig manure	1:3 (weight)	20 d	Average biogas: 0.33 m3/kgVS removed, (0.66% methane)
Two-stage (Batch)			2/18 d	Average biogas: 0.4 m3/kgVS removed, (0.67% methane)
Single-stage	Food waste/ horse manure	NA	20 d	45.4 L cumulative methane production
Two-stage			4/16 d	50.7 L cumulative methane
Three-stage			2/2/16	55.7 L cumulative methane production

[1] Not Available,

[2] Numbers are mean values after 70- day period of phase 1 and phase 2, respectively

2.4. Pretreatment for Improving AnCoD

Pretreatment have been reported to improve the waste stabilization and the methane yield. However, given the additional costs of pretreatment, it mainly requires to be commercially viable (Esposito et al., 2012). Based on the existing literature, the pretreatment methods used for enhancing AnCoD fall into five main categories including mechanical, thermal/hydrothermal, chemical, biological, and hybrid (combined) pretreatment. As such, the different pretreatment methods that have been used for the enhancement of AnCoD of multi feedstocks are presented in the following sections.

2.4.1 Mechanical pretreatment

Mechanical pretreatment as a mean to improve the AnCoD process has been proposed by a number of researchers. The principal of the mechanical pretreatment technique is to breakdown and/or crushing the substrate particles reducing their particle size. The particle size reduction is proportional to the available specific surface area of the substrate constituents and therefore, it causes a more effective contact between the substrate and the anaerobic microorganisms during the AD process. As a result, the hydrolysis stage (as a known rate-limiting stage) is accelerated and the overall AD process is improved (Esposito et al., 2012).
In general, it has been observed that the smaller the size of the substrate particles is, the higher the methane production rate and yield are in the AD process. In addition to decreasing the particle size, the release of the intracellular components of the substrates has been reported by researchers specifically when the pretreatment was applied on waste activated sludge (WAS). Figure 2.4 illustrates different mechanical pretreatment techniques that have been practiced to enhance the AnCoD process.

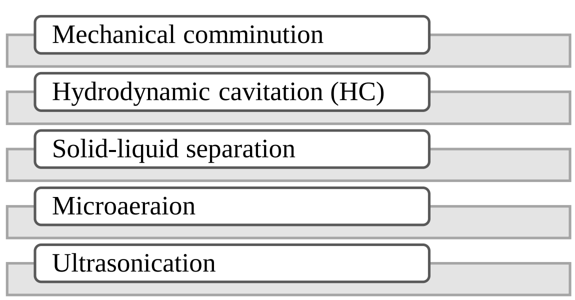


Figure 2.7. Mechanical methods of pretreatment used for AnCoD improvement

2.4.2. Thermal pretreatment

Thermal pretreatment has been commonly applied to both raw and digested substrate to help with dewatering, sludge solublization, viscosity, and pathogen reduction. During thermal pretreatment and as a result of the increase in thermal energy (heat), the structure of the particulate (insoluble) matter changes in a way that it becomes more susceptible to biodegradation (Bougrier et al., 2007). Thermal pretreatment can be applied via conventional heating (conductive heating) or microwave hydrolysis. Edström et al. studied the effect of thermal pretreatment for enhanced co-digestion of animal by-products, food waste from restaurants and food distributors, and sludge from a slaughterhouse wastewater treatment plant. The authors reported a significant increase in the biogas yield from 0.31 to 1.14 L /g VS-added when the animal by-product was exposed to conventional heating at a temperature of 70 ^{o}C for a duration of 1 h before the AnCoD process (Edström et al., 2003). In another study conducted by Paavola et al., thermal pretreatment via conductive heating was applied to a mixture of dairy manure and biowastes. An improvement in methane production at the extent of 14–18% was achieved compared to the process in the absence of thermal pretreatment (Paavola et al., 2006).

Despite these positive effect of thermal pretreatment on AD performance (including biogas production), the result of a study (Cuetos et al., 2010) indicated a 53% decrease in methane production in the co-digestion of a mixture of solid slaughterhouse waste and OFMSW that was thermally pretreated at a temperature of 133 ^{o}C. The reduction of methane production was due to a foaming problem and accumulation of fats in the reactor that most likely occurred due to the formation of recalcitrant compounds at the elevated temperature of 133 ^{o}C, exerting toxic effects on the AD process (Cuetos et al., 2010). Similarly, Guo et al. reported that during the co-digestion of food waste, fruit/vegetable residue, and thermally pretreated dewatered activated sludge, the methanogenesis process was inhibited by the accumulation of VFAs, which reduced the overall biogas production by 6% (Guo et al., 2014).

2.4.3. Chemical pretreatment

Chemical pretreatment can also lead to an improvement in the performance of AnCoD process through enhancement of the rate-limiting hydrolysis stage by facilitating the biodegradation of complex polymers via solubilizing the particulate fraction of the substrate. A variety of chemicals including acids, organic solvents, alkaline, and ionic liquids has shown a positive impact on disintegrating the structure of recalcitrant constituents of the substrates such as lignocellulosic compounds (Zhao et al., 2014).

Various types of alkaline compounds comprising NaOH, KOH, $Ca(OH)_2$, hydrazine, and anhydrous ammonia can increase the internal surface area of biomass resulting in a decreased degree of polymerization. As a result, the complex structures can be decomposed and the strong bounds between carbohydrate molecules can be disrupted, which results in a higher availability of carbohydrates in the hetero matrix and enhanced reactivity of residual carbohydrates polymers in the biochemical process. Pretreatment by alkaline agents is able to eliminate acetyl and other uronic acid substitutions of hemicellulose that obstructs the accessibility of enzymes to cellulose surface. Alkali pretreatment has shown to be most successful for biomasses with lower lignin contents such as agricultural residues (Anwar et al., 2014; Saini et al., 2015).

Acidic pretreatment including dilute solutions of sulfuric acid, hydro-chloric acid, phosphoric acid, and free nitrous acid can be also employed to disintegrate the substrate structure prior to AnCoD process. Compared to the alkaline pretreatment, the application of concentrated acids is limited due to their corrosive nature and elevated costs. Acidic pretreatment has been effective in the hydrolysis of hemicelluloses to its monomeric units and increasing the bioavailability of cellulose. Acidic pretreatment may also be required to neutralize the hydrolysate providing a favorable environment for microbial activities (Lin et al., 2009). Some of the researches have suggested alkaline pretreatment using an ammonia solution or NaOH because of their simplicity, ease of operation, and high methane production efficiency (Bali et al., 2015; Guo et al., 2015; Park and Kim, 2012).

Among different substrates utilized in the co-digestion process, the lignocellulosic biomass as a complex mixture of cellulose, hemicelluloses, and lignin was found to be one of the most difficult to digest by compounds as it contains unbalanced carbon to nitrogen ratio as well as recalcitrant lingocellulosic structure. The lignocellulosic biomass is typically found in plant dry matter such as rice straw. Chemical pretreatment of rice straw prior to AD has been suggested in the literature as an effective way to improve the biodegradability of its constituents (Himmel et al., 2007; Zhao et al., 2014). For instance, the results obtained by Zhao et al. showed that alkali pretreatment using NaOH in co-digestion of rice straw and municipal waste sludge increased biogas yield by 20%. In comparison, 40 and 45% increase in the removal rates of cellulose and hemicellulose were also reported, respectively due to the application of chemical pretreatment (Zhao et al., 2014).

Wei et al. studied the chemical pretreatment using ammonia and NaOH during AnCoD of corn stover and cattle manure, resulting in improved cumulative biomethane production and solids removal rate. In the co-digestion of cattle manure and ammonia solution-treated corn stover, the required time to reach 80% of the ultimate methane yield (T80) was found to be 28 ± 1 days, which was 20 % shorter than that of the control test with a T80 of 35 ± 1 days. Using sodium hydroxide, the T80 was determined to be 22 ± 1 days, 37% shorter as compared to that of the control digester (Wei et al., 2015).

2.4.4. Biological pretreatment

Biological pretreatment, which includes the addition of a particular strain, enzymes, or a consortium of microorganisms to the system, offers certain advantages over mechanical, thermal, and chemical pretreatment methods such as low energy requirements, avoiding toxic compounds generation, high yield of desired products, and the capability to target an specific compounds. Biological pretreatment methods such as bioaugmentation (stage 1) can result in the improvement of AD process by breaking complex polymers into simple monomers in hydrolysis stage which leads to increasing the rate of transformation of organic matter to biogas in the second stage. This process has shown to effectively increase both the methane yield and process stability (Song et al., 2014; Yuan et al., 2012; Zhong et al., 2011). Despite many researchers conducted on the application of biological pretreatment for improving conventional mono-digestion in AD systems, limited studies were performed on the application of these methods for enhancing biogas in AnCoD systems.

Table 2.4 summarizes the results of the studies conducted to enhance the AnCoD performance through biological pretreatment. In a study (Wei et al., 2015), biological pretreatment using liquid fraction of digestate (LFD) for AnCoD of cattle manure and corn stover was investigated. LFD obtained from anaerobic digester contains abundant microbes, inorganic substance as well as an organic substance such as amino acids, protein, a sugar that supplies nutrition substance for the process while acting as a microbial agent. As compared to untreated corn stover, pretreatment using LFD increased cumulative biomethane production (CBP) and VS removal rate by 25.40 and 30.12%, respectively. In addition, it reduced T80 and improved buffer capacity of the anaerobic digestion system (Wei et al., 2015).

Montusiewicz et al. studied the bioaugmentation pretreatment with a commercial product called Arkea® as a method for improving co-digestion of sewage sludge and mature landfill leachate. The co-digestion process was improved due to the enhanced activity of microorganisms involved in bioaugumenting system and their resistance to toxic elements (Montusiewicz, 2014). This was consistent with the results obtained by Duran et al. who used selected strains of Baccillus, Pseudomonas and Actinomycetes species for bioaugmentation (Duran et al., 2006).

In another study by Deng et al., the effect of enzymatic pretreatment on co-digestion of rice straw and soybean straw was evaluated. The research was conducted to investigate the effect of on-site generated cellulase produced by cultivation of Trichoderma reesei RUT C30 on lignocellulose to improve the hydrolysis of cellulose and hemicellulose content of the feedstock. They also investigated the influence of the pretreatment on biogas production via batch experiments. The authors reported more than 300% increase in the cumulative biogas yield compared to the untreated feedstock. They also achieved 40% shorter lag time in co-digestion of pretreated feedstock than the pretreated mono-digestion groups verifying a synergistic effect of rice and soybean straws co-digestion. This study suggested enzymatic pretreatment using Trichoderma reesei RUT C30 as an effective method for improving biogas production (Deng et al., 2018).

Zhang et al, also investigated the effects of biological co-pretreatment on biogas production in AnCoD of food waste (FW) and waste activated sludge (WAS). They used co-pretreatment of FW and WAS followed by anaerobic co-digestion to improve hydrolysis efficiency. Their method was established based on the fact that a biological solublization process by mixing FW, water and microorganisms was effective as a result of size reduction of substrate particles and increase of solublization. They hypothesized that using a mixture of WAS and FW for biological solublization pretreatment (biological co-pretreatment), would improve the hydrolysis of FW and WAS for the reason that: 1) generation of the alkalis from WAS could buffer VFAs and maintain optimum pH for hydrolysis stage, and 2) a lower pH would enhance solublization of WAS through accelerating the hydrolysis of proteins and carbohydrates. Their method of pretreatment included a 2 L glass reactor with a mechanical stirrer at a mixing speed of 150 rpm as a biological co-pretreatment reactor. They applied a blend ratio of 1:1 (weight) with different co-pretreatment time: 0 h, 15 h, 24 h and 35 h. The pretreated mixtures were used as the feed of the subsequent anaerobic digester. A mixture of fresh FW and WAS with 0 h co-pretreatment was used as the feed of the subsequent anaerobic digester as control. They achieved 24.6% higher methane production from co-digestion of co-pretreated substrates compared to control substrates without pretreatment. They also observed an increase of 10.1% in solids reduction under 24 h optimum pretreatment time (Zhang et al., 2017a).

Table 2.4. Biological pretreatment for enhanced AnCoD

Method of Pretreatment	Feedstock	Digester mode	Mixing ratio	Methane yield/ Biogas increase%
Liquid fraction of digestate	Corn stover+ cattle manure	Batch (mesophilic)	3:1 (weight)	25% methane increase
Bioaugmentation by Arkea®	sewage sludge* + mature landfill leachate + Arkea®	CSTR (mesophilic, HRT: 17.4 d)	87:4.3:8.7 (v/v)	5-8% biogas decrease
Biological (Enzymatic)	Rice straw + soybean straw	Batch (mesophilic)	1:1 TS ratio	318% biogas increase
biological co-pretreatment	Food waste and WAS	Semi-continuous	1:1 (weight)	24.6% methane increase

2.4.5. Hybrid pretreatment

Hybrid pretreatment is a combination two or more methods of mechanical, thermal, chemical, and biological pretreatment techniques. Of all the available hybrid pretreatment methods, thermo-alkaline, NaOH/ H_2O_2, and Ozone/NaOH have received more attention to enhance AnCoD. In a recent study performed by Benn et al. (2018), the usage of thermochemical bioplastic pretreatment was investigated in co-digestion with synthetic primary sludge in batch and continuous mode. The pretreatment experiments were conducted by applying different combinations of temperatures ranging from 35–90^{o}C with alkaline conditions in a pH range from 8 to 12 (Benn and Zitomer, 2018). Percent conversion values for bioplastics to biomethane were calculated as the proportion of BMP value divided by the theoretical maximum methane production value indicated by the bioplastic theoretical oxygen demand loading. According to the authors, the thermos-alkaline pretreatment led to an increase in average BMP values up to over 100 %. The batch system co-digesting synthetic primary sludge with bioplastic resulted in 80–100% conversion of bioplastics to biomethane and a 50% biomethane production increase over the non-pretreated substrate. In comparison with the findings of the batch study, less than 20% increase in methane production was obtained by continuous co-digestion of pretreated bioplastics with the synthetic primary sludge in a CSTR system compared to the system fed with non-pretreated feedstock (Benn and Zitomer, 2018).

Another study conducted by Naran et al. revealed that the cumulative methane yield increased from 116.7 to 177.3 mL/g VS added through the co-digestion of thermos-alkaline pretreated food waste from a food waste treatment plant and WAS generated at a municipal treatment compared to the non-pretreated sample (Naran et al., 2016). Similarly, the usage of thermo-alkaline pretreatment method to improve co-digestion of WAS and rice straw was studied and a biogas production of 409 L/kgVS_{added} was obtained under the optimum condition equivalent to a 51% increase compared to the control test. The authors of that work also reported that the degree of WAS solublization was positively correlated with biogas production and VS removal. It was also observed that, following pretreatment, the cellulose and hemicellulose contents of rice straw decreased remarkably. According to the results of their research, the addition of NaOH caused 11%, 32%, and 22% reduction in hemicellulose, cellulose, and lignin contents, respectively. The improvement of digestion performance in this study was related to the increased solublization and reduced particle size of the organic matter (Abudi et al., 2016b).

Abudi et al., employed NaOH/H_2O_2 pretreatment on co-digestion of WAS and rice straw. The applied pretreatment resulted in a remarkable reduction in cellulose and hemicellulose contents of rice straw and hence improved the biogas production. NaOH/H_2O_2 pretreatment was able to decrease hemicellulose, cellulose and lignin contents of rice straw by 16%, 41%, and 7%, respectively. Pretreatment was more effective in the solublization of hemicellulose content than cellulose and lignin contents of rice straw. Consistent results were obtained by others for single digestion of rice and corn straws using NaOH/H_2O_2 pretreatment (He et al., 2009; Song et al., 2014, 2013).

Rajesh Banu et al. employed ozone/NaOH pretreatment for the co-digestion of cow manure and WAS. This resulted in increasing biogas production from 17.9 to 18.8 L/d. Despite the fact that ozone utilization is considered to be costly, the combination of alkali and ozone not only increased the sludge disintegration efficiency but also saved a considerable amount of energy (Rajesh Banu et al., 2015). The increase in biogas production was most likely due to the particles decomposition resulting from ozone reaction with the organic fraction of sludge and the availability of extra carbon source (Ahn et al., 2002).

In the study conducted by Ren et al., the effects of hot alkali pretreatment and mixing ratio on anaerobic co-digestion of duckweed and excess sludge were investigated. The result of their study primarily indicated that through co-digestion the delayed stage of gas generation reduced, and a cumulative gas yield of 2963 mL was obtained which was 11% higher than the calculated value. The methane content of the produced gas was 57%, which was 13% higher than that of the duckweed and 9% higher than that of the excess sludge single digestion. Additionally, pretreatment of the duckweed in the mixture, improved the methane yield by 8% (Ren et al., 2018).

In co-digestion of poultry manure with pig manure in a batch system, the effect of a combined thermochemical pretreatment and ammonia stripping on the digester performance was assessed. The result revealed that the optimal blend ratio of poultry manure to pig manure was 24:76 on volumetric basis which was corresponding to highest methane production. The combined pretreatment improved the co-digestion system achieving an OLR of 4 g COD/L d with a HRT of 20 days (Rodriguez-Verde et al., 2017). The results of these studies are summarized in table 2.5.

Table 2.5. Hybrid pretreatment for enhanced AnCoD

Method of Pretreatment	Feedstock	Digester mode	Mixing ratio	Methane yield/ Biogas increase%
Thermo-alkaline	Synthetic municipal primary sludge + bioplastics	Batch (mesophilic)	10:1 (v/v)	6% methane increase
Thermo-alkaline	WAS + food waste	Batch (mesophilic)	7:3 (v/v)	52% methane increase
Thermo-alkaline	TWAS+ rice straw	Batch (mesophilic)	1:1 (v/v)	51% biogas increase
Thermo-alkaline/H_2O_2	TWAS+ rice straw	Batch (mesophilic)	1:1 (v/v)	56% biogas increase
Ozone/NaOH	Cow manure +dairy wastewater	HUASB***	3:1 (v/v)	5% biogas increase
Thermo-alkaline	duckweed and waste activated sludge	Batch mesophilic	NA	8%
Thermo-alkaline	poultry manure and pig manure	Continuous mesophilic	24:76	37%

In summary, due to various advantages that co-digestion offers over conventional mono digestion, this area is attracted by several researchers and studies are still going on to better understand the system performance and to investigate different methods for improving the AnCoD systems. Such researches along with studies on control parameters, suitability of different feedstocks and their combinations and optimizing procedures would contribute in further improving this technology.

Chapter 3

Materials and methods

3. Materials and methods

3.1. Feedstocks and Inoculum

This research was designed to evaluate the effect of mixing ratio of the feedstocks and its relationship with the lipids: proteins: carbohydrates content on anaerobic co-digestion process. The experiment included two sections including BMP assay and hydrolysis/acidification. The BMP was designed to assess the influence of the mixing ratio on biomethane production. Hydrolysis/acidification experiment was carried out to evaluate the effect of mixing ratio on hydrolysis kinetics. Different feedstocks including dairy manure, TWAS and SSO in different combinations were used as digester feedstocks. TWAS (3.8 % TS) and inoculum were collected from Ashbridges Bay Wastewater Treatment Plant Toronto, Ontario. The inoculum was obtained from the effluent of the anaerobic digesters operating at mesophilic condition at a temperature range of 34-38°C, and receiving approximately 1600 m^3/d TWAS and 6500 m^3/d primary sludge. The average organic loading rate and SRT of the anaerobic digesters are 1.1 kg VS/m^3 and 18 d, respectively.

The Ashbridges Bay Wastewater Treatment Plant is the main wastewater treatment facility among the four treatment plants that service the city of Toronto. After Montreal's Jean-R. Marcotte facility, it is the second largest plant in Canada (Heffez, 2009) The plant treats the wastewater produced by approximately 1.4 million of the city of Toronto's residents and has a capacity of 818,000 m^3/d (City of Toronto, 2018).

The influent to the treatment plant comes from Mid-Toronto, high level, low level and Lakefront interceptor sewers in addition to Coxwell and Queen Street trunk sewers. Biosolids generated at the plant was approximately 149733 wet tones in 2016 with 28.1 % Total Solid (TS). The influent undergoes treatment processes which comprises preliminary treatment i.e. screening and grit removal, primary treatment, secondary treatment, nutrient removal, disinfection, Waste Activated Sludge (WAS) thickening, anaerobic digestion, biosolids dewatering and biosolids management. The influent Total Suspended Solids (TSS) and Biological Oxygen Demand (BOD) concentrations are 318.6 mg/L of 244.6 mg/L, respectively (City of Toronto, 2018; Razavi, 2019).

The unit processes of the plant is shown in figure 3.1. The activated sludge system consists of three main stages including aeration tank, settling tank and return activated sludge. In the aeration tank, the atmospheric air is introduced to the primary treated wastewater using air blowers and the biological mass that produces biological flocs is called waste activated sludge (WAS). The produced sludge then goes through thickening process using different mechanisms to produce thickened waste activated sludge (TWAS).

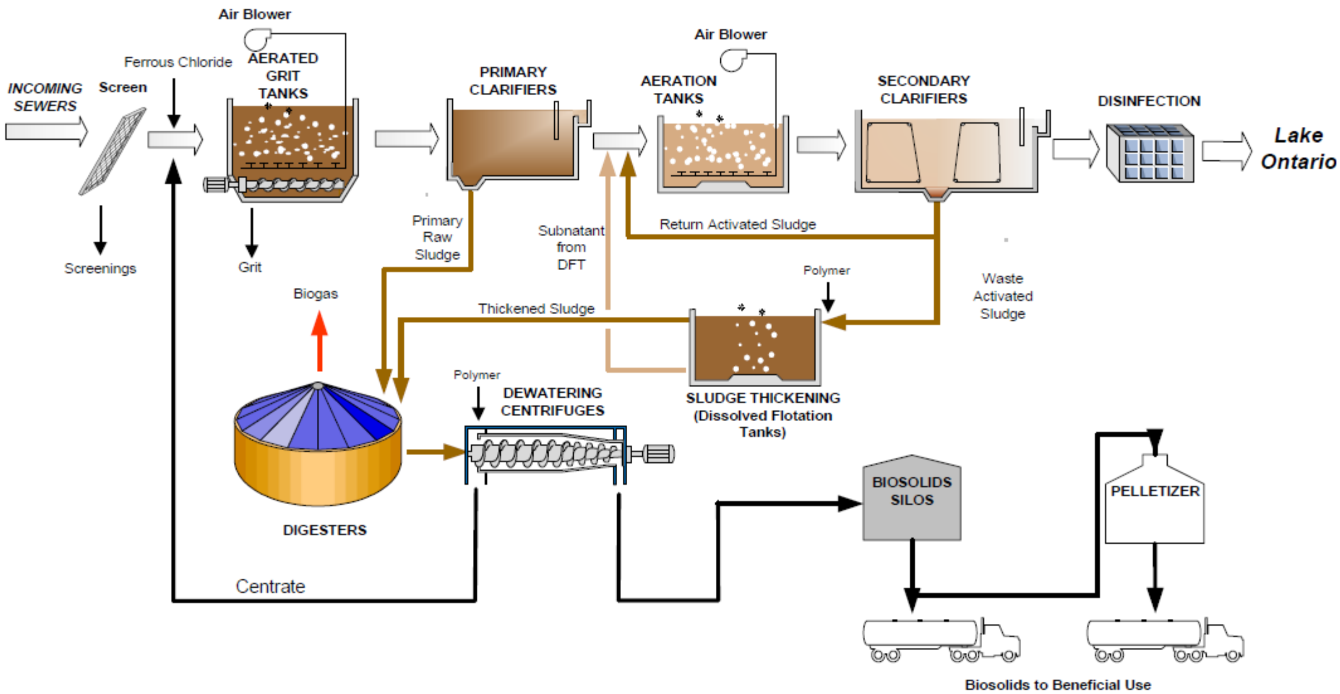


Figure 3.1. The flow diagram of the Ashbridges Bay Wastewater Treatment Plant (City of Toronto, 2018)

SSO was obtained from City of Toronto Disco Road Organics Processing Facility, Toronto, Ontario. The facility is located on 120 Disco Road and on a 1-hectare site and is the first full-scale plant in North America. It has been operated since 2014 for processing the source separated organics by anaerobic digestion. Being one of the municipality's diversion program, it receives almost half of the organics collected in Toronto and is capable of processing up to 75,000 tons of organic waste per year from homes and public buildings. The ultimate capacity of the facility is planned to rise to 130,000 tons in the near future. The acceptable materials to Disco Road facility includes food waste, paper food packaging, pet waste, diapers, houseplants, and biodegradable plastics. The organic processing at the facility is shown in figure 3.2. SSO first is delivered and stored and goes through a visual inspection to remove large unwanted items. At the next stage, the materials undergo the BTA® hydro-mechanical technology through which the organics will convert to a liquid (slurry) pulp. The BTA® consists of screens and hydropulpers for separating the unwanted materials including glass shards, plastic bags, metals and sand from the pulp (Razavi, 2019). In this study, the SSO samples were collected after the hydro-mechanical stage and transferred to the lab in the slurry form.

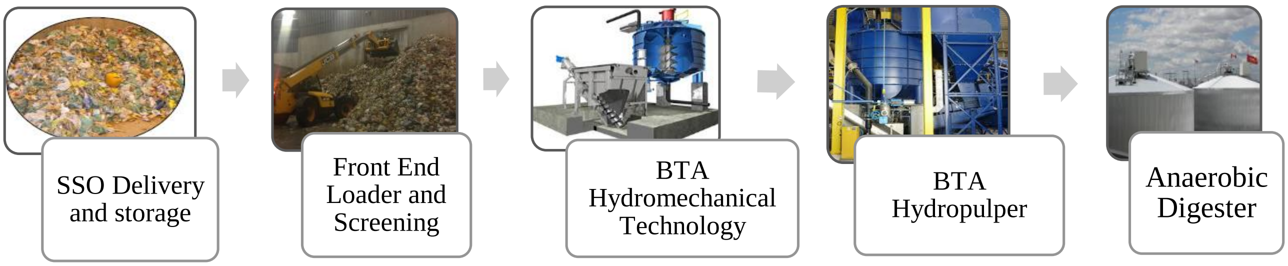


Figure 3.2. Source separated organics processing in Disco Road Facility

Cow manure was collected from a manure pit of a dairy farm located in Newmarket, Ontario. Manure slurry was prepared by addition and homogenization of cow manure with deionized distilled water using a blender followed by a VWR 400 DS bench top homogenizer. The reactors were fed with different combinations of the feedstocks.

The BMP included 3 binary co-digestion experiments using different feedstocks mixtures including different mixing ratios of TWAS/SSO, TWAS/manure, and Manure/SSO. The BMP of TWAS/SSO, TWAS/manure, and Manure/SSO were conducted individually in different periods. It also included a ternary co-digestion using the three feedstocks, TWAS/manure/SSO, at different mixing ratios. Each of the experiments continued until biogas production stopped or was negligible. A series of analysis for characterization of the inoculum, TWAS and SSO and manure was carried out primarily and presented in Table 3.1. Samples were transported and preserved according to Standard Method for Examination of Water and Wastewater (APHA, 2005). Feed of digesters as explained above were mixed at different mixture ratios on a volumetric basis. Batch reactors in working volume of 200 mL containing inoculum and feedstocks at different mixing ratios were prepared for the BMP essay. TWAS, manure, and SSO alone were also used as control reactor to assess the effect of co-digestion on the efficiency of the system in comparison with the single digestion of the feedstocks.

A series of analysis for characterization of the inoculum, TWAS and SSO was carried out primarily and are presented in table 3.1. The mean values are the average of four measurements on each of the parameters for the raw substrates and RSD is the ratio of standard deviation to the mean or relative standard deviation.

Table 3.1. Initial Characteristics of the feedstocks and inoculum used in this study

Parameters	Units	SSO		Manure		TWAS		Inoculum	
		MEAN	RSD	MEAN	RSD	MEAN	RSD	MEAN	RSD
TCOD	mg/L	110000	0.07	198833	0.03	40000	0.07	16400	0.03
SCOD	mg/L	44400	0.002	10933	0.04	360	0.07	362	0.05
TSS	mg/L	53833	0.12	56520	0.03	31450	0.08	17033	0.02
VSS	mg/L	38478	0.095	29998	0.02	25600	0.09	10900	0.02
TS	mg/L	62187	0.02	73727	0.04	38810	0.12	21450	0.03
VS	mg/L	43493	0.02	38647	0.02	31205	0.01	13140	0.02
Ammonia	mg/L	1738	0.003	22	0.07	255	0.12	1495	0.03
pH	-	5.6	0.001	6.4	0.01	6.3	0.005	7.2	0.001
Alkalinity	mg CaCO3/L	7700	0.07	11133	0.05	1953	0.07	3943	0.12
TN	mg/L	4167	0.18	2200	0.12	2900	0.14	2025	0.10
TSN	mg/L	1793	0.02	104	0.09	420	0.15	696	0.16
Total Carbs	mg/L	40360	0.09	7398	0.06	1288	0.08	961	0.08
Total Proteins	mg/L	2021	0.09	5199	0.08	1459	0.18	1448	0.09
Total Lipids	mg/L	18620	0.12	7241	0.09	551	0.08	1920	0.10

3.2. Experimental design and procedure

3.2.1. Co-digestion- BMP assay

The biochemical methane potential (BMP) assays were conducted according to the procedures described in the literature (Angelidaki et al., 2009a; Moody et al., 2009; Owen et al., 1979). The experiment was initiated by feeding the digesters with different mixing ratios of the feedstocks in triplicates. A 100% SSO and 100% TWAS, and 100% manure as control reactors were assessed in triplicates as well. In this research, a substrate-to-biomass ratio (S^0/X^0) of 2 g COD $_{substrate}$/g VSS $_{inoculum}$ was kept in all digesters which is within the range that has been suggested by the literature (Elbeshbishy et al., 2012). Substrate to inoculum ratio was selected for all of the batch reactors according to the procedure used in by Nasr et al., 2011 as following:

$$S^0/X^0 = \frac{g\ TCOD_{substrate}}{g\ VSS_{inoculum}} = \frac{V_{substrate} \times TCOD_{substrate}}{V_{inoculum} \times VSS_{substrate}} = 2 \qquad \text{Eq.3.1}$$

The TCOD of the mixture used in Eq. 3.1 was calculated considering the TCOD of the feedstocks and the mixing ratios. Accordingly, the volumes of the feedstocks were calculated based on the mixing ratios of substrates. Because of the heterogeneous composition of the feedstocks, all of the combinations were prepared in triplicates. The specific amount of the substrates along with the mesophilic inoculum as described above was added to 250 mL glass bottles. The headspaces in the bottles were flushed with nitrogen gas for 3 minutes at 10 psi and subsequently the bottles were sealed to satisfy the anaerobic conditions. In addition, anaerobic systems require a pH within the range of 6.5–7.5 according to the literature (Cioabla et al., 2012; Droste, 1997). Therefore, the pH in each bottle were kept in a range of 7-7.4 using sulfuric acid and sodium hydroxide. The binary co-digestion of TWAS/SSO, TWAS/manure, and manure/SSO were conducted at the mixing ratios as presented in table 3.2.

Table 3.2 Proportions of digesters' feed for binary co-digestion of TWAS/ SSO, TWAS/manure, and manure/ SSO in BMP experiment

Feedstock	TWAS	Manure	SSO
Proportion of the feedstocks in different runs of Binary co-digestion Volume: 200 mL	0	1	0
	1	0	0
	9	1	0
	7	3	0
	5	5	0
	3	7	0
	1	9	0
	0	2	4
	0	0	1
	0	9	1
	0	7	3
	0	5	5
	0	3	7
	0	1	9
	0	0	
	9	0	1
	7	0	3
	5	0	5
	3	0	7
	1	0	9

The ternary co-digestion of TWAS/manure/SSO was carried out at different mixing ratios of TWAS, manure and SSO as presented in Table 3.3. As demonstrated in Table 3.2 and 3.3 reactors containing only manure, only TWAS and only SSO were also used in triplicates as control in each run.

Table 3.3 Proportions of digesters' feed for ternary co-digestion of TWAS, SSO, and manure in BMP experiment

Feedstock	TWAS	Manure	SSO
Proportion of the feedstocks in different runs of ternary co-digestion Volume: 200 mL	8	1	1
	1	8	1
	1	1	8
	5	2.5	2.5
	2.5	5	2.5
	2.5	2.5	5
	4	4	2
	4	2	4
	2	4	4
	1	0	0
	0	1	0
	0	0	1

The sets of 21 bottles for each of the three binary co-digestion experiments, and the 36 bottles for the ternary co-digestion experiment were placed in the Thermo Scientific MAXQ 4000 shakers and a rotational speed of 150 RPM was applied during the entire process. The incubator temperature was set at 37 ^{o}c to satisfy mesophilic condition for the batch reactors.

Figure 3.3- Experimental set-up for biomethane potential experiment

3.2.2. Co-digestion- hydrolysis/acidification experiment

This experiment was conducted to assess the influence of the mixing ratio and its relationship with the lipids: proteins: carbohydrates in co-digestion on hydrolysis/acidification rate. Similar to BMP experiment, the binary co-digestion of TWAS/ SSO, TWAS/manure, and manure/SSO were conducted at different combinations as presented in Table 3.4. The ternary co-digestion of TWAS/manure/SSO was carried out at the mixing ratios that are presented in Table 3.5. For each of the experiment's rectors containing only TWAS, only manure and only SSO were also used in triplicates as control reactors.

The bottles for each of the three binary co-digestion experiments, and for the ternary co-digestion experiment were placed in the Polyscience WB28 water bathes. Bottles were equipped with a mixer which maintained a rotational speed of 150 RPM the entire process. The temperature in water bathes was set at 37 ^{o}c to satisfy mesophilic condition for the batch reactors.

Consistent with the BMP experiment, the substrate-to-biomass ratio (S^0/X^0) of to 2 g COD $_{substrate}$/g VSS $_{inoculum}$ was kept in all digesters. Feed of digesters as explained above were mixed at different mixture ratios on a volumetric basis. Batch reactors in working volume of 2000 mL containing inoculum and feedstocks at different mixing ratios were prepared for the BMP essay. In order to deactivate methanogens and to increase the accuracy of the hydrolysis/acidification experiment, the inoculum was heated to 70 ^{o}c for 30 min and pH was adjusted to a range between 5- 5.5 in all digesters. The experiment continued for a period of three days for each run of the binary co-digestions and the ternary co-digestion experiment. Samples were collected with time to evaluate the solublization and hydrolysis rate of the mixtures and to assess the influence of the mixing ratios on them. Consistent with the BMP assay, The TCOD of the mixture in Eq. 3.1 was calculated considering the TCOD of the feedstocks and the mixing ratios. Accordingly, the volumes of the feedstocks were calculated based on the mixing ratios of substrates. Because of the heterogeneous composition of the feedstocks, all of the combinations were prepared in triplicates. The specific amount of the substrates along with the mesophilic inoculum as described above was added to 2500 mL glass bottles.

Table 3.4 Proportions of digesters' feed for binary co-digestion of TWAS/ SSO, TWAS/manure, and manure/ SSO in hydrolysis/acidification experiment

Feedstock	**TWAS**	**Manure**	**SSO**
Proportion of the feedstocks in different runs of Binary co-digestion Volume: 2000 mL	0	1	0
	1	0	0
	9	1	0
	7	3	0
	5	5	0
	3	7	0
	1	9	0
	0	2	4
	0	0	1
	0	9	1
	0	7	3
	0	5	5
	0	3	7
	0	1	9
	0	0	
	9	0	1
	7	0	3
	5	0	5
	3	0	7
	1	0	9

Table 3.5 Proportions of digesters' feed for ternary co-digestion of TWAS, SSO, and manure in in hydrolysis/acidification experiment

Feedstock	TWAS	Manure	SSO
Proportion of the feedstocks in different runs of ternary co-digestion Volume: 200 mL	8	1	1
	1	8	1
	1	1	8
	5	2.5	2.5
	2.5	5	2.5
	2.5	2.5	5
	4	4	2
	4	2	4
	2	4	4
	1	0	0
	0	1	0
	0	0	1

The headspaces in the bottles were flushed with nitrogen gas for 3 minutes at 10 psi and subsequently the bottles were sealed to satisfy the anaerobic conditions. Gas production during the experiment was monitored using water displacement method and displayed real time. The percentage improvements in the soluble contents concentrations (degree of solubilization) (P) values was calculated using Eq 3.2.

$$P\ (\%) = SC_f - SC_i / PC_i * 100\% \qquad \text{Eq. 3.2}$$

Where SC_i and SC_f are the mass of soluble COD of the digester contents before and after the hydrolysis/acidification phase experiment (mg) and PC_i is the mass of initial particulate COD in the digesters (mg).

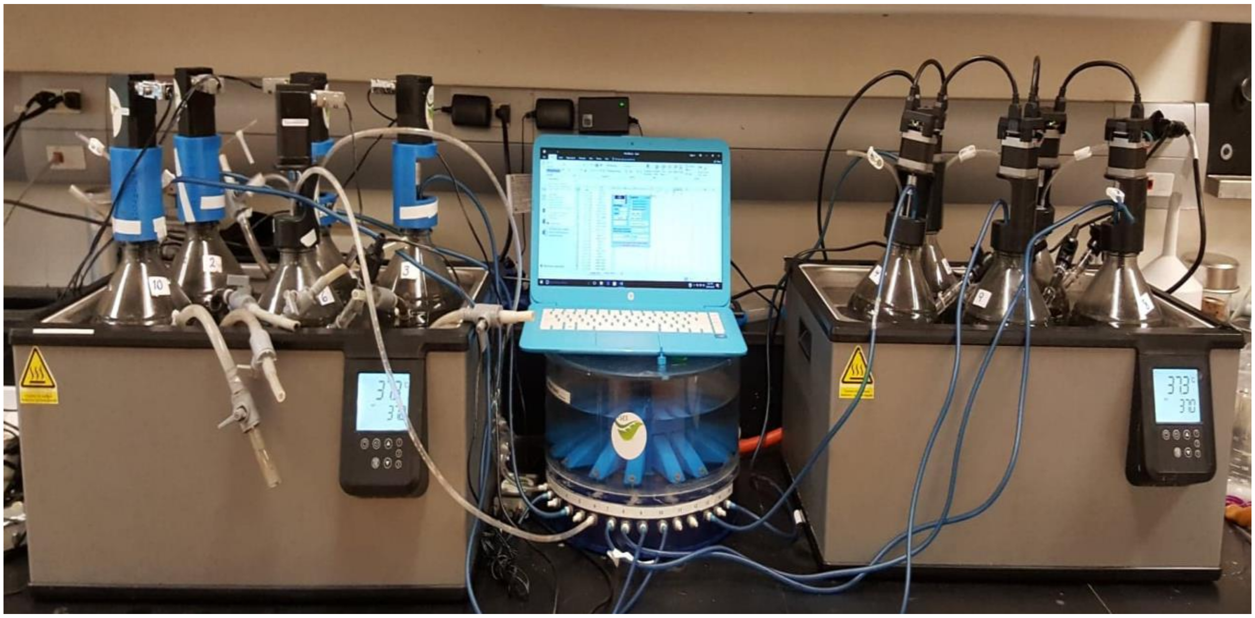


Figure 3.4. Experimental set-up for hydrolysis/acidification experiment

A first-order reaction model was applied using AquaSim 2.0 software to assess the effect of different mixing ratios on biodegradation rates of COD, proteins, lipids, and carbohydrates.

r_{su}= dC/dt= -kC Eq. 3.3

Where C is the concentration (mg/L) of the parameters (TCOD, proteins, lipids, and carbohydrates) at time t, k is first-order specific biodegradation rate constant (1/d) and r_{su} is biodegradation rate (mg/L.d). Eq. 3.4 is derived by integration of Eq. (3.3).

$C_t= C_u e^{-kt}$ Eq. 3.4

Where t, C_t and C_u are time (d), concentration at time t (mg/L), ultimate particulate parameters (TCOD, proteins, lipids, and carbohydrates) in mg/L, respectively.

3.3. Analytical analysis

The analysis of solid contents of the feedstocks including total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) of the inoculum, TWAS, and SSO samples were determined according to the Standard Methods procedures (APHA, 2005). Chemical oxygen demand (TCOD) and (SCOD), ammonia, total nitrogen (TN) and total soluble nitrogen (TSN) were measured using a Hach spectrophotometer model 3900. For the measurement of the soluble content, samples were prepared by centrifuging at 9000 rpm for 45 min and then the supernatant was filtered using microfiber filters with a pore size of 0.45 µm. The absorbance was set at the wavelengths of 600, 560 and 650 nm for the analysis of COD, ammonia, and alkalinity, respectively.

Total lipids concentration was measured by solvatochromatic method that rely upon a dye or mixture of dyes which change optical properties upon a change in condition of the solvent in which they are dissolved. In this test, there is an increase in fluorescence when there is an increased amount of dissolved lipids that form micelles or other structures. Solvatochromatic analysis was carried out at the fluorescence 405 nm mode using Vernier SpectroVis spectrometer and Logger Pro software. The data then exported as CSV files for further analysis. Figure 3.5 shows the graph created by Logger Pro at 405 nm wavelength and the absorbance for different concentrations of total lipids. The peak values of the curves corresponding to the lipids concentration of different standard samples with known concentrations were used to plot the calibration curve and get the curve equation. The measured absorbance values for the actual samples with unknown lipids concentrations were substituted into the calibration equation and was solved for the true value.

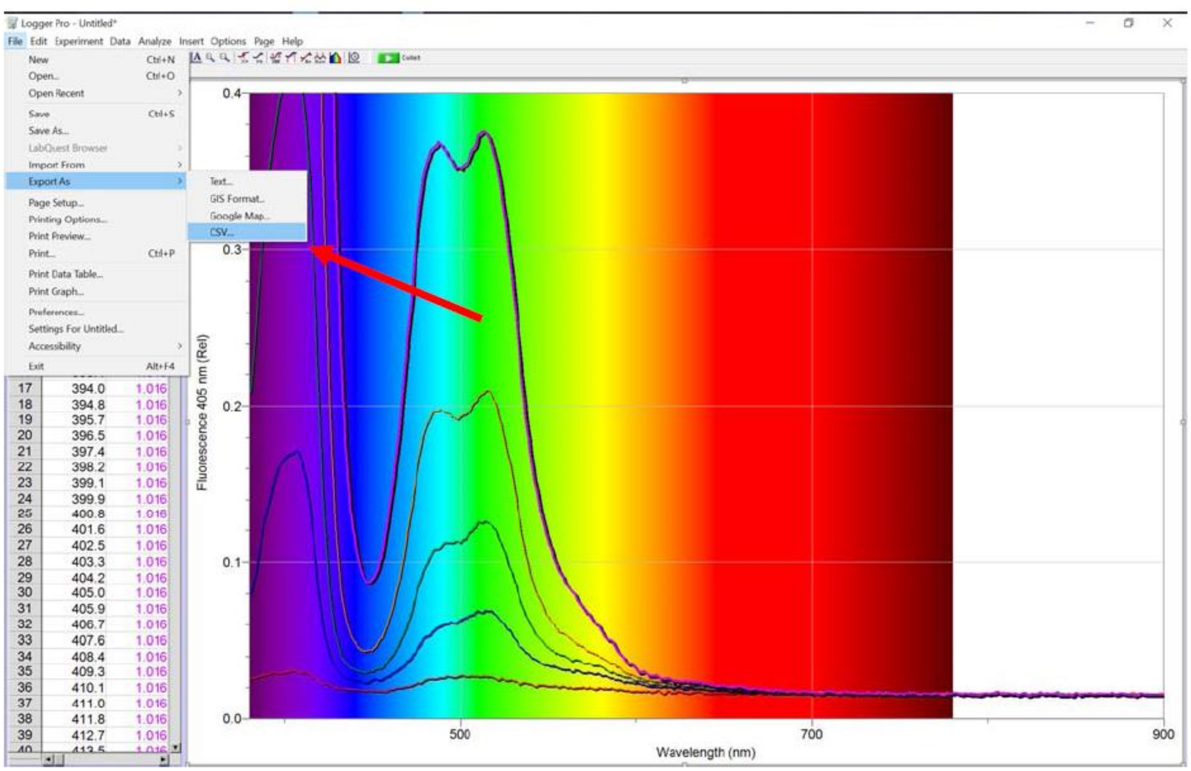


Figure 3.5. The spectra and flourometric data for different total lipids concentrations

The volume of the produced gas was measured manually using a 100-mL Gastight Luer-Lock glass syringe daily at the beginning of the digestion period. The gas measurement was continued every couple of days later on when the gas production rate slowed down over time. The amount of biomethane in the produced biogas during the anaerobic digestion process was measured using a Thermo Scientific Trace 1310 gas chromatograph (GC). The GC was equipped with a thermal conductivity detector and the temperature of the oven, detector, and filament were set to 80, 100, and 250 °C, respectively. The type of column used was a TG-Bond Msieve 5A model with a 30 m length and 0.53 mm diameter.

3.4. Statistical and Kinetics analysis

Statistical data including mean and standard deviation were calculated for the data obtained by the experiment. One way ANOVA for the analysis of variance was used to to find statistically significant differences between the group means and the results are presented in chapter 8.

Gompertz equations provides a wide range of applications in process kinetics of anaerobic digestion and the methane potential studies. In this work, Modified Gompertz model (Elbeshbishy and Nakhla, 2012; Lay et al., 1999) was used to predict the biogas yield and to assess the kinetic parameters and to describe the progress of cumulative methane production through the batch process (eq. 3.52) where CH_4 is the cumulative methane production (mL), P is the ultimate methane production (mL), R_m^e is the maximum methane production rate (mL/d), λ is the lag phase time (d), t is the digestion time (d).

$$CH_4 = p.\exp\{-\exp[\frac{R_m^e}{p}(\lambda - t) + 1]\} \qquad \text{eq. 3.5}$$

Chapter 4
Results and discussion

TWAS and SSO Co-digestion

4. Results and discussion- TWAS/SSO co-digestion

4.1. BMP of TWAS and SSO

This experiment was designed to evaluate the effect of mixing ratio of TWAS with SSO and its relationship with the lipids: proteins: carbohydrates on anaerobic co-digestion process using TWAS and SSO in different combinations as digester feedstocks. Co-digestion of SSO with TWAS was conducted as explained in Chapter 3. The characteristics of the feed in each digester having different mixing ratios of the substrates are summarized in Table 4.1. As presented in Table 4.1, the amount of TCOD of SSO is remarkably higher than that of TWAS. Increasing the fractions of SSO, increased the TCOD of the feed to digesters. The total lipids and total carbohydrates contents of SSO are also significantly more than that of TWAS and therefore, by increasing the proportion of SSO in the co-digesters the concentrations of lipids and carbohydrates increased. pH was kept at neutral level from 7.0- 7.3 in all the mixtures to satisfy the favorable condition for methanogenesis.

Table 4.1. Mean values of the feed characteristics in digesters with different mixing ratios of TWAS and SSO

		TWAS Only	SSO Only	TWAS:SSO 9:1	TWAS:SSO 7:3	TWAS:SSO 1:1	TWAS:SSO 3:7	TWAS:SSO 1:9
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)
TCOD	g/L	40	110	47	61	75	89	103
SCOD	g/L	1.4	44	5.7	14	23	32	40
TSS	g/L	32	54	34	38	43	47	52
VSS	g/L	26	39	27	30	32	35	37
TS	g/L	39	62	41	46	51	55	60
VS	g/L	35	44	35	37	39	41	43
Ammonia	g/L	0.3	1.1	0.3	0.5	0.7	0.9	1.0
pH		7.2	7.1	7.0	7.0	7.2	7.1	7.3
Alkalinity	g CaCO3/L	2.0	6.1	2.4	3.2	4.0	4.8	5.7
TN	g/L	2.9	3.3	2.9	3.0	3.1	3.2	3.2
TSN	g/L	0.4	0.9	0.5	0.6	0.7	0.8	0.9
T-Carbs	g/L	1.1	144	2.4	5.1	7.7	10.4	13.0
T-Proteins	g/L	3.8	2.3	3.6	3.3	3.0	2.7	2.5
T-Lipids	g/L	0.4	1.7	0.5	0.8	1.0	1.3	1.6

As shown in Fig. 4.1, no significant lag-phase occurred in the generation of biogas the reactors. Operation of the digesters continued until no significant biogas was produced. Fig. 4.1 shows the time-course profile of the cumulative biomethane production during the total operation period. As illustrated in the Figure 4.1, no significant lag time was observed for all the digesters. This no sign of significant inhibition would be due to the use of mesophilic inoculum acclimatized to municipal sludge streams similar to the one used in the experiment. The most lag-phase occurred at TWAS:SSO mixing ratios of 1:1, and 3:7, and 1:9 which could be due to the existence of particulate matters introduced by SSO to the mixture which could delay the hydrolysis phase and consequently affect methanogenesis.

The amount of biomethane produced by SSO was significantly higher than that of TWAS. Only 542 mL cumulative methane was produced by TWAS while the amount of cumulative methane obtained by SSO was 1101 mL. This verified the low biodegradability of TWAS compared to SSO and would be due to the composition of TWAS as it mostly consists of proteins and humic substances with some bacterial biomass and carbohydrates. Although proteins, DNA and carbohydrates are anaerobically biodegradable, their biodegradability decreases when they are combined into an organized structure similar to TWAS (Gonzalez et al., 2018; Stuckey and McCarty, 1984). Microbial cells are difficult to break down under anaerobic digestion (Foladori et al., 2015; Wett et al., 2010) and similarly, the presence of humic substances affects enzymatic activity by immobilizing enzymes and as a result, lowers biodegradability (Azman et al., 2015a, 2015b; Fernandes et al., 2015).

Another reason for the low biodegradability of TWAS was investigated by a study, in which low digestibility was attributed to the slow hydrolysis process for the exterior polymeric component of the microbial culture within the sample. Furthermore, the study also found that the ratio of SCOD to TCOD was 34.6% for untreated TWAS compared to 63.6% and 68.1% for thermally and alkaline pretreated samples, respectively. The COD ratio was a clear indicator of the expected biogas production by the TWAS feedstock samples where lower biogas yield was reported for the untreated raw TWAS as compared to pretreated samples (Abudi et al., 2016a).

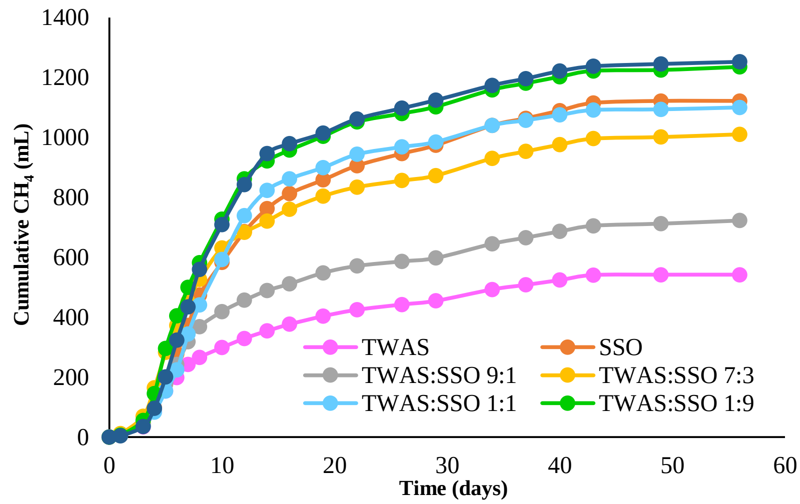


Figure 4.1. Cumulative methane production for different mixing ratios of TWAS and SSO

Addition of SSO to TWAS increased biodegradability and the methane yield compared to TWAS alone. However, as demonstrated in Figure 2, the trend showed an optimal mixing ratio of SSO with TWAS. The TWAS:SSO mixing ratio of 3:7 (V/V) delivered better results compared to TWAS:SSO mixing ratio of 1:9 (V/V) in terms of digestion process and methane enhancement.

Fig 4.2 shows the methane production rate in mL CH_4/d through the digestion period. Comparing TWAS and SSO, the methane production rate obtained by SSO was significantly higher than that of TWAS, although the maximum rate for the rectors digesting only TWAS occurred in earlier stage of the digestion process compared to the ones digested only SSO. Similar trend was observed in the digesters containing mixtures of TWAS and SSO so that increasing the fraction of SSO to the co-digesters, caused maximum methane production rate take place later than the co-digesters containing more fraction of TWAS. This could be as a result of abundant particulate matter that affects the hydrolysis rate and prolongs the entire process.

The biomethane data monitoring as presented in Figure 4.2, revealed that the highest portion of the biomethane was produced within the first month. The digesters generated 31–45%, 65–76% and 83–90% of their ultimate biogas productions during first 7, 14 and 30 days of operation, respectively. This would be due to the availability of sufficient nutrient right after the start of the operation which increases the metabolic activity of the microorganisms causing rapid conversion of substrate to biogas without inhibition in digesters (A. J. Li et al., 2011; Sung and Dague, 1995). The maximum methane production rate for mono and co-digestions is presented in Figure 4.3. The amount of maximum methane production rate of SSO mono digestion was 109 mL CH_4/d which was 60% higher that of TWAS mono digestion corresponding to 68 mL CH_4/d. Comparing to TWAS mono digestion, increasing the percentage of SSO in the co-digesters containing TWAS:SSO mixtures from the ratio of 1:9 ratio to 3:7, increased the maximum methane production rate by 25 % and 75%, respectively. Further increasing the percentage of SSO in the co digesters at TWAS:SSO mixing ratios of 1:1, 1:9 and 3:7, significantly increased the maximum methane production rate both compared to TWAS and SSO mono digestion, although the maximum rate values were almost the same for the three mixing ratios.

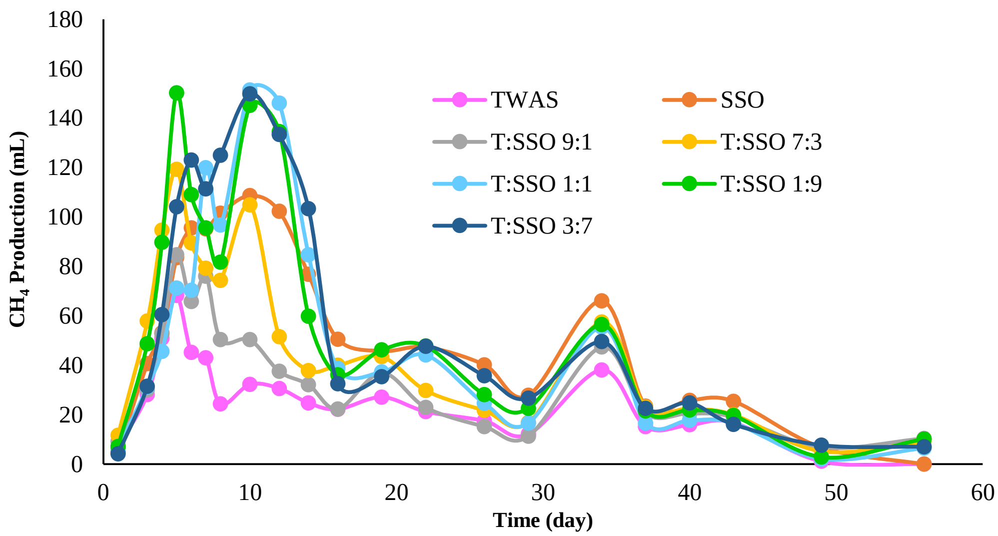


Figure 4.2. Methane production rate (mL/d) for different mixing ratios of TWAS and SSO

These findings as a matter of fact showed a close agreement to a previously conducted study (Abudi et al., 2016a), where the reported cumulative specific biogas yield (CSBY) for alternating ratios of organic fraction of municipal solid waste (OFMSW) and TWAS resulted in increasing biogas yield. As presented in Figure 4.3, the amount of maximum methane production rate was the highest for the TWAS:SSO mixing ratios of 1:1, 3:7, and 1:9 in a range between 150 to 151 mL/d. However, the maximum biomethane production rate for the TWAS:SSO mixing ratio of 3:7 occurred on the day 5 of the operational period while it was observed on the day 10 for the mixing ratios of and 1:1 and 1:9. This would be an evident to the advantage of co-digestion while emphasizing the necessity of a proper mixing ratio of the substrates which fulfills both enhancing the methane production and the process kinetics.

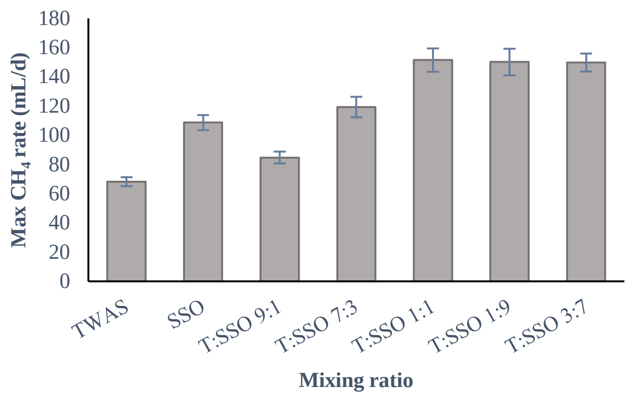


Figure 4.3. Maximum methane production rate (mL/d) for different mixing ratios of TWAS and SSO

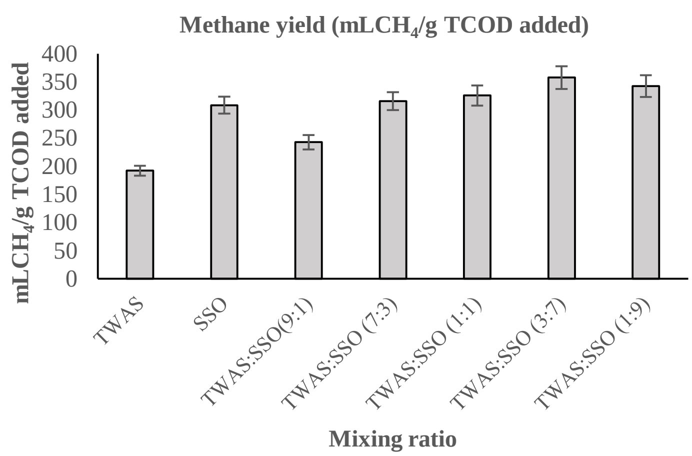
A COD mass balance was conducted for all of the digesters to assess the accuracy of the experiment. The mass balance was carried out with reference to the initial and the final TCOD concentrations of the digester contents, and the theoretical methane production per unit mass of TCOD removed. Comparison between the experimental methane production attained by this research and that of determined by TCOD mass balance, verified a deviation of less than 10% for all the digesters.

4.2. Cumulative methane yields

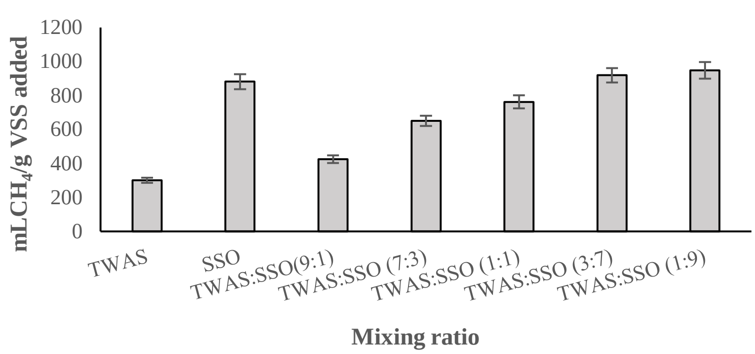
Cumulative methane yields were calculated and presented in Figures 4.4 a), b) and c. The cumulative methane yield was normalized per substrate unit mass COD added (mLCH_4/g TCOD added), per substrate unit mass VSS added (mLCH_4/g VSS added) and per unit volume of substrate added (mLCH_4/mL substrate added).

Figure 4.4 a) shows the result of cumulative methane yield per mass COD of substrate added for mono and co-digestions. Results showed that 192 mLCH_4/g TCOD added was obtained by TWAS mono digestion while a higher yield corresponding to 308 mLCH_4/g TCOD added was achieved by SSO alone. Mixing TWAS with SSO in co-digesters at all ratios excluding TWAS:SSO ratio of 9:1, increased the methane yield in comparison with both TWAS and SSO mono digestion. Even though, the ratio of 9:1 resulted in 27% increase of the CH_4 yield (mL) per mass of TCOD added compared to TWAS alone. The maximum cumulative methane yield was obtained by the 3:7 mixing ratio of TWAS:SSO corresponding to 358 mL CH_4/g TCOD added being 85% and 16% higher than that of TWAS and SSO alone. It was observed that addition of SSO to co-digesters significantly increased the methane yield compared to mono digestion of TWAS while it did not remarkably increased the yield in comparison with SSO mono digestion.

4.4. a)



4.4. b)



4.4. c)

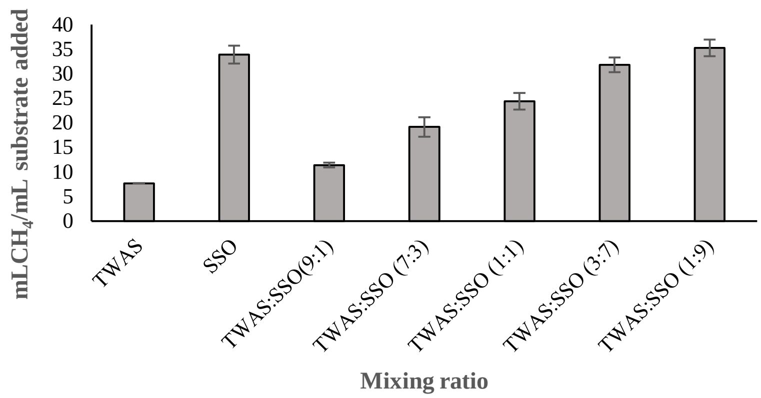


Figure 4.4. Methane yields: a) per unit mass TCOD added, b) per unit mass of VSS added and c) per unit volume of substrate added at different mixing ratio of TWAS and SSO

The yields of cumulative methane in terms of $mLCH_4$/g VSS added have been presented in Figure 4.4. b). Similarly, the methane yield per mass of VSS added by SSO mono digestion was significantly higher than that of TWAS. A 300 $mLCH_4$/g VSS added and 892 $mLCH_4$/g VSS added were attained by TWAS and SSO alone, respectively. All co-digesters produced higher methane yield per mass of VSS added compared to TWAS alone. However only the TWAS:SSO mixing ratios of 1:9 and 3:7 resulted in higher methane yields per mass of VSS added in comparison with both TWAS and SSO alone. The maximum methane yield of 975 $mLCH_4$/g VSS added occurred at the TWAS:SSO mixing ratio of 3:7.

It was revealed that addition of SSO to TWAS significantly increased biomethane production in the rectors co-digesting them compared to the control reactor digesting only TWAS. However, only the TWAS:SSO mixing ratio of 3:7 resulted in the increase of the methane yield compared to both TWAS and SSO mono digesters.

Eq. 4.1 was used for assessing the biodegradable fraction of the feedstocks for each of the rectors and the results are illustrated in Figure 4.5.

$$BF\,(\%) = (BM_{experimental} / BM_{theoretical}) \times 100 \qquad \text{Eq. 4.1}$$

Where BF is biodegradable fraction, $BM_{experimental}$ is the measured biomethane by the experiment and $BM_{theoretical}$ is the theoretical biomethane production in mL CH_4 per g TCOD of the substrate in mesophilic condition and standard pressure.

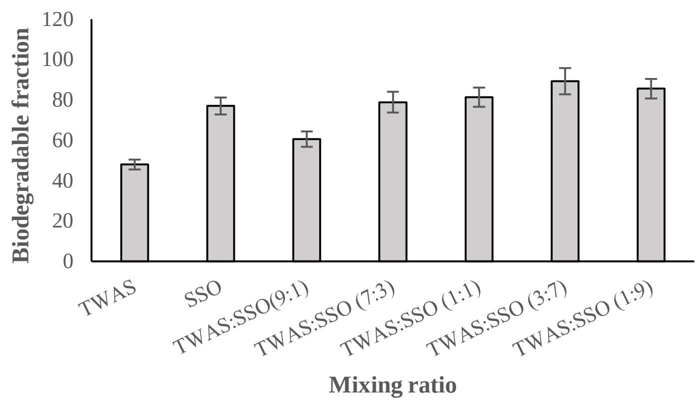


Figure 4.5. Biodegradable fraction of the feedstocks for different mixture ratios of TWAS and SSO

As shown in Figure 4.5, the TWAS:SSO mixing ratio of 3:7 corresponded to the maximum percentage of biodegradable fraction which complies with the maximum methane yield at the same mixing ratio. BF was 48% and 77% for TWAS and SSO, respectively. It was verified that addition of SSO as co-substrate increased biodegradability and enhanced the production of methane in the rectors co-digesting SSO with TWAS. All co-digesters had higher BF than TWAS alone. The reason would be the existence of readily biodegradable compounds in SSO that was introduced to the co-digesters. At the mixing ratio of 3:7, the BF was 89% which was 85% and 16% higher than that of TWAS and SSO alone respectively. The trend of BF changes in digesters complies with the methane yields obtained by the experimental results for the corresponding digesters.

4.3. Synergistic effect

In anaerobic digestion, production of biomethane develops through a syntrophic metabolism between both communities of methanogens including bacteria and archaea (Viotti et al., 2004). It is evident that both communities of bacteria and archaea are present in AnCoD systems. An improvement in the synergy and diverse microbial consortia is obtained when applying co-digestion of multiple feedstocks (Zamanzadeh et al., 2017). The synergistic effect of co-digestion can be estimated as an additional methane production (mL) for co-substrates over the weighted average of the methane production of individual substrates (Parra-Orobio et al., 2016). In this research, in order to investigate the synergetic effect of microbial populations on anaerobic co-digestion of TWAS and SSO at different mixing ratios, the weighted methane production (MP) of co-substrates were calculated using Eq. (4.2):

$$\text{Weighted MP} = MP_{SSO} * P_{SSO} + MP_{TWAS} * P_{TWAS} \qquad \text{Eq. (4.2)}$$

Where weighted MP is the weighted average of methane production for co-substrates (mLCH$_4$); MP_{SSO} and MP_{TWAS} are the experimental methane production (mLCH$_4$/ mL substrate added) for SSO and TWAS; and P_{SSO} and P_{TWAS} are the volume (mL) of SSO and TWAS in the substrate's mixture, respectively. When the percentage difference between experimental methane production for the mixtures and the calculated weighted average of methane production was positive, the synergistic effect could be concluded. Figure 4.6 shows the percentage of additional methane production for co-substrates over the weighted average of the methane production of individual substrates. As revealed in Fig 4.6, the maximum synergetic impact was observed in co-digestion of TWAS with SSO at the mixing ratio of 3:7. This is in good agreement with the maximum methane yield that occurred at the same mixing ratio of TWAS and SSO in their co-digestion.

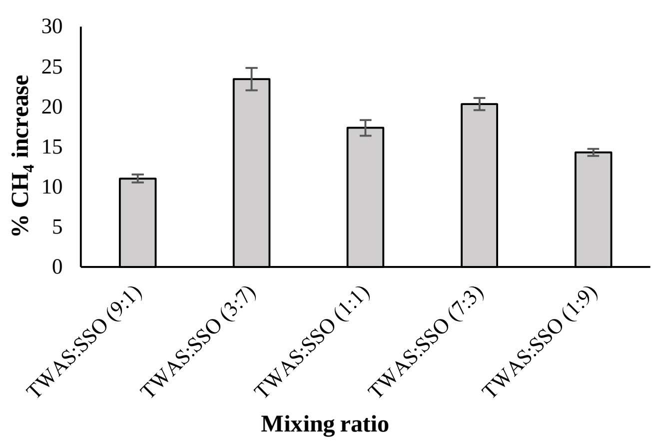


Figure 4.6. Synergetic effect of co-digestion at different mixing ratios of TWAS and SSO

Although all of the mixings demonstrated the synergetic impact of co-digestion on improving biomethane production, no specific trend for the change of the synergetic effect and methane increase corresponding to the fraction of SSO in the co-digestion mixtures was observed. The percentage of methane increase due to the synergetic impact varied from 11 to 23. The most percentage of biomethane increase of 23% as a result of the improved synergy occurred at the mixing ratio of 3:7. This is in good compliance with the results of maximum cumulative methane rate and the maximum methane yield which were achieved at the same mixing ratio. Although introducing fractions of SSO added more amounts of readily biodegradable materials to the co-digesters, the more fractions of SSO did not necessarily satisfied the optimum condition for the process improvement.

The improvement of the synergy and biogas yield could be as a result of diverse microbial consortia introduced by applying co-digestion of multiple feedstocks. Nevertheless, enhanced synergy would be dependent on some factors relying on the proper mixing ratios which satisfy the optimal nutrients balance and effective conditions for microbial syntrophy. This could be the reason for the optimum fraction of TWAS and SSO at the mixing ratio of 3:7 in the co-digestion rather than other ratios.

4.4. COD:N and lipids: proteins: carbohydrates ratios

The relationship between the COD:N ratios as well as lipids: proteins: carbohydrates ratios at different mixing ratios of TWAS and SSO with methane yield and ultimate methane production is presented in Table 4.2. The table also shows a comparison between the COD:N ratios with lipids: proteins: carbohydrates ratios for the digesters fed with different mixings of TWAS and SSO in volumetric basis. In co-digestion of TWAS and SSO, the COD:N ratio above 20 resulted in higher ultimate methane production, however, variation of the ratios from 28 to 34 caused a reduction of the amount of the produced methane. As shown in the table, the amounts of ultimate methane production were in a range between 542 to 1252 mL for different mixing ratios. The lowest ultimate methane production of 542 mL corresponded to a COD:N ratio of 14 and a lipids: proteins: carbohydrates ratio of 1:11:3 which occurred at TWAS:SSO mixing ratio of 1:0 in the reactors digesting TWAS alone. The maximum ultimate CH_4 corresponded to COD:N ratio of 28 associated with the lipids: proteins: carbohydrates ratio of 1:2:8 for the reactors co-digesting TWAS and SSO at the mixing ratio of 3:7 (v/v).

Considering the fact that lipids, proteins and carbohydrates are the main constituents of any organic material, considering the ratios of lipids: proteins: carbohydrates would be a good approach for optimizing the mixing ratios of the substrates. In addition, the optimal C:N or COD:N for some types of the feedstocks have shown to be very different from the generally observed the optimal values.

As presented in Table 4.2, the ratio of COD:N was 14 for TWAS alone while it was higher by 2.4 fold for SSO. The increase of the ratio of COD to N from 14 to 16, increased ultimate methane from 542 to 723 mL. This increase of methane production could be due to the sufficient amounts of nutrient for microbial activities which led to enhanced biomethane production. On the other hand, an optimal nutrient synergy is required to ensure synergetic interactions of the microbial communities as the increase of COD:N ratio from 28 to 34, decreased the amount of ultimate CH_4.

Table 4.2. Ultimate CH_4 at different ratios of the substrates, COD:N, and lipids:proteins:carbohydrates

Digester code	TWAS: SSO (V/V)	COD:N	Feedstock ratios code	Lipids: Proteins: Carbohydrates	Ultimate CH_4 (mL)	mLCH_4/g TCOD added
TWAS Only	1:0	14	AA	1:11:3	542	192
SSO Only	0:1	34	BB	1:1.3:8	1122	308
TWAS:SSO 9:1	9:1	16	A	1:7:5	723	243
TWAS:SSO 7:3	7:3	20	B	1:4:7	1010	316
TWAS:SSO 1:1	1:1	24	C	1:3:7	1100	326
TWAS:SSO 3:7	3:7	28	D	1:2:8	1252	358
TWAS:SSO 1:9	1:9	32	E	1:1.5:8	1235	343

The main effect plot for CH_4 yield data means in response to the feedstock ratios at different lipids:proteins:carbohydrates ratios is shown in Fig 4.7. As the trend shows, both type of the feedstock and their ratios have significant effect on the methane yield. Each of the feedstock ratios correspond to a different lipids:proteins:carbohydrates ratio. As shown in Figure 4.7, the minimum methane yield corresponds to the mono digestion of TWAS which occurred at the lipids:proteins:carbohydrates ratio of 1:11:3. The maximum CH_4 yield corresponded to TWAS:SSO ratio of 3:7 corresponding to the lipids: proteins: carbohydrates ratio of 1:2:8.

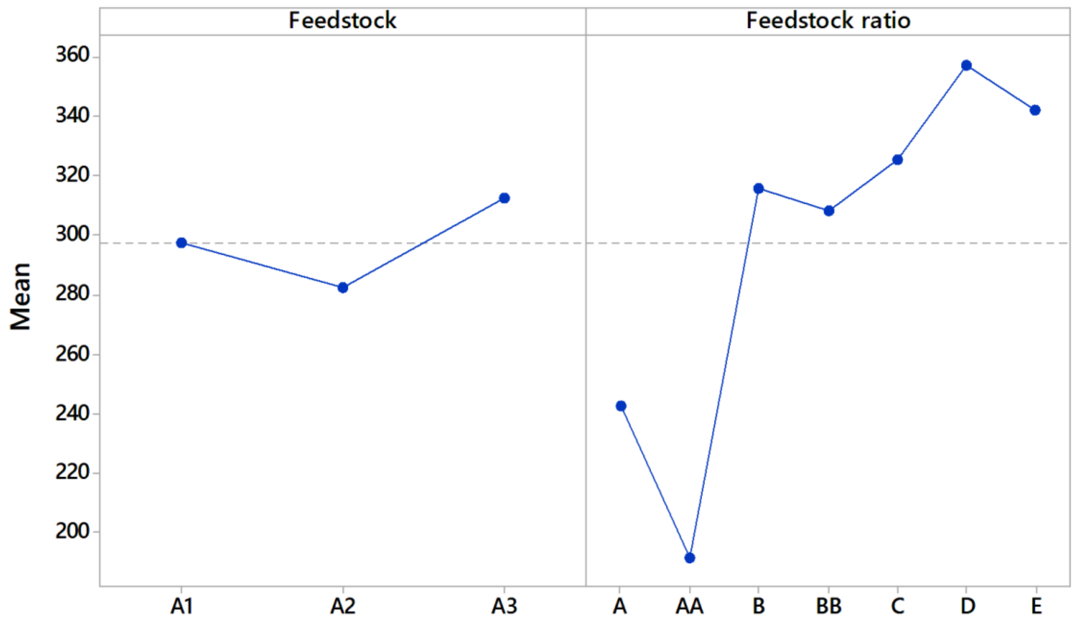
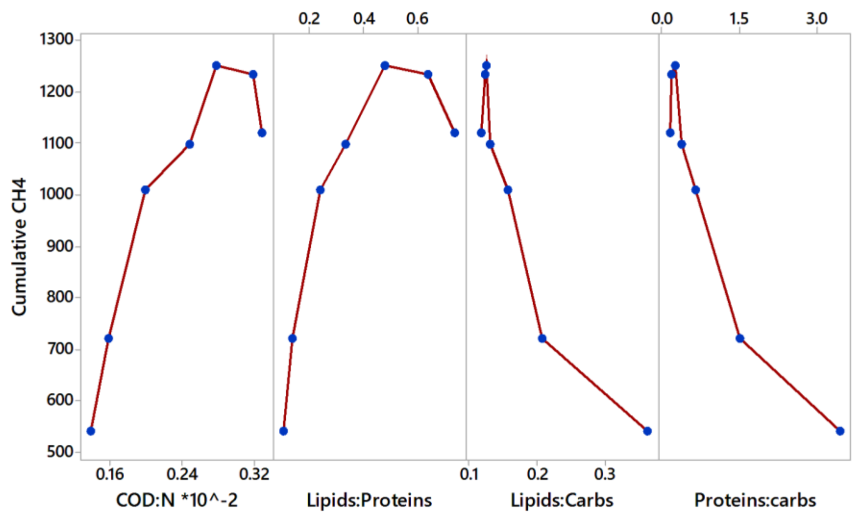
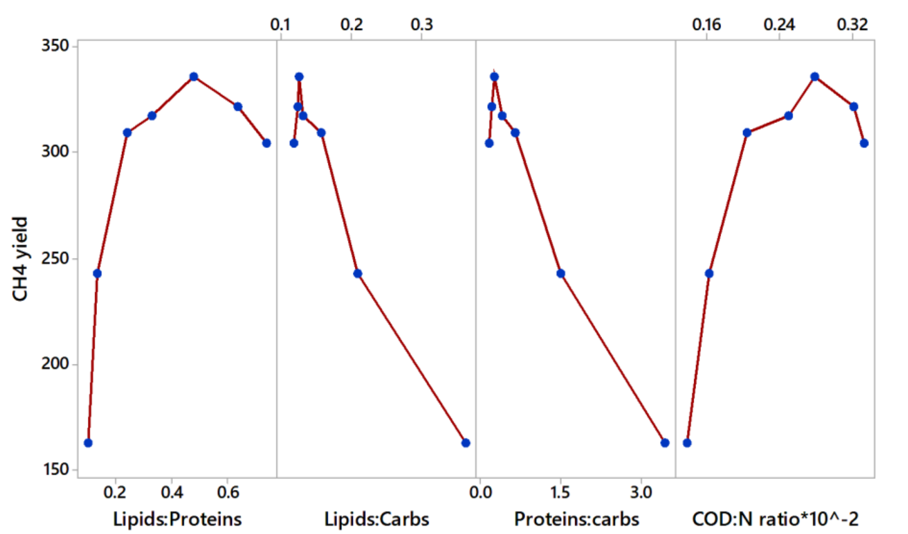


Figure 4.7. Main effects polot for CH4 yield data means in response to feedstock ratios at different lipids:proteins:carbohydrates ratios

A comparison between COD:N and the ratios of lipids: proteins, lipids: carbohydrates, and proteins:carbohydrates is presented in Figure 4.8. As illustrated in Figure 4.8 a) and 4.8 b), a same trend for the ultimate methane production in response to both COD:N ratios and lipids: proteins ratios was observed for the corresponding feedstocks mixing ratios. However, the COD:N ratio did not show the same trend as lipids: carbohydrates and proteins: carbohydrates ratios for the same feedstocks mixings, so that the minimum ultimate CH_4 (542 mL) corresponded to the minimum COD:N ratio of 14 while it corresponded to maximum lipids: carbohydrates of 0.32 as well as the maximum proteins: carbohydrates ratio of 3.48. Both lipids to carbohydrates and proteins to carbohydrates ratios showed the same trends at the corresponding feedstocks mixing ratios so that the maximum ultimate methane of 1252 mL occurred at the minimum lipids: carbohydrates of 0.12 and the minimum proteins: carbohydrates of 0.19. Similar trends were observed for the CH_4 yield (mLCH_4/g TCOD added) in response to the COD:N, lipids: proteins, lipids: carbohydrates and proteins: carbohydrates ratios.



4.8. a



4.8. b

Figure 4.8. Matrix effects plot for: a. ultimate CH_4 and b. CH_4 yield at different COD:N and Lipids: Proteins; lipids:carbohydrates and proteins:cabohydrates for the same corresponding mixing ratios of TWAS:SSO

4.5. Kinetic analysis results

The modified Gompertz model according to Eq. 3.5 was used to determine the coefficients for cumulated methane production and the results have been presented in Table 4.4. The modified Gompertz model could identify significant parameters related to anaerobic digestion including the maximum methane production rate, maximum methane production and lag phase, which underlines the time when the substrate is transformed and its correlation with the methane production phase. The volume data for the controls and for each mixing ratio collected during the experiment were used to apply the modified Gompertz model. The model corresponds to a sigmoid function expressing methane production in the reactor as a function of time (Lay et al., 1999; Parra-Orobio et al., 2016).

Applying the collected data for cumulative methane production (CH_4) per unit substrate in mL/g and the digestion time (t) in days to the Gompertz equation, the values of P, representing the maximum methane production per unit substrate (mL/g), R_m^e, the maximum methane production rate (ml/g h), and λ, the lag phase time (d) were calculated. The determination coefficient (R^2) as presented in Table 4 was used as criterion to evaluate the fitted models. The modified Gompertz model for mono- and co-digestions, respectively showed a good fit to the experimental results and the estimated parameters indicated that the co-digestion of SSO with TWAS improved the biogas production rate (Figure 1). The value of R^2= 0.9998 indicates that the proposed equations can accurately describe the variation of methane yield curves. Typically, S-shaped curves are obtained by the modified Gompertz model which shows a relatively slow upward trend related to the lag phase at the beginning of the curve. Therefore, the lag phase time for all of the TWAS/SSO co-digestion mixtures could verify the suitability of the modified Gompertz model in estimated performance of the process.

Table 4.3. Summary of results of kinetic study using modified Gompertz models.

	P (mL)	$\boldsymbol{R_m^e}$ (mL/d)	λ (d)	R^2
TWAS	510	28	0.1	0.999
SSO	1063	68	2.0	0.999
TWAS:SSO 9:1	661	44	1.0	0.999
TWAS:SSO 7:3	938	68	1.1	0.999
TWAS:SSO 1:1	1046	84	3.1	0.999
TWAS:SSO 3:7	1182	95	2.7	0.999
TWAS:SSO 1:9	1165	89	1.9	0.999

With reference to the adjustment to the Gompertz model, the lag phase varied from 0.1 to 3.1 days for different substrate mixing ratios. The shortest lag phase of 0.1 d corresponded to TWAS alone digestion. This result would be due to the existence of anaerobic inoculum that are easily assimilated by the microorganisms during this phase and cause rapid acclimatization to the substrate, which can be observed in the methane production. In theory, the inoculum activity, the amount of readily degradable constituent, and the initial pH of the feedstock affects the AD start-up time (Kafle and Chen, 2016).

The results showed that with the increase of the proportion of SSO in TWAS/SSO co-digestion, a longer lag phase time occurred. This trend stopped with further increasing the SSO proportion to the mixing ratio of 1:9. However, the mixing ratio of 3:7 resulted in slightly higher value of P which corresponds to the experimental results that showed the maximum methane production occurred at the mixing ratio of 3:7. Although, a long lag phase time is not favorable as it would increase the residence time and consequently, larger reactor volumes and higher costs during implementation and operation would be required.

The values of $P_,$ varied from 509.5 to 1182 mL corresponding to TWAS alone and TWAS:SSO mixing ratio of 3:7. The trend of changes in the P values complies with the experimental results as it showed an increasing trend in methane production by increasing the proportion of SSO. Similar to the experimental results, the trend changed from TWAS:SSO mixing ratio of 3:7 to 1:9 so that the ratio of 3:7 corresponded a higher P value for methane production than that of 1:9. The values obtained for R^e_{max} were within a range from 28 to 95 ml/d. The values of R^e_{max} and P_{max} obtained by Gompertz for the TWAS:SSO ratios of 3:7 were 95 mL/d and 1182 mL, respectively.

In co-digestion of TWAS and SSO, the COD:N ratio above 20 resulted in higher ultimate methane production, however, the difference in the ratios from 21 to 26 did not have significant effect on the amounts of the produced methane. Therefore, considering lipids: proteins: carbohydrates ratios would be a good approach for optimizing the mixing ratios of the substrates. A lipids: proteins: carbohydrates ratio of 7:1:15 corresponding to COD:N ratio of 26, resulted in an ultimate biomethane production of 1252 mL in TWAS and SSO co-digestion. It was verified that co-digestion of TWAS and SSO improved methane production in comparison with conventional single digestion of the feedstocks. It was concluded that cumulative methane production and methane yields varied at different mixing ratios of TWAS and SSO. Co- digestion of TWAS and SSO at the mixing ratio of 3:7 resulted in a biomethane yield of 353 mL CH_4/g $TCOD_{added}$ which is corresponding to an increase of methane yield by 84% and 15% compared to TWAS and SSO alone. A 23% increase in methane yield was observed as a result of synergetic effect of co-digestion at TWAS:SSO mixing ratio of 3:7 (v/v) equivalent to lipids: proteins: carbohydrates ratio of 1:2:8. This study verified the advantage of co-digestion over conventional single digestion of the feedstocks in terms of biomethane improvement and increased microbial synergy resulting higher methane production. Further studies utilizing a range of various feedstocks is required to promote the viability of lipids: proteins: carbohydrates ratio for optimizing the anaerobic digestion process and for optimizing the mixing ratios in anaerobic co-digestion of multiple feedstocks.

4. 6. Hydrolysis/acidification

This experiment was carried out to evaluate the hydrolysis/acidification stage in anaerobic co-digestion of TWAS with SSO. In hydrolysis which is known to be the rate limiting stage in the entire anaerobic digestion process, the large complex organic polymers including lipids, proteins, and carbohydrates break down to simple smaller molecules such as fatty acids, amino acids and sugars. Subsequently, acidogenic microorganisms break down the by-product of hydrolysis to further smaller molecules in acidification step (Gould, 2014). Therefore, this part of the present research was aimed to investigate the degradation of organic compounds in hydrolysis stage through the analysis of the degree of solublization, synergetic effect of co-digestion at different mixing ratios on hydrolysis and liquefaction, volatile fatty acids (VFAs) yield, and kinetics of lipids, proteins, and carbohydrates in co-digestion of TWAS and SSO.

A series of the analysis for the characterization of the feedstocks was primarily carried out in triplicates and the mean values are summarized in Table 4.4. As presented in Table 4.4, the COD concentration of SSO is higher than that of TWAS. Adding SSO to TWAS in the co-digesters, increased the COD concentrations compared to TWAS mono digestion. SSO also contains higher amounts of carbohydrates and lipids than TWAS while the proteins concentration of TWAS is more than SSO by 30%. The concentrations of total, soluble and particulate COD; total, soluble and particulate proteins; and total, soluble and particulate carbohydrates were monitored over time for calculating their hydrolysis rate coefficients.

Table 4.4. Characteristics of the feedstocks at different mixing ratios of TWAS and SSO

		TWAS Only	SSO Only	T/SSO 9:1	T/SSO 7:3	T/SSO 1:1	T/SSO 3:7	T/SSO 1:9
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)
TCOD	g/L	40	110	47	61	75	89	103
SCOD	mg/L	0.4	44.4	4.8	13.6	22.4	31.2	40.0
TSS	mg/L	31.5	53.8	33.7	38.2	42.6	47.1	51.6
VSS	mg/L	18.5	36.0	20.2	23.7	27.2	30.8	34.3
TS	mg/L	38.8	62.2	41.1	45.8	50.5	55.2	59.8
VS	mg/L	34.5	43.5	35.4	37.2	39.0	40.8	42.6
Ammonia	mg/L	0.3	1.1	0.3	0.5	0.7	0.9	1.0
pH	-	5.6	5.6	5.8	5.7	5.7	5.8	5.8
Alkalinity	mg CaCO3/L	2.0	6.8	2.4	3.4	4.4	5.3	6.3
TN	mg/L	2.9	4.0	3.0	3.2	3.4	3.6	3.9
TSN	mg/L	0.4	1.1	0.5	0.6	0.7	0.9	1.0
TotalCarbs	mg/L	0.9	13.5	2.2	4.7	7.2	9.7	12.2
TotalProteins	mg/L	2.8	2.1	2.7	2.6	2.4	2.3	2.2
TotalLipids	mg/L	0.3	1.1	0.4	0.5	0.7	0.9	1.0

The data form total and soluble COD (SCOD) concentrations monitoring with time was used to obtain the degree of COD solublization for each mixture and the results are as follows. The degree of solublization was calculated using Eq. 3.1 and the result is shown in Figure 4.8. The degree of the COD solublization varied from 14% to 30%. The maximum solublization of COD content was 30% for TWAS:SSO combination of 1:9 while the minimum value corresponded to the digester containing only TWAS. Except for TWAS:SSO mixing ratio of 9:1, other co-digesters achieved more than 25% improvement in solublization. The lower degree of solublization in TWAS mono digestion as well as TWAS:SSO mixing ratio of 9:1 with a high portion of TWAS would be due to the existence of slowly biodegradable content which slows down the hydrolysis and liquefaction. SSO contains sufficient amount of carbohydrates which decomposes more rapidly than proteins and lipids. This would be the reason for observing higher degree of solublization for SSO compared to TWAS which contains more proteins than carbohydrates.

However, by calculating the theoretical degree of solublization of each co-digester and comparing them to the ones obtained from the measured values based on the experimental data, it was revealed that co-digestion was effective for improving the solublization due to enhancing microbial synergy. As shown in Figure. 4.10, all co-digesters achieved an improvement in solublization due to synergistic effect of the microbial communities from 23 to 44% corresponding to TWAS:SSO mixing ratios of 3:7 and 7:3, respectively.

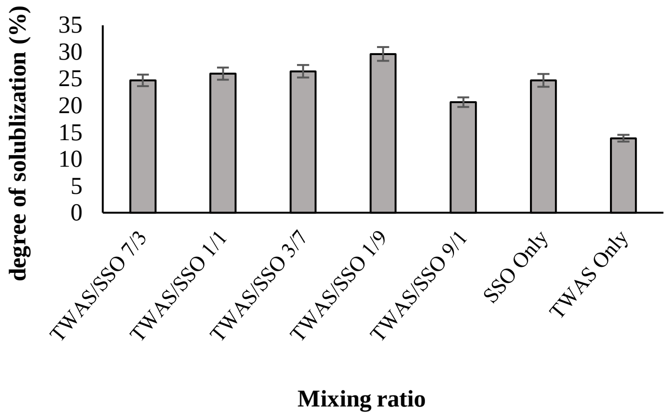


Figure 4.9. Degree of COD solublization at different mixing ratios of TWAS and SSO

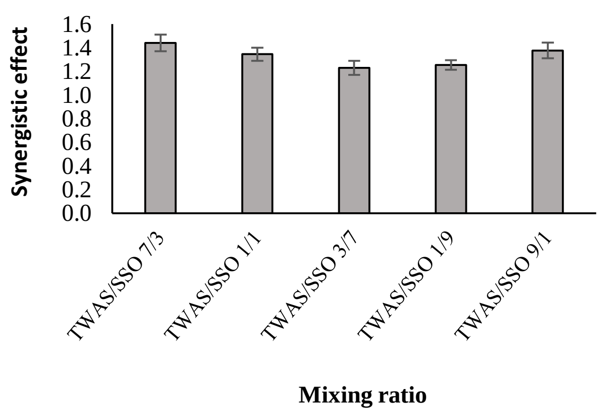


Figure 4.10. Synergistic effect on solublization at different mixing ratios of TWAS and SSO

As discussed earlier, the by-product of hydrolysis is subsequently broken down to smaller molecules by acidogenic bacterial communities. This leads to the formation of volatile fatty acids in acidogenesis. Therefore, monitoring VFAs concentration over time and calculating VFAs yield per mass of VSS added would be a good indicator of acidification progress. The VFAs concentrations monitoring showed an increasing trend during the 72-hr of the hydrolysis/acidification process. The total VFAs concentration was used for calculating the VFAs yield in terms of mass of VFAs produced in mg per mass of VSS added in g. As indicated in Figure

4.11, the VFAs yield decreased with the addition of TWAS in the digesters. This trend is similar to the trend of COD solublization as demonstrated in Figure 4.11. All of the reactors containing the mix of substrates had higher VFAs yield compared to the reactors containing only TWAS. The VFAs yields were 98 mg VFAs/g VSS added for TWAS mono digestion and 213 mg VFAs/g VSS added for TWAS:SSO ratio of 9:1, respectively. Other digesters resulted in higher amounts of VFAs production from 281 to 328 mg VFAs/g VSS added.

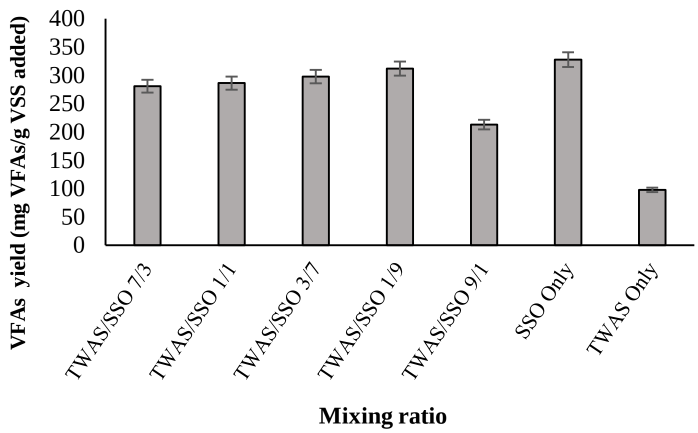


Figure 4.11. Total VFAs yield at different mixing ratios of TWAS/SSO

The analysis of soluble and particulate COD over time during the hydrolysis experimental period, showed an increasing trend in COD solublization and decreasing trend in particulate COD concentrations. Similarly, the analysis of soluble and particulate lipids, proteins, and carbohydrates over time demonstrated an increasing trend in solublization and particulate matter degradation. Although the trend is remarkably slower for lipids and proteins compared to carbohydrates. These results are summarized in the tables that are presented in the appendix. With applying the first order kinetics using equations 3.4., the hydrolysis rate coefficient (K_h) was calculated in AquaSim 2.0 for COD, lipids, proteins and carbohydrates based on the particulate matter degradation and the results are summarized in Table 4.5.

As presented in Table 4.4, the hydrolysis rate for COD content of SSO was higher than that of TWAS by 1.8 folds. The K_h values varied from 0.17 to 0.35 corresponding to TWAS alone and TWAS:SSO mixing ratio of 1:9, respectively. Hydrolysis rate coefficient increased in the reactors containing mixings of TWAS and SSO as compared to the reactors digesting only TWAS. The increase of COD hydrolysis rate could be the result of improved microbial synergy and good syntrophic interactions between the hydrolytic and acidogenic microbial communities through the process. The higher kinetic rate coefficient at the mixing ratio of 1:9 is in good agreement with the higher degree of solublization at the same mixing ratio compared to other co-digestion mixtures. Among lipids, proteins, and carbohydrates, lipids showed the lowest hydrolysis rate. In contrast, hydrolysis proceeded more rapidly for carbohydrates compared to lipids and proteins.

The higher hydrolysis rate of carbohydrates would be the result of more rapid biological metabolism of carbohydrates than lipids and proteins. K_h varied from 0.03 to 0.08 corresponding to hydrolysis of the lipid content of TWAS alone and SSO alone, respectively. All co-digesters had a higher hydrolysis rate of the lipid content in comparison with the digesters containing only TWAS.

As shown in Table 4.5, the hydrolysis rate of proteins content of the digesters was higher than that of lipids content, however it was still lower compared to the hydrolysis rate of carbohydrates content of the feedstocks. K_h for the proteins content was in a range between 0.19 to 0.34 corresponding to TWAS alone and TWAS:SSO mixings of 1:9, respectively. All of the reactors co-digesting TWAS with SSO demonstrated bigger hydrolysis rate coefficient than that of the TWAS mono digesters. The hydrolysis rate coefficient of the carbohydrates content was within a range between 0.32 to 0.68 corresponding to TWAS alone and the co-digestion of TWAS with SSO at the mixing ratio of 1:9. Contrary to expectation, the K_h values of carbohydrates did not increase with the addition of SSO portion in the mixtures.

The variation of hydrolysis rate for the carbohydrates content did not show the same trend as the lipids and proteins contents. This verifies that the hydrolysis of the lipids, proteins and carbohydrates of the feedstocks occurs independently during the hydrolysis/acidification stage. It was observed that co-digestion had an effect on the hydrolysis rate by mostly improving the hydrolysis of proteins and carbohydrates content rather than lipids.

Table 4.5. Hydrolysis rate coefficient for COD, lipids, proteins, and carbohydrate content in co-digesion of TWAS and SSO at different mixing ratios

K_h	TWAS:SSO 7:3	TWAS:SSO 5:5	TWAS:SSO 3:7	TWAS:SSO 9:1	TWAS:SSO 1:9	TWAS	SSO
Kh_{COD}	0.30	0.32	0.32	0.25	0.35	0.17	0.30
Kh_{Lipids}	0.06	0.07	0.07	0.03	0.05	0.03	0.08
$Kh_{Proteins}$	0.28	0.27	0.33	0.25	0.34	0.19	0.28
$K_{h\ Carbohydrates}$	0.56	0.57	0.54	0.46	0.68	0.32	0.55

Chapter 5

Results and discussion

TWAS and Manure Co-digestion

5. Results and discussion- TWAS and manure co-digestion

5.1. BMP of TWAS and manure

This experiment was designed to investigate the effect of mixing ratio of TWAS with manure and its correlation with the lipids: proteins: carbohydrates ratios on anaerobic co-digestion of TWAS and manure. The characteristics of the feed in each digester having different mixing ratios of the substrates are summarized in table 5.1. The COD concentration of manure slurry was significantly higher than that of TWAS. The average TCOD of manure was 122 g/L while it was 45 g/L for TWAS. Manure also contained significantly higher amount of total carbohydrates (27 g/L), proteins (5.1 g/L), and lipids (1.4 g/L) concentrations compared to TWAS. Mixtures of TWAS and manure as described in chapter 3 were prepared in different combinations and used as digester feedstocks. Addition of manure to TWAS increased the concentration of carbohydrates in co-digesters by a large extent as compared to TWAS mono digesters. It also increased lipids and proteins content of the co-digesters in comparison with the digester containing TWAS alone.

Table 5.1. Characteristics of feed to digesters with different mixing ratios

		TWAS	Manure	T*:M** 9:1	T:M 7:3	T:M 1:1	T:M 3:7	T:M 1:9
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)
TCOD	g/L	45	122	53	68	84	99	115
SCOD	g/L	2.0	8.9	2.7	4.1	5.5	6.7	8.2
TSS	g/L	36	80	40	49	58	67	75
VSS	g/L	27	77	38	42	52	62	72
TS	g/L	38	99	44	56	68	80	93
VS	g/L	28	86	33	45	57	68	80
Ammonia	g/L	0.32	0.02	0.29	0.23	0.17	0.11	0.05
pH	-	7.1	7.0	6.9	7.0	7.1	7.2	7.4
Alkalinity	g CaCO3/L	4.8	5.1	4.8	4.9	4.9	5.0	5.1
TN	g/L	2.9	2.2	2.8	2.7	2.5	2.4	2.3
TSN	g/L	0.4	0.1	0.4	0.3	0.3	0.2	0.1
T-Carbs	g/L	1.3	27.1	3.9	9.1	14.2	19.4	24.5
T-Proteins	g/L	3.9	5.1	4.1	4.3	4.5	4.8	5.0
T-Lipids	g/L	0.5	1.4	0.6	0.8	1.0	1.1	1.3

* T: TWAS
** M: Manure

The range of carbohydrates concentrations were within 3.9 to 24 g/L. Total proteins concentrations were 3.9 and 5.1 for TWAS and manure respectively and varied from 4.1 to 5 g/L in the co-digesters. The total lipids concentration of manure was 1.4 g/L and it was higher by 2.8 fold than TWAS. Adding manure to TWAS increased the lipids content. In the reactors containing the mixtures of the TWAS and manure, lipids concentrations varied from 0.6 to 1.3 g/ L.

Operation of the digesters continued until no significant biogas was produced. Fig. 5.1 shows the profile of the cumulative biomethane production versus time during the total digestion period. TWAS resulted in minimum cumulative methane production of 590 ml while manure alone produced 838 mL of cumulative methane during the operation period.

As shown in figure 5.1, the maximum cumulative methane production was obtained by co-digestion of TWAS:manure at the mixing ratio of 3:7 corresponding to the lipids: proteins: carbohydrates ratio of 1:4:17. Compared to mono digestion of manure, co-digestion at the mixing ratio of 9:1 did not increase methane production, although higher cumulative methane was obtained at that ratio in comparison with TWAS mono digestion. In fact TWAS alone with lipids: proteins: carbohydrates ratio of 1:7:2.5, produced the least amount of methane. Other co-digesters produced higher methane than that of TWAS and manure single digestion.

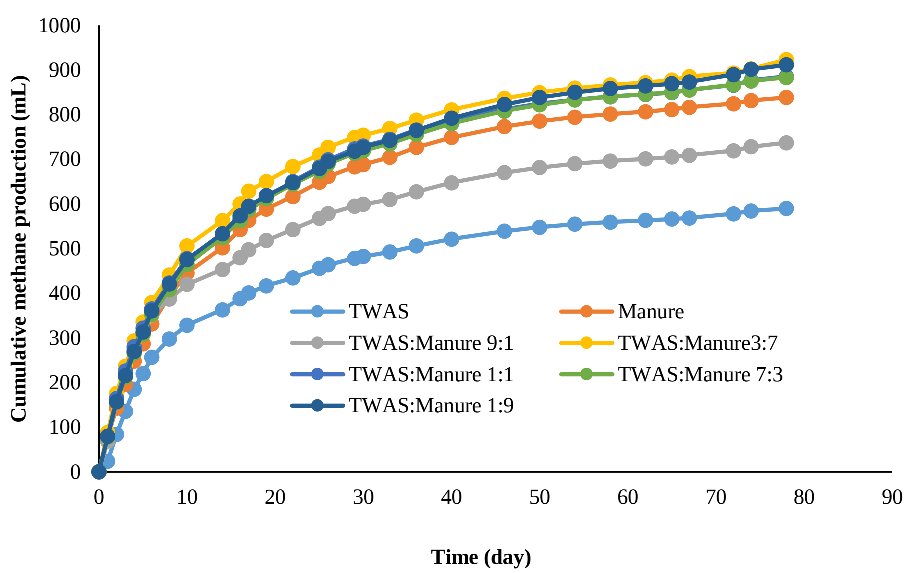


Figure 5.1. Cumulative methane production for different mixing ratios of TWAS and manure

As illustrated in Figure 5.2, for all of the digesters, the maximum methane rate occurred in the first week of the operation period. The amount of maximum methane production rate of the digesters differed from each other, although a similar trend was observed for all of the co-digestion mixtures. The reactors generated 47% to 52% of their ultimate methane production at the first week of the digestion period and 59% to 62% in two weeks operation. Compared to TWAS, manure alone resulted in higher methane rate. Addition of manure to TWAS increased partially the maximum methane production rate so that all of the co-digesters demonstrated slightly higher maximum methane rate compared to TWAS and manure mono digesters except for the reactor containing the TWAS and manure at the mixing ratios of 7:3 and 1:9 which had almost the same maximum methane rate as of manure alone. In comparison with TWAS alone, the maximum methane rate in co-digestion reactors were 30% to 47% higher.

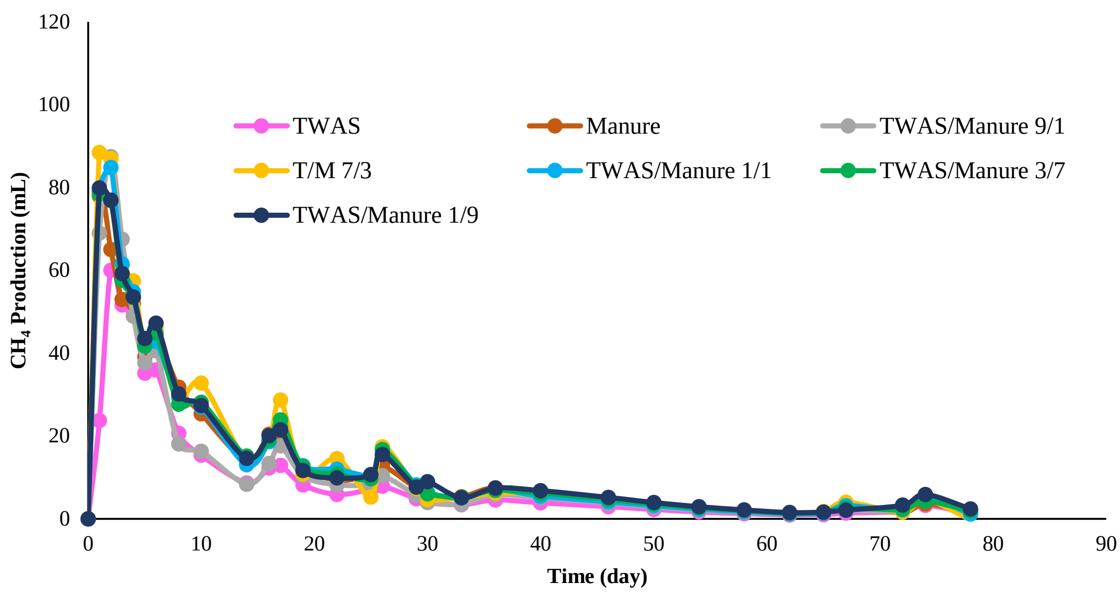


Figure 5.2. Methane production rate (mL/d) for different mixing ratios of TWAS and Manure

The maximum CH_4 production rate at different mixing ratios is presented in Figure 5.3. As shown in Figure 5.3, the maximum rate was 100 mL/day that occurred at the first day of the process for TWAS/manure mixing ratio of 1:1 corresponding to the lipids: proteins: carbohydrates ratio of 1:25:78 and the minimum rate corresponded to TWAS mono digestion at the lipids: proteins: carbohydrates ratio of 1:7:2.5.

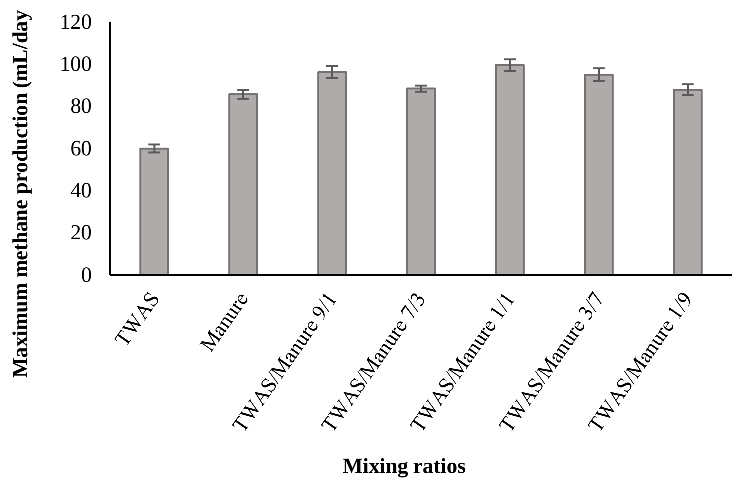


Figure 5.3. Maximum methane production rate (mL/d) for different mixing ratios of TWAS and manure

During the anaerobic process COD is only rearranged meaning that all COD that enters the system converts eventually to the end products methane minus the COD that incorporates in new microbial mass. Therefore, COD is generally taken as a useful control tool for the operation of anaerobic systems (Henze et al., 2015). A COD mass balance was conducted for all of the digesters to assess the accuracy of the experiment. The mass balance was carried out with reference to the initial and the final TCOD concentrations of the digester contents, and the theoretical methane production per unit mass of TCOD removed. Comparison between the experimental methane production attained by this research and that of determined by TCOD mass balance, as presented in figure 5.4 verified a deviation of less than 10% for all the digesters. The COD balance varied from 90 % to 98% in all mono digestion and co-digestion reactors.

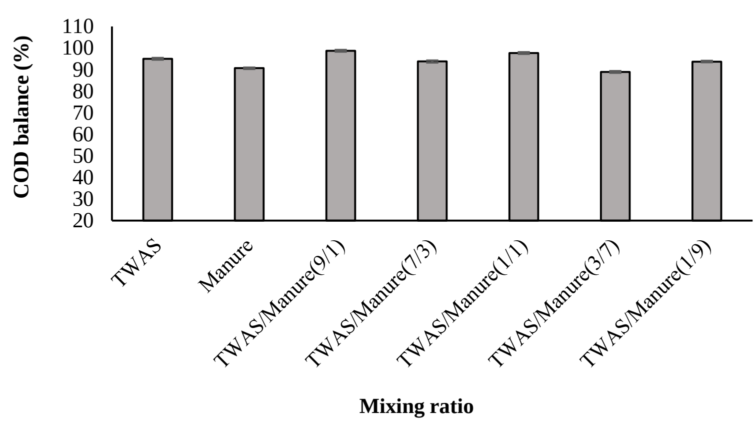


Figure 5.4. COD mass balance in co-digestion of TWAS and manure for different mixing ratios

5.2. Cumulative methane yields

Cumulative methane yields including $mLCH_4$/g TCOD added, $mLCH_4$/g VSS added, and $mLCH_4$/mL substrate added are presented in Figure 5.5. Figure 5.5 a. shows cumulative methane yield per mass COD of substrate added for mono and co-digestions. Manure produced a higher amount of biomethane compared to TWAS. The methane yield was 196 $mLCH_4$/g TCOD added and 298 $mLCH_4$/g TCOD added for TWAS and manure mono digestion, respectively. The addition of manure to TWAS, only increased the methane yield by 15% in co-digesters compared to digestion of manure alone. Even though, it increased the methane yield by 65% in comparison with mono digestion of TWAS. Results showed that 320 and 324 $mLCH_4$/g TCOD added was obtained by co-digestion at the mixing ratios of 1:9 and 3:7. Mixing TWAS with manure in co-digesters except for TWAS:manure ratio of 9:1 and 7:3 enhanced the methane yield in comparison with both TWAS and manure mono digestion. Although, the ratios of 9:1 and 3:7, increased the methane yield per unit mass of COD added by 40 % and 59% compared to TWAS alone. The maximum cumulative methane yield of 324 mL CH_4/g TCOD added at TWAS/SSO ratio of 3:7 corresponded to 1:4:17 lipids: proteins: carbohydrates ratio.

The methane yield per volume of substrate added in co-digesters were lower than that of manure alone. However, all of the co-digesters yielded higher methane per volume substrate added in comparison with TWAS mono digestion. Only 4 mL methane per unit volume of substrate added in single digestion of TWAS was obtained.

As shown in figure 5.5. b, cumulative methane yield in terms of $mLCH_4$/g VSS added significantly increased compared to TWAS alone due to addition of manure as co-substrate. Although the yield ($mLCH_4$/g VSS added) only increased by 12 % compared to manure alone. Co-digestion of manure with TWAS significantly increased the methane yield per unit mass of VSS added compared to TWAS alone. A 136 $mLCH_4$/g VSS added and 401 $mLCH_4$/g VSS added were attained by TWAS and manure alone, respectively. The amount of methane yield per unit mass of VSS added in co-digesters varied from 206 to 448 $mLCH_4$/g VSS added. The maximum CH_4 yield per unit mass of VSS added corresponded to lipids: proteins: carbohydrates ratio of 1:4:17 at TWAS:manure mixing ratio of 3:7 (v/v).

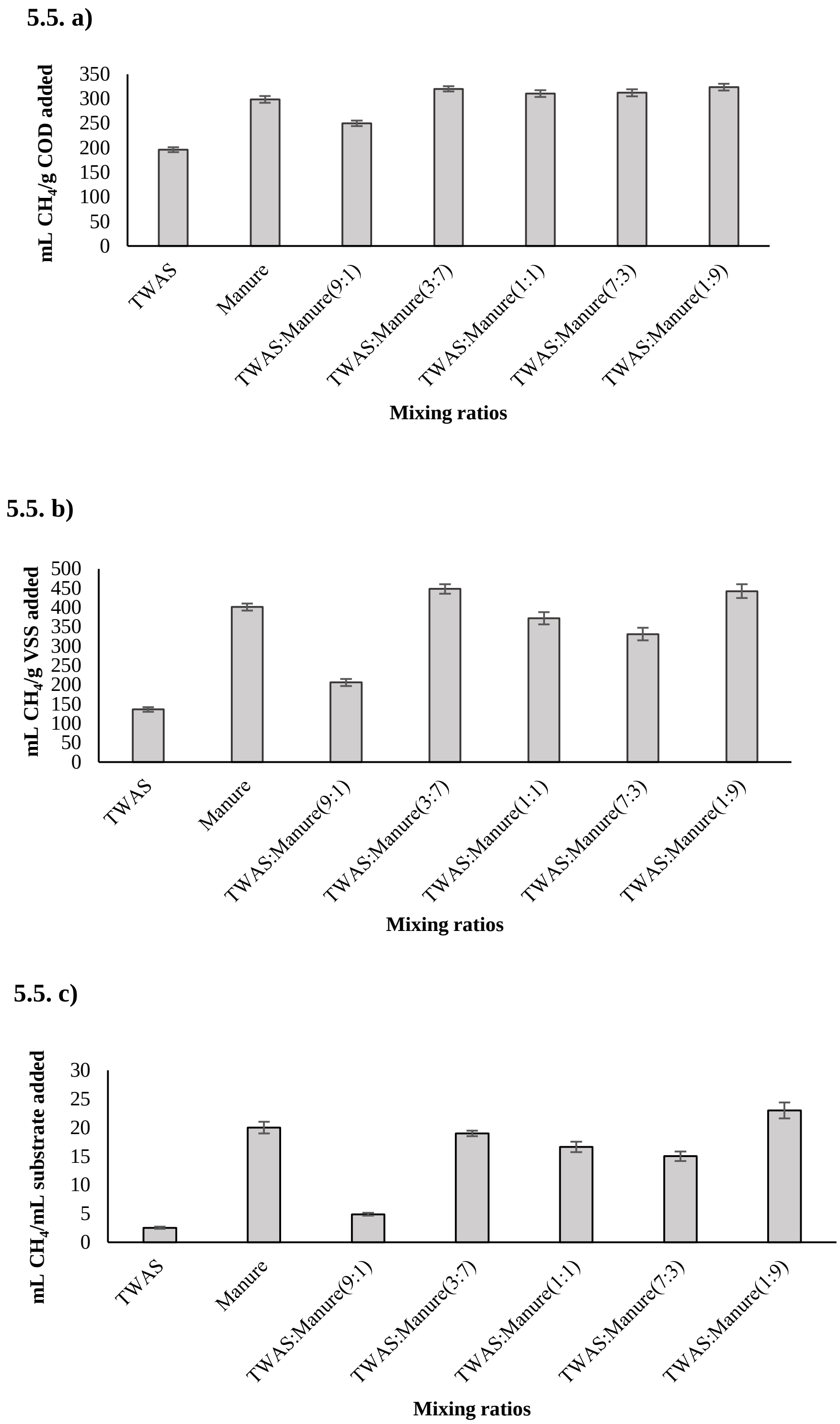


Figure 5.5. Methane yields a) per unit mass TCOD added, b) per unit mass of VSS added and c) per volume substrate added at different mixing ratio of TWAS and manure

Assessing biodegradable fraction of the feedstocks with reference to Eq. 4.1, confirmed a 53% more biodegradable fraction for manure than TWAS. This verifies the higher amount of biomethane obtained by manure compared to TWAS. Co- digestion enhanced biodegradability by 8% and 65% in comparison with the control reactors digesting only manure and TWAS, respectively.

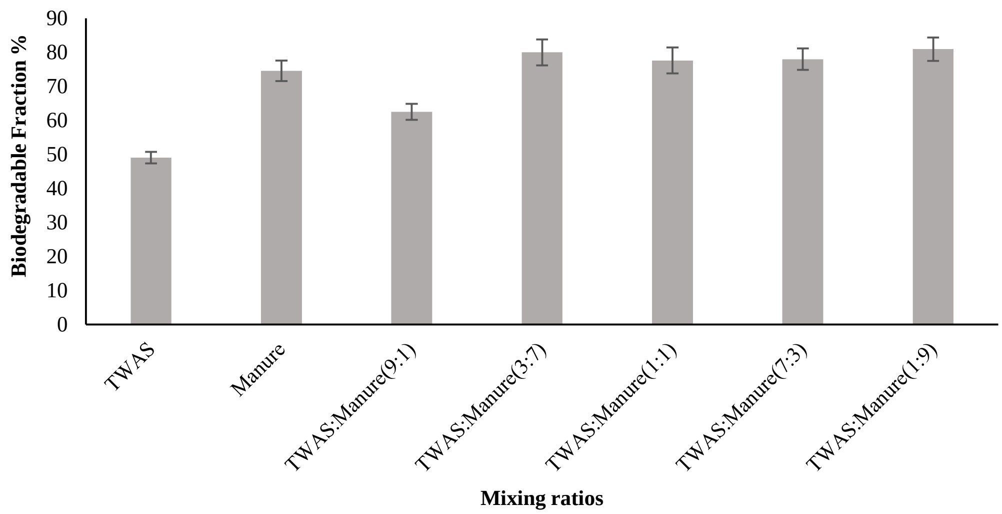


Figure 5.6. Biodegradable fraction of the feedstocks for different mixture ratios of TWAS and manure

As shown in Figure 5.6, biodegradable fraction was 49% for TWAS while it was 75 % for manure. The mixing ratios of 3:7 and 1:9 had the highest biodegradable fraction of almost 80% in TWAS/manure co-digestion. It was verified that addition of manure as co-substrate increased biodegradability and enhanced the production of methane in the rectors co-digesting manure with TWAS. All co-digesters had higher biodegradable fraction than TWAS alone. The reason would be the existence of readily biodegradable compounds in manure that was introduced to the co-digesters. At the mixing ratio of 3:7, the biodegradable fraction was almost 10 % and 0.53 % higher than that of manure and TWAS alone. The trend of biodegradable fraction changes in digesters complies with the methane yields obtained by the experimental results for the corresponding digesters.

5.3. Synergistic effect

Synergistic effect was evaluated with reference to Eq. 4.2 as described in chapter 4. Figure 5.7 shows the percentage of additional methane yield for co-substrates that was measured by the experiment, over the weighted average of the methane production of individual substrates per unit volume of substrate added. As shown in Fig 5.7, the synergistic effect varied from 10 to 24 % in co-digesters. The maximum synergetic impact was observed in co-digestion of TWAS/manure at the mixing ratio of 3:7. This is in compliance with the maximum methane yield that was obtained at the same mixing ratio of TWAS and manure in the reactors co-digesting them. In co-digestion of TWAS and manure, 24% improvement was achieved due to synergistic effect at the mixing ratios of 3:7.

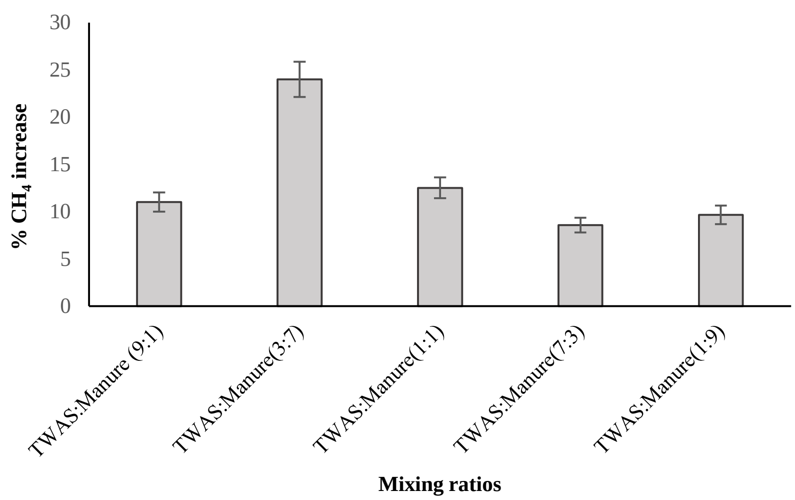


Figure 5.7. Synergetic effect of co-digestion at different mixing ratios of TWAS and SSO

Although all of the mixings demonstrated the synergetic impact of co-digestion on improving biomethane production, no specific trend for the change of the synergetic effect and methane increase corresponding to the fraction of manure in the co-digestion mixtures was observed. Similar to co-digestion of TWAS/SSO, adding fractions of manure as co-substrate enhanced the amounts of readily biodegradable materials to the co-digesters. The ratios with more fraction of carbohydrates produced more methane as the biodegradation of carbohydrate occurs more rapidly than lipids and proteins content. However, increasing the fractions of manure did not essentially resulted in the process improvement.

The improvement of the synergy and biogas yield in TWAS/manure co-digestion could be due to the abundance of methanogenic populations and diversity of archaeal communities present in manure. Nevertheless, improved synergy would also depend on the optimal nutrients balance and effective conditions for microbial growth. This could explain the optimum fraction of TWAS and manure at the mixing ratio of 3:7 in the co-digestion rather than other ratios.

5.4. COD:N and Lipids: Proteins: Carbohydrates ratios

Table 5.2 presents the ultimate methane production and the methane yield per unit mass of COD added, the COD:N and lipids: proteins: carbohydrates ratios of the digesters. Table 5.2 provides a comparison between the COD:N ratios and the lipids: proteins: carbohydrates ratios of the digesters fed with different mixings of TWAS and manure. In co-digestion of TWAS and manure, the COD:N ratios between 33 and 56 resulted in higher ultimate methane production, however, the ratios below 20 resulted in lower methane yields. The ultimate methane production and methane yield ranged between 590 to 1069 mL and 196 to 324 mL/g TCOD added for different mixing ratios, respectively.

As presented in Table 5.2, the ratios of COD:N and lipids: proteins: carbohydrates were 16 and 1:7:2.5 for TWAS alone while they were 56 and 1:4:20 for manure, respectively. The methane yield obtained by manure was 52% more than that of TWAS. The lowest ultimate methane production and methane yield of 590 mL and 196 mL/g TCOD added, corresponded to the TWAS/manure mixing ratio of 1:0 in the control reactors digesting only TWAS. The maximum ultimate CH_4 and CH_4 yield corresponded to the COD:N ratio of 41 and the lipids: proteins: carbohydrates ratio of 1:4:17 for the reactors co-digesting TWAS and manure at the mixing ratio of 3:7 (v/v).

Figure 5.8. shows the main effect plot for CH_4 yield data means in response to feedstock and lipids:proteins:carbohydrates ratios at different feedstock mixing ratios in co-digestion of TWAS/manure. As illustrated in Figure 5.8, both feedstock and lipids:proteins:carbohydrates ratios has significant effect on the methane yield. The minimum methane yield corresponded to TWAS alone with lipids:proteins:carbohydrate ratio of 1:7:2.5 while the maximum yield occurred at the mixing ratio of 3:7 and lipids:proteins:carbohydrate ratio of 1:4:17 in co-digestion of TWAS with SSO. With reference to the results of the ANOVA test, the both COD:N and Lipids: Proteins:

Carbohydrates ratios had statistically significant effects on the ultimate methane production ($P < 0.05$).

Table 5.2. Ultimate CH_4 and yield at different ratios of the substrates, COD:N, and lipids:proteins:carbohydrates

Digester code	TWAS: Manure (V/V)	COD:N	Feedstock ratios code	Lipids: Proteins: Carbohydrates	Ultimate CH_4 (mL)	$mLCH_4$/g TCOD added
TWAS Only	1:0	16	AA	1:7:2.5	590	196
Manure Only	0:1	56	CC	1:4:20	922	298
TWAS/Manure 9/1	9:1	19	A	1:7:6	811	250
TWAS/Manure 7/3	7:3	26	B	1:5.5:12	902	320
TWAS/Manure 1/1	1:1	33	C	1:25:78	1015	310
TWAS/Manure 3/7	3:7	41	D	1:4:17	1069	324
TWAS/Manure 1/9	1:9	51	E	1:4:19	1002	312

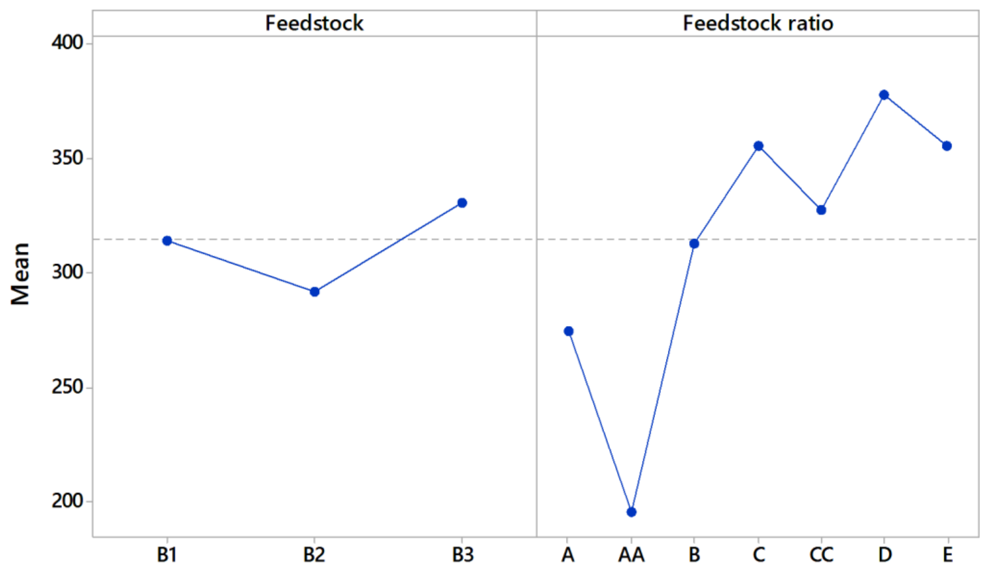


Figure 5.8. Main effect plot for CH_4 yield data means in response to feedstock and lipids:proteins:carbohydrates ratios at different feedstock mixing ratios in AnCoD of TWAS/manure

Figures 5.9. a and 5.9. b show the variation of ultimate methane and the methane yield versus COD:N ratio, lipids:proteins ratio, and proteins:carbohydrates ratios. As illustrated in Figure 5.9, different trends for the variations of methane yield and the ultimate methane versus COD:N, lipids: proteins, lipids: carbohydrates, and proteins: carbohydrates ratios were observed. For the corresponding feedstocks mixing ratios, a similar trend for the variations of methane versus COD:N and lipids: proteins ratios was observed. However, the COD:N ratio did not show the same trend as lipids: carbohydrates and proteins: carbohydrates ratios for the same feedstocks mixings.

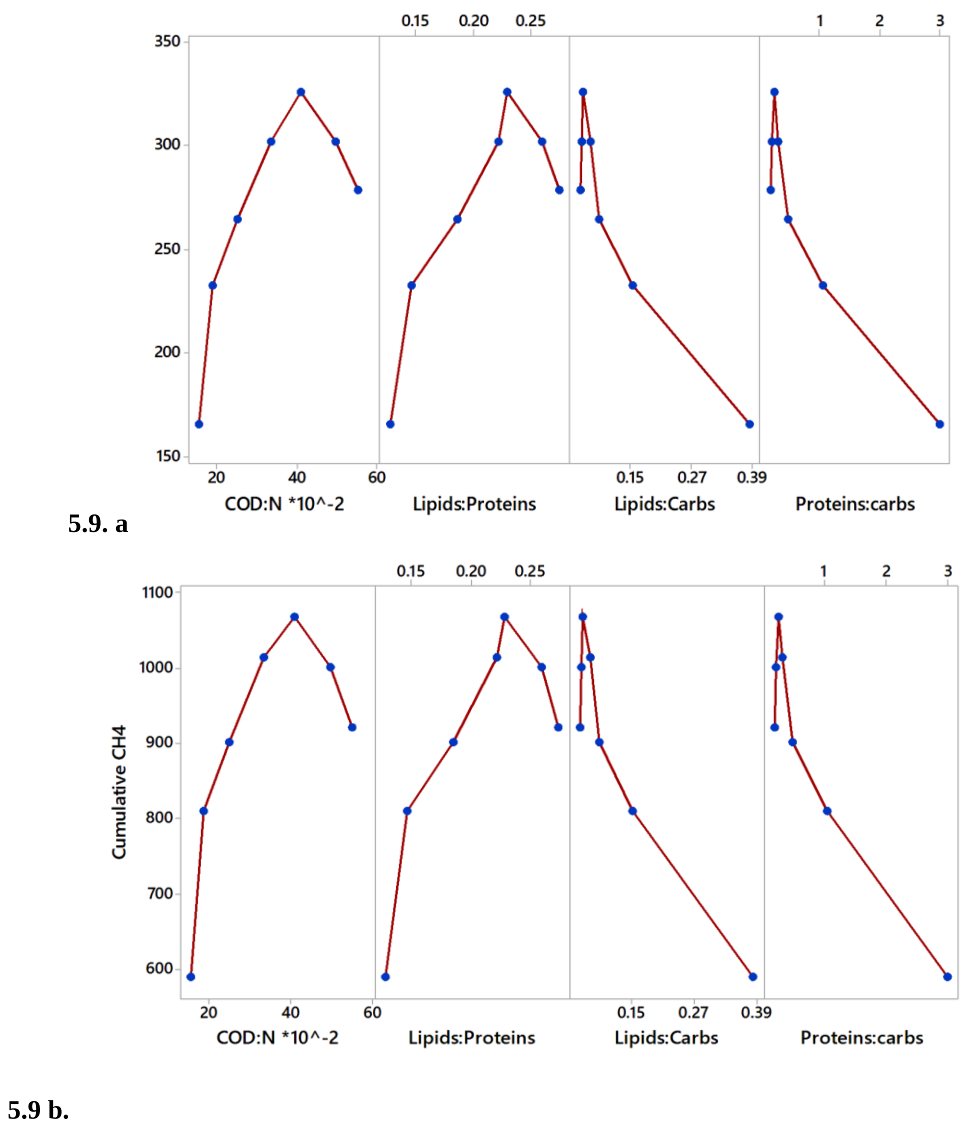


Figure 5.10. Matrix plot for: a. ultimate CH_4 and b. CH_4 yield at different COD/N and lipids: proteins, lipids: carbohydrates, and proteins: carbohydrates ratios

5.5. Kinetic analysis results

Table 5.3 presents the results of analyzing biomethane production according to the modified Gompertz model using Eq. 3.5. The data collected by the experiment from the control reactors and from each co-digester was applied to the model. The experimental data for cumulative methane production (CH_4) per unit substrate in mL/g and the digestion time (t) in days were applied to the Gompertz equation to calculate the values of P, representing the maximum methane production per unit substrate (mL/g), R_m^e, the maximum methane production rate (mL/g h), and λ, the lag phase time (d). The modified Gompertz model for mono- and co-digestions of TWAS and manure, showed a good fit to the experimental results with less than 5% diversion from the experimental values. The estimated parameters indicated that the co-digestion of manure with TWAS enhanced the biogas production rate. Such performance (R^2= 0.9998) shows that the proposed equations can accurately describe the variation of methane yield curves.

Table 5.3. Summary of results of kinetic study using modified Gompertz model

	P (mL)	R_m^e **(mL/d)**	**λ (d)**	R^2
TWAS	554	21	0.09	0.999
Manure	880	31	0.38	0.999
TWAS/Manure 9/1	763	27	0.06	0.999
TWAS/Manure 7/3	861	32	0.04	0.999
TWAS/Manure 5/5	969	36	0.04	0.999
TWAS/Manure 3/7	1016	35	0.04	0.999
TWAS/Manure 1/9	947	31	0.05	0.999

With reference to the adjustment to the nonlinear regression Gompertz model, the lag phase varied from 0.04 to 0.06 days for the co-digesters with different substrate mixing ratios. By increasing the proportion of manure higher values of P was estimated. The mixing ratio of 7:3 and 1:9 corresponded to the higher P values. These findings were in good agreement with the data collected from the experiment. As verified by the experiment, a longer lag phase was observed in co-digestion of TWAS:manure compared to co-digestion of TWAS:SSO. As mentioned before, a long lag phase time is unfavorable as it demands for a higher residence time and consequently, larger reactor volumes which increases the operational costs of the anaerobic system. The values of $P_,$ varied from 554 to 1016 mL corresponding to TWAS alone and TWAS:manure mixing ratio of 3:7. The trend of changes in the P values complies with the experimental data as it showed an increasing trend in methane production by increasing the proportion of manure. The values of R^{e}_{max} were within a range from 21.3 to 36.2 mL/d. The values of R^{e}_{max} and P_{max} obtained by Gompertz for the TWAS/manure ratios of 3:7, were 35.1 mL/d and 1016 mL, respectively.

5. 6. Hydrolysis/acidification

This experiment was conducted to investigate the hydrolysis/acidification phase in anaerobic co-digestion of TWAS and manure. The degradation of organic compounds in hydrolysis stage was evaluated using a series of analysis such as degree of solublization, synergetic effect of co-digestion at different mixing ratios on hydrolysis and liquefaction, volatile fatty acids (VFAs) yield, and hydrolysis kinetics of lipids, proteins, and carbohydrates .

Characterization of the feedstocks was initially carried out in triplicates and the mean values are summarized in table 5.4. As presented in the table, the amount of COD concentration is remarkably higher in manure than TWAS. Adding manure to TWAS in the co-digesters, increased the COD concentrations compared to the reactor digesting only TWAS. Manure also contains higher amounts of carbohydrates, lipids, and proteins than TWAS. The concentrations of total, soluble and particulate COD; total, soluble and particulate proteins; and total, soluble and particulate carbohydrates were monitored over time to obtain their hydrolysis rate coefficients.

The data form total and soluble COD (SCOD) concentrations monitoring over time during a 72- h experimental period was used to obtain the degree of COD solublization for each mixture. The degree of solublization was calculated using Eq. 3.1 and the result is summarized in Figure 5.10. The degree of the COD solublization varied from 21% to 34%. The maximum solublization 34% occurred at TWAS/manure combination of 3:7 while the minimum value corresponded to the digesters containing only TWAS. Except for TWAS/manure mixing ratio of 9:1, other co-digesters demonstrated an increase of solublization compared to both TWAS and manure alone. A 31% and 62% improvement was achieved by TWAS/Manure co-digestion at the mixing ratio of 3:7 compared to manure and TWAS single digestion, respectively. Manure contains sufficient amount of rapidly biodegradable materials than TWAS. The lower degree of solublization in TWAS mono digestion as well as TWAS:manure mixing ratio of 9:1 with a high portion of TWAS could be due to the existence of slowly biodegradable materials which slows down the hydrolysis and liquefaction process and decreases the degree of solublization.

Table 5.4. Characteristics of the feedstocks at different mixing ratios of TWAS and manure

		TWAS	Manure	T*:M** 9:1	T:M 7:3	T:M 1:1	T:M 3:7	T:M 1:9
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)
TCOD	g/L	41	110	48	62	76	90	104
SCOD	g/L	2.2	6.6	2.6	3.5	4.4	5.2	6.1
TSS	g/L	37.3	52.6	38.8	41.9	44.9	48.0	51.1
VSS	g/L	26.5	42.8	28.1	31.4	34.7	37.9	41.2
TS	g/L	39.2	68.0	42.1	47.9	53.6	59.4	65.2
VS	g/L	28.6	58.5	31.6	37.5	43.5	49.5	55.5
Ammonia	g/L	0.3	0.0	0.3	0.2	0.2	0.1	0.0
pH	-	5.8	5.8	5.6	5.7	5.8	5.8	5.8
Alkalinity	g $CaCO_3$/L	5.0	7.7	5.2	5.8	6.3	6.9	7.4
TN	g/L	3.0	1.4	2.8	2.5	2.2	1.9	1.6
TSN	g/L	0.4	0.1	0.4	0.3	0.2	0.2	0.1
T-Carbs	g/L	1.3	27.1	3.9	9.1	14.2	19.4	24.5
T-Proteins	g/L	4.0	5.1	4.1	4.3	4.6	4.8	5.0
T-Lipids	g/L	0.5	1.4	0.6	0.8	0.9	1.1	1.3

* T: TWAS
** M: Manure

Manure contains a large portion of carbohydrates which decomposes more rapidly than proteins and lipids. This would lead to a higher degree of solublization of manure than TWAS which contains more proteins than carbohydrates.

Comparing the theoretical degree of solublization of each co-digester to the ones obtained from the experimental data, showed that co-digestion improved solublization due to enhancing microbial synergy. As shown in Figure. 5.11, all co-digesters demonstrated an improvement in solublization due to synergistic effect of the microbial communities from 11 to 38% corresponding to TWAS:manure mixing ratios of 1:1 and 3:7, respectively.

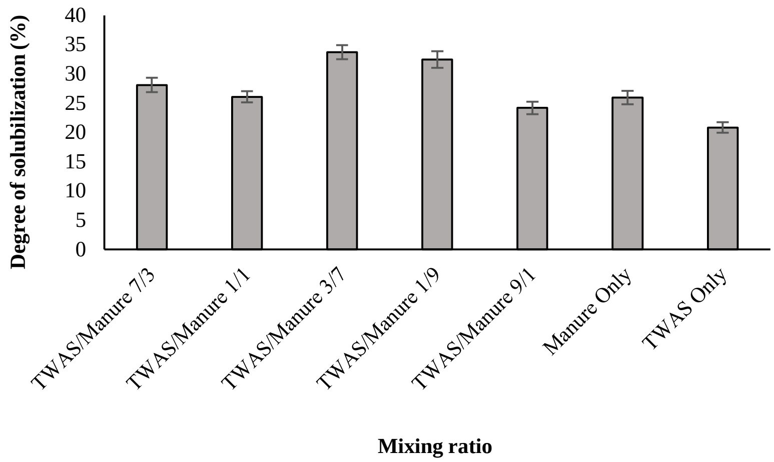


Figure 5.10. Degree of COD solublization at different mixing ratios of TWAS:Manure

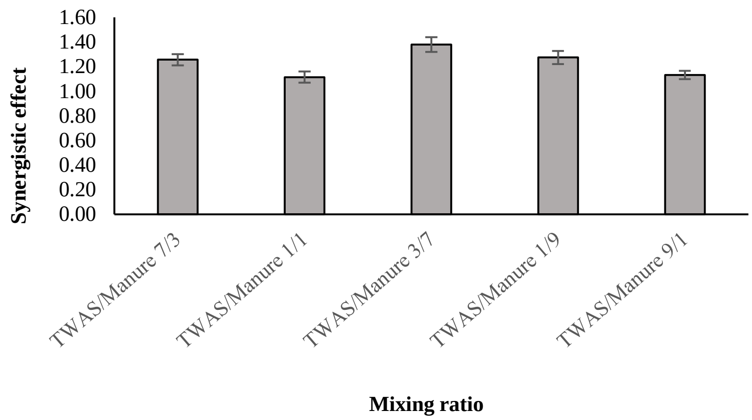


Figure 5.11. Synergistic effect on solublization at different mixing ratios of TWAS:Manure

The VFAs concentrations monitoring showed an increasing trend over the 72-hr of the hydrolysis/acidification experimental period. The total VFAs yield is presented in terms of mass of VFAs produced in mg per mass of VSS added in g. As indicated in Figure 5.12, manure alone had a significantly higher VFAs yields than TWAS alone. The VFAs yield in the co-digester was correlated to the mixing ratios of the feedstocks. The trend of VFAs yield did not conform to the trend of COD solublization of the corresponding mixing ratios (Figure 5.10). Therefore, hydrolysis and liquefaction showed a different trend from acidification. All of the reactors containing the mix of substrates had higher VFAs yield compared to the reactors containing only TWAS. The VFAs yields were 95 mg VFAs/g VSS added and 260 mg VFAs/g VSS added for TWAS and manure mono digestions, respectively. A VFAs yield of 307 mg VFAs/g VSS added was achieved for TWAS:manure ratio of 3:7.

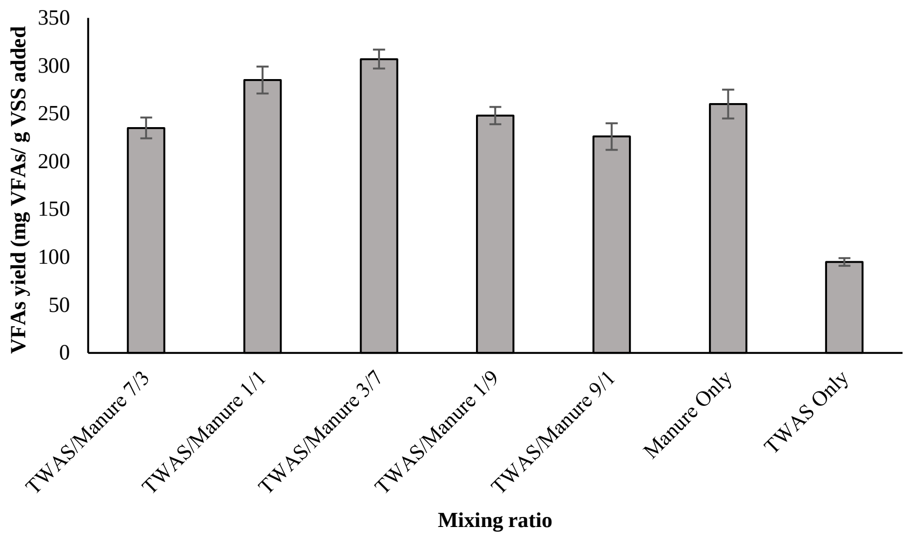


Figure 5.12. Total VFAs yield at different mixing ratios of TWAS/Manure

Monitoring COD over time, showed an increasing trend in solublization of COD and decreasing particulate COD concentrations. In addition, the analysis of soluble and particulate lipids, proteins, and carbohydrates over time showed an increasing trend in solublization and particulate matter degradation. For lipids and proteins, the hydrolysis rate was slower than carbohydrate. These results are summarized in the tables in the appendix. The hydrolysis rate coefficient (K_h) was calculated by applying first order kinetics using AquaSim 2.0 for COD, lipids, proteins and carbohydrates based on the particulate degradation and results are summarized in Table 5.5.

As presented in Table 5.5, the hydrolysis rate for COD content of TWAS was higher than that of manure by 35%. The K_h values in the co-digesters varied from 0.21 to 0.33 corresponding to TWAS and manure mixing ratios of 9:1 and 3:7, respectively. The maximum hydrolysis rate coefficient corresponded to the reactors containing mixings of TWAS and manure at the ratio of 3:7.

Lipids showed the lowest hydrolysis rate compared to proteins, and carbohydrates. On the contrary, the most rapid hydrolysis rate was observed for carbohydrates as a result of more rapid biological metabolism of carbohydrates than lipids and proteins. K_h varied from 0.4 to 0.09 in the reactors co-digesting TWAS with manure. The maximum hydrolysis rate coefficient of the lipids contents also corresponded to TWAS:manure mixing of 7:3. TWAS alone has the minimum K_h for the lipids. TWAS alone and manure alone had K_h values of 0.03 and 0.07, respectively. As presented in Table 5.5, the hydrolysis rate of proteins was slightly higher than that of lipids content of the digesters, although it was still lower than the hydrolysis rate of carbohydrates. K_h for the proteins content of the feedstocks was within a range between 0.22 to 0.27 corresponding to TWAS:manure mixings of 1:9 and 3:7, respectively. The hydrolysis rate coefficient of the carbohydrates varied from 0.38 to 0.59 corresponding to the digestion of TWAS:manure with the mixing ratios of 9:1 and 3:7, respectively. The carbohydrates content of the manure showed more rapid biodegradability than TWAS. The hydrolysis rate variation of lipids did not show the same trend as the proteins and carbohydrates of the feedstocks. This revealed that the hydrolysis of the lipids, proteins and carbohydrates of the feedstocks developed independently during the hydrolysis/acidification stage. This independent hydrolysis of the lipids, proteins, and carbohydrates was observed in co-digestion of TWAS and SSO as well.

Table 5.5. Hydrolysis rate coefficients for COD, lipids, proteins, and carbohydrate content in co-digestion of TWAS and manure at different mixing ratios

K_h	T/M 7/3	T/M 5/5	T/M 3/7	T/M 1/9	T/M 9/1	TWAS	Manure
Kh_{COD}	0.26	0.24	0.33	0.28	0.21	0.17	0.23
Kh_{Lipids}	0.04	0.05	0.09	0.07	0.04	0.03	0.07
$Kh_{Proteins}$	0.23	0.22	0.27	0.25	0.22	0.19	0.21
$K_{h\ Carbohydrates}$	0.49	0.44	0.59	0.48	0.38	0.32	0.43

[*] T: TWAS
[**] M: Manure

Chapter 6

Results and discussion

Manure and SSO Co-digestion

6. Results and discussion- manure and SSO co-digestion

6.1. BMP of manure and SSO

In this experiment co-digestion of manure and SSO was investigated. Manure slurry was prepared as discussed in chapter 3 and was fed to the reactors in different combinations with SSO. The influence of the feedstocks mixing ratios and their correlation with the lipids: proteins: carbohydrates ratios on biomethane production in anaerobic co-digestion of manure and SSO was evaluated. The characteristics of the feed in each digester containing different mixing ratios of the substrates are summarized in table 6.1. The values are the average of each parameter that was measured in triplicate. As presented in Table 6.1, both manure and SSO have high amount of COD concentrations and as a result, the amount of COD in the digesters are high and exceed 100 g/L. Both VSS and COD values did not vary significantly (less than 8%) in the digesters. The amount of carbohydrates and proteins of manure is significantly higher than that of SSO. Therefore, addition of manure increased the carbohydrates and proteins content of the co-digesters compared to the rectors digesting only SSO.

Table 6.1. Characteristics of feed to digesters with different mixing ratios of manure and SSO

		Manure Only	SSO Only	M/SSO 9/1	M/SSO 7/3	M/SSO 5/5	M/SSO 3/7	M/SSO 1/9
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)
TCOD	g/L	105	115	106	108	110	112	114
SCOD	g/L	44	43	44	44	44	43	43
TSS	g/L	54	62	55	57	58	60	61
VSS	g/L	46.4	45.6	46.3	46.2	46.0	45.8	45.7
TS	g/L	70	68	70	69	69	69	68
VS	g/L	59	49	58	56	54	52	50
Ammonia	g/L	0.02	1.07	0.13	0.3	0.5	0.8	1.0
pH	-	6.6	5.8	6.9	7.0	7.2	7.1	7.0
Alkalinity	g CaCO3/L	4.9	6.2	5.0	5.3	5.6	5.8	6.1
TN	g/L	2.5	3.5	2.6	2.8	3.0	3.2	3.4
TSN	g/L	0.1	1.0	0.2	0.4	0.6	0.8	1.0
T-Carbs	g/L	29	13	27	24	21	18	15
T-Proteins	g/L	5.9	2.1	5.5	4.8	4.0	3.2	2.5
T-Lipids	g/L	1.4	1.1	1.4	1.3	1.3	1.2	1.1

* M: Manure

Carbohydrates concentrations were within a range between 13 to 29 g/L in the rectors. Total proteins concentrations of manure and SSO were 2.1 and 5.9, respectively and varied from 2.5 to 5.5 g/L in the co-digesters. The total lipids concentrations varied from 1.1 to 1.4 g/L in the digesters including the controls. Adding manure slightly increased the lipids content in the reactors containing the combination of manure and SSO.

Operation of the digesters proceeded until no significant amount of biogas was generated. Fig. 6.1 shows the profile of the cumulative biomethane production versus time during the digestion period of manure with SSO including the controls. The cumulative methane production generated by SSO was higher than that of manure in the control reactors. All co-digesters produced more biomethane than the control reactors containing only manure and only SSO.

SSO alone produced 15% more methane than manure alone. The amount of ultimate CH_4 obtained by single digestion of SSO and manure was 1063 and 919 mL, corresponding to the lipids: proteins: carbohydrates ratios of 1:2:12 and 1:4.2:21, respectively. As shown in figure 6.1, the maximum cumulative methane production of 1186 mL corresponded to the manure:SSO mixing ratio of 7:3 and lipids: proteins: carbohydrates ratio of 1:3.5:18.5.

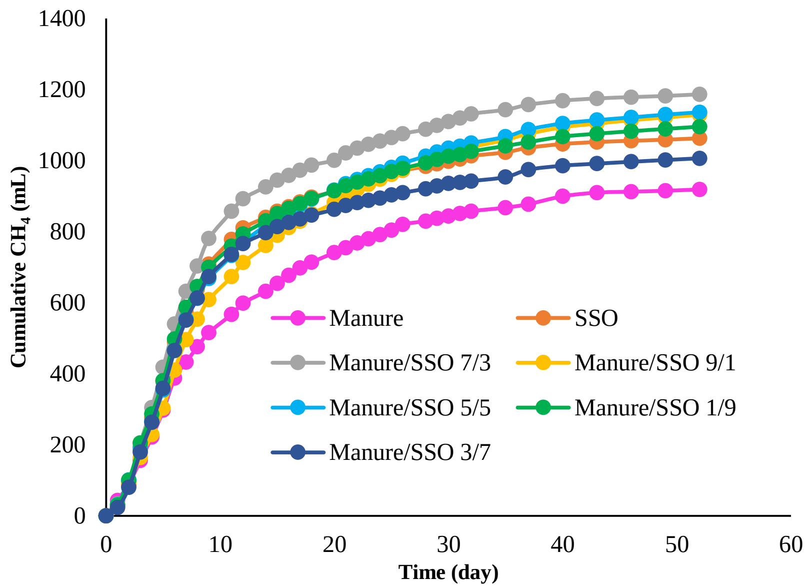


Figure 6.1. Cumulative methane production for different mixing ratios of manure and SSO

Figure 6.2, shows the methane rate in mL/day for all of the feedstocks combinations including the control reactors. For all of the digesters, the maximum methane rate occurred in the first week of the operation period. The maximum methane production rate of the digesters containing the mixings of manure and SSO was higher compared to the control reactors. The reactors generated 44% to 55% of their ultimate methane production at the first week of the digestion period and 69% to 79% of it in two weeks of operation. Compared to SSO alone, a higher CH_4 rate was observed for single digestion of manure. Addition of manure to SSO increased the maximum methane production rate so that all of the co-digester demonstrated a higher maximum methane rate compared to the control rectors. The manure/SSO mixing ratio of 7:3 achieved the highest maximum biomethane rate in comparison with other combinations and the lowest value for the

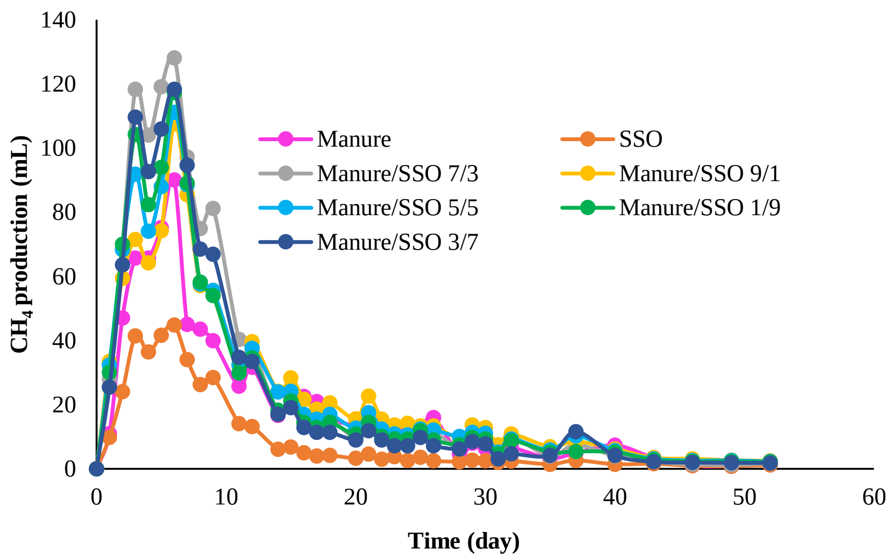


Figure 6.2. Methane production rate (mL/d) for different mixing ratios of Manure and SSO

maximum biomethane rate corresponded to single digestion of SSO. The maximum CH_4 production rate at different mixing ratios is shown in Figure 6.3. As presented in the figure, the most maximum rate was 128 mL/day corresponding to the mixing ratio of 7:3. The lowest value of 45 mL/day corresponded to single digestion of SSO. Manure alone had the maximum CH_4 rate of 90 mL/day which it was higher than that of SSO by 2 fold. The maximum CH_4 rate varied from 108 to 128 mL/day for the digesters containing combinations of manure and SSO.

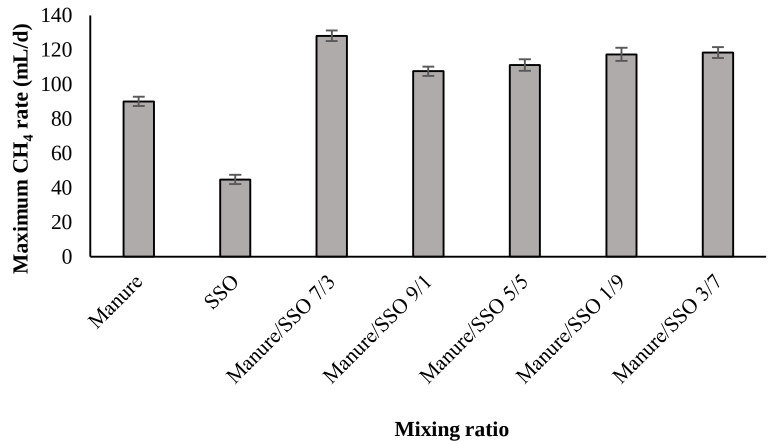


Figure 6.3. Maximum methane production rate (mL/d) for different mixing ratios manure of and SSO

The COD mass balance was conducted for all of the digesters with reference to the initial and the final TCOD concentrations of the digester contents, and the theoretical methane production per unit mass of TCOD removed. Comparison between the experimental methane production data and that of obtained by TCOD mass balance, showed a deviation of less than 8% for all the digesters. As shown in Figure 6.4, the COD balance varied from almost 90% to 97% in all mono and co-digesters.

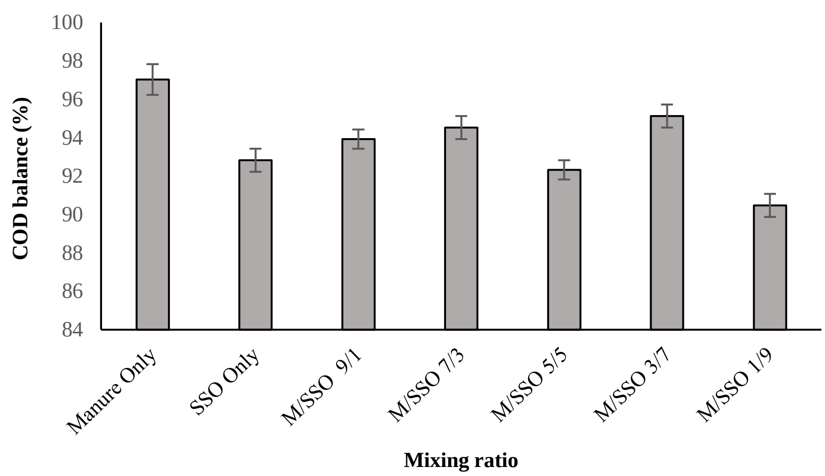
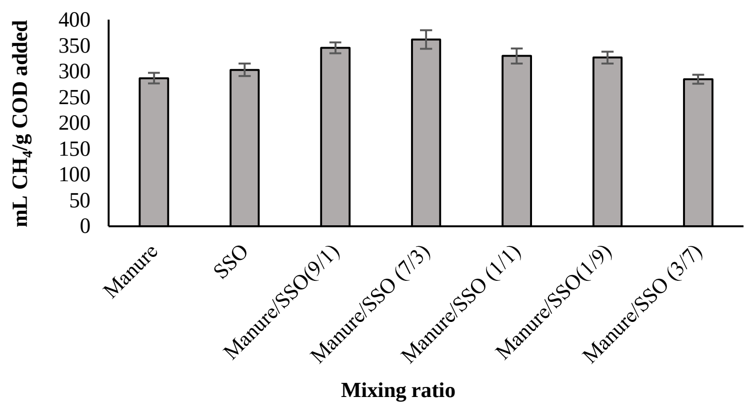


Figure 6.4. COD mass balance in co-digestion of manure and SSO for different mixing ratios

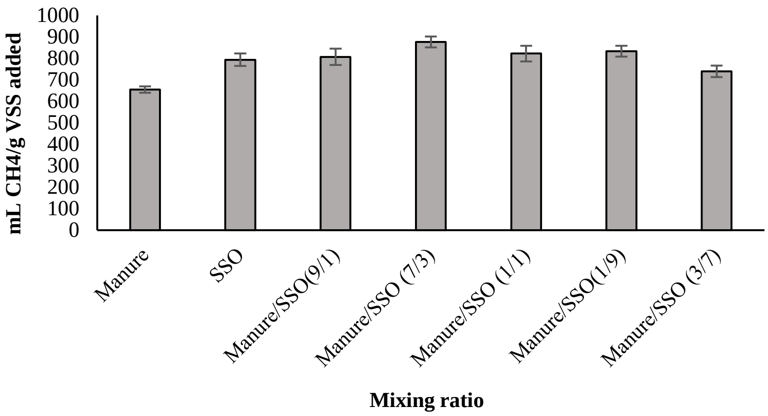
6.2. Cumulative methane yields

Cumulative methane yields including $mLCH_4/g$ TCOD added, $mLCH_4/g$ VSS added, and $mLCH_4/mL$ substrate added are presented in Figure 6.5. It was observed that cumulative methane yield per mass COD of substrate added increased in co-digesters in comparison with the control reactors. As shown in Figure 6.5. a), SSO and manure alone produced 303 $mLCH_4/g$ TCOD added and 287 $mLCH_4/g$ TCOD added, respectively. The addition of manure with SSO, increased the methane yield in the co-digesters. The amounts of the biomethane yields were within a range between 316 and 362 $mLCH_4/g$ TCOD added in the co-digesters. The highest yield of 362 $mLCH_4/g$ TCOD added occurred at the manure/SSO mixing ratio of 7:3. CH_4 yield increased by 26% and 20% compared to single digestion of manure and SSO, respectively.

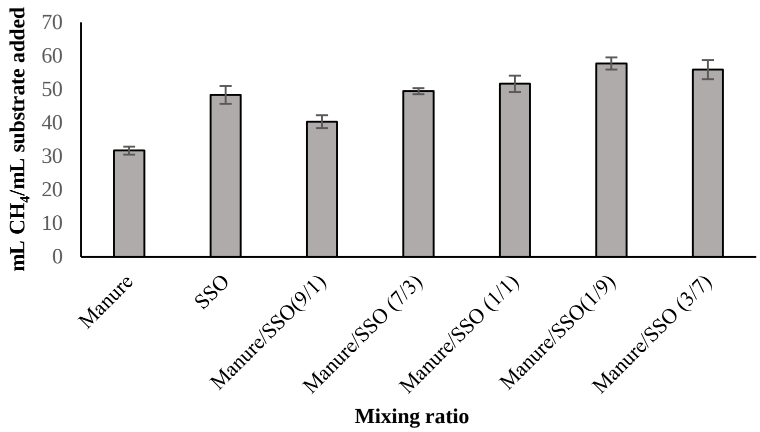
Figure 6.5. b), shows the methane yields in $mLCH_4/g$ VSS added. Manure produced 654 $mLCH_4/g$ VSS added. The yield was higher for SSO corresponding to 793 $mLCH_4/g$ VSS added. The highest yield occurred at the manure/SSO mixing ratio of 7:3 corresponding to 876 $mLCH_4/g$ VSS added. The CH_4 yield improved by 30% compared to the digestion of manure alone. All of the reactors with mixings of manure and SSO resulted in higher amounts of methane yields per unit mass of VSS added. The methane yields of from 819 to 847 $mLCH_4/g$ VSS added were obtained in co-digestion of manure and SSO at different mixing ratios.



6.5. a)



6.5. b)



6.5. c)

Figure 6.5. Methane yields a) per unit mass TCOD added, b) per unit mass of VSS added and c) per volume substrate added at different mixing ratio of TWAS and manure

Figure 6.5. c), shows the methane yield per unit volume of substrate added in mLCH_4/ mL substrate added. Manure and SSO individually produced 32 mLCH_4/mL substrate added and 48 mLCH_4/ mL substrate added, respectively. The reactors with the combinations of manure and SSO resulted in methane yields ranging from 40 mLCH_4/mL substrate added to 58 mLCH_4/mL substrate added. The most values of the CH_4 yield in terms of unit volume of substrate added corresponded to manure/SSO co-digestion at the mixing ratios of 1:9 and 3:7.

Biodegradable fraction of the feedstocks was obtained using the Eq. 4.1 and the result is summarized in Figure 6.6. Manure and SSO individually had biodegradable fractions of 72% and 76 %, respectively. Co- digestion increased biodegradable fraction of the feedstocks by 20% and 26 % in comparison with the control reactors digesting only SSO and manure, respectively. The most percentage of biodegradable fraction occurred at the reactor co-digesting manure with SSO at the mixing ratio of 7:3. This in good compliance with the maximum methane production at the same mixing ratio.

As shown in Figure 6.6, the trend of biodegradable fraction variations in the digesters conforms to the trend of the methane yields obtained by the experimental results for the corresponding digesters. It was verified that addition of manure to SSO as co-substrate increased biodegradability and enhanced methane production in the rectors co-digesting manure and SSO. The reason would be the existence of abundant of methanogenic populations in manure that was introduced to the co-digesters and enhanced degradation of organic matters.

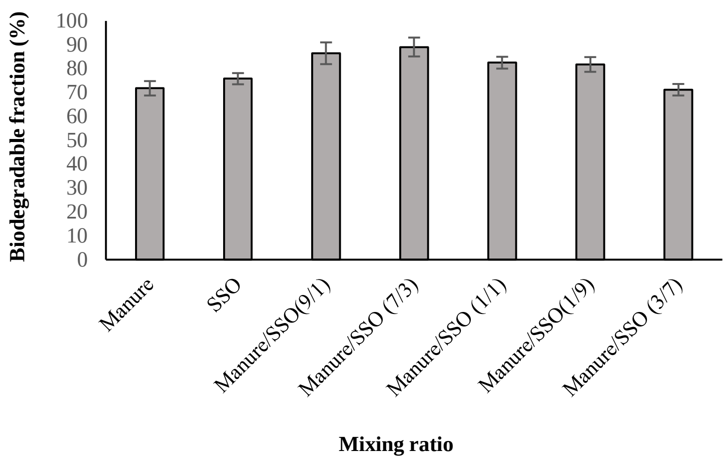


Figure 6.6. Biodegradable fraction of the feedstocks for different mixture ratios of TWAS and manure

6.3. Synergistic effect

Synergistic effect was assessed using Eq. 4.2 as presented in chapter 4 and the result is summarized in Figure 6.7. Synergistic effect represents the percentage of additional methane yield for co-substrates that was measured by the experiment, over the weighted average of the methane yield of individual substrates per unit volume of substrate added. As demonstrated in Fig 6.7, the most synergetic impact corresponds to the co-digestion of manure/SSO at the mixing ratio of 7:3. In co-digestion of manure with SSO, the increase of CH_4 yield due to synergistic effect ranged from 22% to 36 % corresponding to the reactor co-digesting manure and SSO at the mixing ratio of 9:1 and 7:3, respectively. It was revealed that only adding the fraction of manure in co-digesters, did not lead to increasing synergy. Although increasing the fraction of manure would introduce more populations of methanogenic archaea and bacteria, a balance between the microbial populations and nutrient is necessary for the effective microbial growth and enhanced methanogenesis.

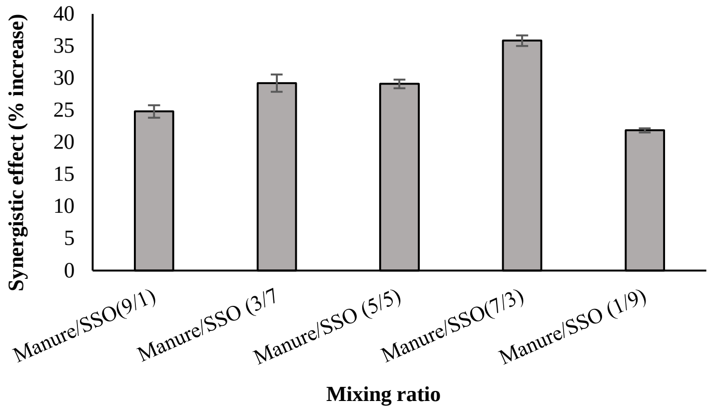


Figure 6.7. Synergetic effect of co-digestion at different mixing ratios of manure and SSO

6.4. COD:N and lipids: proteins: carbohydrates ratios

Table 6.2 presents the COD:N ratios, lipids: proteins: carbohydrates ratios, the ultimate methane production, and the methane yield per unit mass of COD added of the digesters with different mixing ratios. The COD:N ratios were 33 and 42 corresponding to SSO and manure alone, respectively. The values of COD:N varied from 34 to 41 in the co-digesters. The ultimate methane production and methane yield ranged from 919 to 1186 mL, and 287 to 363 mL/g TCOD added for different mixing ratios, respectively.

As shown in Table 6.2 The minimum ultimate methane and methane yield occurred at mono digestion of manure corresponding to the COD:N ratio of 42 and lipids: proteins: carbohydrates ratio of 1:4.2:21. However, the maximum ultimate methane and methane yield occurred at the mixing ratio of 7:3 corresponding to the COD:N ratio of 41 and lipids: proteins: carbohydrates ratio of 1:3.5:18.5. SSO alone with a lipids: proteins: carbohydrates ratio of 1:2:12 produced 15% more ultimate methane than manure alone with the lipids: proteins: carbohydrates ratio of 1:4.2:21. Although, it only resulted in 6% more methane yield per unit mass of COD added than manure. On the other side, the 1:4.2:21 lipids: proteins: carbohydrates ratios for manure alone and 1:4:20 for manure/SSO co-digestion at the mixing ratio of 9:1 had only a minor variation while the ultimate methane and the methane yield were 23% and 20% higher for the latter.

Table 6.2. Ultimate CH_4 and yield at different ratios of the substrates, COD:N, and Lipids:Proteins:Carbohydrates

Digester code	TWAS: Manure (V/V)	COD:N	Feedstock ratio codes	Lipids: Proteins: Carbohydrates	Ultimate CH_4 (mL)	mLCH_4/g TCOD added
Manure	1:0	42	CC	1:4.2:21	919	287
SSO	0:1	33	BB	1:2:12	1063	303
Manure/SSO 7:3	7:3	41	A	1:3.5:18.5	1186	363
Manure/SSO 9:1	9:1	39	B	1:4:20	1129	344
Manure/SSO 5:5	1:1	37	C	1:3:17	1136	330
Manure/SSO 3:7	3:7	35	D	1:2.7:15	1095	327
Manure/SSO 1:9	1:9	34	E	1:2:13	1115	316

Manure alone with lipids:proteins:carbohydrates ratio of 1:4.2:21 corresponded the minimum methane yield while the maximum yield occurred at manure:SSO mixing ratio of 7:3 and lipids:proteins:carbohydrates ratio of 1:3.5:18.5. This increase of the methane would be the result of microbial population diversity introduced by manure to the co-digesters and the synergetic impact of co-digestion rather than the ratio of lipids: proteins: carbohydrates.

Figure 6.8. shows the main effect plot for CH_4 yield data mean in response to feedstock and lipids:proteins:carbohydrates ratios in AnCoD of manure/SSO. As shown in figure 6.8, the different lipids:proteins:carbohydrates ratios for the different mixing ratios of the feedstocks have significant effect on the methane yield.

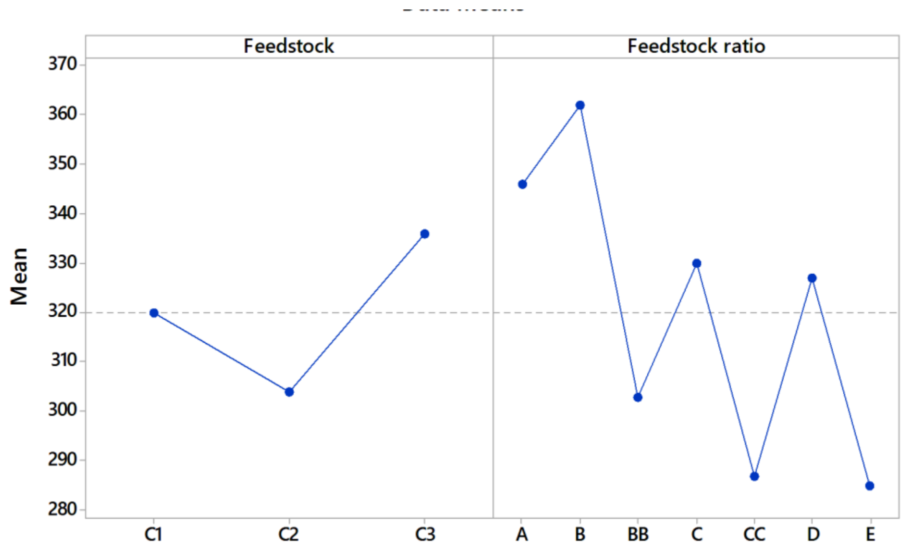
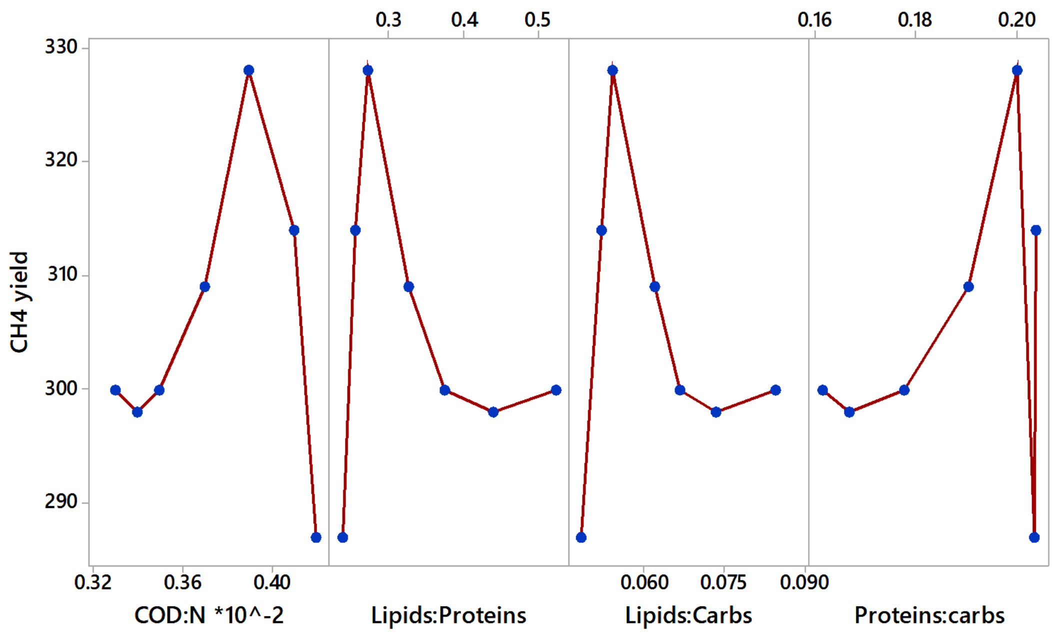
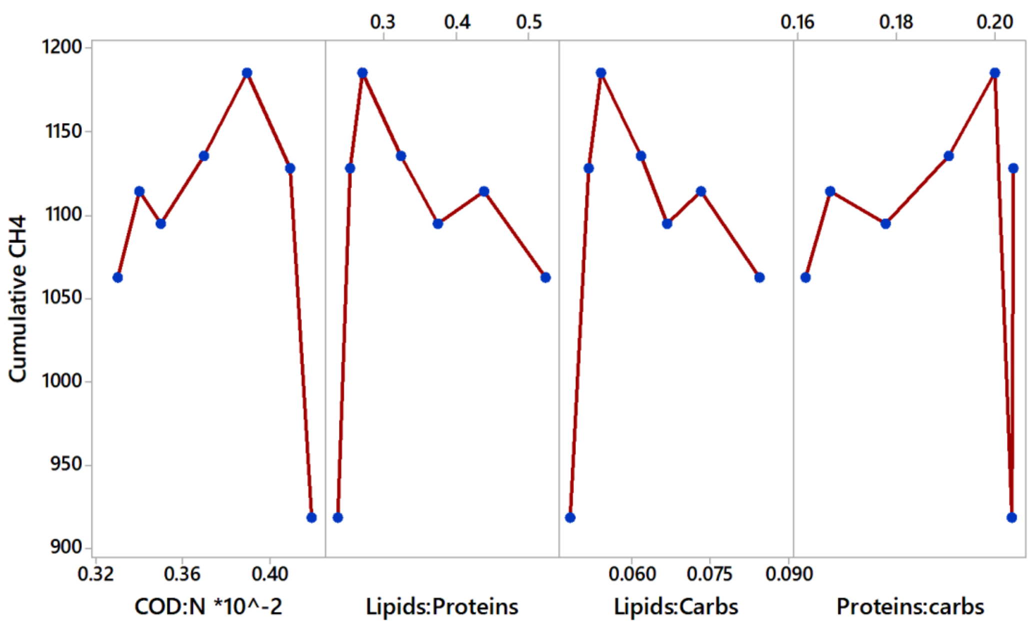


Figure 6.8. Main effect plot for CH_4 yield data mean in response to feedstock and lipids:proteins:carbohydrates ratios in AnCoD of manure/SSO

Figure 6.9 shows the matrix plot for the variations of the methane yield and the ultimate methane versus COD:N ratio, proteins: lipids, carbohydrates: lipids, and carbohydrates: proteins ratios. As illustrated in Figure 6.9. a, the trend of variations of the methane yields versus COD:N and versus proteins: carbohydrates were similar. lipids: proteins and lipids: carbohydrates also showed a similar trend but for both of them the trend was the mirror image of COD:N and proteins: carbohydrates. The ultimate methane as illustrated in Figure 6.9. b also demonstrated a similar response to those ratios. These observations were contrary to the results of TWAS:SSO and TWAS/Manure co-digestion. The reason would be the minor variations of proteins: lipids ratios at the different combinations of manure and SSO. As mentioned earlier, in co-digestion of manure/SSO the methane yield in the co-digesters could more depend on the microbial diversity than the ratio of the lipids: proteins: carbohydrates.



6.9. a



6.9. b

Figure 6.10. Matrix plot for: a. ultimate CH_4 and b. CH_4 yield at different COD:N and Lipids: Proteins, Lipids: Carbohydrates, and Proteins: Carbohydrates Ratios

6.5. Kinetic analysis results

The results of kinetic study by modified Gompertz model using Eq. 3.5 is summarized in Table 6.3. The model was applied to the experimental data from mono and co-digestion of manure and

SSO. The values of P for the maximum methane production per unit substrate (mL/g), R_m^e, the maximum methane production rate (mL/g h), and λ, the lag phase time (d) were calculated and the results values are summarized in Table 6.3. The modified Gompertz model for mono- and co-digestions of manure and SSO, rather showed a good fit to the experimental results with less than 10 % diversion from the measured values. The estimated values and their correlation with the mixing ratio of the feedstock were in good compliance with the data obtained by the experiment. An increasing in P values was observed in co-digestion of manure with SSO which conformed to the experimental results.

Table 6.3. Summary of results of kinetic study using modified Gompertz model

	P (mL)	R_m^e ($\frac{mL}{D}$)	**λ (d)**	**R^2**
Manure	875	48	0.4	0.999
SSO	998	83	1.6	0.999
Manure/SSO 7/3	1112	89	0.9	0.999
Manure/SSO 9/1	1065	57	0.7	0.999
Manure/SSO 5/5	1060	66	0.9	0.999
Manure/SSO 1/9	1018	76	1.1	0.999
Manure/SSO 3/7	936	81	0.8	0.999

The trend of P variations complied with the experimental data as it showed the same trend in response to the corresponding mixing ratios. For instance, the highest P value of 1112 mL corresponded to the mixing ratio of 7:3. The lag phase varied from 0.01 to 0.7 days for different substrate mixing ratios. The lag phase time was quite short and less than 1 day for all the digesters. A short lag phase is advantageous as it does not demand for a long residence time and therefore, it does not require a large reactor volume which reduces the operational costs of the system. The values of R_{max}^e ranged from 48 to 89 mL/d corresponding to manure mono digestion and manure/SSO mixing ratio of 7:3, respectively.

6. 6. Hydrolysis/acidification

This experiment was carried out for evaluating the hydrolysis/acidification phase in anaerobic co-digestion of manure and SSO. A series of analysis such as degree of solublization, synergetic effect of co-digestion at different mixing ratios on hydrolysis and liquefaction, volatile fatty acids (VFAs) yield, and hydrolysis kinetics of lipids, proteins, and carbohydrates in co-digestion of manure and SSO was carried out and the results are summarized below.

Initially, characterization of the feedstocks in triplicates was conducted and the mean values are presented in Table 6.4. As shown in Table 6.4, both manure and SSO have high amount of COD concentration which is above 100 g/L. Therefore, COD concentrations of the feed for all of the reactors containing the combinations of manure and SSO were above 100 g/L. Manure contains high amount of carbohydrates and proteins. Adding manure to SSO increased carbohydrates and proteins concentrations in the co-digesters compared to the reactor digesting SSO alone. In order to obtain the hydrolysis rate coefficients of COD, lipids, proteins, and manure, the concentrations of total, soluble and particulate COD; total, soluble and particulate proteins; and total, soluble and particulate carbohydrates were monitored over time.

The data form total and soluble COD (SCOD) concentrations monitoring in all the digesters over time during a 72-h hydrolysis/acidification period was used to calculate the degree of COD solublization for each digester. As explained earlier, the degree of solublization was calculated using Eq. 3.1 and the result is summarized in Figure 6.10.

As illustrated in figure 6.10, the degree of the COD solublization ranged from 24% to 35%. The most solublization of the COD content was 35% which corresponded to manure:SSO combination of 3:7 and 1:9. The COD solublization in mono digestion of manure and SSO were 24% and 28%, respectively. The reactors containing only manure had the lowest degree of solublization. Manure resulted in a relatively lower solublization than SSO which could be due to the presence of some recalcitrant contents such as fibers and cellulosic compounds in manure that delay its hydrolysis and liquefaction.

Table 6.4. Characteristics of the feedstocks at different mixing ratios of manure and SSO

		Manure	SSO	M*/SSO 9/1	M/SSO 7/3	M/SSO 5/5	M/SSO 3/7	M/SSO 1/9
Parameters	Units	Mixture (1) Ave.	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)
TCOD	g/L	101	115	103	105	108	111	114
SCOD	g/L	10	43	14	20	27	33	40
TSS	g/L	55	47	54	52	51	49	48
VSS	g/L	32.1	39.9	32.9	34.5	36.0	37.6	39.2
TS	g/L	69	78	69	71	73	75	77
VS	g/L	59	64	59	60	61	62	63
Ammonia	g/L	0.02	1.40	0.16	0.4	0.7	1.0	1.3
pH		5.6	5.8	5.9	5.7	5.7	5.6	5.8
Alkalinity	g CaCO3/L	7.2	6.7	7.2	7.1	7.0	6.9	6.8
TN	g/L	1.7	4.2	2.0	2.5	3.0	3.5	4.0
TSN	g/L	0.1	1.1	0.2	0.4	0.6	0.8	1.0
T-Carbs	g/L	26.8	14	25.5	23.0	20.5	17.9	15.4
T-Proteins	g/L	5.4	2	5.1	4.4	3.8	3.1	2.5
T-Lipids	g/L	1.7	1.5	1.7	1.6	1.6	1.5	1.5

* M: Manure

It was observed that co-digestion increased solublization as all of the reactors containing the mixings of manure and SSO achieved a higher degree of solublization than the control reactors.

A 46% and 25% improvement in solublization was achieved in the co-digestion of manure and SSO in comparison with the control reactors digesting only manure and only SSO. Although SSO demonstrated more solublization than manure, no correlation between the portion of SSO and the degree of solublization at different mixing ratio was observed. This could verify that a proper mixing ratio is requires to enhance the microbial synergy for the process improvement.

The synergistic effect on the solublization of the feedstocks was evaluated by comparing the theoretical degree of solublization of each co-digester to the measured data from the experiment. The results indicated that co-digestion improved solublization by improving microbial synergy. As shown in Figure 6.11, all co-digesters achieved an improvement in solublization due to the synergistic effect of the microbial communities. The synergistic effect varied from 18 to 34% corresponding to manure/SSO mixing ratios of 5:5 and 9:1, respectively.

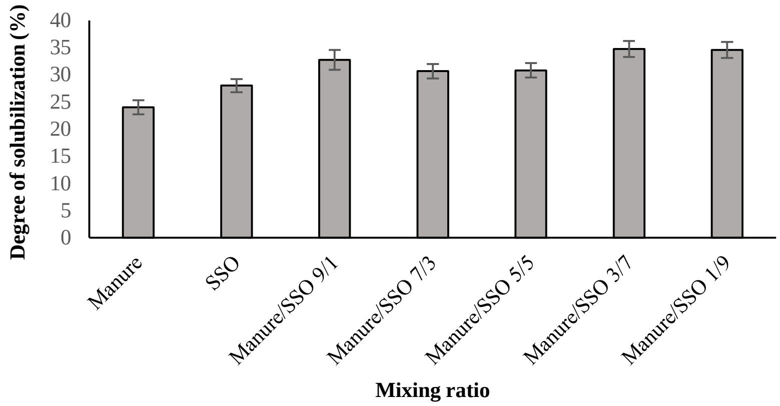


Figure 6.10. Degree of COD solublization at different mixing ratios of Manure/SSO

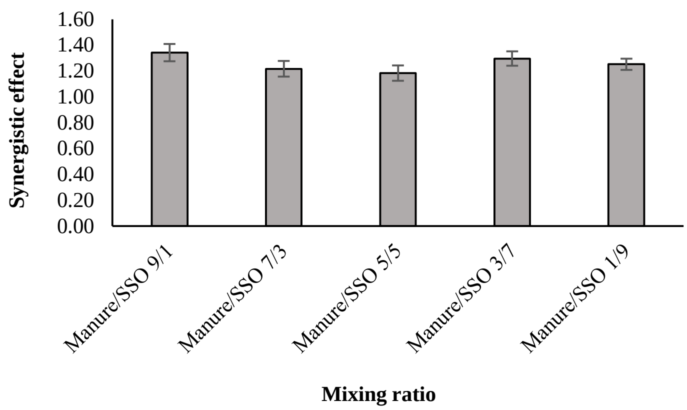


Figure 6.11. Synergistic effect on solublization at different mixing ratios of Manure/SSO

The monitoring of VFAs concentrations over time showed an increasing trend during the 72-hr of the hydrolysis/acidification process. The total VFAs yields were calculated in terms of mass of produced VFAs per mass of VSS added (mg VFAs/g VSS added). As illustrated in Figure 6.12, SSO alone had more VFAs yields than manure alone. The VFAs yield in the co-digester was correlated to the mixing ratios of the feedstocks so that the VFAs yield increased by increasing the fraction of SSO in the co-digesters. The trend of VFAs yield did not comply with the trend of COD solublization of the corresponding mixing ratios (Figure 6.10). Hence, hydrolysis/liquefaction occurred independently of acidification and followed a different trend from acidification. All of the reactors containing the mix of manure and SSO had higher VFAs yield than the control reactors. The VFAs yields were 231 mg VFAs/g VSS added and 325 mg VFAs/g VSS added for the manure and SSO mono digestions, respectively. The maximum VFAs yield of 400 mg VFAs/g VSS added was achieved by manure/SSO ratio of 1:9.

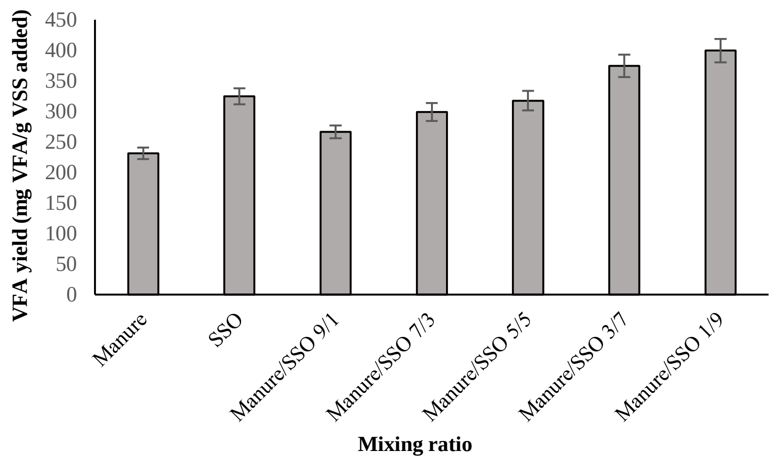


Figure 6.12. Total VFAs yield at different mixing ratios of Manure/SSO

Monitoring soluble and particulate COD over time, indicated an increasing trend in solublization of COD and particulate COD degradation. Furthermore, the monitoring of soluble and particulate lipids, proteins, and carbohydrates over time also demonstrated an increasing trend in solublization and particulate matter degradation. Similar to the previous experiments, in codigestion of manure and SSO, the hydrolysis and solublization rates of the lipids and proteins, were slower than carbohydrate. These results are summarized in the tables presented in the appendix. The hydrolysis rate coefficient (K_h) based on first order kinetics (equations 3.3 and 3.4) was calculated using AquaSim 2.0 for COD, lipids, proteins and carbohydrates based on the particulate degradation and results are summarized in Table 6.5.

In manure and SSO co-digestion, hydrolysis did not show the same trend as methanogenesis. The hydrolysis kinetics showed a higher rate in the manure:SSO mixing ratio of 3:7 while the most methane yield occurred at the mixing ratio of 7:3 with more fraction of manure. This would be due to the more microbial population of methanogens which was introduced by more fraction of manure in the co-digester. Similar to the co-digestion of TWAS with SSO and TWAS with manure, the improvement of the hydrolysis in co-digestion of manure with SSO resulted from the improvement of proteins and carbohydrates hydrolysis than that of lipids contents of the feedstocks. Unlike TWAS/manure and TWAS/SSO co-digestion, the fluctuations of hydrolysis rate coefficient of lipids and carbohydrates at different feedstocks mixing ratios showed similar trend in manure/SSO co-digestion. Nevertheless, in co-digestion of manure with SSO the variations of K_h for proteins demonstrated a different trend from that of lipids and carbohydrates. The maximum hydrolysis rate of proteins corresponded to the manure:SSO mixing ratio of 1:9. However, the maximum K_h values for lipids and carbohydrates were 0.11 and 0.68 corresponding to the mixing ratio of 3:7.

Table 6.5. Hydrolysis rate coefficients for COD, lipids, proteins, and carbohydrate content in co-digestion of SSO and manure at different mixing ratios

$\mathbf{K_h}$	M*/SSO 1/9	M/SSO 3/7	M/SSO 5/5	M/SSO 7/3	M/SSO 9/1	Manure	SSO
$K_{h\ COD}$	0.37	0.38	0.31	0.32	0.35	0.23	0.30
$K_{h\ Lipids}$	0.09	0.11	0.08	0.07	0.08	0.07	0.08
$K_{h\ Proteins}$	0.35	0.33	0.25	0.29	0.32	0.21	0.28
$K_{h\ Carbohydrates}$	0.63	0.68	0.57	0.55	0.61	0.43	0.55

* M: Manure

Chapter 7

Results and discussion

TWAS, Manure, SSO Co-digestion

7. Results and discussion- TWAS, manure, SSO co-digestion

7.1. BMP of TWAS, manure, SSO

This experiment was conducted on ternary co-digestion of TWAS, manure, and SSO. Manure slurry was prepared as discussed in chapter 3 and was fed to the reactors in different combinations with SSO and TWAS in triplicates. Control reactors containing TWAS, manure, and SSO individually were also used in triplicates. The influence of feedstocks mixing ratios and their correlation with the lipids: proteins: carbohydrates ratios on biomethane production in anaerobic co-digestion of TWAS/manure/SSO was evaluated. The characteristics of the feed in each digester with different mixing ratios of the substrates for the average of three measurement of each parameter are summarized in Table 7.1.

As presented in Table 7.1, both manure and SSO have high amount of COD concentrations compared to TWAS. The COD concentrations of the feedstocks were 100, 109, and 101 g/L for the digesters fed with manure, SSO and mixture of TWAS/manure/SSO at 1:1:8 ratio, respectively. Other reactors which contained the combinations of TWAS, manure, and SSO had a COD concentration ranging from 53 to 95 g/L. VSS concentrations were 26.5, 45.4, and 47.0 for TWAS, manure, and SSO respectively and ranged from 30.4 to 44.8 for the reactors containing the combinations of them. The amount of carbohydrates and lipids of manure and SSO were significantly more than that of TWAS. Therefore, addition of manure and SSO to TWAS increased the carbohydrates and lipids content of the co-digesters compared to the rectors digesting only TWAS.

Carbohydrates concentrations were within a range between 5.4 to 23.8 g/L in the co-digesters. Total proteins concentrations of TWAS, manure, and SSO were 3.9, 5.8, and 2.4 respectively and varied from 2.9 to 4.5 g/L in the co-digesters. The total lipids concentrations of TWAS was 0.4 g/L while manure and SSO had lipids concentrations of 1.4 and 1.5 g/L respectively. Adding manure and SSO increased the lipids content in the reactors containing the combination of TWAS, manure, and SSO compared to the reactors containing only TWAS. The total lipids concentrations varied from 0.6 to 1.3 g/L in the co-digesters containing the mix of the three feedstocks. pH was around neutral point varying from 7 to 7.2 for all of the reactors.

Table 7.1. Characteristics of feed to digesters with different mixing ratios of TWAS/ manure/SSO

		TWAS	Manure	SSO	T*:M**:SSO 8:1:1	T:M:SSO 1:8:1	T/M/SSO 1:1:8	T/M/SSO 5:2.5:2.5	T/M/SSO 2.5:5:2.5	T/M/SSO 2.5:2.5:5	T/M/SSO 4:4:2	T/M/SSO 2:4:4	T/M/SSO 4:2:4
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)	Mixture (8)	Mixture (9)	Mixture (10)	Mixture (11)	Mixture (12)
TCOD	g/L	40	100	109	53	95	101	72	87	89	78	71	79
SCOD	g/L	1.4	42	41	9	38	37	21	32	31	26	25	25
TSS	g/L	31	52	62	37	51	58	44	50	52	46	42	48
VSS	g/L	26.5	45.4	47.0	30.4	43.7	44.8	36.3	41.1	41.5	38.2	33.2	38.5
TS	g/L	38.9	67.8	67.0	44.6	64.8	64.3	53.2	60.4	60.2	56.1	48.1	55.9
VS	g/L	35.2	55.6	49.6	38.6	52.9	48.7	43.9	49.0	47.5	46.2	38.0	45.0
Ammonia	g/L	0.2	0.0	1.3	0.3	0.2	1.1	0.4	0.4	0.7	0.4	0.6	0.6
pH	-	7.0	7.0	7.2	7.1	7.0	7.0	7.1	7.1	7.1	7.0	7.0	7.1
Alkalinity	g $CaCO_3$/L	1.9	5.2	6.2	2.7	5.0	5.7	3.8	4.6	4.9	4.1	3.9	4.3
TN	g/L	2.8	2.1	4.0	2.8	2.4	3.7	2.9	2.7	3.2	2.7	2.6	3.1
TSN	g/L	0.4	0.1	1.0	0.4	0.2	0.8	0.5	0.4	0.6	0.4	0.5	0.6
Total carbs	g/L	1.5	27.8	14.1	5.4	23.8	14.2	11.3	17.8	14.4	14.6	11.5	11.8
Total proteins	g/L	3.9	5.8	2.4	3.9	5.2	2.9	4.0	4.5	3.6	4.3	2.9	3.7
Total lipids	g/L	0.4	1.4	1.5	0.6	1.3	1.4	0.9	1.2	1.2	1.0	0.9	1.0

* T: TWAS

** M: Manure

Operation of the digesters carried on until no significant amount of biogas was produced. Fig. 7.1 shows the profile of the cumulative biomethane production versus time during the co-digestion of TWAS, manure and SSO including the control rectors. SSO alone produced more cumulative methane than TWAS in the control reactors. The amount of methane produced by SSO alone was 13% more methane than manure alone.

All co-digesters produced more biomethane than the control reactors containing only TWAS. The amounts of ultimate CH_4 obtained by single digestion of SSO and manure were higher than that of TWAS alone by 3.2 and 2.9 fold. As illustrated in figure 7.1, all of the co-digesters generated higher amounts of CH_4 than the control reactors containing only manure. The reactors with the mix of the three feedstocks at the ratios of 8:1:1 and 5:2.5:2.5 produced more methane than TWAS alone however, they did not show any improvement in comparison with single digestion of manure and SSO. Other combinations produced more methane than TWAS and SSO alone, although only the three of them with the mixing ratios of 2.5:2.5:5, 4:2:2, and 4:2:4 resulted in higher cumulative CH_4 production comparing to all of the control reactors. The maximum cumulative methane production of 1424 mL corresponded to the TWAS/manure/SSO mixing ratio of 2:4:4 and lipids: proteins: carbohydrates ratio of 1:3:12.

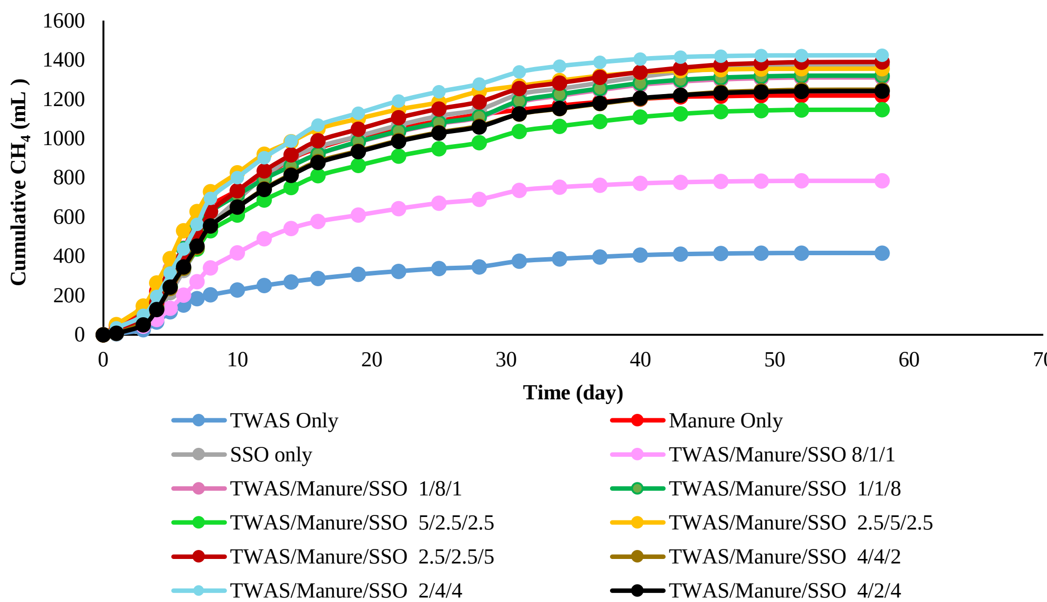


Figure 7.1. Cumulative methane production for different mixing ratios of TWAS/manure/SSO

Figure 7.2, illustrates the methane rate in mL/day for all of the feedstocks combinations including the control reactors. The lowest maximum CH_4 rate corresponded to TWAS mono digestion. Among control reactors, manure had the highest maximum CH_4 production rate. All of the digesters, reached their maximum methane rate in less than 10 days of the process operation. The digesters containing the mixings of TWAS/manure/SSO at the ratio of 1:8:1 achieved the highest maximum methane production rate at the first week of the operational period. The digesters produced 32% to 47% of their ultimate CH_4 production during the first week and 65% to 73% of it during the two weeks of the digestion period.

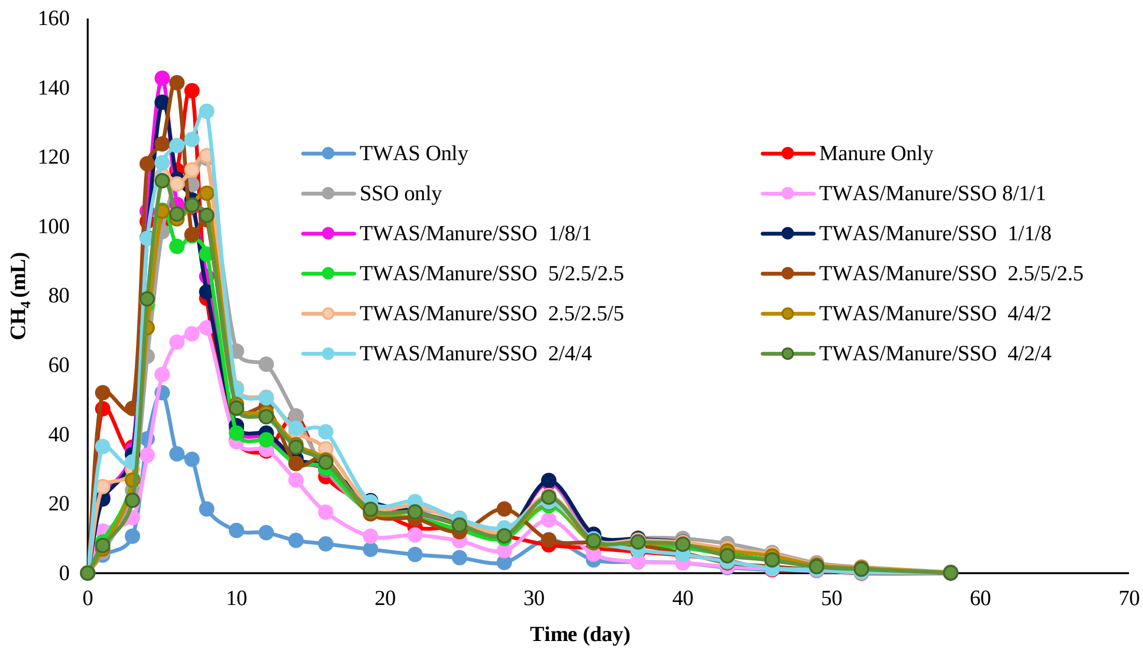


Figure 7.2. Methane production rate (mL/d) for different mixing ratios of TWAS/Manure/SSO

The maximum CH_4 production rates at different mixing ratios including the controls are shown in Figure 7.3. The lowest maximum rate of 52 mL/day corresponded to single digestion of TWAS. The maximum CH_4 rates of manure and SSO alone were 139 mL/day and 120 mL/day, respectively. The maximum CH_4 rates of the co-digesters including the mix of the three feedstocks varied from 71 to 143 mL/day. As presented in the figure, the highest maximum rate of 143 mL/day corresponded to the TWAS/manure/SSO mixing ratio of 1:8:1.

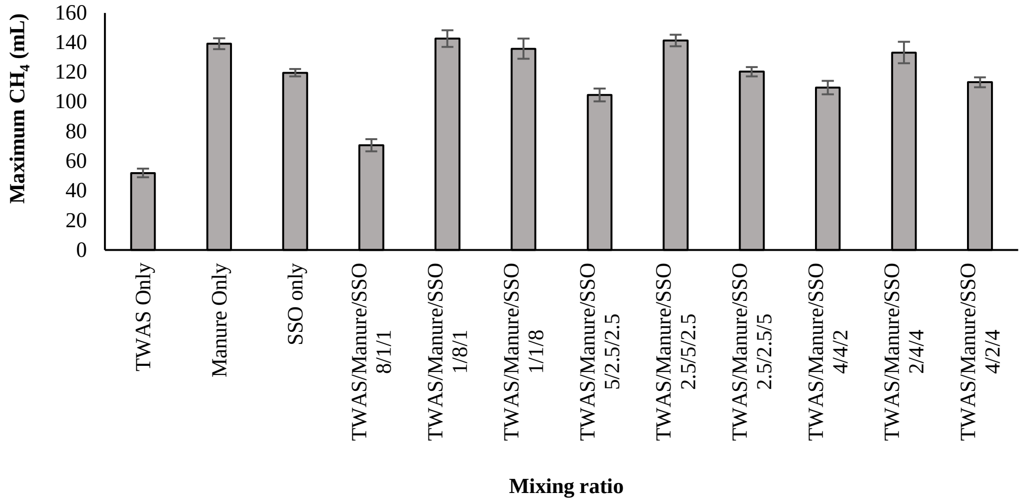


Figure 7.3. Maximum methane production rate (mL/d) for different mixing ratios TWAS/manure/SSO

The COD mass balance was conducted for all of the digesters and the result is summarized in Figure 7.4. The mass balance was calculated considering the initial and the final TCOD concentrations of the reactors' contents, and the theoretical methane production per unit mass of TCOD removed. Comparing the experimental methane production data with the ones obtained by the calculations, showed a deviation of less than 5% for all of the digesters.

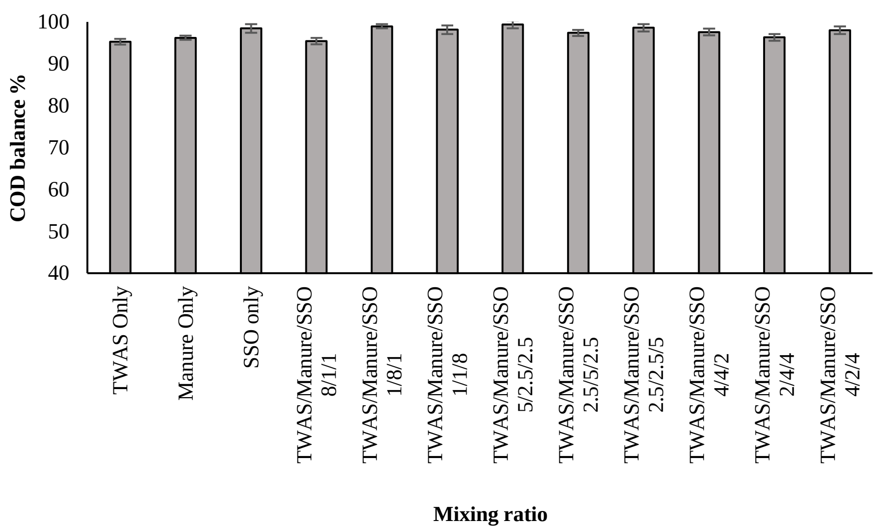


Figure 7.4. COD mass balance in co-digestion of manure and SSO for different mixing ratios 4

7.2. Cumulative methane yields

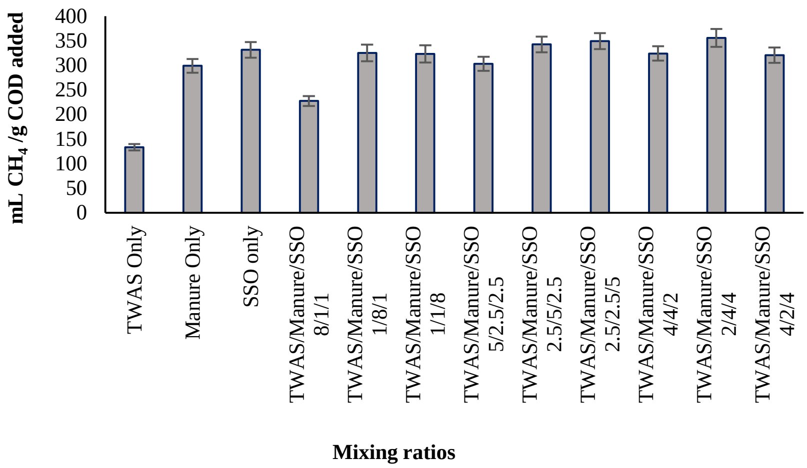
Figure 7.5 shows the cumulative methane yields of the digesters including $mLCH_4$/g TCOD added, $mLCH_4$/g VSS added, and $mLCH_4$/mL substrate added. The cumulative methane yield per mass COD of substrate added were 134, 299, and 332 $mLCH_4$/g TCOD added for the control reactors digesting only TWAS, manure, and SSO, respectively. As shown in Figure 7.5. a), the minimum and the maximum yield corresponded to mono digestion of TWAS and TWAS/manure/SSO mixing ratio of 2:4:4, respectively. The biomethane yields ranged from 228 to 356 $mLCH_4$/g TCOD added in the co-digesters. CH_4 yield increased by 165%, 19% and 7% compared to single digestion of TWAS, manure, and SSO, respectively.

The methane yields in $mLCH_4$/g VSS added are presented in Figure 7.5. b). The CH_4 yields of 202, 659, and 766 $mLCH_4$/g VSS added corresponded TWAS, manure, and SSO for the control reactors, respectively. The CH_4 yield varied from 395 to 982 $mLCH_4$/g VSS added in the reactors containing the combinations of the three feedstocks. The maximum CH_4 yield of 395 $mLCH_4$/g VSS added was achieved by the co-digestion of TWAS/manure/SSO at the mixing ratio of 2:4:4.

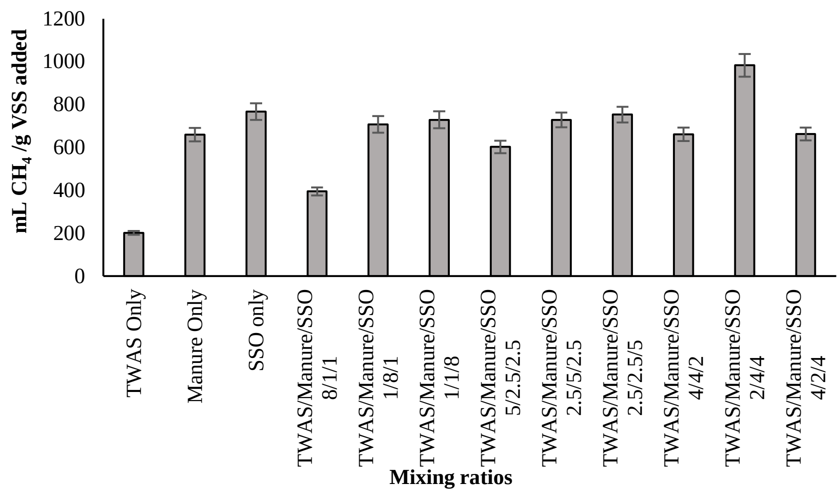
Figure 7.5. c), illustrates the methane yield per unit volume of substrate added. TWAS, Manure and SSO individually resulted in 5.3, 30 and 36 $mLCH_4$/mL substrate added, respectively. The reactors with the combinations of manure and SSO resulted in methane yields ranging from 12 $mLCH_4$/mL substrate added to 32.6 $mLCH_4$/mL substrate added. The most CH_4 yield per unit volume of substrate added corresponded to single digestion of SSO and TWAS/manure/SSO co-digestion at the mixing ratios of 2:4:4.

Biodegradable fraction as mentioned earlier was calculated using the Eq. 4.1 and the result is summarized in Figure 7.6. The biodegradable fraction of the digesters ranged from 33% to 89%. TWAS, manure and SSO alone, corresponded to biodegradable fractions of 33%, 75 % and 83%, respectively. The most percentage of biodegradable fraction occurred at the reactor co-digesting TWAS, manure and SSO at the mixing ratio of 2:4:4. This in good compliance with the maximum methane production that occurred at the same mixing ratio. The trend of variation in the methane yields also conforms to the trend that was observed for the variations of biodegradable fractions at the different mixing ratios. All of the co-digesters demonstrated a more biodegradable fraction than TWAS alone, nevertheless not all of the combinations resulted in more biodegradable fraction than manure and SSO alone.

7.5. a)



7.5. b)



7.5. c)

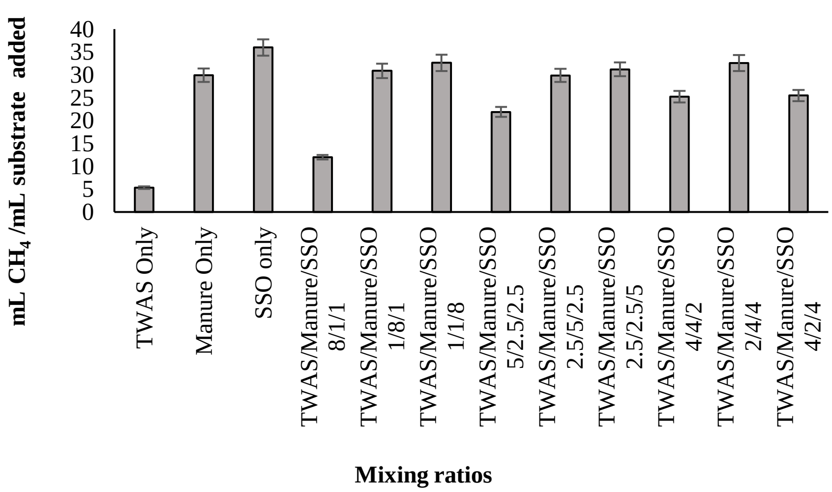


Figure 7.5. Methane yields a) per unit mass TCOD added, b) per unit mass of VSS added and c) per volume substrate added at different mixing ratio of TWAS and manure

The reason would be that the balance of the nutrient with the microbial communities was not necessarily ideal for methanogenic populations in the reactors containing the mix of the three feedstocks at all of the mixing ratios.

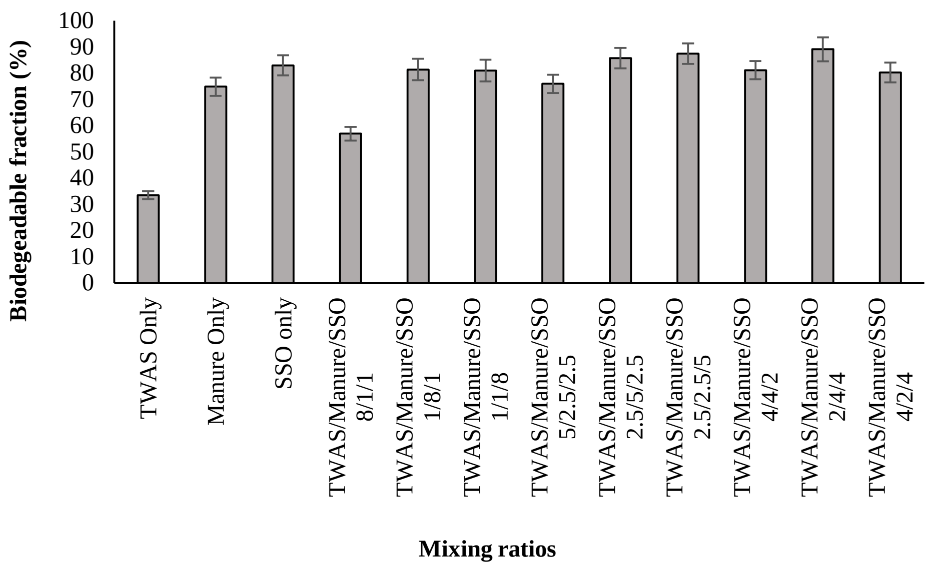


Figure 6.6. Biodegradable fraction of the feedstocks for different mixture ratios of TWAS and manure

7.3. Synergistic effect

Synergistic effect was obtained by calculating the percentage of additional methane yield achieved in co-digestion. This was done by dividing the measured yields, over the weighted average of the methane yield of individual substrates per unit volume of substrate added. Figure 7.7 shows the percentage improvement of biomethane production due to the synergistic impact. As demonstrated in Fig 7.7, in the ternary co-digestion of TWAS/manure/SSO, the most synergetic impact corresponds to the mixing ratio of 2:4:4. No significant improvement due to synergy was observed at the mixing ratio of 1:1:8. The maximum synergistic effect that was achieved by this experiment was 19 % which was slightly lower than the result obtained by some of digesters in the binary co-digestion.

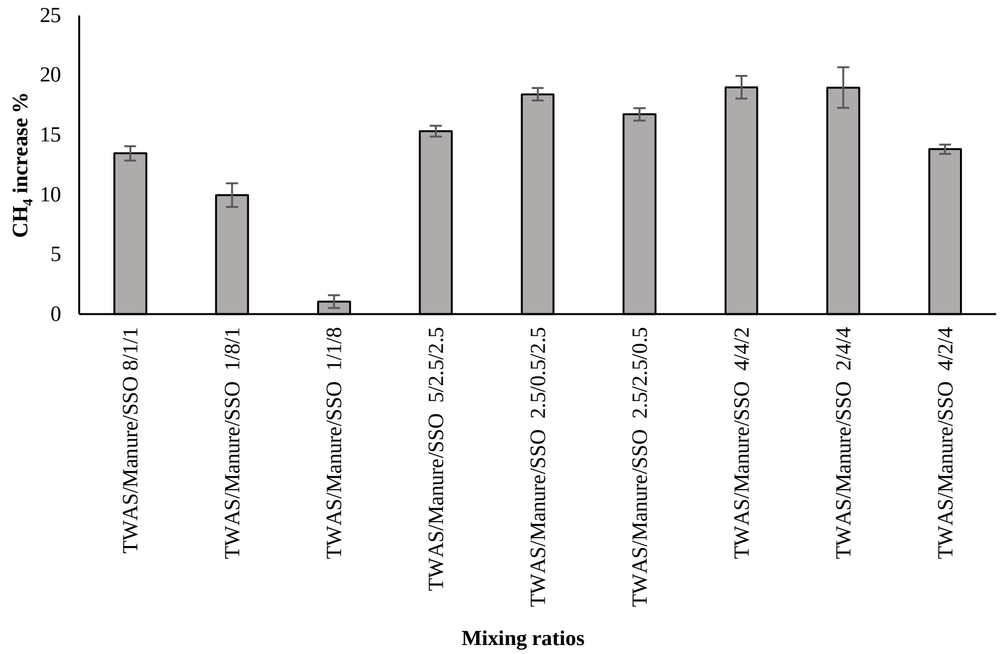


Figure 7.7. Synergetic effect of co-digestion at different mixing ratios of manure and SSO

7.4. COD:N and Lipids: Proteins: Carbohydrates ratios

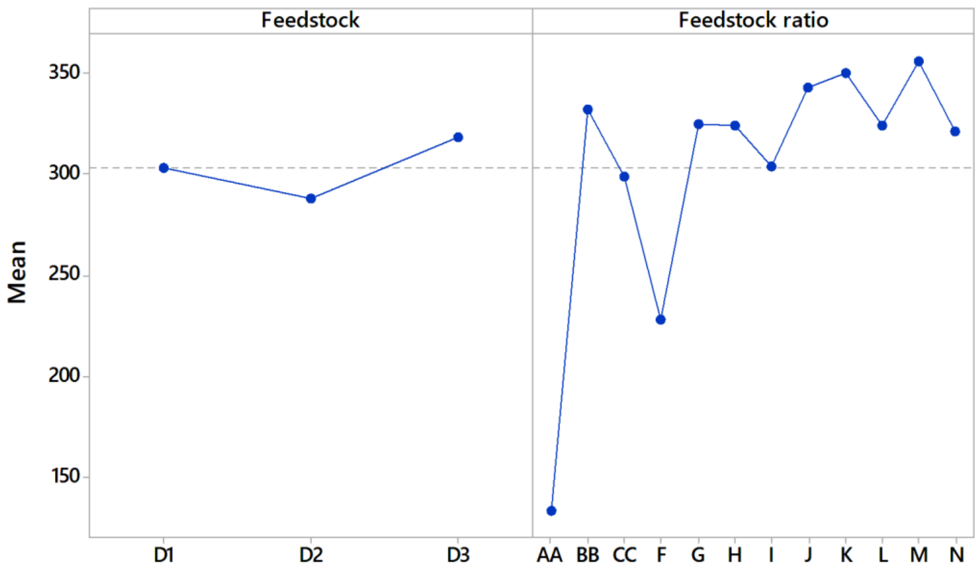
The values of COD:N ratios, lipids: proteins: carbohydrates ratios, the ultimate methane production and the methane yield per unit mass of COD added at different mixing ratios are presented in Table 7.2. The COD:N ratios of TWAS, manure and SSO were 15, 47, and 27 respectively. For the co-digesters, COD:N varied from 19 to 40. The lipids: proteins: carbohydrates ratios were 1:10:4, 1:4:20, and 1:1.6:9 for TWAS, manure, and SSO respectively. Among them SSO had the most ultimate methane production and methane yield corresponding to 1373 mL and 332 mL CH_4/g COD added. As shown in Table 7.2, the minimum ultimate methane production and the methane yield occurred at TWAS mono digestion corresponding to the COD:N ratio of 15 and lipids: proteins: carbohydrates ratio of 1:10:4. On the other side, the maximum ultimate methane production and yields occurred at the mixing ratios of 2:4:4 corresponding to the COD:N ratio of 28 and lipids: proteins: carbohydrates ratio of 1:3:12.

Table 7.2. Ultimate CH_4 and yield at different ratios of the substrates, COD:N, and lipids:proteins:carbohydrates

Digester code	TWAS: Manure: SSO (V/V)	COD:N	Feedstock ratio codes	Lipids: Proteins: Carbohydrates	Ultimate CH_4 (mL)	mLCH_4/g TCOD added
TWAS Only	1:0:0	15	AA	1:10:4	417	134
Manure Only	0:1:0	47	CC	1:4:20	1218	299
SSO only	0:0:1	27	BB	1:1.6:9	1373	332
T/M/SSO 8/1/1	8:1:1	19	F	1:6.5:9	784	228
T/M/SSO 1/8/1	1:8:1	40	G	1:4:19	1311	325
T/M/SSO 1/1/8	1:1:8	28	H	1:2:10	1320	324
T/M/SSO 5/2.5/2.5	5:2.5:2.5	25	I	1:4:12	1146	304
T/M/SSO 2.5/5/2.5	2.5:5:2.5	32	J	1:3.8:15.5	1355	343
T/M/SSO 2.5/2.5/5	2.5:2.5:5	28	K	1:3:12	1390	350
T/M/SSO 4/4/2	4:4:2	28	L	1:4:15	1248	324
T/M/SSO 2/4/4	2:4:4	28	M	1:3:12	1424	356
T/M/SSO 4/2/4	4:2:4	26	N	1:3.7:11.8	1241	321

Figure 7.8. illustrates the main effect plot for CH_4 yield data mean in response to feedstock and lipids:proteins:carbohydrates ratios in AnCoD of TWAS/manure/SSO. As shown in Figure 7.8, feedstocks and lipids:proteins:carbohydrates ratios have significant effect on CH_4 yield. TWAS only with lipids:proteins:carbohydrates ratio of 1:10:4 has the minimum methane yield. TWAS/manure/SSO co-digestion at the mixing ratio of 2:4:4 and lipids:proteins:carbohydrates ratio of 1:3:12 corresponded to the maximum methane yield.

The matrix plot in Figure 7.9 shows the relationship of COD:N, proteins: lipids, carbohydrates: lipids, and carbohydrates: proteins ratios with the ultimate methane production (mL) and with the yield (mg CH_4/g TCOD added). The responses of the ultimate methane production and the methane yield to the different ratios is illustrated in figures 7.9. a) and 7.9. b), respectively.



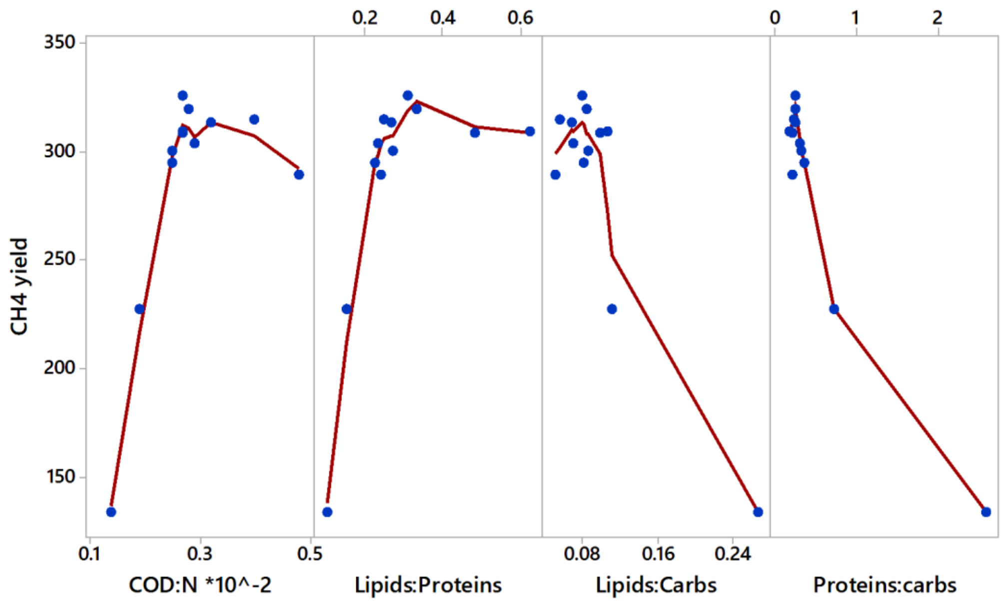
Figure

Figure 7.8. Main effect plot for CH_4 yield data mean in response to feedstock and lipids:proteins:carbohydrates ratio in AnCoD of TWAS/manure/SSO

As shown in Figures 7.9. a) and 7.9. b) the trends of variations of methane versus COD:N, and lipids: proteins were similar. However, lipids: carbohydrates and proteins: carbohydrates did not conform to COD:N ratio. As illustrated in the Figures 7.8. a), the minimum ultimate methane production corresponds to the minimum COD:N ratio and the minimum lipids: proteins ratio while it corresponded to the maximum lipids: carbohydrates and maximum protein: carbohydrates ratios. The trend of the ultimate methane production variations in response to the COD:N and to the lipids: proteins ratios relatively conform to each other excluding some of the ratios. The ultimate methane production was higher at the COD:N ratios between 26 and 47 and at the lipids: proteins ratios between 0.23 and 0.62. On the contrary, the increase of the lipids: carbohydrates and proteins: carbohydrates ratios, reduced the ultimate methane production. The most ultimate methane production occurred at the minimum lipids: carbohydrates and proteins: carbohydrates ratios of 0.05 and 0.17, respectively. In contrast the minimum ultimate methane production corresponded to the maximum lipids: carbohydrates and proteins: carbohydrates ratios of 0.26 and 2.55, respectively. The lipids: carbohydrates ratios above 0.1 and proteins: carbohydrates ratios above

0.3 resulted in significant decrease in the ultimate methane production. Similar trend was observed for the methane yield in response to the COD:N, lipids: proteins and lipids: carbohydrates, and proteins: carbohydrates ratios.

7.9. a)



7.9. b)

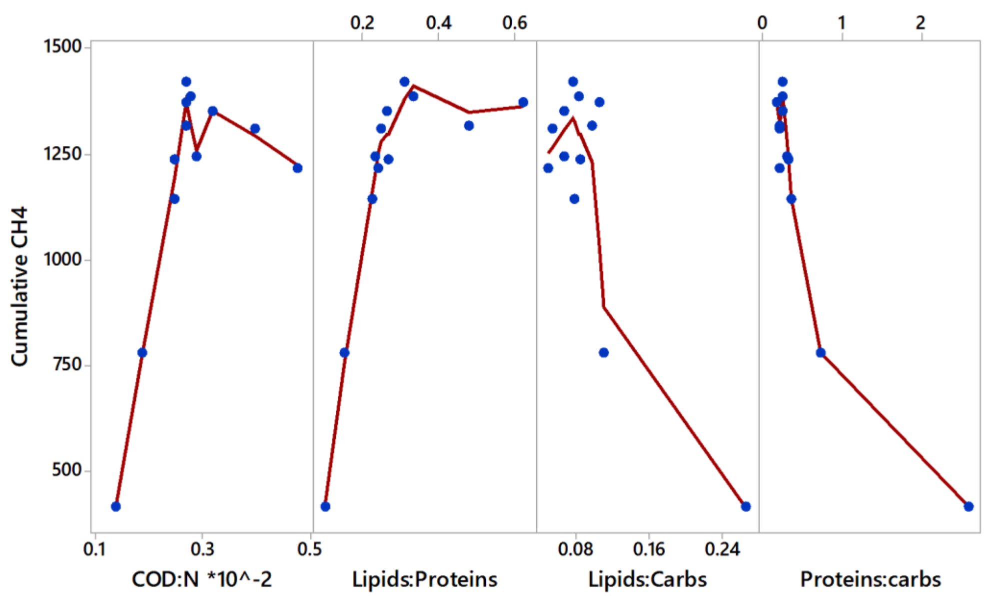


Figure 7.9. Matrix plot for: a. ultimate CH_4 and b. CH_4 yield at different COD/N and Lipids: Proteins, Lipids: Carbohydrates, and Proteins: Carbohydrates Ratios

7.5. Kinetic analysis results

The results of kinetic by modified Gompertz model using Eq. 3.5 is summarized in Table 7.3. The model was applied to the experimental data from mono and co-digestion of manure and SSO. The values of P for the maximum methane production (mL), R_m^e, the maximum methane production rate (mL/g day), and λ, the lag phase time (d) were calculated and the estimated values are presented in Table 7.3. The modified Gompertz model for mono- and co-digestions of TWAS, manure and SSO, showed a good fit to the experimental results with less than 10 % diversion from the measured values. The estimated values and their correlation with the mixing ratio of the feedstock showed compliance with the data obtained by the experiment. The trend of P variations showed the same trend as the trend observed by the experimental data in response to the corresponding mixing ratios.

Table 7.3. Summary of results of kinetic study using modified Gompertz model for TWAS/manure/SSO co-digestion

Digester codes	P (mL)	R_m^e (mL/d)	λ (d)	R^2
TWAS Only	400	20.3	0.02	0.999
Manure Only	1180	78.4	0.7	0.999
SSO only	1307	73.5	1.76	0.999
T*:M**:SSO 8:1:1	760	46.6	1.7	0.999
T:M:SSO 1:8:1	1257	66.9	0.2	0.999
T:M:SSO 1:1:8	1267	66.1	0.2	0.999
T:M:SSO 5:2.5:2.5	1099	60.7	0.8	0.999
T:M:SSO 2.5:5:2.5	1308	84.9	0.5	0.999
T:M:SSO 2.5:2.5:5	1330	74.4	1.0	0.999
T:M:SSO 4:4:2	1193	67.2	1.2	0.999
T:M:SSO 2:4:4	1382	84.6	1.1	0.999
T:M:SSO 4:2:4	1191	66.8	1.2	0.999

* T: TWAS
** M: Manure

The Maximum P value for the predicted cumulative methane production was 1382 mL and corresponded to the mixing ratio of 2:4:4. The lag phase varied from 0.0 to 1.7 days for different substrate mixing ratios. The lag phase time was less than 1 day for half of the digesters. The other half had a lag phase time from 1 to 1.7 days. The values of R^{e}_{max} ranged from 20 to 84.9 mL/g d corresponding to TWAS mono digestion and TWAS/manure/SSO mixing ratio of 2:4:4, respectively.

7.6. Hydrolysis/acidification

This experiment was carried out for evaluating the hydrolysis/acidification phase in anaerobic co-digestion of TWAS, manure, and SSO. The analysis of degree of solublization, synergistic effect of co-digestion at different mixing ratios, volatile fatty acids (VFAs) yield, and hydrolysis kinetics of lipids, proteins, and carbohydrates was conducted by monitoring the soluble and particulate contents during a 72-hr period. Initially, characterization of the feedstocks in triplicates was carried out and the mean values are presented in Table 7.4. As shown in the table, both manure and SSO have higher amount of COD concentration than TWAS. Manure contains high amount of carbohydrates and proteins. The lipids and carbohydrates content of manure and SSO are also remarkably higher than that of TWAS. pH values of the digesters were adjusted to satisfy the required condition for hydrolysis/acidification and ranged from 5.6 to 5.8 in this experiment.

The data form total and soluble COD (SCOD) concentrations monitoring in all of the digesters over time during a 72-h hydrolysis/acidification period was used to obtain the degree of COD solublization for each digester. As explained earlier, the degree of solublization was calculated using Eq. 3.1 and the result is summarized in Figure 7.10.

As illustrated in figure 7.10, the degree of the COD solublization varied from 15% to 30%. The most solublization of the COD content of 30% corresponded to TWAS/manure/SSO combinations of 1:8:1 and 2:4:4. For the control reactors, the COD solublization in mono digestion of TWAS, manure and SSO were 15% and 23%, and 26% respectively. The reactors containing only TWAS had the lowest degree of solublization. Similar to the results of previous experiments, manure resulted in a relatively lower solublization than SSO which would be due to the presence of some recalcitrant contents such as fibers and cellulosic compounds that delay hydrolysis and liquefaction.

Table 7.4. Characteristics of the feedstocks at different mixing ratios of TWAS/manure/SSO

		TWAS	Manure	SSO	T*:M**:SSO 8:1:1	T:M:SSO 1:8:1	T:M:SSO 1:1:8	T:M:SSO 5:2.5:2.5	T:M:SSO 2.5:5:2.5	T:M:SSO 2.5:2.5:5	T:M:SSO 4:4:2	T:M:SSO 2:4:4	T:M:SSO 4:2:4
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)	Mixture (8)	Mixture (9)	Mixture (10)	Mixture (11)	Mixture (12)
TCOD	g/L	37	100	102	50	94	95	69	85	85	75	68	76
SCOD	g/L	1.9	10	37	6	12	31	13	15	22	12	17	17.7
TSS	g/L	29	54	50	34	51	48	40	47	46	43	36	42
VSS	g/L	23	45	44	27	43	42	34	39	39	36	31	36
TS	g/L	36	68	77	43	65	72	54	62	64	57	51	59
VS	g/L	32	58	63	37	56	59	46	53	54	48	43	49
Ammonia	g/L	0.2	0.0	1.3	0.3	0.2	1.1	0.5	0.4	0.7	0.4	0.6	0.6
pH	-	5.6	5.7	5.8	5.6	5.7	5.8	5.7	5.7	5.7	5.7	5.8	5.7
Alkalinity	g CaCO3/L	1.8	7.0	6.4	2.8	6.4	6.0	4.2	5.5	5.4	4.8	4.3	4.7
TN	g/L	2.7	1.7	4.1	2.7	2.0	3.7	2.8	2.5	3.1	2.6	2.5	3.0
TSN	g/L	0.4	0.1	1.1	0.4	0.2	0.9	0.5	0.4	0.7	0.4	0.5	0.6
T-Carbs	g/L	0.8	26.0	13.6	4.6	22.2	13.5	10.3	16.6	13.5	13.4	10.8	11.0
T-Proteins	g/L	2.5	5.1	2.1	2.8	4.6	2.4	3.1	3.7	3.0	3.5	2.4	2.9
T-Lipids	g/L	0.3	1.7	1.4	0.5	1.5	1.3	0.9	1.2	1.2	1.0	0.9	1.0

*T: TWAS

**M: Manure

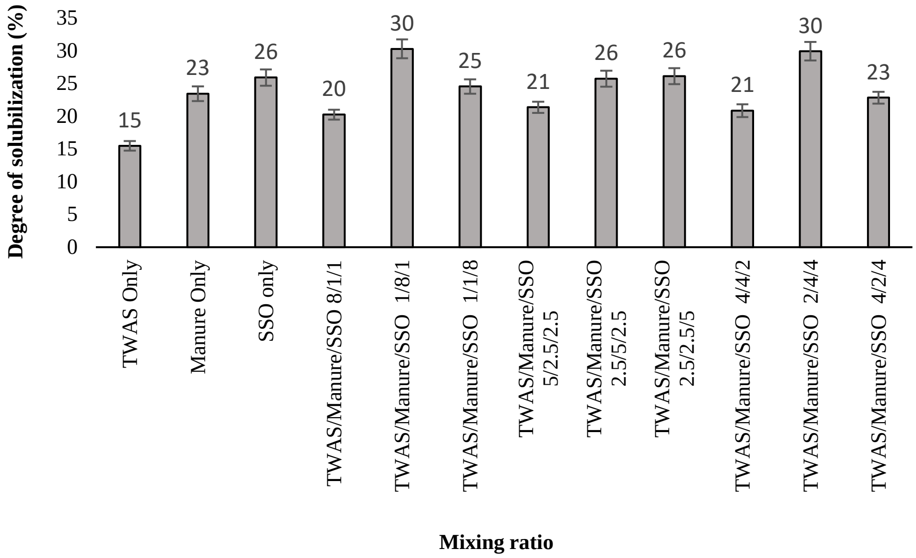


Figure 7.10. Degree of COD solublization at different mixing ratios of TWAS/Manure/SSO

It was observed that all of the reactors containing the mixings of TWAS, manure and SSO achieved a higher degree of solublization than the control reactors containing only TWAS. Solublization increased by 100%, 30% and 15% in the co-digestion of TWAS/manure/SSO in comparison with the control reactors digesting only TWAS, manure and SSO, respectively. Although SSO demonstrated more solublization than manure, no correlation between the portion of SSO and the degree of solublization at different mixing ratio was observed. This could verify that a proper mixing ratio is required to enhance the microbial synergy of the hydrolytic and acidogenic microbial communities for the process improvement.

The synergistic effect on the solublization of the feedstocks was evaluated by comparing the theoretical degree of solublization of each co-digester to the measured data from the experiment and the result is summarized in Figure 7.11. Although the improvement of synergy was not significant at some combinations, most of the co-digesters resulted in an increase of solublization due to the synergistic effect of the microbial communities. The synergistic effect varied from 2% to 35% in hydrolysis/acidification of TWAS/manure/SSO at different mixing ratios.

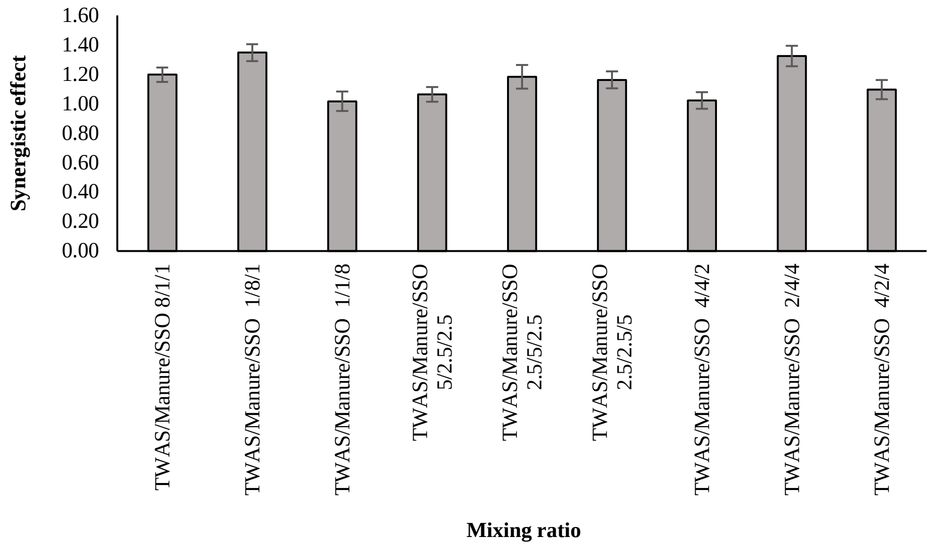


Figure 7.11. Synergistic effect on solublization at different mixing ratios of TWAS/Manure/SSO

The monitoring of VFAs concentrations over time indicated an increasing trend during the 72-hr of the hydrolysis/acidification period. The total VFAs yields were calculated in terms of mass of produced VFAs per mass of VSS added (mg VFAs/g VSS added). As illustrated in Figure 7.12, SSO alone had more VFAs yields than manure alone. Both manure and SSO had a higher VFAs yield than TWAS. The VFAs yield in the co-digester was correlated to the mixing ratios of the feedstocks so that the VFAs yield increased by increasing the fractions of manure and SSO in the co-digesters. Similar to the result of the previous experiments, the trend of VFAs yield did not comply with the trend of COD solublization of the corresponding mixing ratios (Figure 7.10). Therefore, it was assumed that hydrolysis/liquefaction would occur independently of acidification. All of the reactors containing the mix of manure and SSO had higher VFAs yield than the control reactors containing TWAS alone. The VFAs yields were 231 mg VFAs/g VSS added and 325 mg VFAs/g VSS added for the manure and SSO mono digestions, respectively. The maximum VFAs yield of 400 mg VFAs/g VSS added was achieved by manure:SSO ratio of 1:9.

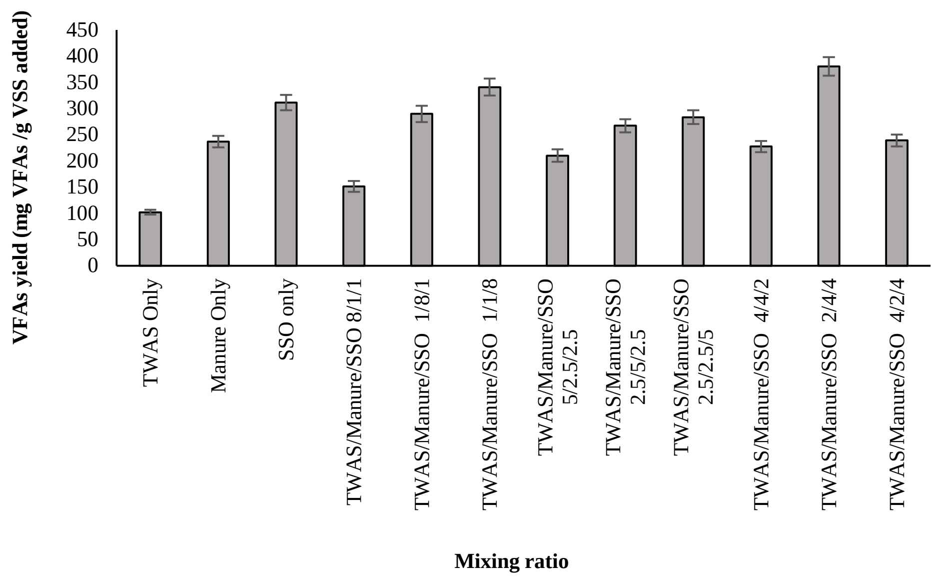


Figure 7.12. Total VFAs yield at different mixing ratios of TWAS/Manure/SSO

In co-digestion of TWAS/manure/SSO, by monitoring soluble and particulate COD over time, an increasing trend in solublization of COD and decreasing particulate COD concentrations was observed. Furthermore, monitoring soluble and particulate lipids, proteins, and carbohydrates over time also showed an increasing trend in solublization and particulate matter degradation of the feedstocks. Similar to the previous experiments, the hydrolysis rates of the lipids and proteins, were slower than carbohydrate. These results are summarized in the tables presented in the appendix. The hydrolysis rate coefficient (K_h) was calculated according to first order kinetic (equations 3.3 and 3.4) for COD, lipids, proteins and carbohydrates based on the particulate degradation and results are summarized in Table 7.5.

Although the first order kinetics was compatible with the trend of particulate matters degradation, the hydrolysis kinetics coefficient did not show the same trend as solublization and acidification as well as methanogenesis. Hydrolysis rate coefficients of the co-digesters increased in the co-digesters although, it did not change significantly in all of them compared to the single digestion of SSO. In some co-digesters the K_h values were even lower than that of SSO alone. The maximum VFAs yield corresponded to TWAS:manure:SSO 2:4:4 and 1:1:8. However, the Kh for the mixing ratio of 1:8:1 was higher than that of 1:1:8. The maximum solublization corresponded to

TWAS:manure:SSO mixing ratios of 2:4:4 and 1:8:1. However, the maximum methane yield occurred at the mixing ratios of 2:4:4 and 2.5:2.5:5 in the reactors co-digesting TWAS, manure, and SSO. Only the mixing ratio of 2:4:4 resulted in improving both methanogenesis and hydrolysis which would be due to the balance of the nutrients that favors both methanogenic and hydrolytic microbial consortia.

Table 7.5. Hydrolysis rate coefficients for COD, lipids, proteins, and carbohydrate content in co-digestion of TWAS, Manure, and SSO at different mixing ratios

K_h	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 5/2.5/2.5	T/M/SSO 2.5/5/2.5	T/M/SSO 2.5/2.5/5	T/M/SSO 4/4/2	T/M/SSO 2/4/4	T/M/SSO 4/2/4
Kh_{COD}	0.17	0.23	0.3	0.21	0.37	0.27	0.24	0.31	0.3	0.24	0.39	0.26
Kh_{Lipids}	0.03	0.07	0.08	0.05	0.12	0.06	0.05	0.09	0.08	0.09	0.14	0.05
$Kh_{Proteins}$	0.19	0.21	0.28	0.23	0.31	0.24	0.2	0.23	0.24	0.21	0.31	0.21
$K_{h\ Carbs}$	0.32	0.43	0.55	0.36	0.66	0.51	0.46	0.63	0.58	0.26	0.69	0.52

* T: TWAS
** M: Manure

Chapter 8

Correlation of CH_4 with lipids, proteins, and carbohydrates

8.1 Correlation of organic compositions and biomethane yield

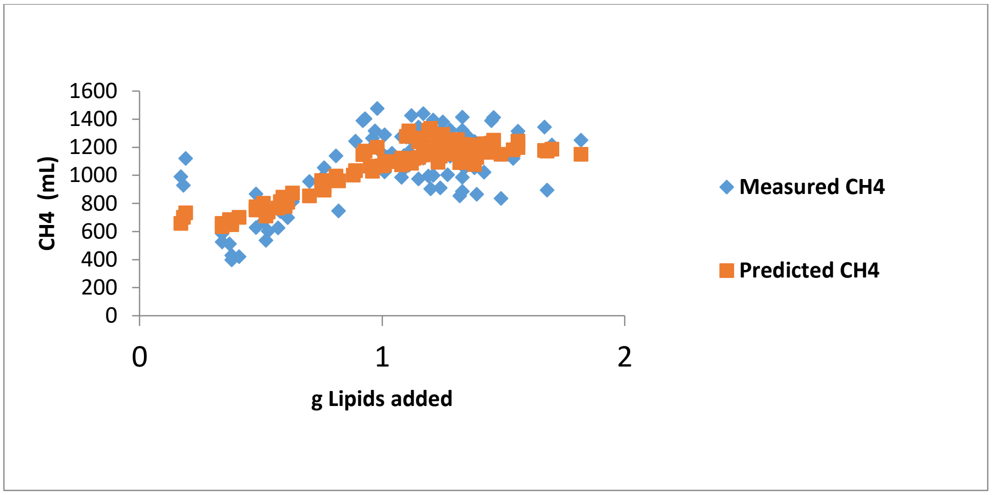
Lipids, proteins and carbohydrates make up the principal constituents of organic waste. Currently, there is limited information on how the three main components of organics impacts the AD performance. Therefore, assessing the correlation between the performance of anaerobic digestion system and the three main organic composition is required in order to enhance the efficiency of the AD process and to provide a tool for the prediction of the system performance.

In addition, in designing a co-digestion system this would provide a tool to optimize the system based on lipids, proteins, and carbohydrates fractions along with C:N ratio as they are both important design parameters. In this work the influence of lipids, proteins and carbohydrates content on the anaerobic digestion of TWAS, SSO and manure was investigated. The feedstocks and their mixtures with different ratios as presented in Table 3.2- Table 3.5, were analysed to determine their total lipids, proteins, and carbohydrates contents. The results of biogas monitoring and the methane in biogas using GC from the sets of BMP essay were used to determine an empirical model based on the relationship between lipids, proteins, carbohydrates components and the system efficiency in terms of methane production and yield. The functional relationship between responses (CH_4) and the factors (Lp, Pr, and Cr) were described by estimating the coefficients of the second-order polynomial model based on the experimental data according to Eq. 8.1 where B_0 is a constant, B_1, B_3, and B_4 are linear coefficients and B_2 is quadratic coefficient. The empirical model was used to predict the methane yield ($mLCH_4/ gCOD_{added}$) in terms of lipids, proteins, and carbohydrates components and the results are depicted by fit plots and residual plots as presented in figures 8.1 and 8.2.

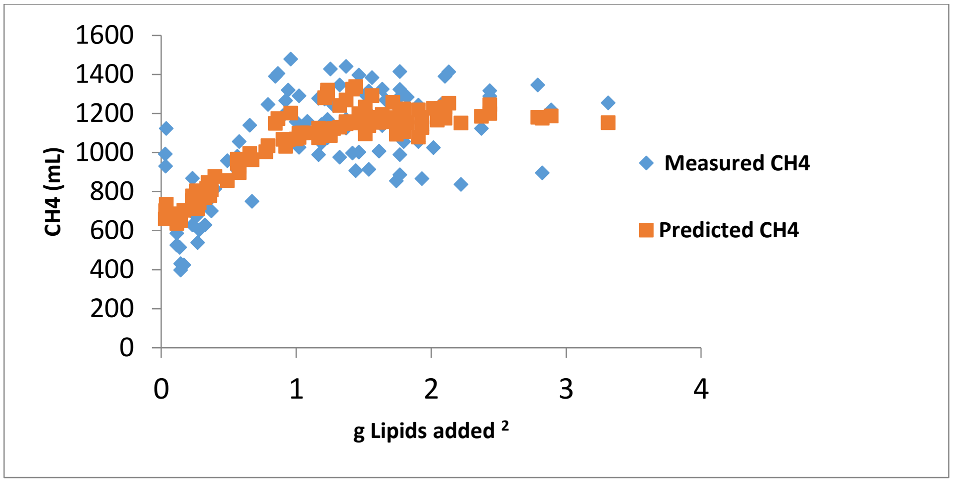
$$CH_4 = B_0 + B_1 Lp + B_2 Lp^2 + B_3 Pr + B_4 Cr \quad \text{Eq. 8.1}$$

Each of the fit plots as shown in Figure 8.1 contains adequate number of observations throughout the entire range of the predictor values. Although for the lipids a few points on the top left corner of the plot seems to be outlier, the multiple R value of 0.7 indicates the model properly fits in the data.

8.1. a)


1600
1400
1200
1000
800
600
400
200
0
CH4 (mL)
0
1
2
g Lipids added
Measured CH4
Predicted CH4

8.1.b)


1600
1400
1200
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800
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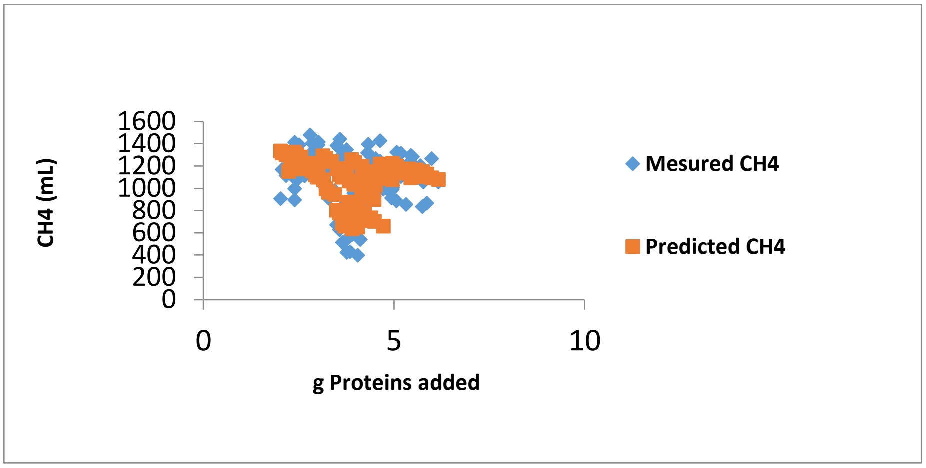
0
CH4 (mL)
0
1
2
3
4
g Lipids added ²
Measured CH4
Predicted CH4

8.1.c)



8.1. d)

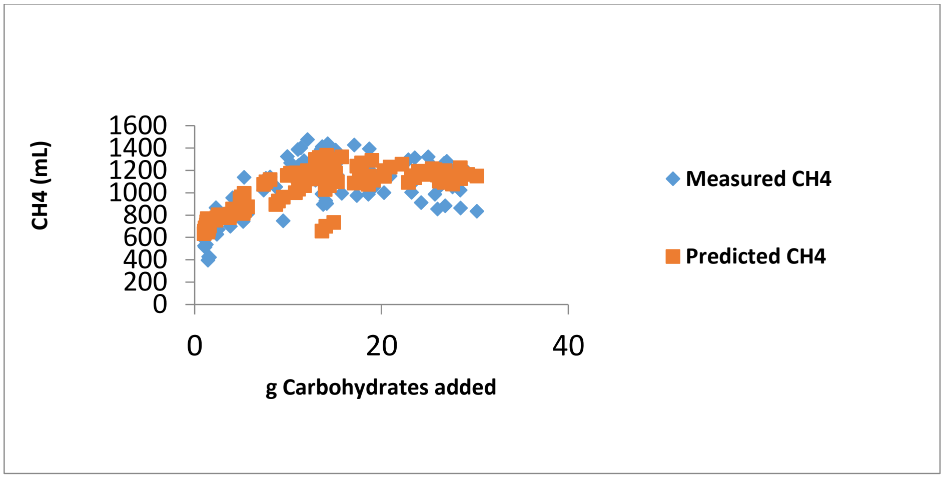
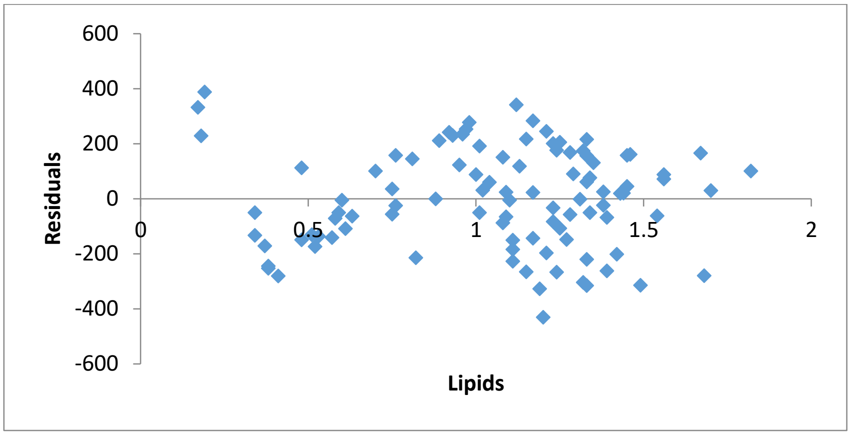


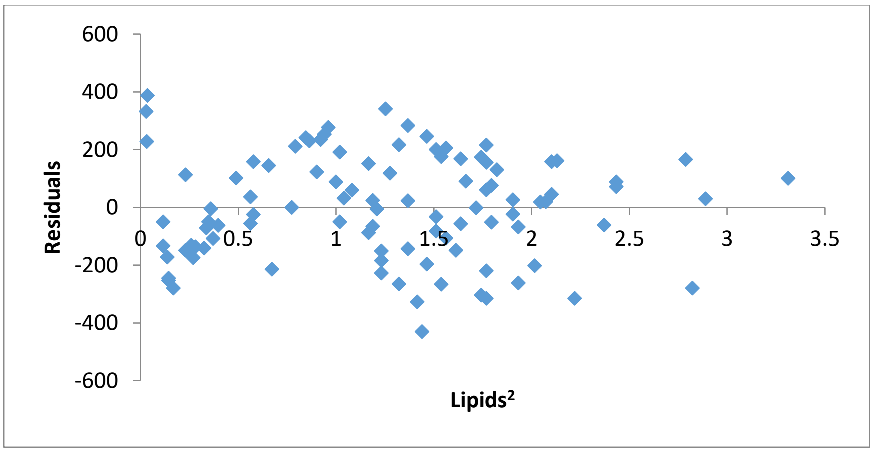
Figure 8.1. Fitted plot of response variable to lipids, Lipids^2, proteins, and carbohydrate

The residual plot was used to determine whether the model adequately meets the assumptions of the model. As the patterns in the points show, the points on the residual plots are randomly on both side of 0 which indicate that the model meets the model assumptions.

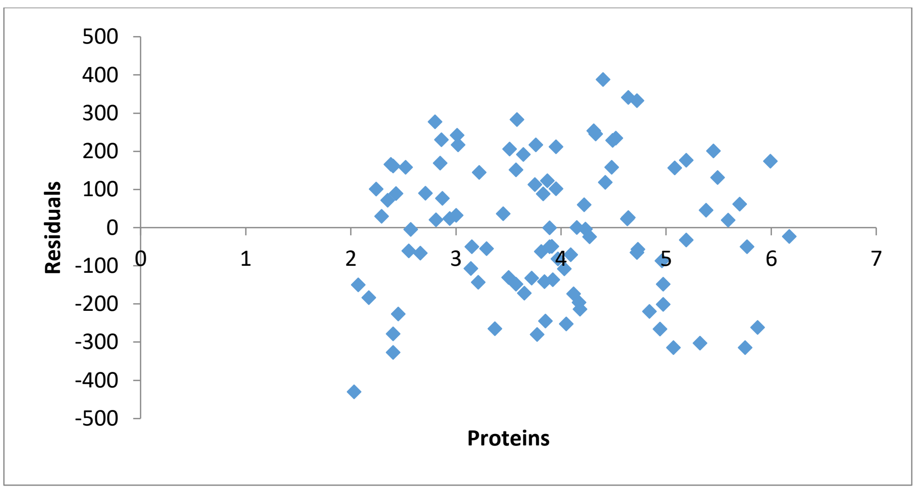
8.2. a)


600
400
200
0
-200
-400
-600
Residuals
0
0.5
1
1.5
2
Lipids

8.2. b)


600
400
200
0
-200
-400
-600
Residuals
0
0.5
1
1.5
2
2.5
3
3.5
Lipids²

8.2. c)


500
400
300
200
100
0
-100
-200
-300
-400
-500
Residuals
0
1
2
3
4
5
6
7
Proteins

8.2.d.)

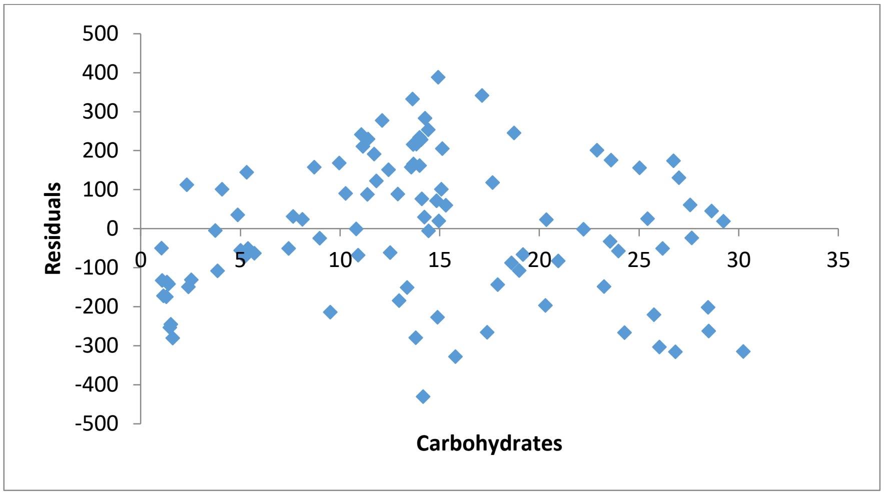


Figure 8.2. Residuals versus fits plot (residuals versus estimated response)

Table 8.1 includes the intercept, linear and quadratic coefficients of the Eq. 8.1 and summarizes some of the results of ANOVA analysis. The P-values < 0.05 as shown in the table verifies that the correlation between the response and the variables is statistically significant. The findings suggest that the amount of lipids in substrate is directly proportional to the methane yield and its impact is significantly more than carbohydrates. While the lower fraction of proteins than lipids and carbohydrates increase the methane yield. Y. Li et al, 2017 also used a second-order polynomial model as a quick estimation of AD parameters, however their model only included proteins and lipids as variables. Their findings though verified that the more fraction of lipids significantly increases the methane production compared to proteins.

Table 8.1.Coefficients of variables in the second-order polynomial model and the results of ANOVA analysis

	Coefficients	**t Stat**	**P-value**	**Lower 95%**	**Upper 95%**	**Lower 95.0%**	**Upper 95.0%**
Intercept	825.279203	5.12631511	1.576E-06	505.632211	1144.926	505.6322	1144.926
Lipids	862.890011	3.63241266	0.0004573	391.223242	1334.557	391.2232	1334.557
Lipids^2	-380.17074	-3.2738253	0.0014856	-610.738303	-149.603	-610.738	-149.603
Proteins	-117.12258	-3.7852707	0.0002703	-178.557985	-55.6872	-178.558	-55.6872
Carbs	18.4329527	3.36910525	0.0010949	7.56981336	29.29609	7.569813	29.29609

8.2- Statistical Analysis

Table 8.2 shows the experimental set-ups and the corresponding feedstock ratios and lipids: proteins: carbohydrates ratios. ANOVA was carried out for the CH_4 yields in response to the feedstock ratios and lipids: proteins: carbohydrates ratios. The main effect plot for CH_4 yield data mean is presented in Figure 8.3. The main effect plot shows the mean response of each level factors connected by the line. The steeper slope in the line explains the greater scale of the main effect. An effect is the variation in the methane yield when the factors i.e. the feedstock and lipids: proteins: carbohydrates ratios change from one level to another. The P value from the ANOVA results showed that the feedstock and lipids: proteins: carbohydrates ratios have significant effects on the CH_4 yield.

Table 8.2. Experimental set-ups and the corresponding ratios of the feedstock and lipids: proteins: carbohydrates

Experiment code	Feedstock ratios	Feedstock ratios code	Lipids: Proteins: Carbs
A	TWAS Only	AA	1:11:3
A	SSO Only	BB	1:1.3:8
A	TWAS/SSO 9/1	A	1:7:5
A	TWAS /SSO 7/3	B	1:4:7
A	TWAS /SSO 1/1	C	1:3:7
A	TWAS /SSO 3/7	D	1:2:8
A	TWAS /SSO 1/9	E	1:1.5:8
B	TWAS	AA	1:7:2.5
B	Manure	CC	1:4:20
B	TWAS / Manure 9/1	A	1:7:6
B	TWAS / Manure 7/3	B	1:5.5:12
B	TWAS / Manure 1/1	C	1:25:78
B	TWAS / Manure 3/7	D	1:4:17
B	TWAS / Manure 1/9	E	1:4:19
C	Manure	CC	1:4.2:21
C	SSO	BB	1:2:12
C	Manure/SSO 9/1	A	1:3.5:18.5
C	Manure /SSO 7/3	B	1:4:20
C	Manure /SSO 5/5	C	1:3:17
C	Manure /SSO 3/7	D	1:2.7:15
C	Manure /SSO 1/9	E	1:2:13

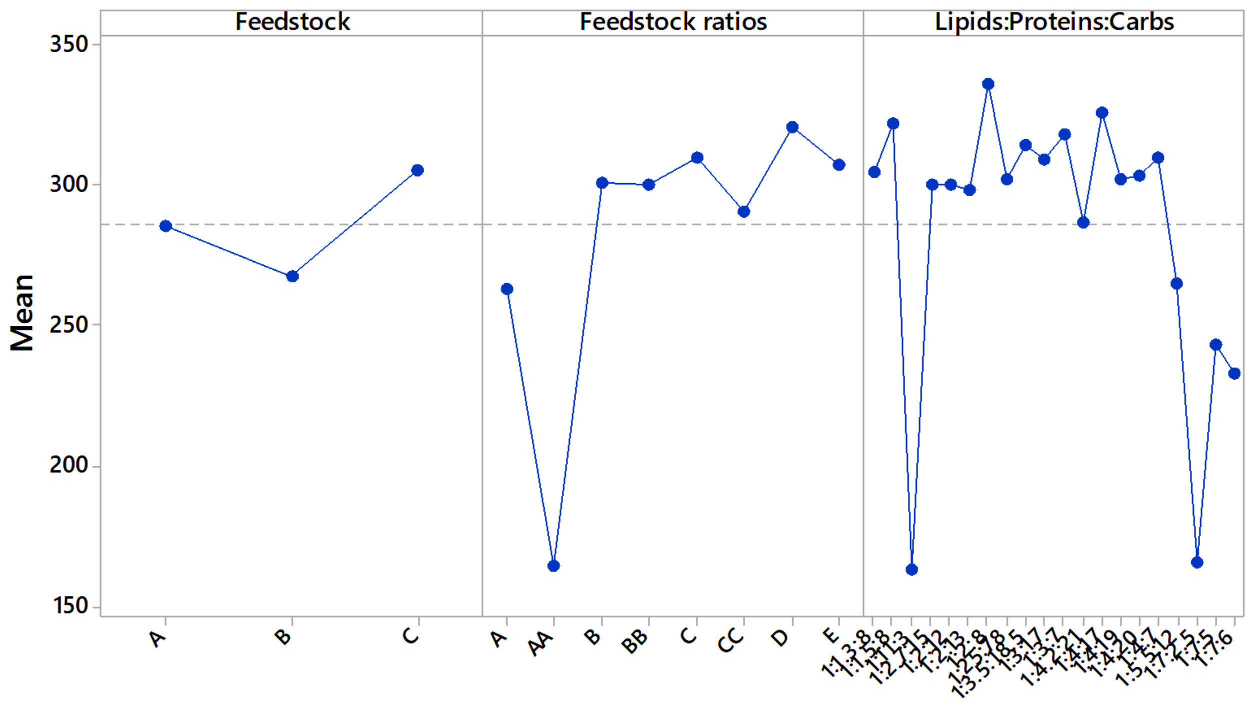


Figure 8.3. a. Main effect plot for CH_4 yield data mean

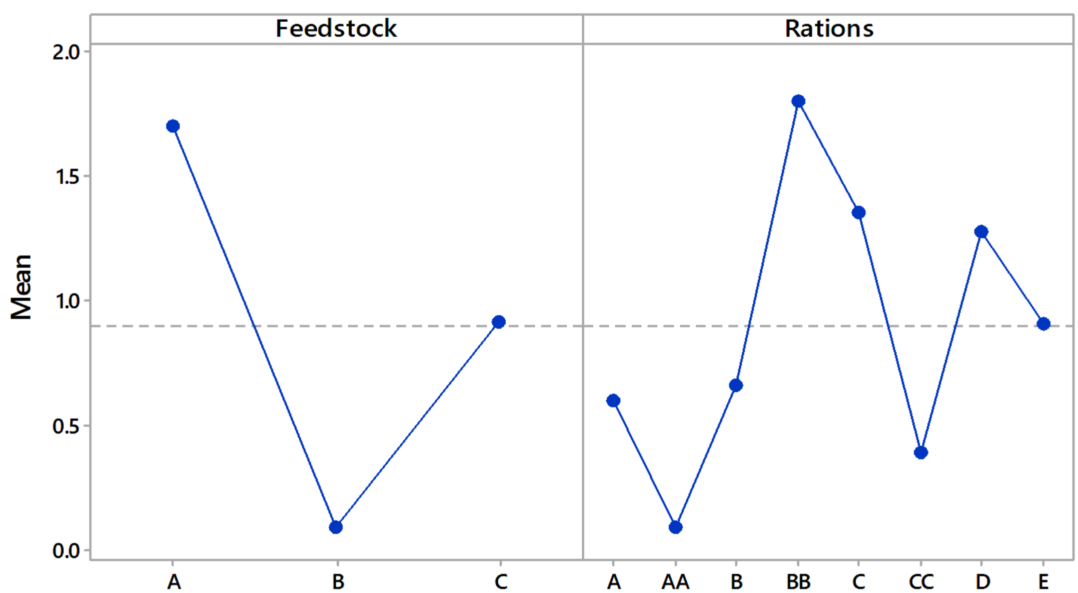


Figure 8.3. b. Main effect plot for CH_4 yield data mean for the lag phase data mean

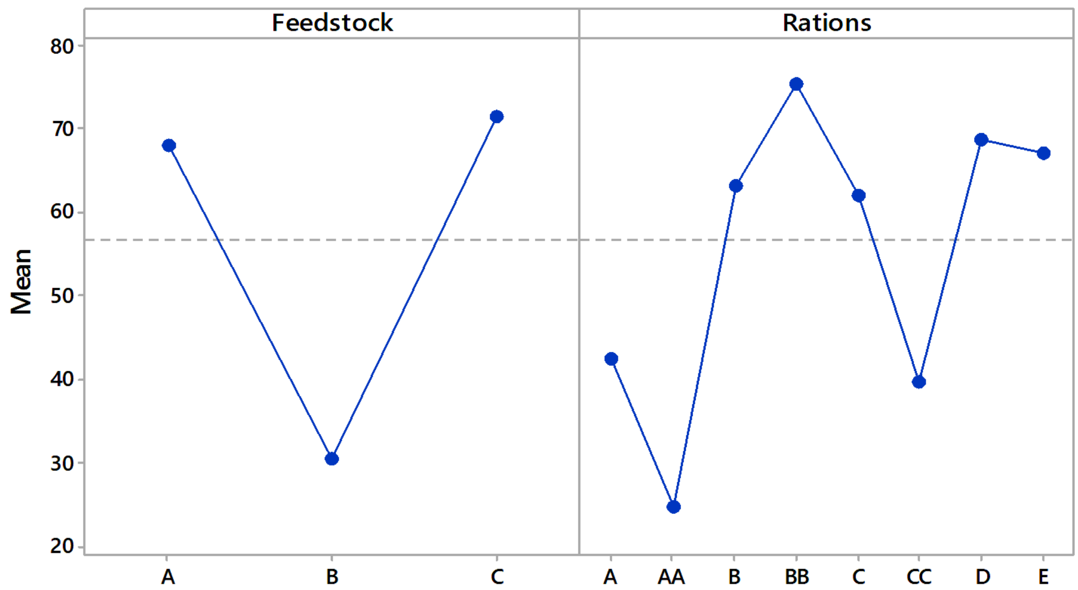


Figure 8.3. c. Main effect plot for CH_4 yield data mean for the CH_4 maximum rate data mean

The interval plot of CH_4 yield with 95% confidence interval for the mean using individual standard deviations to calculate the intervals is shown in Figure 8.4. The width of the interval plot represents the extent of variation in the data. A small interval would indicate more consistent data and less variation. As shown in Figure 8.4 the variation is more for some ratios than the other ones. The variation in the methane yield for the feedstock ratios of 1:9 and 7:3 were more than the other feedstock mixing ratios. The data was quite consistent with that of SSO alone. As shown in Figure 8.5.a, all of the intervals include 0 which means that the corresponding CH_4 yield data means are not significantly different in response to the feedstocks. However, according to Figure 8.5.b., for some of the feedstock mixing ratios, the corresponding means are significantly different.

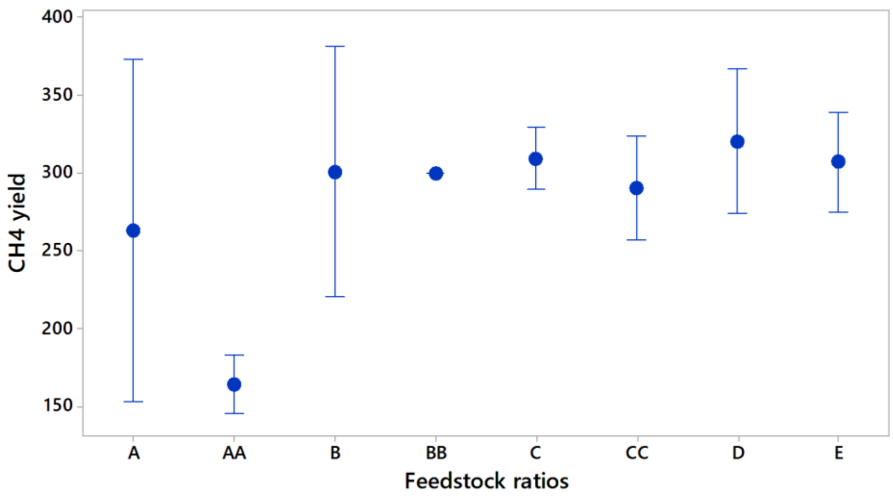


Figure 8.4. Interval plot of CH_4 yield with 95% confidence interval for the mean

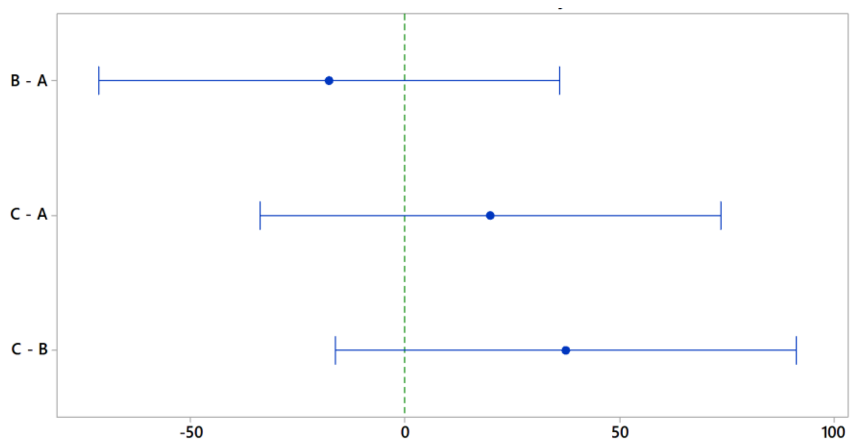


Figure 8.5. a. Fisher individual 95% confidence interval differences of means for CH_4 yields in response to the feedstocks

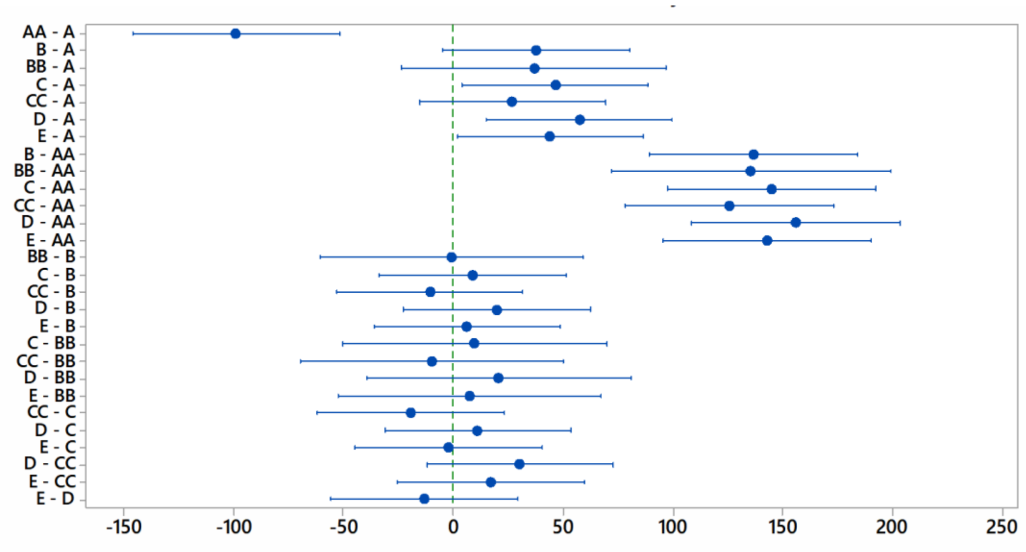


Figure 8.5. b. Fisher individual 95% confidence interval differences of means for CH_4 yields in response to the ratios

Chapter 9

Conclusions

9. Conclusions

9.1. Biomethane potential

A series of binary and ternary anaerobic co-digestion was designed to evaluate the biomethane potential and to investigate the correlation between the biomethane production and the mixing ratios as well as lipids: proteins: carbohydrates ratios of the feedstock. Gompertz model was used to assess the compatibility of the model to the data obtained by the experiment. A first order kinetic model was applied using AquaSim 2.0 to assess the hydrolysis kinetics and its influence on the process. A simple empirical model was derived based on the correlation of biomethane production to the lipids, proteins and carbohydrates content of the feedstocks which included TWAS, manure and SSO.

9.1.1. Binary co-digestion of TWAS, manure, and SSO

- The three sets of the binary co-digestion primarily verified the advantages of co-digestion over mono-digestion of TWAS, manure and SSO.
- Among the three binary co-digestion including TWAS/SSO, TWAS/manure, and Manure/SSO, the most amount of the methane yield was achieved by co-digestion of Manure/SSO at the mixing ratio of 7:3 corresponding to 363 mL CH_4/g COD added.
- The modified Gompertz model showed a good fit to the experimental data by a diversion ranging from 4% to 6%.
- The optimum ratios of the feedstocks as well as the lipids: proteins: carbohydrates varied by the different feedstocks.
- In co-digestion of TWAS/SSO the maximum ultimate CH_4 of 1252 mL and CH_4 yield of 357 mL CH_4/g COD added corresponded to the mixing ratio of 3:7 and lipids: proteins: carbohydrates ratio of 1:2:8.
- TWAS/manure co-digestion resulted in 1069 mL ultimate CH_4 production and 324 mL CH_4/g COD added biomethane yield at the mixing ratio of 3:7 and lipids: proteins: carbohydrates ratio of 1:4:17.
- For Manure/SSO co-digestion an ultimate CH_4 of 1186 mL and a CH_4 yield of 363 mL CH_4/g COD added was achieved corresponding to the mixing ratio of 7:3 and lipids: proteins: carbohydrate ratio of 1: 3.5: 18.5.

9.1.2. Ternary co-digestion of TWAS, manure and SSO

- Among co-digestion of the three feedstocks at different ternary combinations, the most ultimate methane production and yield occurred at TWAS/manure/SSO mixing ratio of 2:4:4 and lipids: proteins: carbohydrate ratio of 1:3:12.
- The maximum methane production and yield in co-digestion of TWAS/manure/SSO were 1424 mL 356 mL CH_4/g COD added, respectively.
- The modified Gompertz model showed a good fit to the results obtained by the experiment with less than 5% diversion.
- A higher maximum ultimate methane and methane yield was achieved by ternary co-digestion of TWAS/manure and SSO, however at some ratios the ternary co-digestion did not produce higher methane than some of the combinations in the binary co-digestion.
- Synergistic effect did not demonstrate further improvement in the ternary co-digestion of TWAS, manure, and SSO compared to their combinations in the binary co-digestion.
- The most synergistic effect in co-digestion of TWAS/manure/SSO was 19% while the maximum synergistic effect was 36 % in manure/SSO co-digestion.

9.2. Hydrolysis/acidification

Along with the biomethane potential assay, a series of hydrolysis/ acidification of the binary and ternary combinations of the feedstocks was designed to evaluate the hydrolysis and acidification process in co-digestion of TWAS, manure, and SSO. The binary and ternary anaerobic co-digestion were set up with the same combination as the BMP experiments for investigating the correlation between the solublization, VFAs yield, and hydrolysis rate, with the mixing ratios as well as lipids: proteins: carbohydrates ratios of the feedstock. First-order kinetic model was used to assess the compatibility of the model to the data obtained by the experiment. The model was used to obtain the kinetic rare coefficients of COD, lipids, proteins and carbohydrates of the feedstocks at different mixing ratios.

9.2.1. Co-digestion of TWAS/SSO

- An improvement of hydrolysis/acidification was observed in co-digestion of the feedstocks at different mixing ratios
- In TWAS/SSO co-digestion the maximum degree of solublization was 30% at the mixing ratio of 1:9.
- The synergistic effect was the highest at TWAS/SSO mixing ratio of 7:3 corresponding to 44% improvement as a result of using co-substrate and improving synergy.
- A maximum VFAs yield of 312 mg/ g VSS added was attained by TWAS/SSO co-digestion at the mixing ratio of 1:9.
- The hydrolysis kinetic rate coefficient was compatible to the first order kinetic model with a diversion less than 10%.
- The maximum hydrolysis rate coefficient of COD, proteins, and carbohydrates in co-digestion of TWAS/SSO were 0.35, 0.07, 0.34 and 0.68 d^{-1}, corresponding to the mixing ratios of 1:9, 3:7, 1:9, respectively.
- The maximum hydrolysis rate coefficient of lipids was 0.07 which corresponded to the TWAS:SSO mixing ratios of 5:5 and 3:7.

9.2.2. Co-digestion of TWAS/manure

- The percentage improvement of hydrolysis/acidification varied by different mixing ratios of TWAS and manure.
- In co-digestion of TWAS/manure, the maximum degree of solublization was 34% corresponding to the TWAS/manure mixing ratio of 3:7.
- A synergistic effect of 38% was achieved by TWAS/manure co-digestion at the mixing ratio of 3:7.
- The maximum VFAs yield of 507 mg/ g VSS added was achieved by TWAS/manure mixing ratio of 3:7.
- The hydrolysis kinetic rate coefficient was compatible to the first order kinetic model with a diversion less than 10%.

- The maximum hydrolysis rate coefficient of COD, lipids, proteins, and carbohydrates in co-digestion of TWAS/manure were 0.33, 0.09, 0.27, and 0.59 d^{-1}, corresponding to the mixing ratio of 3:7.

9.2.3. Co-digestion of manure /SSO

- The hydrolysis/acidification improved to different extents in response to the different mixing ratios in co-digestion of TWAS and manure.
- The most degree of solublization was 35% corresponding to manure/SSO mixing ratios of 3:7 and 1:9.
- The highest synergistic effect of 34% in co-digestion of manure and SSO occurred at the mixing ratio of 9:1.
- In manure/SSO co-digestion, the maximum VFAs yield of 400 mg/ g VSS added was achieved at the mixing ratio of 1:9.
- The hydrolysis kinetic rate coefficient was compatible to the first order kinetic model with a diversion less than 10%
- The maximum hydrolysis rate coefficient of COD, lipids, proteins, and carbohydrates in co-digestion of manure/SSO were 0.38, 0.11, 0.33, and 0.68 d -1, corresponding to the mixing ratio of 3:7.

9.2.4. Co-digestion of TWAS/manure /SSO

- In the ternary co-digestion of TWAS, manure, and SSO the maximum solublization was 30% corresponding to TWAS/manure/SSO mixing ratio of 2:4:4 and 1:8:1.
- The maximum synergistic effect of 35% corresponded to TWAS/manure/SSO mixing ratio of 1:8:1.
- In co-digestion of TWAS/manure/SSO the highest VFAs yield of 380 mg VFAs/g VSS added was achieved at the mixing ratio of 2:4:4.
- The hydrolysis kinetic rate coefficient was compatible to the first order kinetic model with a diversion less than 10%
- The hydrolysis rate coefficient of COD were within the range of 0.21 to 0.39 for all of the co-digesters.

- The maximum hydrolysis rate coefficients of COD, lipids, and carbohydrates in co-digestion of TWAS/manure/SSO were 0.39, 0.14, and 0.69 d^{-1} corresponding to the mixing ratio of 2:4:4.
- In co-digestion of TWAS/manure/SSO, the maximum hydrolysis rate coefficient for the proteins content was 0.23 which corresponded to the mixing ratios of 8:1:1 and 2.5:5:2.5.

9.3 Correlation of biomethane production with the organic content

9.3.1. COD:N and lipids:proteinscarbohydrates ratios

- In TWAS/SSO co-digestion the maximum ultimate CH_4 corresponded to COD:N ratio of 28 corresponding to the lipids: proteins: carbohydrates ratio of 1:2:8. The trend of variations of the methane yield versus COD:N was similar to the trend that was observed for its variation vursus lipids:proteins. The changes of methane yield versus lipids:carbohydrates and proteins:carbohydrates showed a similar trend while it did not comply with that of COD:N ratio.
- In TWAS/manure co-digestion, the maximum CH_4 yield occurred at COD:N ratio of 41 corresponding to the lipids: proteins: carbohydrates ratio of 1:4:17. Similar to the co-digestion of TWAS/SSO, in TWAS/manure co-digestion the trend of variations of the methane yield versus COD:N was similar to the trend that was observed for its variation versus lipids:proteins. The changes of methane yield versus lipids:carbohydrates and proteins:carbohydrates did not comply with that of COD:N ratio although they both showed a simiar trend.
- In Manure/SSO co-digestion, the maximum methane yield corresponded to the COD:N ratio of 41 and lipids: proteins: carbohydrates ratio of 1:3.5:18.5. The variations of methane yield versus COD:N and lipids: proteins did not show a similar trend. Although, in terms of the changes of the CH_4 yield, both lipids: carbohydrates and proteins: carbohydrates ratios demonstrated similar trend.
- In TWAS/manure/SSO co-digestion the maximum methane yield corresponded to COD:N tatio of 28 and the lipids:proteins:carbohydrates ratio of 1:3:12. The trend of variations of the methane yield vursus COD:N was similar to the trend that was observed for its variation versus lipids:proteins. The changes of methane yield vursus lipids:carbohydrates and

proteins:carbohydrates showed a similar trend while it did not comply with that of COD:N. TWAS/manure/SSO co-digestion.

9.3.2. Anaerobic co-digestion model

An empirical model would be a useful approach to predict the methane yield ($mLCH_4$/ $gCOD_{added}$) based on the lipids, proteins, and carbohydrates components of the feedstock in anaerobic co-digestion of multi substrate. As the three main components of any type of feedstocks in biowaste are lipids, proteins, and carbohydrates, an empirical model was presented that correlates the CH_4 yields with lipids, proteins and carbohydrates content of the feedstock. The functional relationship between responses which was CH_4 yield and the factors including lipids, proteins and carbohydrates (Lp, Pr, and Cr) were described by estimating the coefficients of the second-order polynomial model based on the experimental data. The fit plots together with the residual plots for lipids, proteins and carbohydrates showed that the model adequately fits to the data and meet the model assumption.

9.4. Suggested future works

- Validating the batch study results for AnCoD of Manure/SSO and TWAS/Manure/SSO at 7:3 and 2:4:4 mixing ratios in CSTR mode
- Investigating the validity of the proposed empirical model for a variety of the feedstocks including the industrial wastes
- Investigating the validity of the proposed empirical model using CSTR mode.
- Implementing a cost-benefit analysis for applying AnCoD of Manure/SSO and TWAS/Manure/SSO for GTA versus individual AD of manure and SSO.
- Analyzing carbon footprint reduction by AnCoD of manure with TWAS as compared to conventional AD and composting

Appendices

A. Analytical results for AnCoD of TWAS and SSO

Table A. 1. Characteristics of raw feedstocks in AnCoD of TWAS and SSO

Parameters	Units	SSO		TWAS		Seed	
		MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	110000	7795	40000	2053	16400	444
SCOD	mg/L	44400	100	1360	26	762	19
TSS	mg/L	53833	6252	31450	1006	15033	400
VSS	mg/L	38478	3649	25600	2216	10900	200
TS	mg/L	62187	1025	38810	1859	17450	582
VS	mg/L	43493	761	34477	1027	13140	328
Ammonia	mg/L	1138	6	255	9	795	40
pH	-	5.6	0.2	6.3	0.2	7.2	0.2
Alkalinity	mg CaCO3/L	6080	522	1953	148	4943	465
TN	mg/L	3267	751	2900	400	1425	203
TSN	mg/L	910	38	420	64	696	112
Total Carbs	mg/L	14360	3580	1080	112	549	82
Total Proteins	mg/L	2308	198	3759	298	1648	56
Total Lipids	mg/L	1731	2420	351	27	163	52

Table A. 2. Average CH_4 production in AnCoD of TWAS and SSO

Average CH_4 Measurement (mL)													
Time (day)	TWAS	SD	SSO	SD	T/SSO 9/1	SD	T/SSO 7/3	SD	T/SSO 1/1	SD	T/SSO 1/9	SD	T/SSO 3/7
1	6	0.3	3	0.1	6	0.3	7	0.3	3	0.1	4	0.1	3
3	24	1.2	20	1.0	20	1.0	33	1.7	17	0.7	27	1.0	20
4	43	2.3	27	1.3	35	1.7	54	2.8	25	1.1	49	1.9	38
5	58	3.0	42	2.0	57	2.8	68	3.5	39	1.7	82	3.1	65
6	39	2.0	48	2.3	44	2.2	51	2.6	39	1.6	60	2.3	76
7	37	1.9	48	2.3	51	2.5	45	2.3	66	2.8	52	2.0	69
8	21	1.1	51	2.5	34	1.7	42	2.2	53	2.2	45	1.7	78
10	28	1.4	55	2.7	34	1.7	60	3.0	84	3.5	80	3.0	93
12	26	1.4	51	2.5	25	1.2	29	1.5	81	3.4	74	2.8	83
14	21	1.1	39	1.9	22	1.1	22	1.1	47	2.0	33	1.2	64
16	19	1.0	25	1.2	15	0.7	23	1.2	21	0.9	20	0.8	20
19	23	1.2	23	1.1	25	1.2	25	1.3	21	0.9	25	1.0	22
22	18	0.9	24	1.2	15	0.8	17	0.9	24	1.0	26	1.0	30
26	15	0.8	20	1.0	10	0.5	12	0.6	14	0.6	15	0.6	22
29	10	0.5	14	0.7	8	0.4	10	0.5	9	0.4	12	0.5	17
34	33	1.7	33	1.6	32	1.6	33	1.7	31	1.3	31	1.2	31
37	13	0.7	12	0.6	14	0.7	13	0.7	9	0.4	12	0.4	14
40	14	0.7	13	0.6	14	0.7	13	0.7	10	0.4	12	0.5	15
43	14	0.7	13	0.6	13	0.6	11	0.6	9	0.4	11	0.4	10
49	1	0.1	3	0.2	5	0.2	3	0.2	1	0.1	2	0.1	5
56	0	0.0	0	0.0	7	0.3	5	0.3	4	0.2	6	0.2	4

Table A. 3. Cumulative CH_4 production in AnCoD of TWAS and SSO

	Cumulative CH_4 Production (mL)													
Time (day)	TWAS	SD	SSO	SD	T/SSO 9/1	SD	T/SSO 7/3	SD	T/SSO 1/1	SD	T/SSO 1/9	SD	T/SSO 3/7	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
1	7	0	5	0	9	0	12	1	6	0	7	0	4	0.2
3	35	2	46	2	39	2	70	3	37	2	56	3	36	1.4
4	86	4	99	5	92	5	164	8	83	4	146	8	96	3.8
5	154	8	182	8	177	8	284	14	154	8	296	16	200	8.0
6	199	10	278	13	243	13	373	18	225	11	405	22	323	12.9
7	242	12	373	17	319	17	452	22	345	18	500	27	435	17.4
8	267	13	475	22	369	22	527	26	441	23	582	31	560	22.3
10	299	15	583	27	419	27	631	31	593	30	727	39	710	28.3
12	330	16	685	31	457	31	683	34	739	38	862	46	843	34.5
14	354	17	762	35	489	35	721	35	823	42	922	49	946	37.8
16	377	18	813	37	511	37	761	37	862	44	958	51	979	39.1
19	404	20	859	39	548	39	804	40	899	46	1004	54	1014	40.5
22	425	21	906	42	571	42	834	41	944	48	1052	56	1062	42.4
26	443	22	946	43	586	43	856	42	968	49	1080	58	1098	43.8
29	455	22	974	45	598	45	873	43	985	50	1102	59	1124	44.9
34	493	24	1040	48	645	48	930	46	1040	53	1159	62	1174	46.8
37	508	25	1064	49	666	49	953	47	1057	54	1180	63	1196	47.7
40	524	26	1089	50	686	50	976	48	1075	55	1202	64	1221	48.7
43	541	26	1115	51	705	51	996	45	1091	56	1222	65	1237	45.4
49	542	26	1122	48	712	49	1001	49	1094	55	1225	62	1245	49.7
56	542	26	1122	47	723	50	1010	50	1100	57	1235	66	1252	50.0

Table A. 4. Cumulative methane yield per unit mass of COD added in AnCoD of TWAS and SSO

					Cumulative CH_4 yield (mLCH$_4$/COD added)									
Time (day)	TWAS	SD	SSO	SD	T/SSO 9/1	SD	T/SSO 7/3	SD	T/SSO 1/1	SD	T/SSO 3/7	SD	T/SSO 1/9	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	2	0.1	1	0.1	3	0.2	4	0.2	2	0.1	2	0.1	1	0.1
3	12	0.6	13	0.7	13	0.6	22	1.1	11	0.5	16	0.9	10	0.4
4	30	1.6	27	1.5	31	1.5	51	2.5	25	1.1	42	2.3	27	1.2
5	55	2.8	50	2.7	59	2.9	88	4.3	46	2.1	84	4.6	56	2.4
6	71	3.7	76	4.1	81	4.0	116	5.7	66	3.0	116	6.4	90	3.9
7	86	4.5	103	5.5	107	5.2	141	6.9	102	4.6	143	7.9	121	5.2
8	94	4.9	130	7.0	124	6.1	164	8.0	131	5.9	166	9.1	156	6.7
10	106	5.5	160	8.7	141	6.9	197	9.6	175	7.9	208	11.4	197	8.5
12	117	6.1	188	10.2	153	7.5	213	10.4	219	9.8	246	13.5	234	10.1
14	126	6.5	209	11.3	164	8.0	224	11.0	244	11.0	263	14.5	263	10.5
16	133	6.9	223	12.1	172	8.4	237	11.6	255	11.5	273	15.0	272	11.7
19	143	7.4	236	12.7	184	9.0	250	12.3	266	12.0	287	15.8	282	12.1
22	151	7.8	249	13.4	192	9.4	260	12.7	279	12.6	300	16.5	295	11.3
26	157	8.2	260	13.8	197	9.6	266	13.1	287	12.9	308	17.0	305	13.1
29	161	8.4	268	14.5	201	9.8	272	13.3	292	13.1	315	17.3	312	13.7
34	175	9.1	286	15.4	217	10.6	290	14.2	308	13.9	331	18.2	326	14.0
37	180	9.4	292	15.8	223	11.0	297	14.5	313	14.1	337	18.5	332	14.3
40	186	9.7	299	14.8	230	10.3	304	14.9	318	12.7	343	18.9	339	13.5
43	192	10.2	306	16.5	237	12.2	310	16.1	323	14.5	349	17.2	344	14.8
49	192	9.8	308	15.5	239	11.7	312	15.3	324	13.2	350	16.3	346	15.2
56	192	9.5	308	16.2	243	10.6	315	14.4	326	14.7	353	15.8	348	14.4

Table A. 5. Cumulative methane yield per unit mass of VSS added in AnCoD of TWAS and SSO

Cumulative CH_4 yield (mLCH$_4$/VSS added)														
Time (day)	TWAS	SD	SSO	SD	T/SSO 9/1	SD	T/SSO 7/3	SD	T/SSO 1/1	SD	T/SSO 3/7	SD	T/SSO 1/9	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	4	0.2	1	0.0	5	0.3	8	0.4	4	0.2	5	0.3	3	0.2
3	19	0.9	10	0.4	23	1.2	45	2.2	26	1.3	41	2.0	28	1.3
4	47	2.3	21	0.8	54	2.8	106	5.2	58	2.8	107	5.2	74	3.6
5	85	4.1	39	1.6	104	5.4	183	9.0	107	5.2	217	10.6	154	7.6
6	110	5.4	60	2.4	142	7.4	240	11.9	156	7.6	297	14.6	249	12.2
7	134	6.5	81	3.2	187	9.7	291	14.4	239	11.6	367	18.0	335	16.4
8	148	7.2	102	4.1	216	11.3	339	16.8	306	14.9	427	20.9	431	21.1
10	165	8.0	126	5.0	246	12.8	407	20.1	411	20.0	534	26.2	546	26.8
12	182	8.9	148	5.9	268	14.0	440	21.8	512	24.9	633	31.0	649	31.8
14	196	9.5	165	6.6	287	15.0	465	23.0	571	27.8	677	33.2	728	35.7
16	208	10.1	175	7.0	300	15.6	490	24.3	597	29.1	703	34.5	753	36.9
19	224	10.9	185	7.4	322	16.8	518	25.7	623	30.4	737	36.1	781	38.2
22	235	11.4	196	7.8	335	17.5	538	26.6	654	31.8	772	37.8	817	40.0
26	245	11.9	204	8.1	344	17.9	552	26.5	671	32.7	793	38.8	845	41.4
29	252	12.2	210	8.4	351	18.3	563	27.8	683	33.2	809	39.7	865	42.4
34	273	13.3	225	8.9	379	19.7	600	29.7	721	35.1	851	41.7	903	44.3
37	281	13.7	230	9.1	391	20.4	615	30.4	732	35.7	867	42.5	921	45.1
40	290	15.4	235	9.4	403	21.0	629	32.3	745	36.3	883	43.3	940	46.1
43	299	14.6	241	10.0	414	20.2	642	31.8	756	36.8	897	43.2	952	46.7
49	300	13.8	242	9.9	418	19.8	645	30.5	758	35.4	899	45.4	958	45.1
56	300	15.1	242	9.2	424	21.6	651	32.2	762	37.1	907	44.4	964	47.2

Table A. 6. Cumulative methane yield per unit volume of VSS added in AnCoD of TWAS and SSO

Cumulative CH_4 yield ($mLCH_4$/mL substrate added)														
Time (day)	TWAS	SD	SSO	SD	T/SSO 9/1	SD	T/SSO 7/3	SD	T/SSO 1/1	SD	T/SSO 3/7	SD	T/SSO 1/9	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.10	0.00	0.15	0.01	0.14	0.01	0.22	0.01	0.14	0.01	0.18	0.01	0.12	0.00
3	0.49	0.02	1.38	0.07	0.62	0.03	1.32	0.06	0.83	0.04	1.42	0.07	1.02	0.04
4	1.22	0.05	2.99	0.14	1.45	0.08	3.12	0.15	1.84	0.09	3.70	0.18	2.76	0.10
5	2.18	0.09	5.51	0.27	2.79	0.15	5.38	0.26	3.43	0.17	7.52	0.36	5.74	0.22
6	2.82	0.12	8.40	0.41	3.83	0.21	7.08	0.34	4.99	0.25	10.29	0.49	9.26	0.35
7	3.43	0.14	11.28	0.55	5.03	0.27	8.59	0.41	7.65	0.39	12.72	0.61	12.45	0.47
8	3.78	0.16	14.34	0.70	5.82	0.32	10.00	0.48	9.80	0.50	14.79	0.71	16.02	0.61
10	4.24	0.18	17.63	0.85	6.62	0.36	11.99	0.58	13.16	0.67	18.48	0.88	20.31	0.77
12	4.67	0.20	20.72	1.00	7.21	0.39	12.97	0.62	16.40	0.84	21.90	1.05	24.12	0.92
14	5.02	0.21	23.04	1.12	7.72	0.42	13.69	0.66	18.28	0.93	23.42	1.12	27.08	1.03
16	5.34	0.22	24.57	1.19	8.07	0.44	14.45	0.69	19.14	0.98	24.34	1.16	28.01	1.06
19	5.72	0.24	25.95	1.26	8.65	0.47	15.27	0.73	19.97	1.02	25.51	1.22	29.03	1.10
22	6.02	0.25	27.39	1.33	9.01	0.49	15.84	0.76	20.95	1.07	26.73	1.28	30.39	1.15
26	6.27	0.26	28.60	1.39	9.25	0.51	16.25	0.78	21.50	1.10	27.44	1.31	31.42	1.19
29	6.45	0.27	29.44	1.43	9.43	0.51	16.57	0.80	21.87	1.12	28.02	1.34	32.18	1.22
34	6.99	0.29	31.44	1.52	10.18	0.56	17.67	0.85	23.10	1.18	29.45	1.41	33.60	1.28
37	7.20	0.30	32.15	1.56	10.50	0.57	18.11	0.87	23.47	1.20	30.00	1.48	34.24	1.30
40	7.43	0.31	32.93	1.60	10.83	0.59	18.53	0.89	23.86	1.22	30.56	1.46	34.95	1.33
43	7.67	0.29	33.70	1.63	11.13	0.61	18.91	0.78	24.23	1.24	31.06	1.48	35.42	1.45
49	7.68	0.22	33.91	1.56	11.24	0.52	19.01	0.88	24.28	1.35	31.13	1.39	35.64	1.25
56	7.68	0.32	33.91	1.70	11.40	0.65	19.18	0.92	24.42	1.25	31.39	1.50	35.84	1.36

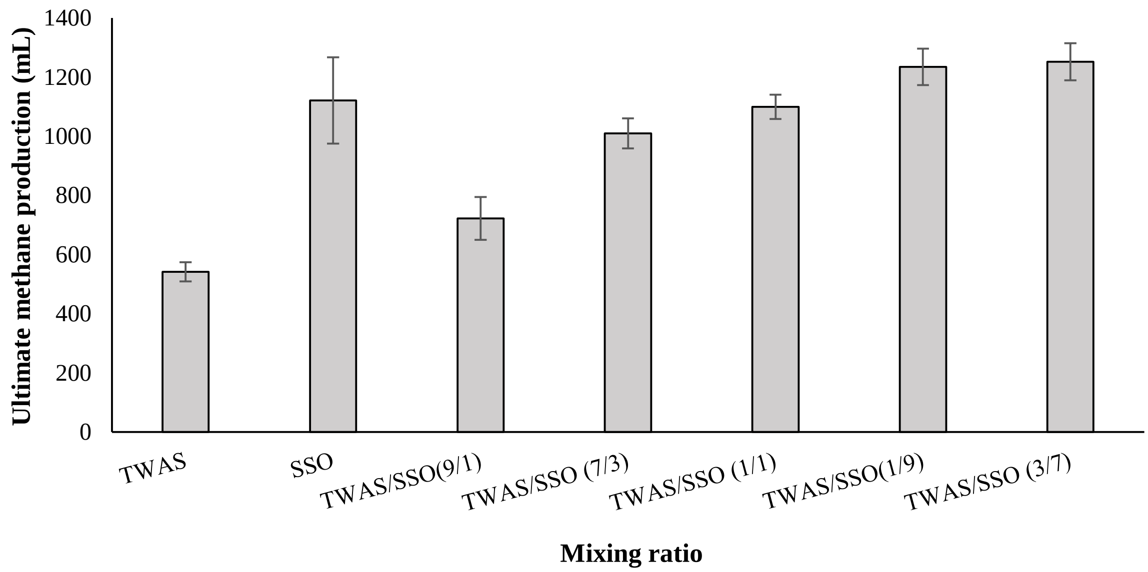


Figure A.1. Ultimate CH_4 production in AnCoD of TWAS and SSO

Table A. 7. Characteristics of raw feedstocks for hydrolysis/acidification in AnCoD of TWAS/SSO

Parameters	Units	SSO		TWAS		Seed	
		MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	110000	7795	40000	2853	16400	444
SCOD	mg/L	44400	100	2360	26	960	19
TSS	mg/L	53833	6252	31450	1006	17033	400
VSS	mg/L	36030	3649	18460	2216	10900	200
TS	mg/L	62187	1025	38810	6859	21450	582
VS	mg/L	43493	761	34477	27	13140	328
Ammonia	mg/L	1138	6	255	30	1495	40
pH	-	5.6	0.006	6.3	0.03	7.2	0.1
Alkalinity	mg CaCO3/L	6800	522	1953	148	3943	465
TN	mg/L	3967	751	2900	400	2025	203
TSN	mg/L	1055	38	420	64	696	112
Total Carbs	mg/L	13495	3580	923	112	545	82
Total Proteins	mg/L	2087	198	2769	298	1635	56
Total Lipids	mg/L	1103	2420	289	27	164	52

Table A. 8. Soluble and particulate COD concentrations (mg/L) over time

Soluble COD concentrations (mg/L) over time							
Time	T/SSO 7/3	T/SSO 1/1	T/SSO 3/7	T/SSO 1/9	T/SSO 9/1	SSO Only	TWAS Only
0	4590	5527	6525	7318	2281	7630	1905
2	4698	6087	6959	7857	2529	8123	2254
4	5036	6527	7233	8216	2727	8416	2377
6	5284	6767	7557	8655	3345	8689	2524
8	5802	7017	8011	8994	3503	9082	2548
10	5990	7487	8275	9353	3961	9355	2597
12	6198	7787	8599	9712	4209	9858	2769
24	6616	8047	8933	10221	4397	10261	2988
48	7534	8427	9337	10540	4715	11094	3087
72	7732	9067	9981	11079	5435	10817	3120

Particulate COD concentrations (mg/L) over time								
Time	T/SSO 7/3	T/SSO 1/1	T/SSO 3/7	T/SSO 1/9	T/SSO 9/1	SSO Only	TWAS Only	
0	24029	24473	24182	24237	23558	13979	23635	
2	23817	24256	23905	24105	23416	13717	22604	1
4	23681	23968	23495	23987	23203	13527	22518	
6	23432	22832	22843	23602	22825	13256	22418	
8	22948	22683	22747	23360	22635	12967	22325	
10	22629	22410	22684	23020	22438	12392	22223	
12	22379	22112	21558	22844	22289	12228	22125	
24	21256	21110	21222	21163	20870	11287	21897	
48	20684	20371	21021	20815	20376	10951	21581	
72	20484	20233	20574	20471	20154	10623	21508	

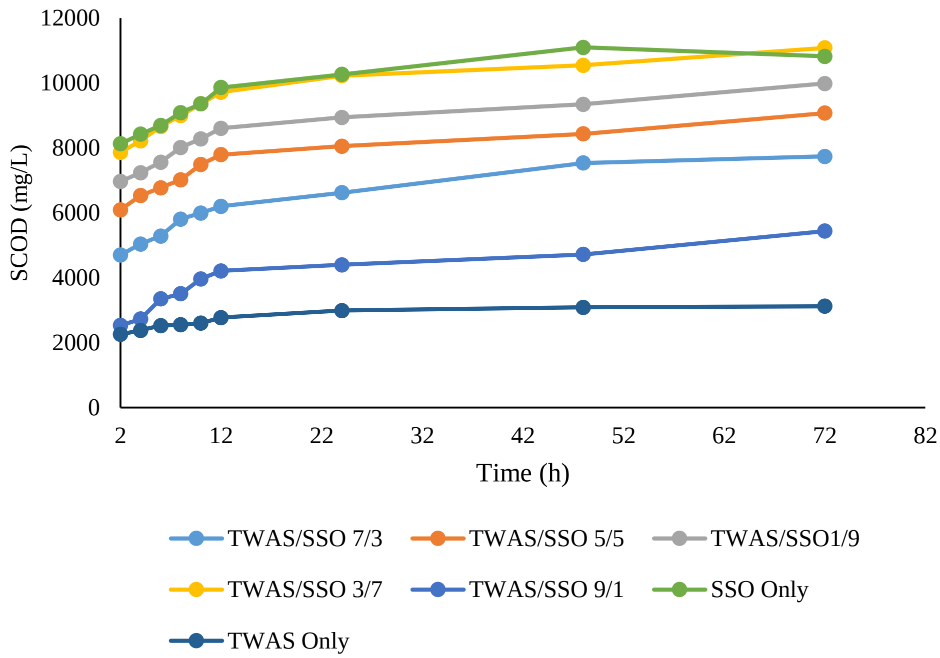


Figure A.2. Concentration of soluble COD over time for different mixing ratios

B. Analytical results for AnCoD of TWAS and manure

Table B.1 Characteristics of raw feedstocks in AnCoD of TWAS and manure

Parameters	Units	Manure		TWAS		Inoculum	
		MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	122833	19553	45600	500	17167	306
SCOD	mg/L	8933	503	2020	265	1250	173
TSS	mg/L	79920	19060	36150	538	15580	459
VSS	mg/L	76998	1789	26710	349	10313	284
TS	mg/L	98727	1035	38053	1832	16590	302
VS	mg/L	85647	1123	27730	1092	10180	286
Ammonia	mg/L	22	3	317	20	497	68
pH	-	6.4	0.1	6.8	0.1	7	0.1
Alkalinity	mg CaCO3/L	5133	902	4820	452	5517	306
Total N	mg/L	2200	100	2883	278	2050	278
Total Soluble N	mg/L	104	15	410	22	747	15
Total Carbs	mg/L	27100	958	1322	104	508	23
Total Proteins	mg/L	5115	244	3958	276	1498	50
Total Lipids	mg/L	1355	54	536	44	205	8

Table B. 2. Cumulative CH_4 production in AnCoD of TWAS and manure

Cumulative methane production (mL)														
time (day)	TWAS	SD	Manure	SD	T/M 9/1	SD	T/M 7/3	SD	T/M 1/1	SD	T/M 3/7	SD	T/M 1/9	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	24	1	86	5	76	3	88	5	100	5	95	5	88	4.39
2	84	4	157	9	172	7	175	9	197	7	188	9	172	8.62
3	135	7	215	12	246	11	237	12	266	12	258	13	238	11.9
4	185	9	273	15	300	13	294	15	331	15	322	16	297	14.8
5	220	11	316	17	341	15	336	17	378	17	373	18	344	17.2
6	256	12	364	20	386	17	382	20	430	20	427	21	396	19.8
8	298	14	434	24	426	19	441	23	496	23	494	24	463	23.1
10	328	16	490	27	462	20	506	26	570	26	562	28	523	26.1
14	363	18	552	30	498	22	562	29	633	29	635	31	587	29.4
16	388	19	597	33	528	23	600	31	675	31	681	33	631	31.6
17	400	19	620	34	547	24	629	33	708	33	710	35	655	32.7
19	417	20	648	36	570	25	650	34	732	34	740	36	680	34
22	434	21	678	37	597	26	694	36	781	36	780	38	713	35.6
25	456	22	713	39	625	27	710	37	799	37	815	40	748	37.4
26	464	22	728	40	636	28	727	38	818	38	835	41	765	38.2
29	478	23	752	41	655	28	749	39	843	39	863	42	790	39.5
30	482	23	757	42	659	29	754	39	848	39	870	43	800	39
33	492	24	775	43	671	29	769	40	865	40	889	44	817	40.8
36	506	24	799	44	690	30	788	41	886	41	914	45	841	42.1
40	521	25	824	45	712	31	811	42	912	42	944	46	871	43.5
46	539	26	851	47	737	32	836	43	941	43	978	48	905	45.2
50	548	27	864	48	750	33	850	44	956	44	995	49	922	46.1
54	555	27	874	48	759	33	859	45	967	42	1007	49	935	46.7
58	559	27	881	48	766	33	866	42	975	45	1017	48	944	47.2
62	563	27	887	49	771	34	872	44	981	41	1023	51	951	45
65	566	27	893	49	776	34	877	46	987	43	1029	50	956	47.8
67	569	28	898	49	780	34	885	45	996	44	1035	52	961	42
72	578	28	907	50	791	34	893	46	1005	42	1049	48	979	48.9
74	584	28	915	50	801	33	903	47	1016	45	1059	49	992	43
78	590	29	922	51	811	35	902	43	1015	47	1069	51	1002	50.1

Table B. 3. Cumulative methane yield per unit mass of COD added in AnCoD of TWAS and manure

Cumulative methane yield (mL CH_4/g COD added)														
time (day)	TWAS	SD	Manure	SD	T/M 9/1	SD	T/M 7/3	SD	T/M 1/1	SD	T/M 3/7	SD	T/M 1/9	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	8	0.3	30	1.6	26	1.2	31	1.4	35	1.3	34	1.8	34	1.2
2	28	1.2	56	2.9	58	2.7	61	2.9	69	2.6	66	3.7	67	2.5
3	45	1.9	77	4.0	84	3.8	82	3.9	93	3.5	91	5.0	91	3.4
4	62	2.7	97	5.0	102	4.7	102	4.8	116	4.4	114	6.3	114	4.2
5	73	3.2	112	5.8	116	5.3	116	5.5	133	5.0	132	7.2	132	4.9
6	85	3.7	130	6.7	131	6.0	132	6.2	151	5.7	151	8.3	152	5.6
8	99	4.3	154	8.0	144	6.6	153	7.2	174	6.6	175	9.6	175	6.5
10	109	4.7	174	9.1	157	7.2	175	8.2	200	7.6	199	10.9	200	7.4
14	121	5.2	196	10.2	169	7.8	195	9.2	222	8.4	224	12.3	225	8.3
16	129	5.5	212	11.0	179	8.2	208	9.8	237	9.0	241	13.2	242	8.9
17	133	5.7	221	11.5	186	8.5	218	10.2	248	9.4	251	13.8	252	9.3
19	139	6.0	230	12.0	193	8.9	225	10.6	257	9.8	262	14.4	263	9.7
22	145	6.2	241	12.6	202	9.3	241	11.3	274	10.4	275	15.2	277	10.2
25	152	6.5	254	13.2	212	9.7	246	11.6	280	10.6	288	15.8	289	10.7
26	154	6.6	259	13.5	216	9.9	252	11.8	287	10.9	295	16.2	296	11.0
29	159	6.8	267	13.9	222	10.2	260	12.2	296	11.2	305	16.8	306	11.3
30	160	6.9	269	14.0	224	10.3	261	12.3	298	10.2	307	16.9	309	11.4
33	164	7.0	276	14.3	227	10.5	267	12.5	303	11.5	314	17.3	316	11.7
36	168	7.2	284	14.8	234	10.8	273	12.8	311	11.8	323	17.8	325	13.3
40	173	7.5	293	15.2	242	11.1	281	13.2	320	12.2	334	18.3	335	12.4
46	179	7.7	303	15.7	250	11.5	290	13.6	330	12.5	345	17.6	347	12.8
50	182	7.8	307	16.0	254	11.7	294	13.8	335	10.7	351	19.3	353	14.1
54	184	7.9	311	16.2	257	11.8	298	14.0	339	12.9	356	17.5	358	13.2
58	186	8.0	314	16.3	260	11.9	300	13.5	342	13.5	359	19.8	361	14.2
62	187	7.5	316	15.7	261	12.0	302	14.2	344	12.9	362	19.3	363	13.4
65	188	8.1	318	16.5	263	11.8	304	14.3	346	13.2	364	20.0	365	12.8
67	189	7.9	320	15.5	265	12.2	307	13.8	349	14.1	366	19.2	368	14.5
72	192	8.3	323	16.8	268	10.9	310	14.6	353	12.8	371	20.4	372	11.7
74	194	7.6	326	15.3	272	11.5	313	13.5	356	13.5	374	18.9	376	13.9
78	196	8.4	328	17.1	275	12.6	313	14.7	356	11.6	378	20.8	379	14.0

Table B. 4. Cumulative methane yield per unit mass of VSS added in AnCoD of TWAS and manure

Cumulative methane yield (mL CH4/g VSS added)														
time (day)	TWAS	SD	Manure	SD	T/M 9/1	SD	T/M 7/3	SD	T/M 1/1	SD	T/M 3/7	SD	T/M 1/9	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	16	0.7	127	5.8	62	2.4	92	4.2	119	5.1	136	4.8	117	4.208
2	58	2.6	233	10.7	142	5.5	182	8.4	237	10.2	270	9.4	229	8.258
3	94	4.2	320	14.7	203	7.9	245	11.3	319	13.7	370	13.0	316	11.38
4	128	5.8	404	18.6	247	9.6	304	14.0	397	17.1	463	16.2	394	14.2
5	153	6.9	468	21.5	281	11.0	348	16.0	453	19.5	536	18.7	458	16.49
6	178	8.0	540	24.9	318	12.4	396	18.2	516	22.2	613	21.5	527	18.98
8	206	9.3	644	29.6	351	13.7	456	21.0	595	25.6	710	24.8	615	22.15
10	227	10.2	727	33.4	380	14.8	524	24.1	683	29.4	807	28.3	695	25.03
14	251	11.3	819	37.7	410	15.6	582	26.8	759	32.6	912	31.9	781	28.11
16	268	12.1	885	40.7	435	16.9	621	28.6	810	34.8	979	34.3	839	30.22
17	277	12.5	919	42.3	451	17.6	651	29.9	849	36.5	1020	35.7	871	31.35
19	289	13.0	961	44.2	469	18.3	673	31.0	878	37.7	1064	37.2	905	32.58
22	301	13.5	1006	45.3	492	19.2	719	33.1	937	40.3	1120	39.2	948	34.13
25	316	14.2	1058	48.7	514	20.1	735	33.8	958	39.1	1170	41.0	995	35.81
26	321	14.5	1079	46.7	524	18.7	753	34.6	981	42.2	1199	42.0	1017	36.63
29	331	14.9	1115	51.3	539	21.0	776	35.7	1011	43.5	1240	43.4	1051	39.2
30	334	13.8	1123	49.5	543	22.4	781	34.2	1017	42.8	1250	43.8	1064	38.3
33	341	15.4	1149	52.9	552	21.5	796	36.6	1038	44.6	1277	44.7	1086	39.1
36	351	15.8	1186	54.5	568	20.3	815	37.5	1063	45.7	1313	46.0	1119	38.6
40	361	16.3	1222	50.6	586	22.9	840	38.6	1094	47.1	1356	44.8	1158	41.7
46	373	14.5	1261	58.0	607	23.7	866	39.8	1129	48.5	1404	49.2	1204	43.33
50	380	17.1	1281	54.5	617	24.1	880	40.5	1147	45.2	1429	50.0	1226	44.15
54	384	16.9	1296	59.6	625	23.8	890	39.3	1160	49.9	1447	48.7	1243	44.76
58	387	15.2	1307	58.3	631	24.6	897	41.3	1169	50.3	1461	51.1	1256	46.3
62	390	17.6	1316	60.5	635	25.2	903	41.5	1177	48.6	1470	52.6	1265	45.52
65	392	16.8	1324	56.4	639	24.9	908	39.7	1184	50.9	1478	49.8	1272	45.78
67	394	17.7	1332	61.3	642	23.5	917	40.9	1195	49.7	1487	51.3	1278	46
72	400	16.7	1345	59.2	651	25.4	925	42.6	1206	51.8	1507	52.7	1302	45.2
74	405	17.5	1357	58.1	660	24.8	935	43.0	1218	47.4	1522	50.2	1319	47.48
78	408	18.4	1368	60.8	667	26.7	934	41.5	1217	50.2	1535	53.7	1333	47.98

Table B. 5. Cumulative methane yield per unit volume of substrate added in AnCoD of TWAS and manure

Cumulative methane yield (mL CH_4/mL substrate added)														
time (day)	TWAS		Manure		T/M 9/1		T/M 7/3		T/M 1/1		T/M 3/7		T/M 1/9	
0	0	0	0	0	0	0	0	0.00	0	0.00	0	0.00	0	0.00
1	0.3	0.01	2.2	0.10	0.7	0.03	1.1	0.04	1.4	0.08	1.8	0.10	2.0	0.10
2	1.0	0.04	4.0	0.18	1.6	0.10	2.2	0.08	2.9	0.16	3.5	0.19	4.0	0.19
3	1.6	0.06	5.5	0.24	2.2	0.09	3.0	0.11	3.9	0.21	4.8	0.26	5.5	0.26
4	2.2	0.08	7.0	0.31	2.7	0.11	3.7	0.14	4.8	0.26	6.0	0.33	6.9	0.32
5	2.6	0.09	8.1	0.36	3.1	0.12	4.2	0.16	5.5	0.30	6.9	0.38	8.0	0.38
6	3.1	0.11	9.3	0.41	3.5	0.14	4.8	0.18	6.2	0.34	7.9	0.43	9.2	0.43
8	3.5	0.13	11.1	0.49	3.9	0.13	5.5	0.21	7.2	0.40	9.1	0.50	10.8	0.51
10	3.9	0.14	12.6	0.55	4.2	0.16	6.3	0.24	8.3	0.45	10.4	0.57	12.2	0.57
14	4.3	0.16	14.2	0.62	4.5	0.18	7.0	0.27	9.2	0.50	11.8	0.65	13.7	0.64
16	4.6	0.17	15.3	0.67	4.8	0.19	7.5	0.29	9.8	0.54	12.6	0.69	14.7	0.69
17	4.8	0.15	15.9	0.70	5.0	0.15	7.9	0.30	10.3	0.56	13.1	0.72	15.2	0.72
19	5.0	0.18	16.6	0.73	5.2	0.20	8.1	0.31	10.6	0.58	13.7	0.75	15.8	0.74
22	5.2	0.19	17.4	0.77	5.4	0.21	8.7	0.33	11.3	0.62	14.4	0.79	16.6	0.78
25	5.4	0.20	18.3	0.80	5.7	0.22	8.9	0.34	11.6	0.64	15.1	0.83	17.4	0.82
26	5.5	0.19	18.7	0.82	5.8	0.23	9.1	0.35	11.9	0.65	15.5	0.85	17.8	0.84
29	5.7	0.20	19.3	0.85	6.0	0.23	9.4	0.36	12.2	0.67	16.0	0.88	18.4	0.86
30	5.7	0.21	19.4	0.85	6.0	0.27	9.4	0.36	12.3	0.68	16.1	0.89	18.6	0.87
33	5.9	0.21	19.9	0.87	6.1	0.24	9.6	0.41	12.5	0.69	16.5	0.91	19.0	0.89
36	6.0	0.20	20.5	0.90	6.3	0.20	9.8	0.37	12.8	0.71	16.9	0.93	19.6	0.92
40	6.2	0.22	21.1	0.93	6.5	0.25	10.1	0.39	13.2	0.73	17.5	0.96	20.3	0.95
46	6.4	0.23	21.8	0.96	6.7	0.26	10.5	0.40	13.6	0.75	18.1	1.00	21.0	0.99
50	6.5	0.21	22.2	0.97	6.8	0.29	10.6	0.36	13.9	0.76	18.4	1.01	21.4	1.01
54	6.6	0.24	22.4	0.99	6.9	0.27	10.7	0.39	14.0	0.77	18.7	1.03	21.7	0.99
58	6.7	0.33	22.6	0.88	7.0	0.25	10.8	0.41	14.1	0.78	18.8	1.04	22.0	1.03
62	6.7	0.24	22.7	1.22	7.0	0.27	10.9	0.45	14.2	0.78	19.0	1.04	22.1	1.04
65	6.7	0.31	22.9	1.01	7.1	0.22	11.0	0.42	14.3	0.79	19.1	1.05	22.2	1.05
67	6.8	0.24	23.0	1.35	7.1	0.24	11.1	0.36	14.4	0.79	19.2	0.98	22.3	0.97
72	6.9	0.25	23.3	0.98	7.2	0.28	11.2	0.42	14.6	0.80	19.4	1.07	22.8	1.07
74	7.0	0.29	23.5	1.23	7.3	0.25	11.3	0.39	14.7	0.81	19.6	1.08	23.1	1.08
78	7.0	0.25	23.6	1.04	7.4	0.29	11.3	0.43	14.7	0.81	19.8	1.09	23.3	1.10

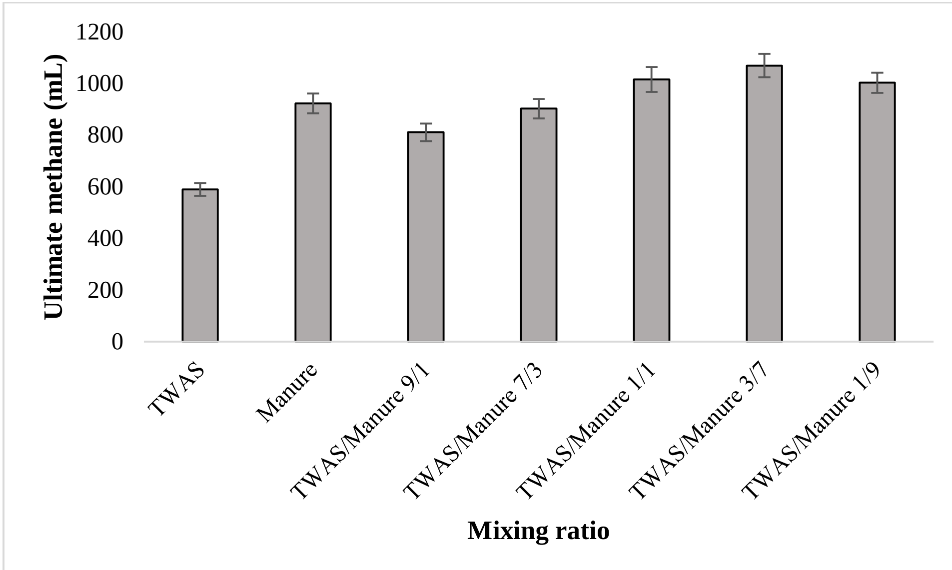


Figure B. 1. Ultimate methane production in AnCoD of TWAS and manure

Table B. 6. Characteristics of the raw feedstocks for hydrolysis/acidification in AnCoD of TWAS and manure

Parameters	Units	Manure		TWAS		Seed	
		MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	110433	4422	41200	1236	16652	552
SCOD	mg/L	6560	228	2161	65	1255	50
TSS	mg/L	52600	2530	37254	1118	15112	604
VSS	mg/L	42800	2040	26521	796	10067	403
TS	mg/L	68040	3202	39198	1176	36882	1475
VS	mg/L	58520	2526	28562	857	26874	1075
Ammonia	mg/L	13	1	327	10	307	12
pH	-	6.5	0.2	6.8	0.204	6	0.2
Alkalinity	mg CaCO3/L	7689	384	4964	149	4671	187
TN	mg/L	1435	72	2969	89	2794	112
TSN	mg/L	68	3	422	13	397	16
Total Carbs	mg/L	27116	1355.8	1327	40	503	20
Total Proteins	mg/L	5123	256.15	3978	119	1510	60
Total Lipids	mg/L	1360	68	530	16	201	8

Table B. 7. Measured and theoretical VFAs concentrations over time

Measured VFAs over time							
	Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5	Mixture 6	Mixture 7
Time (hr)	TWAS/Manure 7/3	TWAS/Manure 1/1	TWAS/Manure 3/7	TWAS/Manure 1/9	TWAS/Manure 9/1	Manure Only	TWAS Only
0	584	686	718	790	581	810	432
6	596	719	793	831	593	849	458
12	786	892	827	958	640	974	504
24	912	1105	971	1230	681	1141	514
48	942	1131	1299	1378	735	1366	528
72	965	1175	1530	1491	775	1396	542
Theoretical VFAs over time							
Time (hr)	TWAS/Manure 7/3	TWAS/Manure 1/1	TWAS/Manure 3/7	TWAS/Manure 1/9	TWAS/Manure 9/1	Manure Only	TWAS Only
0	545	621	697	772	470	810	432
6	575	654	732	810	497	849	458
12	645	739	833	927	551	974	504
24	702	827	953	1078	576	1141	514
48	780	947	1115	1283	612	1366	528
72	798	969	1140	1311	627	1396	542

Table B. 8. Soluble and particulate COD concentrations over time

Soluble COD concentrations (mg/L) over time							
Time (hr)	TWAS/Manure 7/3	TWAS/Manure 5/5	TWAS/Manure 3/7	TWAS/Manure 1/9	TWAS/Manure 9/1	Manure only	TWAS only
0	1880	1920	1611	1940	1929	1628	2181
2	1920	1960	1817	2020	1972	1661	2254
4	2040	2100	1868	2060	2471	1744	2377
6	2260	2260	2005	2180	2536	1861	2524
8	2380	2360	2159	2300	2644	1960	2548
10	2420	2480	2228	2360	2904	1977	2597
12	2540	2560	2451	2400	2969	2010	2769
24	2780	2580	2622	2540	3099	2126	2988
48	2920	2940	2896	2780	3208	2193	3087
72	3990	3690	4190	4020	3860	3530	3120
Particulate COD concentrations (mg/L) over time							
Time (hr)	TWAS/Manure 7/3	TWAS/Manure 5/5	TWAS/Manure 3/7	TWAS/Manure 1/9	TWAS/Manure 9/1	Manure only	TWAS only
0	23155	22556	22486	21637	24388	21836	23635
2	23015	22326	22467	21427	24545	21582	23115
4	22895	22156	22219	21257	24186	21489	22696
6	22675	22215	22081	21227	23712	21223	22468
8	22555	22050	21527	20787	23893	21010	22276
10	22515	21690	21399	20297	23543	20527	22083
12	22485	21310	21016	20127	23228	20324	21945
24	21155	20496	19691	19787	22801	20117	21697
48	20615	20336	19300	19187	22203	19856	21563
72	20445	20186	19056	19107	22177	19434	21508

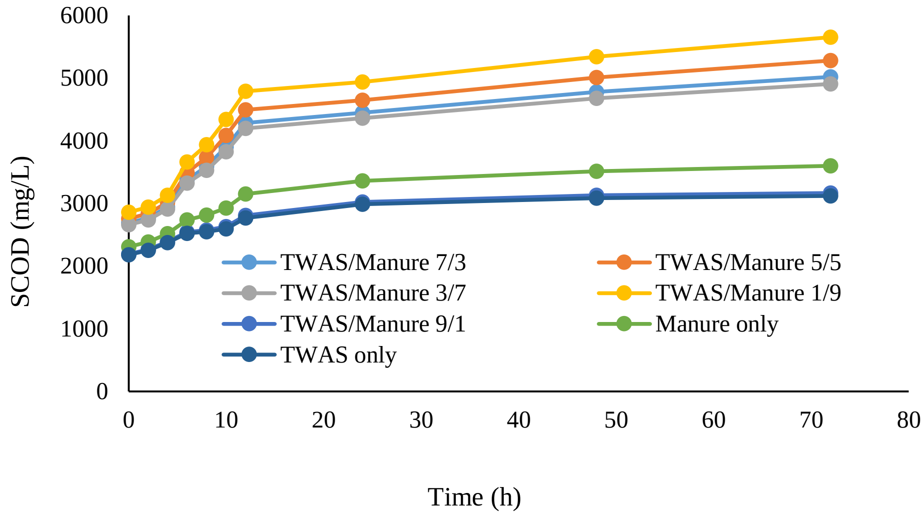


Figure B.2. Concentration of soluble COD over time at different mixing ratios of TWAS and manure

C. Analytical results for AnCoD of manure and SSO

Table C. 1. Characteristics of raw feedstocks in AnCoD of manure and SSO

Parameters	Units	Manure		SSO		Inoculum	
		MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	105120	4795	115163	6090	17980	400
SCOD	mg/L	44116	489	42932	92	722	16
TSS	mg/L	54380	2252	62125	3063	15128	330
VSS	mg/L	46420	2649	45584	2283	11687	160
TS	mg/L	69785	3025	67987	1011	16160	503
VS	mg/L	58590	2761	48535	682	12380	308
Ammonia	mg/L	20	68.0	1073	5.87	745	36
pH		6.8	0.030	5.8	0.050	7.2	0.05
Alkalinity	mg CaCO3/L	4924	522	6176	339	4186	460
TN	mg/L	2510	751	3498	202	1940	186
TSN	mg/L	105.00	38	1046	21	716	93
Total Carbs	mg/L	28796	3580	13495	687	453	68
Total Proteins	mg/L	5930	198	2087	98	2598	38
Total Lipids	mg/L	1400	2420	1103	92	225	43

Table C. 2. Average CH_4 measurements in AnCoD of manure and SSO

Average CH_4 Measurement (ml)														
Time (day)	Manure	SD	SSO	SD	M/SSO 9/1	SD	M/SSO 7/3	SD	M/SSO 5/5	SD	M/SSO 1/9	SD	M/SSO 3/7	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	44	1.7	24	1.0	28	1.2	33	1.5	32	1.7	30	1.6	25	1.1
2	47	1.8	59	2.5	69	3.0	59	2.7	68	3.7	70	3.6	64	2.7
3	66	2.5	102	4.3	118	5.1	72	3.3	92	5.0	104	5.4	110	4.7
4	66	2.5	90	3.8	104	4.5	64	3.0	74	4.0	82	4.3	93	4.0
5	75	2.9	103	4.3	119	5.1	74	3.4	88	4.7	94	4.9	106	4.6
6	90	3.4	111	4.6	128	5.5	108	4.9	111	6.0	117	6.1	118	5.1
7	45	1.7	84	3.5	97	4.2	85	3.9	89	4.8	89	4.6	95	4.1
8	44	1.7	65	2.7	75	3.2	57	2.6	58	3.1	58	3.0	68	2.9
9	40	1.5	70	2.9	81	3.5	56	2.6	56	3.0	54	2.8	67	2.9
11	51	2.0	70	2.9	81	3.5	64	3.0	65	3.5	60	3.1	69	3.0
12	32	1.2	33	1.4	38	1.6	40	1.8	38	2.0	34	1.8	33	1.4
14	33	1.3	30	1.3	35	1.5	48	2.2	48	2.6	37	1.9	34	1.5
15	23	0.9	17	0.7	19	0.8	28	1.3	24	1.3	21	1.1	19	0.8
16	23	0.9	12	0.5	14	0.6	22	1.0	17	0.9	14	0.7	13	0.6
17	21	0.8	14	0.6	16	0.7	19	0.9	15	0.8	13	0.7	11	0.5
18	16	0.6	14	0.6	16	0.7	21	0.9	17	0.9	14	0.7	11	0.5
20	27	1.0	16	0.7	19	0.8	31	1.4	25	1.4	22	1.1	18	0.8
21	14	0.5	11	0.5	13	0.6	23	1.0	18	0.9	14	0.7	12	0.5
22	13	0.5	11	0.5	13	0.6	15	0.7	12	0.7	10	0.5	9	0.4
23	12	0.5	9	0.4	11	0.5	14	0.6	11	0.6	9	0.5	7	0.3
24	12	0.5	9	0.4	10	0.4	14	0.7	11	0.6	9	0.5	7	0.3
25	13	0.5	9	0.4	10	0.4	13	0.6	12	0.7	12	0.6	10	0.4
26	16	0.6	10	0.4	11	0.5	13	0.6	12	0.7	9	0.5	7	0.3
28	9	0.3	11	0.5	13	0.5	19	0.9	20	1.1	15	0.8	12	0.5
29	8	0.3	7	0.3	9	0.4	14	0.6	11	0.6	10	0.4	8	0.4
30	6	0.2	7	0.3	9	0.4	13	0.6	11	0.6	9	0.5	8	0.3
31	7	0.3	6	0.3	7	0.3	7	0.3	5	0.3	5	0.2	3	0.1
32	7	0.3	9	0.4	10	0.4	11	0.5	9	0.5	9	0.5	5	0.2
35	10	0.4	10	0.4	12	0.5	21	0.9	18	0.9	15	0.8	12	0.5
37	10	0.4	13	0.5	15	0.6	17	0.8	21	1.1	11	0.6	23	1.0
40	22	0.8	10	0.4	12	0.5	19	0.9	17	0.9	15	0.8	12	0.5
43	10	0.4	6	0.2	7	0.3	10	0.5	9	0.5	8	0.4	6	0.3
46	3	0.1	3	0.1	4	0.2	9	0.3	8	0.4	7	0.4	6	0.2
49	2	0.1	3	0.1	3	0.1	8	0.4	8	0.4	7	0.3	6	0.1
52	4	0.2	4	0.2	5	0.2	7	0.3	7	0.4	6	0.3	5	0.2

*M: manure

Table C. 3. Cumulative CH_4 production in AnCoD of manure and SSO

Cumulative CH_4 production (mL)														
Time (day)	Manure	SD	SSO	SD	M*/SSO 9/1	SD	M/SSO 7/3	SD	M/SSO 5/5	SD	M/SSO 1/9	SD	M/SSO 3/7	SD
0	0	0	0	0	0	0	0	0	0	0.0	0	0.0	0	0.0
1	44	2.0	24	1.3	28	1.3	33	1.8	32	1.5	30	1.3	25	1.0
2	90	4.1	83	4.6	93	4.5	93	5.0	101	4.5	100	4.2	89	3.4
3	156	7.0	186	10.2	206	9.9	164	8.9	193	8.7	204	8.6	199	7.6
4	222	10.0	276	15.2	305	14.6	229	12.3	267	12.0	287	12.0	291	11.1
5	297	13.4	379	20.8	418	20.1	303	16.4	355	16.0	381	16.0	397	15.1
6	387	17.4	489	26.9	540	25.9	410	22.2	466	21.0	498	20.9	516	19.6
7	432	19.4	573	31.5	632	30.3	496	26.8	554	24.9	587	24.7	611	23.2
8	476	21.4	638	35.1	703	33.7	553	29.9	612	27.5	645	27.1	679	25.8
9	516	23.2	708	38.9	780	37.5	609	32.9	668	30.0	699	29.4	746	28.3
11	567	25.5	778	42.8	857	41.1	673	36.3	732	33.0	759	31.9	815	31.0
12	599	26.9	810	44.6	893	42.9	713	38.5	770	34.7	793	33.3	849	32.3
14	632	28.4	840	46.2	926	44.4	761	41.1	818	36.8	830	34.9	883	33.5
15	654	29.5	857	47.1	944	45.3	789	42.6	842	37.9	851	35.7	902	34.3
16	677	30.5	870	47.8	958	46.0	811	43.8	859	38.7	866	36.4	915	34.8
17	698	31.4	883	48.6	973	46.7	829	44.8	875	39.4	878	36.9	926	35.2
18	714	32.1	897	49.3	987	47.4	850	45.9	892	40.1	893	37.5	937	35.6
20	741	33.3	913	50.2	1001	48.0	881	47.6	917	41.3	914	38.4	955	36.3
21	755	34.0	924	50.8	1021	49.0	904	48.8	934	42.0	929	39.0	967	36.8
22	768	34.5	936	51.5	1035	49.7	919	49.6	947	42.6	939	39.4	976	37.1
23	780	35.1	945	52.0	1045	50.2	933	50.4	957	43.1	948	39.8	984	36.3
24	792	35.6	954	52.4	1055	50.6	947	51.1	968	43.6	957	40.2	991	37.6
25	804	36.2	962	52.9	1065	51.1	960	51.9	980	44.1	969	40.7	1001	38.0
26	820	36.9	972	53.5	1076	51.6	974	52.6	992	44.7	978	41.1	1008	35.4
28	829	37.3	983	54.1	1088	52.2	993	53.6	1013	45.6	993	41.7	1020	38.8
29	837	37.7	991	54.5	1099	52.7	1007	54.4	1024	46.1	1003	42.1	1029	39.1
30	844	38.0	998	54.9	1110	53.3	1019	55.0	1035	46.6	1012	42.5	1036	40.6
31	851	38.3	1004	55.2	1120	53.7	1027	55.5	1040	46.8	1017	42.7	1039	39.5
32	858	38.6	1013	55.7	1132	54.3	1038	56.0	1049	47.2	1026	43.1	1044	38.4
35	867	39.0	1023	56.3	1143	54.9	1058	57.1	1067	48.0	1041	43.7	1056	39.7
37	877	39.5	1036	57.0	1157	55.5	1075	58.1	1087	48.9	1052	44.2	1080	41.0
40	899	40.5	1046	57.6	1168	56.1	1094	59.1	1104	49.7	1067	44.8	1092	40.2
43	910	40.9	1052	57.9	1175	56.4	1104	59.6	1114	50.1	1076	43.8	1098	41.7
46	912	41.1	1056	58.1	1179	56.6	1113	60.1	1121	49.2	1082	45.5	1104	42.0
49	915	41.2	1059	58.2	1182	56.7	1121	58.3	1129	50.8	1089	44.3	1110	43.5
52	919	41.3	1063	58.5	1186	56.9	1129	61.0	1136	51.1	1095	46.0	1115	42.4

*M: manure

Table C. 4. Cumulative CH_4 production per mass of COD added in AnCoD of manure and SSO

Cumulative CH_4 yield (mL CH4/g COD added)														
Time (day)	Manure	SD	SSO	SD	M*/SSO 9/1	SD	M/SSO 7/3	SD	M/SSO 5/5	SD	M/SSO 1/9	SD	M/SSO 3/7	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	14	0.6	7	0.3	9	0.3	10	0.4	9	0.3	9	0.5	7	0.3
2	28	1.2	24	0.9	29	1.1	28	1.0	29	1.0	30	1.5	25	0.9
3	49	2.0	53	2.0	63	2.5	50	1.8	56	2.0	61	3.1	56	2.0
4	69	2.9	79	3.0	93	3.7	70	2.4	77	2.7	86	4.4	82	3.0
5	93	3.9	108	4.1	128	5.1	92	3.2	103	3.6	114	5.8	112	4.0
6	121	5.1	140	5.3	165	6.6	125	4.4	135	4.7	149	7.6	146	5.3
7	135	5.7	163	6.2	193	7.7	151	5.3	161	5.6	175	8.9	173	6.2
8	149	6.2	182	6.9	215	8.6	169	5.9	178	6.2	193	9.8	192	6.9
9	161	6.8	202	7.7	239	9.6	186	6.5	194	5.8	209	10.6	211	7.6
11	177	7.4	222	8.4	262	10.5	205	7.2	213	7.4	226	11.5	231	8.3
12	187	7.9	231	8.8	273	10.6	217	7.6	224	7.8	237	12.1	240	8.6
14	197	8.3	240	9.1	283	11.3	232	8.1	238	8.3	248	11.6	250	9.0
15	204	7.5	244	9.3	289	10.4	241	8.6	245	7.6	254	13.0	255	8.2
16	211	8.9	248	9.4	293	11.7	247	7.7	250	8.7	258	12.2	259	9.3
17	218	9.2	252	10.1	298	10.3	253	8.9	254	8.9	262	13.4	262	9.8
18	223	9.4	256	9.7	302	11.2	259	9.1	259	9.1	266	12.3	265	8.6
20	231	8.6	260	9.9	306	12.3	269	9.4	266	8.3	273	13.9	270	7.7
21	236	9.9	264	10.0	312	12.5	276	8.7	271	9.5	277	14.1	274	8.9
22	240	10.1	267	10.1	317	12.7	280	9.8	275	8.6	280	13.2	276	9.9
23	244	9.7	269	9.4	320	11.6	285	10.0	278	9.7	283	14.4	278	9.3
24	247	10.4	272	10.3	323	12.9	289	10.6	281	9.8	286	13.6	280	9.1
25	251	10.6	274	9.7	326	13.0	293	10.3	285	10.0	289	14.7	283	10.5
26	256	10.8	277	10.5	329	13.2	297	9.8	288	9.1	292	12.9	285	9.3
28	259	10.9	280	9.8	333	13.3	303	10.6	294	10.3	296	15.1	289	10.4
29	262	11.0	283	10.7	336	13.4	307	10.7	297	9.4	299	14.3	291	9.5
30	264	10.4	285	9.3	340	13.6	311	10.9	301	10.5	302	15.4	293	10.6
31	266	11.2	286	10.9	343	12.7	313	11.0	302	9.6	303	13.5	294	9.6
32	268	10.7	289	12.3	346	13.9	317	9.7	305	10.7	306	15.6	295	10.6
35	271	11.4	292	11.1	350	14.0	323	11.3	310	9.8	311	13.8	299	9.8
37	274	11.5	295	10.5	354	13.8	328	9.8	316	11.1	314	14.9	306	11.0
40	281	11.8	298	11.3	358	14.5	334	11.7	321	9.8	318	16.2	309	10.1
43	284	11.9	300	10.2	360	13.2	337	10.2	323	11.3	321	15.1	311	11.2
46	285	11.6	301	11.4	361	14.4	340	11.9	326	10.5	323	16.5	312	10.4
49	286	10.2	302	10.6	362	13.1	342	10.9	328	9.2	325	14.6	314	9.7
52	287	12.1	303	11.5	363	14.5	344	11.6	330	11.5	327	16.7	316	10.6

*M: manure

Table C. 5. Cumulative CH_4 production per mass of VSS added in AnCoD of manure and SSO

					Cumulative CH_4 yield (mL CH4/g VSS added)									
Time (day)	Manure	SD	SSO	SD	M*/SSO 9/1	SD	M/SSO 7/3	SD	M/SSO 5/5	SD	M/SSO 1/9	SD	M/SSO 3/7	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
1	31	1.4	18	0.6	20	0.8	25	0.9	23	0.8	23	0.9	19	0.9
2	64	2.9	62	2.2	67	2.8	69	2.5	73	2.5	76	2.9	65	3.1
3	111	5.0	139	4.9	147	6.2	121	4.5	139	4.9	155	5.9	146	6.9
4	158	7.1	206	7.2	218	9.1	169	6.2	193	6.8	218	8.3	214	10.1
5	212	9.5	282	9.9	298	12.5	224	8.3	257	9.0	289	11.0	292	13.7
6	276	12.4	365	12.8	385	16.2	303	11.2	337	11.8	379	14.4	379	17.8
7	308	13.9	428	15.0	451	19.0	366	13.6	401	14.0	446	17.0	448	21.1
8	339	15.2	476	16.7	502	21.1	408	15.1	443	15.5	491	18.6	499	23.4
9	367	16.5	528	18.5	557	23.4	450	16.6	483	16.9	532	20.2	548	25.8
11	404	18.2	580	20.3	612	25.7	497	18.4	530	18.5	577	21.9	599	28.2
12	426	19.2	605	21.2	638	26.8	526	19.5	557	19.5	603	22.9	624	29.3
14	450	20.3	627	21.9	661	27.8	562	20.8	592	20.7	631	24.0	648	30.5
15	466	21.0	640	22.4	674	28.3	583	21.6	609	21.3	647	24.6	662	31.1
16	482	21.7	649	22.7	684	28.7	599	22.2	622	21.8	658	23.7	672	31.6
17	497	20.5	659	22.1	695	28.2	613	23.7	633	19.3	668	25.4	680	29.5
18	508	22.9	669	23.4	705	29.6	628	23.2	645	22.6	679	25.8	689	32.4
20	528	23.7	681	23.8	715	30.0	651	24.1	663	21.5	695	26.4	702	33.0
21	538	24.2	690	24.1	729	29.6	668	24.7	676	23.7	706	26.8	710	31.9
22	547	24.6	698	24.4	739	31.1	679	25.1	685	24.0	714	27.1	717	33.7
23	555	25.0	705	23.5	747	30.4	689	25.5	693	23.2	721	25.8	722	34.0
24	564	24.4	711	24.9	753	31.6	700	24.2	700	24.5	728	27.7	728	34.2
25	573	25.8	718	25.1	760	30.9	709	26.2	709	24.8	737	28.0	735	33.5
26	584	26.3	725	25.4	768	32.3	719	25.6	718	25.1	744	26.8	740	34.8
28	591	24.6	734	24.7	777	32.6	733	27.1	733	24.6	755	28.7	749	35.2
29	596	26.8	739	25.9	785	33.0	743	26.5	741	25.9	763	29.0	755	34.5
30	601	25.5	745	25.1	792	33.3	753	27.9	749	26.2	770	29.2	761	35.8
31	606	27.3	749	26.2	800	33.6	758	28.1	753	25.7	773	27.9	763	35.9
32	611	26.5	756	24.4	808	32.9	766	27.4	759	26.6	780	29.6	767	34.9
35	618	27.8	763	26.7	816	34.3	782	28.9	772	25.3	792	30.1	776	36.5
37	625	28.1	773	27.1	826	34.7	794	28.4	787	27.5	800	28.4	793	37.3
40	641	28.8	781	27.3	835	35.1	808	29.9	799	28.0	811	30.8	802	36.8
43	648	29.2	785	255.0	839	34.2	815	30.2	806	26.2	818	29.3	807	37.9
46	650	27.9	788	27.6	842	35.4	822	30.4	811	28.4	823	31.3	811	38.1
49	652	28.3	790	25.6	844	34.5	828	29.7	817	27.6	828	30.5	815	37.3
52	654	29.4	793	27.8	847	35.6	834	30.8	822	28.8	833	31.6	819	38.5

*M: manure

Table C. 6. Cumulative CH_4 production per volume of substrate added in AnCoD of manure and SSO

Time (day)	Manure	SD	SSO	SD	Cumulative CH_4 yield (mL CH4/mL substrate added) M*/SSO 9/1	SD	M/SSO 7/3	SD	M/SSO 5/5	SD	M/SSO 1/9	SD	M/SSO 3/7	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1.45	0.06	1.4	0.05	1.0	0.04	1.4	0.06	1.5	0.07	1.6	0.07	1.4	0.08
2	3.02	0.12	4.9	0.16	3.3	0.14	3.9	0.18	4.6	0.21	5.3	0.22	5.0	0.27
3	5.21	0.20	10.9	0.35	7.3	0.31	6.8	0.32	8.8	0.40	10.8	0.45	11.0	0.61
4	7.40	0.29	16.2	0.52	10.9	0.46	9.5	0.44	12.1	0.56	15.1	0.63	16.2	0.89
5	9.90	0.39	22.3	0.71	14.9	0.63	12.6	0.58	16.1	0.74	20.0	0.84	22.1	1.21
6	12.91	0.50	28.8	0.92	19.3	0.81	17.1	0.79	21.2	0.97	26.2	1.10	28.7	1.58
7	14.41	0.56	33.7	1.08	22.6	0.95	20.7	0.95	25.2	1.16	30.9	1.30	33.9	1.87
8	15.86	0.62	37.5	1.20	25.1	1.05	23.0	1.06	27.8	1.28	34.0	1.43	37.7	2.07
9	17.19	0.67	41.7	1.33	27.9	1.17	25.4	1.17	30.3	1.40	36.8	1.55	41.4	2.28
11	18.90	0.74	45.7	1.46	30.6	1.29	28.0	1.29	33.3	1.53	39.9	1.88	45.3	1.94
12	19.96	0.78	47.7	1.53	31.9	1.34	29.7	1.37	35.0	1.61	41.8	1.75	47.2	2.59
14	21.06	0.82	49.4	1.58	33.1	1.39	31.7	1.46	37.2	1.71	43.7	2.03	49.0	1.97
15	21.81	0.95	50.4	1.61	33.7	1.42	32.9	1.51	38.3	1.76	44.8	1.88	50.1	2.76
16	22.57	0.88	51.2	1.64	34.2	1.44	33.8	1.55	39.1	1.80	45.6	1.91	50.8	1.80
17	23.27	0.91	52.0	1.66	34.7	1.46	34.6	1.59	39.8	1.83	46.2	1.94	51.5	2.83
18	23.79	0.83	52.8	1.69	35.2	1.48	35.4	1.63	40.5	1.86	47.0	1.97	52.1	1.86
20	24.69	0.96	53.7	1.72	35.7	1.50	36.7	1.69	41.7	1.92	48.1	2.02	53.1	2.92
21	25.16	0.98	54.4	1.74	36.5	1.53	37.7	1.73	42.5	1.95	48.9	2.05	53.7	1.96
22	25.59	1.00	55.0	1.76	37.0	1.55	38.3	1.76	43.0	1.98	49.4	2.08	54.2	2.98
23	25.99	1.01	55.6	1.78	37.3	1.57	38.9	1.79	43.5	2.00	49.9	2.75	54.6	3.01
24	26.39	9.83	56.1	1.79	37.7	1.58	39.5	1.82	44.0	2.02	50.4	2.12	55.0	2.93
25	26.81	1.05	56.6	1.81	38.0	1.60	40.0	1.84	44.6	2.05	51.0	2.53	55.6	3.06
26	27.35	9.87	57.2	1.83	38.4	1.61	40.6	1.87	45.1	2.08	51.5	2.16	56.0	2.98
28	27.65	1.08	57.8	1.85	38.8	1.63	41.4	1.90	46.0	2.12	52.3	1.92	56.7	3.12
29	27.91	1.09	58.3	1.86	39.2	1.65	41.9	1.93	46.5	2.14	52.8	2.22	57.1	2.94
30	28.13	1.10	58.7	1.88	39.6	1.66	42.5	1.95	47.0	1.98	53.3	1.94	57.6	3.17
31	28.36	1.11	59.1	1.89	40.0	1.68	42.8	1.97	47.3	2.17	53.5	2.25	57.7	2.98
32	28.58	1.11	59.6	1.91	40.4	1.70	43.2	1.99	47.7	1.92	54.0	1.27	58.0	3.19
35	28.92	9.83	60.2	1.93	40.8	1.71	44.1	2.03	48.5	2.23	54.8	2.30	58.7	3.23
37	29.25	1.14	60.9	1.95	41.3	1.74	44.8	2.06	49.4	1.72	55.4	1.33	60.0	2.30
40	29.98	1.17	61.6	1.97	41.7	1.75	45.6	2.10	50.2	2.31	56.2	2.36	60.7	3.34
43	30.32	1.18	61.9	1.98	42.0	1.76	46.0	2.12	50.6	1.63	56.6	1.83	61.0	2.36
46	30.41	1.19	62.1	1.99	42.1	1.77	46.4	2.13	51.0	2.34	57.0	2.39	61.3	3.37
49	30.49	1.23	62.3	1.99	42.2	1.77	46.7	2.15	51.3	1.36	57.3	1.94	61.6	3.39
52	30.63	1.19	62.5	2.00	42.4	1.78	47.0	2.16	51.6	2.37	57.6	2.42	61.9	2.41

*M: manure

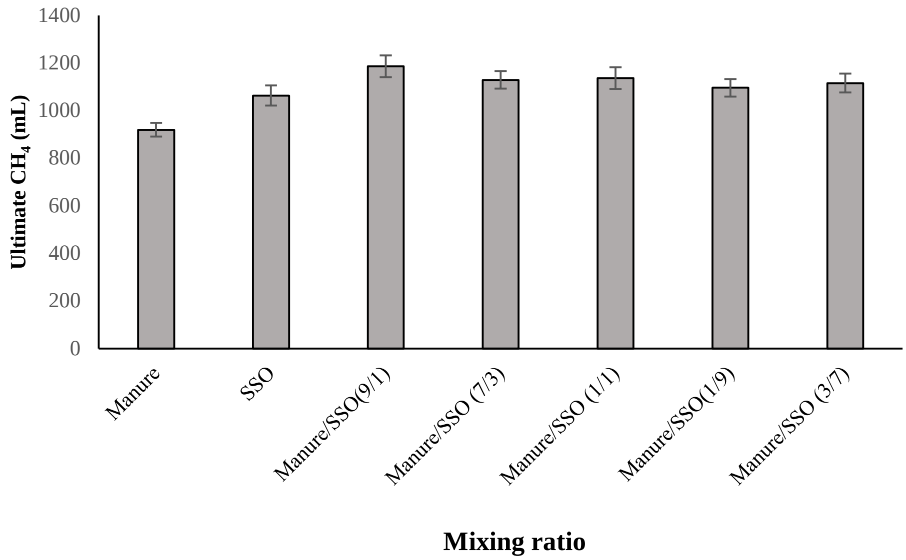


Figure C. 1. Ultimate methane production in AnCoD of TWAS and manure

Table C. 7. Characteristics of the raw feedstocks for hydrolysis/acidification of manure and SSO

Parameters	Units	Manure		SSO		Inoculum	
		MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	101125	4551	115163	4607	17100	400
SCOD	mg/L	10480	472	42932	1717	986	16
TSS	mg/L	54600	2457	47112	1884	17143	330
VSS	mg/L	32115	1445	39940	1598	11200	160
TS	mg/L	68540	3084	77987	3119	21870	503
VS	mg/L	58520	2633	63537	2541	13380	308
Ammonia	mg/L	24.3	1	1396	55.84	1505	36
pH	-	6.7	0.302	5.8	0.2	7.2	0.1
Alkalinity	mg CaCO3/L	7224	325	6720	269	3986	460
TN	mg/L	1740	78	4198	167.92	2048	186
TSN	mg/L	107	5	1146	46	716	93
Total Carbs	mg/L	26796	1206	14125	565	595	68
Total Proteins	mg/L	5408	243	2156	86.24	1680	38
Total Lipids	mg/L	1703	77	1468	59	168	93

Table C. 8. Measured and theoretical VFAs over time in hydrolysis/acidification of manure and SSO

Measured VFAs concentrations (mg/L)over time							
	Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5	Mixture 6	Mixture 7
Time (hr)	Manure	SSO	Manure/SSO 9/1	Manure/SSO 7/3	Manure/SSO 5/5	Manure/SSO 3/7	Manure/SSO 1/9
0	668	635	646	649	666	740	722
6	783	790	661	688	749	861	803
12	803	1000	864	72	855	917	973
24	1119	1178	983	843	1092	1054	1178
48	1204	1365	1025	975	1143	1299	2756
72	1114	1102	1227	1237	1258	1338	1408
Theoretical VFAs concentrations (mg/L)over time							
Time (hr)	Manure	SSO	Manure/SSO 9/1	Manure/SSO 7/3	Manure/SSO 5/5	Manure/SSO 3/7	Manure/SSO 1/9
0	668	635	665	658	651	645	638
6	783	790	784	785	786	788	789
12	803	1000	823	862	902	941	980
24	1119	1178	1125	1137	1148	1160	1172
48	1204	1365	1220	1252	1284	1317	1349
72	1114	1102	1113	1110	1108	1106	1103

Table C. 9. Concentration of soluble and particulate COD over time in hydrolysis/acidification of manure and SSO

Soluble COD concentrations (mg/L) ocer time							
Time (hr)	Manure/SSO 7/3	Manure/SSO 5/5	Manure/SSO 3/7	Manure/SSO 1/9	Manure/SSO 9/1	Manure only	SSO only
0	3986	4710	5624	6555	3460	2860	6980
2	4580	5159	5941	6771	3860	2940	6992
4	5336	6273	6273	6887	4440	3131	7009
6	5559	6379	6379	7004	4725	3659	7015
8	5751	6469	6469	7105	4927	3936	7026
10	5974	6500	6469	7105	5280	4338	7040
12	6127	6530	6509	7149	5525	4790	7043
24	6326	6547	6547	7193	6268	4935	7052
48	6543	6639	6639	7295	6675	5340	7061
72	7189	7120	6934	7418	7355	5650	7080
Particulate COD concentrations (mg/L) over time							
Time (hr)	Manure/SSO 7/3	Manure/SSO 5/5	Manure/SSO 3/7	Manure/SSO 1/9	Manure/SSO 9/1	Manure only	SSO only
0	23605	22489	20787	19466	25746	26535	18565
2	23298	21940	20539	19250	25160	26155	18446
4	22780	21426	20176	19085	24726	25940	18279
6	22489	21270	20099	18957	24141	25766	18165
8	22385	21130	19887	18895	23749	25665	18050
10	22205	21045	19830	18796	23470	25560	17955
12	21935	20989	19695	18752	23255	25340	17915
24	21325	20542	19280	18508	22410	24795	17755
48	20895	20255	18965	18430	21978	24535	17646
72	20809	20195	18925	18405	21860	24400	17614

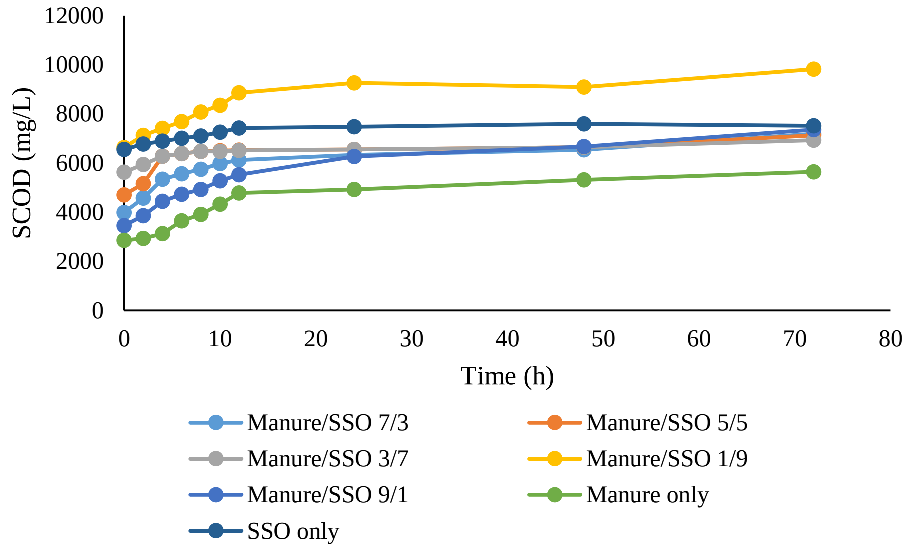


Figure C.2. Concentration of soluble COD over time at different mixing ratios

D. Analytical results for AnCoD of TWAS, manure and SSO

Table D. 1. Characteristics of raw feedstocks in AnCoD of TWAS, Manure, and SSO

Parameters	Units	TWAS		Manure		SSO		Seed	
		MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	39977	1199	100126	3004	108560	3257	17105	513
SCOD	mg/L	1354	41	42116	1263	40855	1225.65	761	23
TSS	mg/L	31445	943	52380	1571	62136	1864	16055	482
VSS	mg/L	26480	794	45420	1363	46990	1410	12781	383
TS	mg/L	38944	1168	67785	2034	66985	2010	17125	514
VS	mg/L	35155	1055	55590	1668	49585	1488	14380	431
Ammonia	mg/L	218	7	18	1	1289	39	800	24
pH		6.5	0.2	6.7	0.2	5.7	0.17	7.2	0.2
Alkalinity	mg CaCO3/L	1888	57	5224	157	6227	187	4215	126
TN	mg/L	2754	83	2110	63	3970	119	1975	59
TSN	mg/L	376	11	125	4	965	29	716	21
Total Carbs	mg/L	1524	46	27842	835	14126	424	839	25
Total Proteins	mg/L	3892	117	5762	173	2420	73	1917	58
Total Lipids	mg/L	396	12	1356	41	1494	45	191	6

Table D. 2. Average CH_4 measurements (mL) in AnCoD of TWAS/Mnaure/SSO (T: TWAS, M: Manure)

Time (day)	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 0.5/0.25/0.25	T/M/SSO 0.25/0.5/0.25	T/M/SSO 0.25/0.25/0.5	T/M/SSO 0.4/0.4/0.2	T/M/SSO 0.2/0.4/0.4	T/M/SSO 0.4/0.2/0.4
0	0	0	0	0	0	0	0	0	0	0	0	0
1	5	47	6	12	22	21	9	52	25	7	37	8
3	21	73	48	32	71	68	55	95	60	54	64	42
4	39	101	63	34	104	97	77	118	79	71	97	79
5	52	101	98	57	143	136	105	124	115	104	118	113
6	34	116	113	67	106	114	94	141	112	102	123	104
7	33	139	112	69	105	108	97	98	116	106	125	106
8	19	79	120	71	86	81	92	102	120	110	133	103
10	25	77	128	76	83	85	81	95	107	97	106	95
12	23	70	121	71	79	81	77	96	101	92	101	90
14	19	91	90	54	65	66	63	63	81	74	84	73
16	17	56	60	35	60	61	61	65	72	65	81	64
19	21	58	54	32	61	63	52	51	59	53	61	55
22	16	40	56	33	52	53	49	47	58	53	62	53
25	13	40	47	28	42	43	38	36	46	41	47	42
28	9	32	33	19	31	32	30	56	36	33	39	32
31	29	24	78	46	78	80	58	29	67	61	62	66
34	12	22	28	16	33	34	26	27	29	27	30	28
37	10	19	30	10	29	30	24	24	29	26	20	27
40	9	15	30	9	26	27	22	18	27	24	16	25
43	5	11	26	6	17	17	16	9	21	19	10	15
46	3	3	18	4	11	11	12	6	16	15	4	11
49	2	3	9	2	6	6	6	3	8	7	3	6
52	1	0	5	1	3	4	4	1	5	4	1	3
58	0	0	2	0	0	0	1	1	1	1	1	1

*T: TWAS

* M: Manure

Table D. 3. Cumulative CH_4 production (mL) in AnCoD of TWAS/Mnaure/SSO (T: TWAS, M:Manure)

Time (day)	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 0.5/0.25/0.25	T/M/SSO 0.25/0.5/0.25	T/M/SSO 0.25/0.25/0.5	T/M/SSO 0.4/0.4/0.2	T/M/SSO 0.2/0.4/0.4	T/M/SSO 0.4/0.2/0.4
0	0	0	0	0	0	0	0	0	0	0	0	0
1	5	48	6	12	18	24	9	52	25	8	35	8
3	27	120	54	44	89	93	64	147	85	62	99	50
4	65	222	116	78	194	189	141	265	164	132	196	129
5	117	323	215	135	336	325	246	389	278	237	314	242
6	152	440	327	202	442	439	340	530	391	339	437	346
7	185	579	440	271	548	547	437	628	507	445	562	452
8	203	658	559	342	633	628	529	730	627	554	696	555
10	228	735	687	417	717	713	610	825	734	652	802	650
12	251	805	808	489	796	793	687	921	836	744	903	741
14	270	895	898	542	860	859	750	984	917	818	986	813
16	287	951	958	577	920	921	811	1049	989	883	1068	877
19	307	1010	1012	609	981	983	862	1101	1047	937	1129	932
22	324	1049	1067	642	1034	1037	911	1148	1105	989	1191	985
25	337	1089	1115	670	1075	1079	948	1184	1151	1031	1238	1027
28	346	1121	1148	690	1107	1111	978	1239	1187	1063	1277	1059
31	375	1146	1225	736	1185	1191	1037	1268	1254	1125	1339	1125
34	387	1167	1253	752	1218	1225	1063	1295	1283	1151	1369	1153
37	397	1186	1284	762	1247	1255	1087	1319	1312	1177	1389	1180
40	406	1201	1314	771	1273	1281	1109	1336	1338	1202	1405	1205
43	411	1212	1339	777	1290	1298	1125	1345	1360	1221	1415	1220
46	414	1215	1357	781	1301	1310	1137	1351	1376	1236	1419	1232
49	416	1218	1366	783	1307	1316	1142	1354	1384	1243	1422	1237
52	417	1218	1371	784	1310	1320	1146	1355	1388	1247	1423	1241
58	417	1218	1373	784	1311	1320	1146	1355	1390	1248	1424	1241

*T: TWAS

* M: Manure

Table D. 4. Cumulative CH_4 production per mass of COD added in AnCoD of TWAS/Mnaure/SSO (T: TWAS, M: Manure)

Time (day)	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 0.5/0.25/0.25	T/M/SSO 0.25/0.5/0.25	T/M/SSO 0.25/0.25/0.5	T/M/SSO 0.4/0.4/0.2	T/M/SSO 0.2/0.4/0.4	T/M/SSO 0.4/0.2/0.4
0	0	0	0	0	0	0	0	0	0	0	0	0
1	2	12	1	4	4	6	2	13	6	2	9	2
3	9	30	13	13	22	23	17	37	21	16	25	13
4	21	54	28	23	48	46	37	67	41	34	49	33
5	38	79	52	39	83	80	65	98	70	62	79	63
6	49	108	79	59	110	108	90	134	98	88	109	89
7	59	142	106	79	136	134	116	159	128	116	141	117
8	65	162	135	99	157	154	140	185	158	144	174	144
10	73	180	166	121	178	175	162	209	185	169	201	168
12	80	198	195	142	198	195	182	233	210	193	226	191
14	87	220	217	157	214	211	199	249	231	213	247	210
16	92	234	231	168	228	226	215	265	249	230	267	227
19	99	248	244	177	244	241	228	278	263	243	283	241
22	104	258	258	186	257	254	241	290	278	257	298	255
25	108	267	269	195	267	265	251	299	290	268	310	266
28	111	275	277	200	275	272	259	314	299	276	320	274
31	120	281	296	214	294	292	275	321	315	292	335	291
34	124	287	303	218	302	300	282	328	323	299	343	298
37	127	291	310	221	310	308	288	334	330	306	348	305
40	130	295	317	224	316	314	294	338	337	312	352	312
43	132	298	324	225	320	318	298	340	342	317	354	315
46	133	298	328	227	323	321	301	342	346	321	355	318
49	133	299	330	227	324	323	303	343	348	323	356	320
52	134	299	331	228	325	324	303	343	349	324	356	321
58	134	299	332	228	325	324	304	343	350	324	356	321

*T: TWAS

* M: Manure

Table D. 5. Cumulative CH_4 production per mass of VSS added in AnCoD of TWAS/Mnaure/SSO (T: TWAS, M: Manure)

Time (day)	TWAS Only	Manure Only	SSO only	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 0.5/0.25/0.25	T/M/SSO 0.25/0.5/0.25	T/M/SSO 0.25/0.25/0.5	T/M/SSO 0.4/0.4/0.2	T/M/SSO 0.2/0.4/0.4	T/M/SSO 0.4/0.2/0.4
0	0	0	0	0	0	0	0	0	0	0	0	0
1	3	26	27	6	10	13	5	28	14	4	24	4
3	13	65	67	22	48	51	34	79	46	33	68	27
4	32	120	124	39	104	105	74	142	89	70	135	69
5	57	175	181	68	181	180	129	209	151	125	217	129
6	73	238	245	102	239	242	179	285	212	179	302	185
7	89	313	323	137	296	302	230	337	275	235	388	241
8	98	356	367	172	342	347	278	392	340	294	480	296
10	110	398	410	210	387	394	321	443	398	345	553	347
12	122	436	449	246	429	438	361	494	453	394	623	395
14	131	485	500	273	464	475	395	528	497	433	681	434
16	139	515	531	291	496	508	426	563	536	468	737	468
19	149	546	564	307	530	543	454	591	567	496	779	498
22	157	568	586	324	558	572	479	616	599	524	822	526
25	163	589	608	338	580	596	499	636	623	546	854	548
28	168	607	626	348	597	614	515	665	643	563	881	566
31	182	620	640	371	639	658	545	681	679	596	924	601
34	187	632	652	379	657	676	559	695	695	610	944	616
37	192	642	662	384	673	693	572	708	711	623	958	630
40	196	650	671	389	687	708	583	718	725	636	969	643
43	199	656	677	392	696	717	592	722	737	647	976	651
46	200	658	678	394	702	723	598	726	745	654	979	657
49	201	659	680	395	705	727	601	727	750	658	981	660
52	202	659	680	395	707	729	603	727	752	661	982	662
58	202	659	680	395	707	729	603	728	753	661	982	663

*T: TWAS

* M: Manure

Table D. 6. Cumulative CH_4 production per volume of substrate added in AnCoD of TWAS/Mnaure/SSO (T: TWAS, M: Manure)

Time (day)	TWAS Only	Manure Only	SSO only	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 0.5/0.25/0.25	T/M/SSO 0.25/0.5/0.25	T/M/SSO 0.25/0.25/0.5	T/M/SSO 0.4/0.4/0.2	T/M/SSO 0.2/0.4/0.4	T/M/SSO 0.4/0.2/0.4
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	0	1	0	1	1	0	1	0
3	0	3	1	1	2	2	1	3	2	1	2	1
4	1	5	3	1	5	5	1	6	4	3	4	3
5	2	8	6	2	8	8	2	9	6	5	7	5
6	2	11	9	3	10	11	3	12	9	7	10	7
7	2	14	12	4	13	14	4	14	11	9	13	9
8	3	16	15	5	15	16	5	16	14	11	16	11
10	3	18	18	6	17	18	6	18	16	13	18	13
12	3	20	21	7	19	20	7	20	19	15	21	15
14	3	22	24	8	20	21	8	22	21	17	23	17
16	4	23	25	9	22	23	8	23	22	18	24	18
19	4	25	27	9	23	24	9	24	24	19	26	19
22	4	26	28	10	24	26	9	25	25	20	27	20
25	4	27	29	10	25	27	10	26	26	21	28	21
28	4	28	30	11	26	27	10	27	27	21	29	22
31	5	28	32	11	28	29	10	28	28	23	31	23
34	5	29	33	12	29	30	11	29	29	23	31	24
37	5	29	34	12	29	31	11	29	29	24	32	24
40	5	30	34	12	30	32	11	29	30	24	32	25
43	5	30	35	12	30	32	11	30	31	25	32	25
46	5	30	36	12	31	32	11	30	31	25	32	25
49	5	30	36	12	31	33	11	30	31	25	33	25
52	5	30	36	12	31	33	12	30	31	25	33	25
58	5	30	36	12	31	33	12	30	31	25	33	25

*T: TWAS

* M: Manure

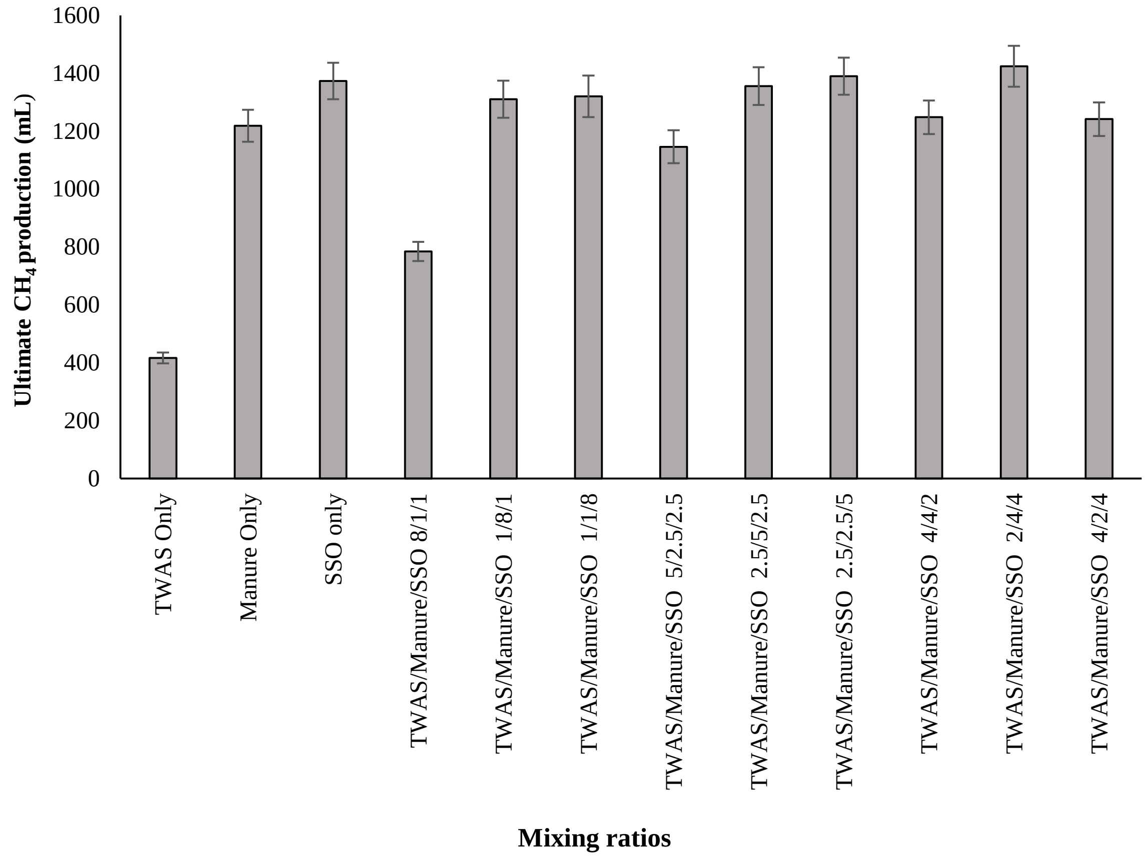


Figure D. 1. Ultimate CH4 production in AnCoD of TWAS/Manure/SSO

Table D. 7. Characteristics of raw feedstocks for hydrolysis/acidification in AnCoD of TWAS/Manure/SSO

Parameters	Units	TWAS		Manure		SSO		Inoculum	
		MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	36875	2853	100240	4014	102180	4120	16854	389
SCOD	mg/L	1931	26	10145	405	37350	1861	786	22
TSS	mg/L	28934	1006	54100	2164	49510	2093	15022	326
VSS	mg/L	22983	2216	45216	1289	44130	1743	10175	298
TS	mg/L	35705	6859	67680	2707	76878	3460	20650	425
VS	mg/L	31719	27	57830	2313	62948	2833	12988	396
Ammonia	mg/L	235	30	22.3	1	1326	60	1478	32
pH	-	6	0.1	6.8	0.2	5.8	0.2	7.1	0.2
Alkalinity	mg $CaCO_3$/L	1797	148	6984	279	6415	289	3867	135
TN	mg/L	2668	400	1690	68	4086	184	1983	69
TSN	mg/L	386	64	119	4	1096	49	679	24
Total Carbs	mg/L	849	112	25968	1039	13575	611	548	19
Total Proteins	mg/L	2547	298	5126	205	2087	94	1596	56
Total Lipids	mg/L	266	27	1652	66	1395	63	153	5

Table D. 8. Measured and theoretical VFAs concentrations (mg/L) over time in hydrolysis/acidification of TWAS/Manure/SSO

Measured VFAs concentrations (mg/L) over time												
	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 5/2.5/2.5	T/M/SSO 2.5/5/2.5	T/M/SSO 2.5/2.5/5	T/M/SSO 4/4/2	T/M/SSO 2/4/4	T/M/SSO 4/2/4
Time (hr)	1	2	3	4	5	6	7	8	9	10	11	12
0	428	1096	1538	673	1353	1660	977	1258	1357	1055	1402	1127
6	340	1286	1914	657	1580	2032	1086	1459	1609	1188	1658	1297
12	441	1319	2423	807	1690	2537	1295	1664	1949	1367	1949	1578
24	672	1837	2854	1117	2296	3041	1690	2178	2424	1811	2474	1991
48	744	1976	3309	1247	2503	3503	1897	2422	2755	2012	2783	2258
72	925	2196	2671	1361	2667	2939	1881	2417	2497	2050	2622	2103
Theoretical VFAs concentrations (mg/L) over time												
time (hr)	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/SSO 1/8/1	T/SSO 1/1/8	T/SSO 5/2.5/2.5	T/SSO 2.5/5/2.5	T/SSO 2.5/2.5/5	T/SSO 4/4/2	T/SSO 2/4/4	T/SSO 4/2/4
0	428	1096	1538	606	1074	1383	873	1040	1150	917	1140	1006
6	340	1286	1914	592	1254	1694	970	1206	1363	1033	1348	1158
12	441	1319	2423	727	1341	2114	1156	1375	1651	1188	1585	1409
24	672	1837	2854	1007	1823	2534	1509	1800	2054	1575	2011	1778
48	744	1976	3309	1124	1986	2919	1693	2001	2334	1750	2263	2016
72	925	2196	2671	1227	2117	2449	1679	1997	2116	1783	2132	1878

*T: TWAS * M: Manure

Table D. 9. Soluble and particulate COD concentrations (mg/L) over time in hydrolysis/acidification of TWAS/Manure/SSO

Soluble COD concentrations (mg/L) ocer time

Time	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 5/2.5/2.5	T/M/SSO 2.5/5/2.5	T/M/SSO 2.5/2.5/5	T/M/SSO 4/4/2	T/M/SSO 2/4/4	T/M/SSO 4/2/4
0	1050	2803	9906	2322	4106	8975	4109	4886	7040	3945	5955	5536
2	1700	3169	9933	2936	4548	9100	4576	5299	7353	4404	6274	5917
4	1968	3375	9948	3198	4787	9172	4790	5507	7509	4622	6438	6094
6	2175	3945	9956	3443	5374	9263	5065	5906	7745	4972	6745	6319
8	2557	4243	9972	3814	5716	9350	5364	6200	7956	5280	6972	6564
10	2868	4677	9992	4137	6184	9448	5662	6553	8189	5618	7246	6808
12	3147	5163	9996	4437	6697	9534	5953	6923	8420	5962	7530	7044
24	3282	5320	10009	4574	6870	9576	6075	7059	8514	6095	7636	7146
48	3317	5757	10121	4665	7317	9725	6247	7361	8721	6332	7891	7310
72	3368	6837	11168	4944	8516	10751	6866	8322	9681	7074	8145	8044

Particulate COD concentrations (mg/L) over time

Time	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/SSO 1/8/1	T/SSO 1/1/8	T/SSO 5/2.5/2.5	T/SSO 2.5/5/2.5	T/SSO 2.5/2.5/5	T/SSO 4/4/2	T/SSO 2/4/4	T/SSO 4/2/4
0	23602	31182	23728	25372	29385	23980	26557	27793	25255	27501	22242	25756
2	22053	30915	23581	24958	28895	23746	26090	27380	24983	27042	21923	25576
4	21784	30659	23497	24496	28656	23573	25877	27072	24787	26824	21779	25198
6	21577	30240	23319	24350	28265	23482	25601	26472	24451	26574	21452	24974
8	21395	29942	23165	23990	27923	23395	25502	26279	24339	25966	21225	24329
10	21184	29788	22885	23837	27555	22998	25375	25726	23906	25828	20951	24084
12	20985	29586	22605	23755	27382	22770	25130	25455	23856	25584	20467	23948
24	20780	28875	21920	23120	25960	22349	24320	24919	23182	24550	19461	22750
48	20515	27730	21590	22776	25141	21880	23950	24108	22774	24124	19181	22110
72	20404	27511	21496	22665	24990	21794	23830	23978	22675	23972	19055	21955

*T: TWAS

* M: Manure

Soluble COD concentrations (mg/L) over time												
Time	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 5/2.5/2.5	T/M/SSO 2.5/5/2.5	T/M/SSO 2.5/2.5/5	T/M/SSO 4/4/2	T/M/SSO 2/4/4	T/M/SSO 4/2/4
0	1050	2803	9906	2322	4106	8975	4109	4886	7040	3945	5955	5536
2	1700	3169	9933	2936	4548	9100	4576	5299	7353	4404	6274	5917
4	1968	3375	9948	3198	4787	9172	4790	5507	7509	4622	6438	6094
6	2175	3945	9956	3443	5374	9263	5065	5906	7745	4972	6745	6319
8	2557	4243	9972	3814	5716	9350	5364	6200	7956	5280	6972	6564
10	2868	4677	9992	4137	6184	9448	5662	6553	8189	5618	7246	6808
12	3147	5163	9996	4437	6697	9534	5953	6923	8420	5962	7530	7044
24	3282	5320	10009	4574	6870	9576	6075	7059	8514	6095	7636	7146
48	3317	5757	10121	4665	7317	9725	6247	7361	8721	6332	7891	7310
72	3368	6837	11168	4944	8516	10751	6866	8322	9681	7074	8145	8044

Particulate COD concentrations (mg/L) over time												
Time	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/SSO 1/8/1	T/SSO 1/1/8	T/SSO 5/2.5/2.5	T/SSO 2.5/5/2.5	T/SSO 2.5/2.5/5	T/SSO 4/4/2	T/SSO 2/4/4	T/SSO 4/2/4
0	23602	31182	23728	25372	29385	23980	26557	27793	25255	27501	22242	25756
2	22053	30915	23581	24958	28895	23746	26090	27380	24983	27042	21923	25576
4	21784	30659	23497	24496	28656	23573	25877	27072	24787	26824	21779	25198
6	21577	30240	23319	24350	28265	23482	25601	26472	24451	26574	21452	24974
8	21395	29942	23165	23990	27923	23395	25502	26279	24339	25966	21225	24329
10	21184	29788	22885	23837	27555	22998	25375	25726	23906	25828	20951	24084
12	20985	29586	22605	23755	27382	22770	25130	25455	23856	25584	20467	23948
24	20780	28875	21920	23120	25960	22349	24320	24919	23182	24550	19461	22750
48	20515	27730	21590	22776	25141	21880	23950	24108	22774	24124	19181	22110
72	20404	27511	21496	22665	24990	21794	23830	23978	22675	23972	19055	21955

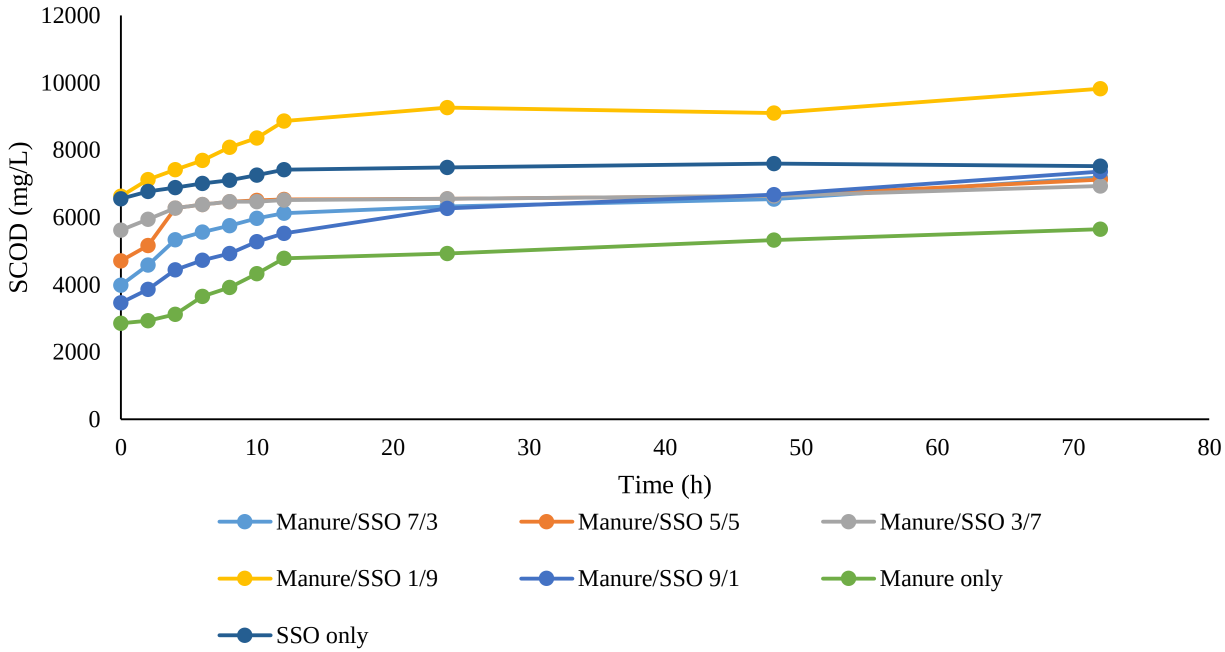


Figure D.2. Concentration of soluble COD over time at different mixing ratios

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There will come a time when you believe everything is finished.

That will be the beginning…

Luis L'Amour

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ANAEROBIC CO-DIGESTION OF MULTIPLE FEEDSTOCKS FOR BIOMETHANE RECOVERY- THE IMPACT OF LIPIDS:PROTEINS:CARBOHYDRATES RATIO

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ABSTRACT

Municipalities are facing increasing challenges regarding management and disposal of solid waste. Anaerobic digestion (AD) of municipal biowaste enables waste reduction and biogas production that can be utilized as a renewable source of energy for heat and power generation. Anaerobic co-digestion (AnCoD) enhances the performance of conventional mono-digestion. The mixing ratio of the feedstocks is an important criterion in AnCoD design which is typically determined based on the optimum carbon to nitrogen (C:N) ratio within the range of 25-30 or COD:N ratio in the range of 50-140. However, literature has shown contradictory results for the optimum C:N and COD:N ratios. Therefore, the main objective of this study was to primarily investigate the influence of the mixing ratio of the feedstocks including thickened waste activated sludge (TWAS), manure and source separated organics (SSO) on improving biomethane production and introducing a new methodology for optimizing the mixing ratio in AnCoD based on the lipids, proteins, and carbohydrates contents as the three main compounds existing in biowaste. The hydrolysis/acidification performance in AnCoD of manure, TWAS and SSO individually and in different combinations was also investigated. This study has introduced an empirical model to explain the relationship between the biomethane production and lipids: proteins: carbohydrates ratio of the feedstocks in anaerobic co-digestion of TWAS, manure and SSO. Among the binary ad ternary combinations, the ternary co-digestion of TWAS/manure/ SSO at the mixing ratio of 2:4:4 and lipids: proteins: carbohydrates ratio of 1:3:12 resulted in the maximum ultimate methane production. The maximum methane yield of 363 ml CH_4/g COD added corresponded to co-digestion of manure/SSO at the mixing ratio of 7:3. The maximum hydrolysis rate corresponded to the co-digestion of TWAS/manure at the ratio of 9:1. Overall, the best performance in both hydrolysis and methanogenesis was achieved by the co-digestion of TWAS with SSO at the ratio of 3:7 as well as TWAS/manure/SSO at the ratio of 2:4:4 compared to other feedstock mixes. It was observed that the proposed second order polynomial model could describe the relationship between biomethane production and lipids, proteins, and carbohydrates content of the feedstock.

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Dedication

To the light of my eyes

To my heavenly gift

And to all of my

inspiration

Armin

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List of Nomenclature

AD	Anaerobic digestion
AnCoD	Anaerobic co-digestion
TWAS	Thickened waste activated sludge
SSO	Source separated organics
F:M	Food to microorganism ratio
COD	Chemical Oxygen Demand
TCOD	Total Chemical Oxygen Demand
SCOD	Soluble Chemical Oxygen Demand
VSS	Volatile suspended solids
TSS	Total suspended solids
VS	Volatile solids
TS	Total solids
VFAs	Volatile fatty aids
GC	Gas chromatograph
S Proteins	Soluble proteins
S Lipids	Soluble lipids
S Carbohydrates	Soluble carbohydrates
LCFAs	Long chain fatty acids
TN	Total nitrogen
TSN	Total soluble nitrogen
C:N	Carbon to nitrogen ratio
COD:N	COD to nitrogen ratio

Chapter 1
Introduction

1. INTRODUCTION

Waste materials including biowaste are constantly being generated due to inevitable human activities. Different techniques have been employed to manage and reduce the growing amount of biowaste. However, such technologies result in secondary environmental impact. Landfilling can lead to soil and groundwater contamination imposing further actions and cost to remediate the secondary contamination. If not well managed and maintained, incineration will cause air pollution and subsequent environmental and health impact (Elbeshbishy et al., 2017; Feng et al., 2018). Mitigation of climate change and fossil fuel consumption demands a shift to alternative, renewable energy sources (Cornelissen et al., 2012; Kwietniewska and Tys, 2014). As reported by International Energy Outlook, 2011, total world energy consumption will increases from 505 quadrillion British thermal units (Btu) in 2008 to an estimated number of 619 quadrillion Btu in 2020, and it is expected to rise to 770 quadrillion Btu in 2035 which is equivalent to 53% increase in spam of 27 years (OECD/EIA, 2011). Energy obtained from biomass is regarded as an important future renewable source, as it is capable of providing a continuous power generation and it is also an essential part of the current CO_2-mitigation policy (Appels et al., 2011; Kwietniewska and Tys, 2014).

Production of biofuel from biomass, has received increasing attention during recent years. Several treatment processes and technologies have been established to obtain sustainable and affordable biofuel which includes syngas (SNG). SNG is a synthetic gas produced by gasification of a carbon containing fuel that has some energy value. However, production of SNG is narrowly practiced due to its cost (Guo et al., 2015; Schuetzle et al., 2015). Anaerobic digestion (AD) which is widely used for the treatment of wet residual biomass is considered to be one of the most favorable processes for biofuel production from biomass.

In Canada, more than 27 million tons of food waste are disposed yearly. The produced organic waste can lead to serious health and environmental issues. Municipalities have taken different management actions to manage the organic fraction of municipal solid waste (OFMSW). Biological processes for the treatment and/or conversion of OFMSW to value-added products has aroused significant attention due to its financial benefits and less environmental impacts compared to the other waste disposal methods such as landfilling, incineration, gasification, etc. (Luk and Bekmuradov, 2014; Naroznova et al., 2016; Razavi et al., 2019). Organic matters in waste includes food scraps, yard trimmings, wood waste, paper and cardboard products which normally make up around 33% by weight of the municipal solid waste. Source Separated Organics (SSO) refers to the organic waste which is segregated from other waste materials at the source for separate collection. SSO comprises mostly of food waste which is separated from the residential waste (Kelleher Robins, 2013).The study by Kelleher Environmental have indicated that almost 23% of waste from non-residential industrial, commercial and institutional (IC&I) sector is food waste which is generated by institutions and businesses in communities across Canada. The majority of food waste from this sector is created by restaurants, hotels, food processing facilities and hospitals. A considerable amount of biogas through anaerobic digestion process can be produced by food wastes from all of these sources (Government of Canada, 2013; Kelleher Robins, 2013).

In areas with vast numbers of large-scale livestock farms, the development of a treatment process for manure is necessary to properly handle the high amount of produced waste. Animal manure provides adequate nutrients for anaerobic digestion to produce biogas as a source of renewable energy. High degradability of manure when flushed and fibrous materials are separated makes its anaerobic digestion a good treatment option to minimize waste above and beyond bioenergy recovery. However, in some farms manure is collected in lagoons or in the open areas which can result in air and water pollution. Manure stockpiling can lead to serious health environmental issues as a result of emissions such as methane, nitrous oxide, ammonia, hydrogen sulfide, volatile organic compounds and particulate matter (Lin et al., 2018; Peter Wright et al., 2013). Co-digestion of manure with additional substrates provided that appropriate mixture ratios are applied can improve digestion process and increase biogas production.

In addition, in Canada more than 660,000 metric tons of dry stabilized biosolids is produced each year. Biosolids are produced during the treatment of wastewater due to the removal of the solids content (sludge) from the liquid effluent. Biosolids management is responsible for almost 50% of the total operating annual cost of wastewater management. According to Canadian Council of Ministers of the Environment (CCME), a Canada-wide approach for the management of wastewater biosolids was approved on October 11, 2012 which encourages the favorable and thorough management of biosolids. Biosolids are the nutrient-rich, organic materials which makes them a useful resource, containing necessary plant nutrients, and organic matter. They can be recycled as a fertilizer and soil improvement for agricultural utilization. Annually, around 195,000 tons of biosolids is processed by the Ashbridges Bay and Highland Creek wastewater treatment plants in City of Toronto. The City's biosoilds management can be classified as the following options (City of Toronto, 2019):

- Land application for agricultural and other purposes
- Pelletization for fertilizer production
- Alkaline stabilization for producing fertilizer, landfill cover, or for the pH adjustment of acidic soil
- Landfilling
- Incineration

There are some pros and cons associated with any of the above options. However, Anaerobic Digestion (AD) for producing bioenergy from organic waste has shown to be an energy-efficient technology while causing less environmental footprint compared to other technologies such as composting that requires large land and can release uncontrolled odorous volatile organic compounds and pathogens (Bordeleau and Droste, 2011; Lin et al., 2018; Razavi et al., 2019). Nevertheless, in conventional anaerobic digestion of single feedstock some problems such as nutrient imbalance, low biodegradability, toxic substances, etc. can hinder the process causing low production of biogas. Such problems can be modified by simultaneous digestion of two or more feedstocks referred to as anaerobic co-digestion (AnCoD).

1.2. Research objective

Anaerobic digestion involves sequential phases comprising hydrolysis, acidogenesis, acetogenesis and methanogenesis. Anaerobic digestion of multi feedstocks has been advantageous compared to conventional anaerobic digestion of single feedstock. Literature has showed contradictory values for the optimum range of the C:N or COD:N ratio for optimizing the mixing ratio of the feedstocks in anaerobic co-digestion systems. In addition, hydrolysis of particulate organic matter which is the rate-limiting stage, remains as the least well-defined phase of the process. Therefore, this research focused on the three main following objectives:

4- Study biomethane potential in AnCoD of TWAS, Manure, and SSO. This part of the study included the following purposes:

- ➢ Evaluate the influence of the mixing ratio on improving biomethane production in anaerobic co-digestion of TWAS, manure, and SSO.
- ➢ Investigate the impact of lipids: proteins: carbohydrates on improving biomethane production in anaerobic co-digestion of TWAS, manure, and SSO.
- ➢ Compare the impacts of COD:N ratio, lipids:proteins, lipids:carbohydrates, and proteins:carbohydrates on biomethane production in anaerobic co-digestion of TWAS, manure, and SSO.

5- Study the hydrolysis/acidification in AnCoD of TWAS, Manure, and SSO. This part of the present research comprised the following areas:

- ➢ Monitor the dynamic changes of soluble and particulate COD, lipids, proteins, and carbohydrate content in co-digestion of TWAS, manure, and SSO.
- ➢ Evaluate the influence of the mixing ratio on hydrolysis/acidification performance in co-digestion of TWAS, manure, and SSO.
- ➢ Study the hydrolysis kinetics of lipids, proteins, carbohydrates and COD and calculate their hydrolysis rate coefficients for co-digestion of TWAS, manure, and SSO.

6- Investigate the relationship between biomethane production and the lipids, proteins, and carbohydrates contents of the feedstocks aiming to:

- ➢ Propose an empirical model that explains the relationship between biomethane yield and concentration of lipids, proteins, and carbohydrates at different mixing ratios in anaerobic digestion of manure, TWAS and SSO individually and in their co-digestion.

an empirical model that explains the relationship between biomethane yield and concentration of lipids, proteins, and carbohydrates at different mixing ratios in anaerobic digestion of manure, TWAS and SSO individually and in their co-digestion.

Chapter 2

Literature review

2. LITERATURE REVIEW

2.1. Anaerobic digestion

Biological treatment is an integral part of any wastewater treatment plant that treats wastewater from either municipality or industry which contains soluble organic contaminants or a mix of both source types. There are clear economic advantages of biological systems in terms of capital investment and operating costs when compared to other methods such as chemical oxidation, thermal oxidation, etc. In addition, mostly biological treatment converts toxic contaminants to end products that are less harmful or non-harmful with the help of microorganisms (Mittal, 2011). The two types of biological treatment: aerobic and anaerobic processes are directly related to first the type of microorganisms involved in the degradation of organic compounds in a particular wastewater and second the operating conditions of the bioreactor. Aerobic treatment processes occur in the presence of air and consume those microorganisms also called aerobes, which use molecular/free oxygen to assimilate organic contaminant. They convert them in to carbon dioxide, water and biomass (Eddy, 2003). The anaerobic processes, on other side occur in the absence of air (molecular/free oxygen) by those microorganisms also called anaerobes which do not require air or molecular/free oxygen to assimilate organic contaminants. The end products of organic assimilation in anaerobic treatment contains methane (CH_4) and carbon dioxide (CO_2) and biomass.

Anaerobic digestion of municipal wastewater sludge has been widely developed since the early 1900s and is the most applied sludge treatment method. Throughout the process about 40% to 60% of the organic solids is converted to methane and carbon dioxide. The chemical composition of the produced gas contains 60-65% methane, 30-35% carbon dioxide, and small quantities of H_2, N_2, H_2S, and H_2O. Among these, methane has the most value since it is a hydrocarbon fuel producing 36.5 MJ/m^3 in combustion (Lema and Suarez, 2017). The residual organic matter is chemically stable, nearly odorless, and contains considerably reduced levels of pathogens.

Hydrogen production by the use of anaerobic microbial communities also referred to as dark fermentation from organic waste has raised attention due to its ability to produce an environmentally benign energy source, while it stabilizes waste material simultaneously (Sung et al., 2003). Albert Lea facility in Minnesota with 12 million gallons/day of sewage and 4.5 million gallons/day sludge produces 75,000 ft^3/day of biogas. Their facility which consists of 4 Capstone microturbines, each of them 30 kW, is producing 2,500 kWh per day electricity at peak production in addition to 28,000 Btu/day of heat.

According to Canadian Biogas Study conducted by Canadian Biogas Association in 2013, biogas production has significant potential for extension and development. All biogas sources excluding energy crops, could meet nearly 3% of Canada's natural gas demand as biogas contribution is 2,420 Mm^3 /year of renewable natural gas (RNG) or 1.3% of its electricity demand where biogas contribution is 810 MW. Anaerobic digestion (AD) is a multi-step process during which organic material is converted to biogas and digestate in the absence of oxygen and presence of anaerobic microorganisms. The AD process occurs in four stages including hydrolysis/liquefaction, acidogenesis, acetogenesis, and methanogenesis. The pathways in anaerobic degradation is shown in figure 1.

The first stage of the process is hydrolysis/liquefaction. This stage is very important in AD process since polymers cannot be directly consumed by the fermentative microorganisms. Therefore, hydrolysis makes the substrate available for the following conversion steps. In hydrolysis stage, insoluble complex organic materials are decomposed into their constituents. This allows them transport through microbial cell membrane (Madigan, 2014). Hydrolysis is accomplished due to the function of hydrolytic enzymes. In the first step of hydrolysis, or liquefaction, fermentative bacteria convert the insoluble complex organic compounds like cellulose, into soluble molecules such as sugars, fatty acids, and amino acids.

Hydrolysis/Liquefaction reactions

Lipids → Fatty Acids

Polysaccharides → Monosaccharides

Protein → Amino Acids

Nucleic Acids → Purines and Pyrimidines

The next stage is acidogenesis/fermentation through which facultative and anaerobic bacteria convert sugars, amino acids and fatty acids to hydrogen, acetate, carbon dioxide, VFAs such as propionic, butyric and acetic acid, ketones, alcohols and lactic acid. Although a simple substrate like glucose can be fermented, various products are created by the diverse bacterial community.

Conversion of glucose to acetate, ethanol and propionate are shown in the reactions 1, 2 and 3 below respectively (Kangle et al., 2012).

$$C_6H_{12}O_6 + 2\ H_2O \rightarrow 2\ CH_3COOH + 2\ CO_2 + 4\ H_2 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2\ CO_2 \quad (2)$$

$$C_6H_{12}O_6 + 2\ H_2 \rightarrow 2CH_3CH_2COOH + 2\ H_2O \quad (3)$$

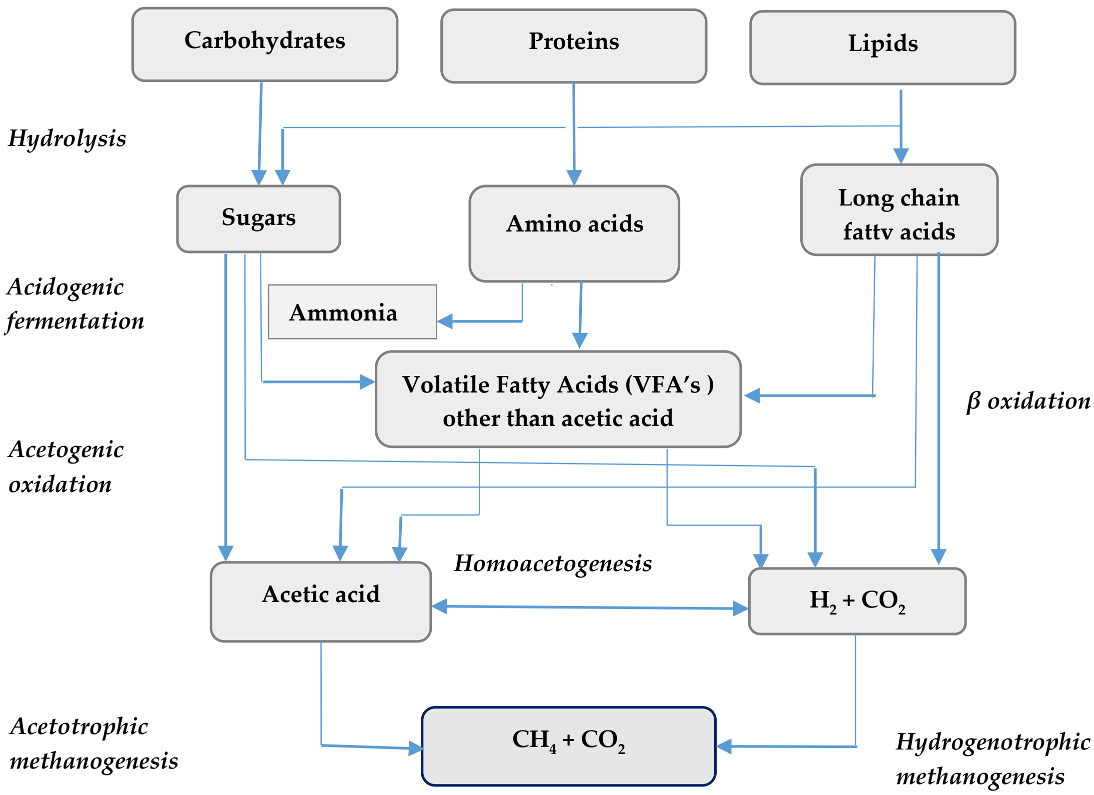


Figure 2.1. Pathways of anaerobic digestion (Salminen and Rintala, 2002)

In equilibrium condition, most of the organic material is converted into substrates such as acetate, hydrogen and carbon dioxide that are readily available for methanogenic microbes. However, a significant portion of about 30% is converted to short chain fatty acids or alcohols. Degradable organic material is eliminated in this step (Angelidaki et al., 2009a; Kangle et al., 2012). The byproduct of amino acids fermentation, ammonia and hydrogen sulfide are released. These compounds can be inhibitory for AD process (Kangle et al., 2012; Salminen and Rintala, 2002).

During the third stage, acetogenesis, certain fermentation products including VFAs with more than two atoms of carbon, alcohols and aromatic fatty acids are converted into acetate and hydrogen these conversions take place via obligate hydrogen producing bacteria (Boe, 2006, Kangle et al., 2012). In this step, the products of the first phase are converted to simple organic acids, carbon dioxide and hydrogen by acetogenic bacteria, also called acid formers. The main acids produced include acetic acid (CH_3COOH), propionic acid (CH_3CH_2COOH), butyric acid ($CH_3CH_2CH_2COOH$), and ethanol (C_2H_5OH). The activities of different microorganisms cause the formation of the products that are created during acetogenesis. These microorganisms include syntrophobacter wolinii, a propionate decomposer and sytrophomonos wolfei, a butyrate decomposer. Also other acid formers include clostridium spp., peptococcus anerobus, lactobacillus, and actinomyces (Kangle et al., 2012; Themelis, 2002). Hydrogen-producing acetogenic bacteria yield acetate, H_2 and CO_2 from volatile fatty acids and alcohol, whereas, homoacetogenic bacteria produce acetate from CO_2 and H_2 (Sterling et al., 2001). However, most of the acetate is formed via hydrogen-producing acetogenic bacteria (Angelidaki et al., 2009b). A reaction that occurs during acetogenesis is presented below:

$$C_6H1_2O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

The final stage of AD process is methanogenesis. Various methane-forming bacteria are required in an AD system. That is because a single species is not able to degrade all the existing substrates. The methanogenic bacteria comprise methanobacterium, methanobacillus, methanococcus, and methanosarcina. Methanogenesis can be classified into two groups. These two groups involves acetate and H_2/CO_2 consumers. *Methanosarcina spp*. and *methanothrix spp*. Also known as *methanosaeta* are important in AD process both as H_2/CO_2 and acetate consumers. Almost 70% of the methane is produced from acetate. The remaining 30% is produced due to the reduction of carbon dioxide via hydrogen and other electron donors (Smith and Mah, 1966; Varel et al., 1980). With regard to the type of the substrate consumed by the methanogens, methanogenesis can be classified into two main types (Bitton, 2010; Kangle et al., 2012):

- Hydrogenotrophic methanogenesis

In this type of methanogenesis, hydrogen and carbon dioxide are converted into methane through the following reaction:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

- Acetotrophic or aceticlastic methanogenesis

This type of methanogenesis involves the formation of methane from the conversion of acetate by the following reaction:

$$CH_3COOH \rightarrow CH_4 + CO_2$$

Any substrate that can be converted to methane by anaerobic bacteria is referred to as feedstock. The main components of organic wastes (feedstock) are carbon, oxygen, nitrogen, hydrogen and phosphorus, and microbial cell material of those elements is approximately 50, 20, 12, 8 and 2 % respectively (Gerardi, 2003). Sulfur is also required to synthesize vital proteins in metabolic and anabolic pathways (Madigan, 2014). Feedstocks can be a range of different waste materials from easily degradable wastewater to complex high-solid waste.

AD technologies have shown sufficient adaptability to different feedstocks (Bordoloi et al., 2014). Although AD is a commercial reality for a range of wastes, anaerobic digestion of single waste may be associated with certain drawbacks such as unbalanced nutrients, rapid acidogenesis, poor buffering capacity, high ammonia nitrogen concentration, inhibition of long chain fatty acids which can inhibit methanogenesis and lead to severe instability and process disruption (Bayr et al., 2014; Silvestre et al., 2014).

The growth rate of anaerobic microorganisms and subsequent biogas production depends highly on the composition of the organic matter in feedstock. The constituents of the feedstock added to the digester are consumed selectively by a range of different microbial consortia. In addition, the existence of nitrogen in the feedstock is necessary for the synthesis of amino acids, proteins and nucleic acids. It is also required for ammonia formation to neutralize VFAs produced during the fermentation process and to maintain neutral pH condition for cell growth. However, an excess of nitrogen in the feedstocks can result in toxic effects to bacteria by extreme ammonia formation. Therefore, a suitable amount of nitrogen is required to provide sufficient nutrient while avoiding ammonia toxicity (Hagos et al., 2017; P. et al., 2014).

During anaerobic digestion, a series of complex biological degradation pathways are involved which are influenced by numerous factors. Therefore, a profound understanding of the biochemical activities of anaerobic microorganisms in the AD system is required to support an effective control of the governing factors in anaerobic digestion process (Viotti et al., 2004).

2.2. Process parameters of anaerobic digestion

A number of parameters affect the rate of the different steps of the digestion within the anaerobic environment. Overall, two groups of parameters can affect the anaerobic digestion performance including environmental and operational parameters. Environmental factors comprise temperature, pH, alkalinity, waste characteristics such as the amounts of volatile solids (VS) carbon to nitrogen (C:N) ratio, total solids (TS), nutrients, organic loading rate (OLR), and volatile fatty acids (VFAs). The performance of anaerobic digesters can be reduced by various environmental factors including low pH, accumulation of ammonia and volatile fatty acids (VFAs) which inhibit the activity of methanogenic microorganisms (Heo et al., 2004; Towey, 2013). VS consists of both biodegradable volatile solids (BVS) and the refractory volatile solids (RVS) fractions. BVS fraction of substrate is helpful in better biodegradability of the waste, organic loading rate, C:N ratio, and biogas production. Apart from the environmental factors, a number of operational factors including solid retention time (SRT), hydraulic retention time (HRT), digestion mode in terms of single or multistage approaches, digester design being batch or continuous types, and digester mixing also affect the AD performance. These parameters are individually discussed in the following sections.

2.2.1. Waste composition and volatile solids

The wastes to be treated by AD process can be comprised of a biodegradable organic fraction, a combustible and an inert fraction. Waste materials containing high VS and low non- biodegradable material, or refractory volatile solids, are the most suited to AD treatment. Kitchen waste, food waste, and garden waste are biodegradable organic fraction of the waste. The combustible fraction can be slowly degrading lignocellulosic organic compounds such as coarser wood, paper, and cardboard. The lignocellulosic organic content does not readily degrade under anaerobic conditions. Therefore, they are more suitable for waste-to-energy plants. The inert fraction of the waste may contain stones, glass, sand, metal, etc. that can ideally be removed, recycled or used at land fill. It is important to remove the inert fraction before digestion, otherwise it causes the increase of digester volume and wear of equipment. The Volatile Solids (VS) of organic wastes are measured as total solids excluding the ash content which is obtained by complete combustion of the feed wastes. The VS contains the Biodegradable Volatile Solids (BVS) fraction and the Refractory Volatile Solids (RVS). Knowing the BVS fraction of substrate can be helpful in better estimation of the biodegradability of the waste, of biogas production, organic loading rate and C:N ratio. Lignin is a complex organic compound which is not easily degraded by anaerobic bacteria and forms refractory volatile solids (RVS) in organic matter. Waste materials containing high VS and low non-biodegradable material, or RVS, are the most suited to AD treatment (Elbeshbishy et al., 2017; Kangle et al., 2012).

2.2.2. Carbon to Nitrogen Ratio (C:N)

The amount of carbon and nitrogen present in feedstock or C:N ratio is a very important parameter of the AD process. A high C:N ratio indicates rapid consumption of nitrogen by methanogens and leads to lower gas production. On the contrary, a lower C:N ratio results in accumulation of ammonia and exceeding pH values which is toxic to methanogens. Low C:N ratios occur when too much nitrogen is present. On the other side, a high C:N ratio leads to deficiency in AD system (Mata-Alvarez et al., 2000a; Poliafico and Murphy, 2007). According to a study (Gerardi, 2003), the optimal gas production can be achieved by feedstock with C:N ratio of 25:1. Other studies reported that the optimum range falls within 25-30 and can be achieved by the co-digestion of different waste streams (Gonzalez-Avila et al., 2011; Monnet, 2003). Some other studies even though reported that the optimum C:N ratios in anaerobic digesters are between 20 and 30 while some other studies even reported lower values than 20. Although, a very low C:N ratios occurs when too much nitrogen is present and leads ammonia (NH_3) to be accumulated which leads to either high pH values or methanogenic inhibition (Salminen et al., 2002, Kangle et al, 2012). On the contrary, the high C:N ratio indicates that nitrogen is rapidly depleted by methanogens and leads to lower gas production. A lower C:N ratio results in accumulation of ammonia and exceeding pH values to over 8.5, which is toxic to methanogens. Mixing materials of high and low C:N ratios, such as organic solid waste mixed with animal manure or sewage can help achieve optimum C:N ratios (Poliafico, 2007, Kangle et al, 2012).

2.2.3. Nutrients

Some nutrient elements are needed for the growth of methane-forming bacteria. Particular metals comprising nickel, iron, cobalt, and molybdenum are essential for optimal growth and methane production. Trace metals stimulate methanogenic activity. Some metals including selenium, molybdenum, manganese, aluminum, and boron have been suggested as additional components in media. Addition of metal ions solutions to anaerobic digesters can improve the performance of the AD system. The amounts of 0.002, 0.004, 0.003, 0.02 mg/g are suggested for iron, cobalt, nickel, and zinc respectively. The requirement for nickel is quite unusual for biological systems and can exclusively characterize methanogenic bacteria. Addition of metal ions solutions to anaerobic digesters can improve the performance of the AD system (Azbar and Speece, 2001; Kangle et al., 2012; Mata-Alvarez et al., 2000a).

2.2.4. Total solids content (TS)/Organic loading rate (OLR)

The increase of the total solids (TS) fraction leads a corresponding decrease in the reactor volume. High solids (HS) AD system contain 22% to 40% TS, medium solids (MS) system about 15 to 20% and low solids (LS) AD systems contain less than 10 % of TS. The organic loading rate (OLR) is defined as the organic matter flowing into the digester per time which is expressed as mass of organic matter over digester volume over time (Appels et al., 2008; Kangle et al., 2012). OLR is also defined as measure of the biological conversion capacity of the AD system. Typical values of OLR ranges between 0.5 and 3 kg VS/m^3/d. OLR is particularly important parameter in continuous systems. System failures as a result of overloading have been reported by numerous plants (Kangle et al., 2012; Poliafico and Murphy, 2007).

When feeding the system above its sustainable OLR, low biogas yield is obtained. This is caused by the accumulation of the inhibiting substances such as fatty acids in the digester slurry. Any substrate that can be converted to methane by anaerobic bacteria is referred to as feedstock. The main components of feedstock are carbon, oxygen, nitrogen, hydrogen and phosphorus, and microbial cell material of those elements is reported to be approximately 50, 20, 12, 8 and 2 % respectively. Feedstocks can be a range of different waste materials from easily degradable wastewater to complex high-solid waste (Gerardi, 2003).

2.2.5. pH, alkalinity and volatile acids/alkalinity ratio

There is a different optimum pH range for each group of micro-organisms. Methanogenic bacteria are very sensitive to pH. The optimum range for them is between 6.5 and 7.2. The fermentative microorganisms are relatively less sensitive and can tolerate a wider range of pH between 4.0 and 8.5. At low pH, mainly acetic and butyric acids are produced, while at a pH of around 8.0, acetic and propionic acids are mainly produced. The VFAs produced during AD process result in a pH reduction. This reduction is normally adjusted by methanogenic bacteria, which produce alkalinity in the form of ammonia, carbon dioxide, and bicarbonate (Appels et al., 2008).

The pH of the system is controlled by the CO_2 in the gas phase and the HCO_3-alkalinity of the liquid phase. If the concentration of CO_2 remains constant, the addition of HCO_3-alkalinity can increase the pH of digester. In order to maintain a stable and well-buffered digestion process a buffering capacity of 70 meq $CaCO_3$/L or a molar ratio of at least 1.4:1 of bicarbonate/VFA is required. However, previous studies have shown that particularly the stability of the ratio is very significant and not so much its level.

Except for ammonia, other factors such as sulfide, sodium and potassium, heavy metals, volatile fatty acids, long-chain fatty acids, and hydrogen can also affect the activity of methanogens. Molecular hydrogen is formed throughout different stages of anaerobic digestion. Inhibition can occur due to the lack of balance between the rates of hydrolysis and methanogenesis. A suitable balance between those rates is essential for higher methane production. Rapid methanogenesis is required to prevent accumulation of organic acid lowering pH to an extent that inhibits methanogenesis (Appels et al., 2008; Pouget et al., 2012).

2.2.6. Temperature

The temperature has an important effect on the physicochemical properties of the substrate. Moreover, it is effective on the growth rate and metabolism of micro-organisms and the population dynamics in the reactor. A stable operating temperature is very important to be maintained in the digester, since fluctuations in temperature affect the bacteria particularly the methanogens. Acetotrophic methanogens are one of the most sensitive groups of bacteria to increasing temperatures. The degradation of propionate and butyrate is also sensitive to temperatures above 70 ˚C. (The propionate or propanoate ion is $C_2H_5COO^-$ the conjugate base of propionic acid- A propionic or propanoic compound is a small salt or ester of propionic acid. Butyric acid, also known under the systematic name butanoic acid, abbreviated BTA, is a carboxylic acid with the structural formula $CH_3CH_2CH_2$-COOH. Salts and esters of butyric acid are known as butyrates or butanoates) (Appels et al., 2008; Turovskiy and Mathai, 2005).

The temperature has also a substantial effect on the partial pressure of H_2 in digesters and therefore, it affects the kinetics of the syntrophic metabolism. Thermodynamic studies show that endergonic reactions under standard conditions, for example the decomposition of propionate into acetate, CO_2, and H_2, would be more favorable energetically at higher temperature, whereas the exergonic reactions such as hydrogenotrophic methanogenesis are less favored at higher temperatures. Increasing temperature can be favorable for several reasons. It can increase solubility of the organic compounds, enhance chemical and biological reaction rates, and an increase the rate of pathogens' death in thermophilic conditions. Although, the application of high temperatures (thermophilic) has some adverse effects as there will be an increase of the fraction of free ammonia which can be an inhibiting factor for the microorganisms. The increasing pKa of the VFA will make the process more susceptible to inhibition. Therefore, temperature control is a very important concern for thermophilic digestion in comparison with mesophilic digestion. A stable operating temperature is very important to be maintained in the digester, since fluctuations in temperature affect the bacteria particularly the methanogens. Process failure can occur at changes in temperature over 1℃/day and changes of more than 0.6℃/day in temperature should be avoided Process failure can occur at changes in temperature over 1℃/day, and changes of more than 0.6℃/day in temperature should be avoided (Appels et al., 2008; Turovskiy and Mathai, 2005).

2.2.7. Solids and hydraulic retention time

The average time that the solids spend in a digester is referred to as solids retention time (SRT), and the average time that the liquid sludge is held in the digester is referred to as hydraulic retention time (HRT). SRT and HRT are an important design and operating parameter for all anaerobic processes. Reduction of SRT decreases the extent of the reactions and vice versa. A fraction of the bacterial population is removed each time when the sludge is withdrawn. Therefore, the cell growth must at least compensate the cell removal to maintain steady state and to prevent from process failure.

The ratio between the solid content of the reactor by the solid flow rate indicates the average SRT. HRT specifies the average time that waste and wastewater are exposed to microorganisms for degradation. In a conventional mixed reactor, the SRT and HRT are equal while in a retained biomass reactor the SRT is usually higher than HRT. Higher SRT can be achieved by increasing the digester volume, increasing the solid content or using a retained biomass reactor. Equation (1.1) shows the calculation for HRT and equation (1.2) shows the calculation for SRT in continues stirred tank reactor (CSTR).

HRT= Volume of the reactor (V)/ Influent flow rate= L/L/d= d Eq. 2.1

SRT= Mass of the biomass in reactor/ mass of the biomass leaving

$= (V \times VSS)/(Q_{out} \times VSS) = V/Q_{out} = HRT\ d$ Eq. 2.2

The effect of the retention time on the gas production is mostly studied on laboratory scale. Studies indicated that in a semi-CSTR system retention times shorter than 5 days are insufficient for a stable digestion. VFA concentrations are increasing due to washout of methanogenic bacteria. VFA concentrations are relatively high for 5–8 days SRT. There is an incomplete breakdown of compounds, especially of the lipids for this SRT. It has also been indicated that stable digestion is obtained after 8–10 days. There is low VFA concentrations and the breakdown of lipids starts. According to the studies, the breakdown curve stabilizes at SRT>10 days as all sludge compounds are significantly reduced. Figure 2 shows a schematic relationship between SRT and degree of digestion (Appels et al., 2008).

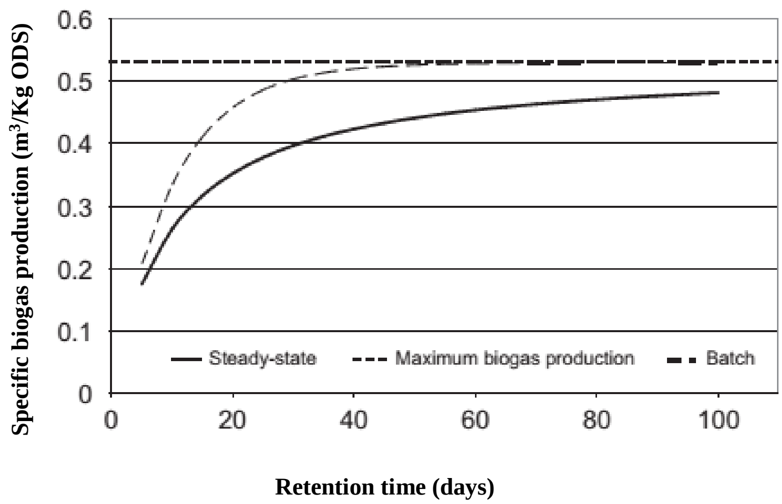


Figure 2.2. Schematic relationship between SRT and degree of digestion (Appels et al., 2008)

2.2.8. Digester mixing

To attain an optimum performance for an AD system, it is essential to maintain a proper mixing. Mixing causes near contact between the feed sludge and active biomass, yielding uniformity of several parameters including temperature, substrate concentration, other chemical, physical and biological aspects all through the digester. Mixing also prevents from the formation of surface scum layers and the sludge deposition on the bottom of the tank (Appels et al., 2008). The rise of gas bubbles and the thermal convection currents caused by the addition of heated sludge, results in some degree of natural mixing in the digestion tank. However, this is not adequate for an optimum performance and auxiliary mixing is required. There are some methods of auxiliary mixing including external pumped recirculation, internal mechanical mixing and internal gas mixing as it is shown in Fig. 3.

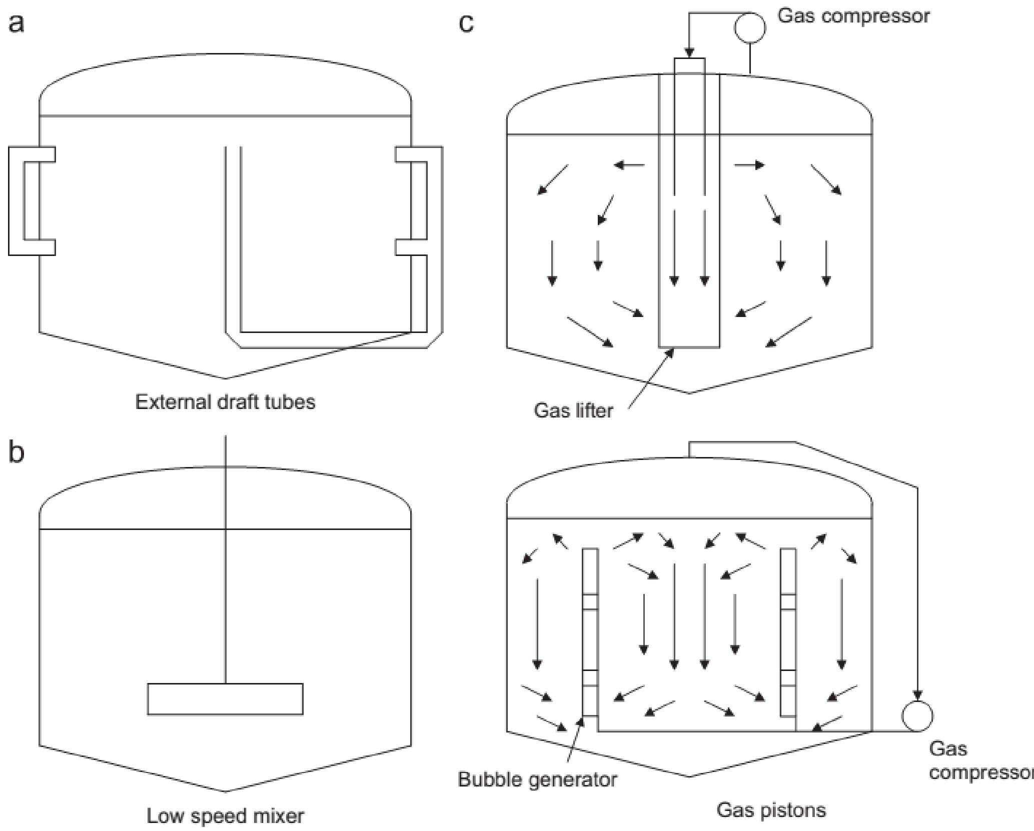


Figure 2.3. Types of mixing methods for digesters (a) external, pumped recirculation mixing, (b) internal mechanical mixing, and (c) external gas recirculation mixing (Appels et al., 2008).

2.3. Anaerobic Co-Digestion (AnCoD)

Anaerobic Co-digestion (AnCoD) involves the simultaneous anaerobic digestion of two or more organic waste feedstocks. The anaerobic co-digestion can be referred to as the simultaneous treatment of two or more organic biodegradable waste streams by anaerobic digestion. It provides a proper method of disposal for the organic fraction of solid waste which comes from source or from separate collection systems. This method of treatment, makes it possible to use the existing anaerobic reactors in wastewater treatment plants, with minor modifications and some additional requirements. By combining the treatments of two problematic wastes including for instance organic part of municipal solid waste and paper pulp sludge, higher yield in the production of biogas can be attained. Conventionally, anaerobic digestion was a single substrate and single purpose treatment. Recently, it has been indicated that when an increased variety of substrates applied at the same time, more stable AD is achieved. The most common state is when a major amount of a main basic substrate for example manure or sewage sludge is mixed and digested accompanied by minor amounts of a single, or a variety of additional substrate. The usage of co-substrates usually improves the biogas yields from anaerobic digester due to positive interaction established in the digestion medium and the supply of missing nutrients by the co-substrates (Alvarez et al., 2008, Kangle et al, 2012). Anaerobic co-digestion offers several benefits including: improved nutrient balance and digestion, possible gate fees for waste treatment, additional biogas collection, and additional fertilizer i.e. soil conditioner (Elbeshbishy and Nakhla, 2012; Viotti et al., 2004).

Substrates with high C:N ratio have the poor buffering capacity and produce excessive amounts of VFAs during the fermentation. In contrast, substrates characterized by low C:N ratio have high buffer capacity and the increased concentration of ammonia in the fermentation process leads to microbial growth inhibition. Anaerobic co-digestion (AnCoD), which entails the simultaneous digestion of two or more feedstocks has shown to be beneficial for its economic viability and increasing methane yields (Gelegenis et al., 2007; Karagiannidis and Perkoulidis, 2009; Kwietniewska and Tys, 2014). These problems have made AnCoD of multi feedstock to become a hot research area in the enhancement of conventional AD technology. The publications on AnCoD significantly increased within the last fifteen years indicating its capability for improving biogas production (Hagos et al., 2017). The main goal of anaerobic co-digestion is to increase biogas mainly biomethane for heat and electricity. As shown in Figure 2, a range of feedstocks can be co-digested at suitable blend ratio to maintain optimum condition required for metabolic activity and improved biogas production for thermal energy and power generation. Anaerobic co-digestion has shown to be a viable option to alleviate the drawbacks of mono-digestion while enhancing economic feasibility of the existing AD plants by increasing methane yields (Bayr et al., 2014; Mata-Alvarez et al., 2014, 2000b; Shah et al., 2015),(Mata-Alvarez et al., 2000a).

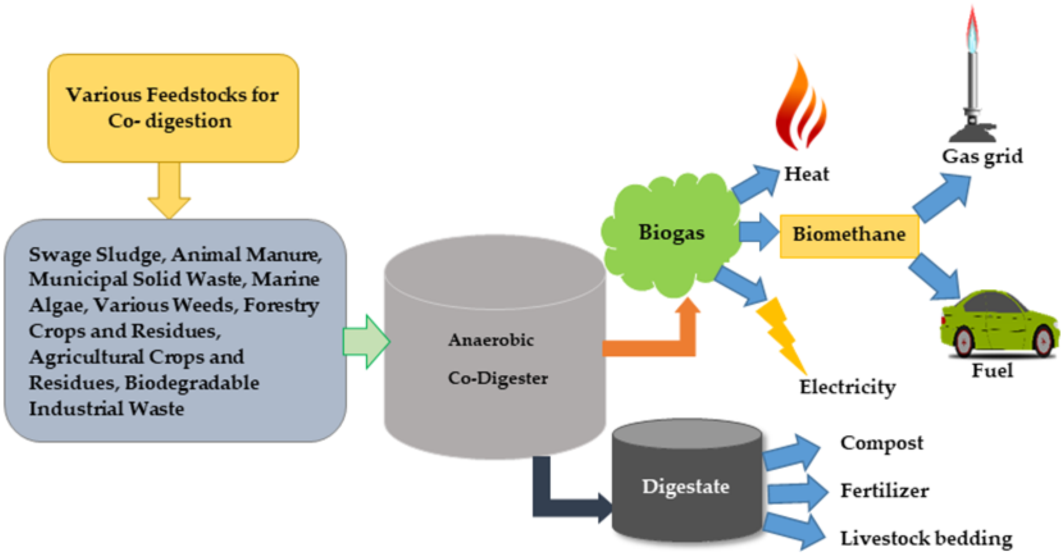


Figure 2.4. Co- digestion of multi feedstocks for waste reduction and energy recovery

Co-digestion can increase biogas production from 25% to 400% compared to the mono-digestion of the same substrates. Feedstocks characterized by higher C:N ratio (>50) such as rice and wheat straws, corn stalks, seaweed and algae can be co-digested by the feedstocks of lower C:N ratio for instance pig manure, poultry manure, food and kitchen wastes to achieve nutrient balance and to avoid the inhibitions which leads to system instability and reduced biogas production as a result of unsuited C:N ratio (Hagos et al., 2017; R et al., 2017; Sosnowski et al., 2003). Table 2.1 shows the possible feedstocks for co-digestion with regard to C:N ratio.

Various advantages of AnCoD systems are presented in Figure 3. Multiple aspects are considered when applying AnCoD. Cost of transporting the co substrate from the generation point to the AD plant seems to be the primary consideration, the selection of the best co-substrate and blend ratio in order to enhance synergism, dilute disruptive compounds, optimization of methane production and digestate quality, are also important consideration that treatment plants need to evaluate when using the AnCoD (Divya et al., 2015; Mata-Alvarez et al., 2014). Operational and environmental factors as mentioned previously are factors that influence methane yield in anaerobic co-digestion of multi feedstocks (Long et al., 2012).

Table 2.1. Potential feedstocks for co-digestion to balance nutrient with regard to C/N ratio [(Hagos et al., 2017),(R et al., 2017)–(Sosnowski et al., 2003)]

Feedstocks with max C/N ratio <20	C/N ratio	Feedstocks with max C/N ratio ≤40	C/N ratio	Feedstocks with C/N ratio around or >50	C/N ratio
TWAS[1]	6-9	OFMSW[3]	24	Potatoes	35-60
CSW[2]	11	Cow dung	16-25	Oat straw	48-50
Poultry manure	5-15	Horse manure	20-25	Corn stalks/straw	50-56
Pig manure	6-14	Kitchen Waste	25-29	Fallen leaves	50-53
Goat manure	10-17	Peanut shoots/ hulls	20-31	Rice straw	51-67
Grass/Grass trimmings	12-16	Slaughterhouse waste	22-37	Seaweed	70-79
Alfalfa	12-17	Mixed food waste	15-32	Algae	75-100
Food Waste	3-17	Waste cereal	16-40	Sugar cane/ bagasse	140-150
		Sugar beet/ Sugar foliage	35-40	Sawdust	200-500
		Waste cereals	16-40		

[1] Thickened Waste Activated Sludge,

[2] Caned Seafood Waste,

[3] Organic Fraction of Municipal Solid Wastes

However, for the implementation of AnCoD, other than aforementioned factors that govern overall AD process, additional factors including the selection of co-substrates and their mixing ratio should be taken into consideration. For instance, mixing materials of high and low C:N ratios, such as organic solid waste mixed with animal manure or sewage can help achieve optimum C:N ratios (Akyol et al., 2016). In order to attain an improved co-digestion process, some precautions and suitable procedures are necessary. There may be requirements for supplementary digester equipment depending on the size of the operation, quality of waste, and characteristics of the wastes to be co-digested. Mainly, precautions or supplementary equipment would be required for: homogenization and mixing of co-substrates, delivery of the waste, prevention of excessive foaming and scum layer formation, and removal of sediments from the digester.

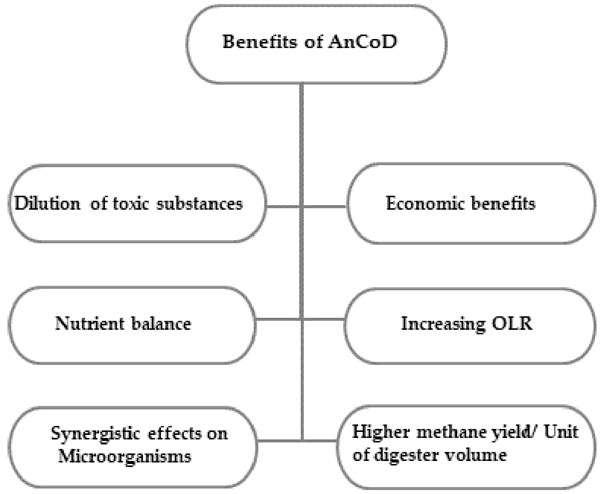


Figure 2.5. Advantages of anaerobic co-digestion systems

Furthermore, proper monitoring parameters should be determined to control and regulate the AnCoD digesters to help maintain an efficient performance when it is under operation. Applying suitable monitoring and control procedures when running the AnCoD process, allows for utilizing the full capacity of the system without overload risks. Monitoring can be performed by measuring indirectly the activity of different groups of organisms for example by measuring the rate of gas production, or the accumulation of intermediates of anaerobic degradation which reflect the existing metabolic status of the active organisms in the system (Gujer and Zehnder, 1983).

Several recommendations in the literature has been proposed specifying what control parameters should be chosen to be measured for this purpose. Some of the more common ones include pH, alkalinity, volatile fatty acids (VFA), gas production rate and the amounts of hydrogen, methane and carbon dioxide in the gas (Ahring et al., 1995; Moletta et al., 1994).

Partial alkalinity (PA) has been considered as a reliable monitoring parameter (Jenkins et al., 1991; Rozzi et al., 1994; Supaphol et al., 2011; Wilcox et al., 1995). The applicability of pH as a process indicator was reported to be intensely dependent on the buffering capacity making it an unreliable monitoring parameter (Ahring et al., 1995). It is expected that the selection and applicability of a specific parameter could not be generalized depending on the individual process configuration and the waste characteristics. For a full-scale municipal system, co-digested excess sludge from the municipal wastewater treatment plant with carbohydrate-rich food processing waste, different parameters were assessed for monitoring and control of the system performance (Björnsson et al., 2000). Those parameters included the volume of gas produced, pH, VFA, and alkalinity. In addition, gas composition and the degradation of organic matter were also measured at steady state and during process changes. Both full-scale and lab-scale experiments were carried out by that research to evaluate the suitability of those parameters. The digester was run below maximum capacity in order to avoid overload. The only operational limit set for the plant, was that the pH should not have been below 6.8. So, the pH was compared with alkalinity, VFA concentration, gas production rate, and the gas composition. Alkalinity was measured as partial alkalinity (PA). OLR changes were monitored both in the full-scale digester and in the lab-scale models (Björnsson et al., 2000).

As indicated by the results of Björnsson et al's study, the load's fluctuations were reflected in the pH, PA, and VFAs concentration. At overload condition, all the three parameters clearly demonstrated the process imbalance. The VFA concentrations proved to be a better indicator for an overload of the microbial system, although alkalinity and pH showed to be good monitoring parameters as well. The results indicated that gas-phase parameters demonstrated a slow response to load changes. The response of gas production and gas composition was behind and significant change occurred only after severe overload. Prior studies had observed that change in the gas phase parameters only takes place after well-developed imbalance. For that reason, the gas-phase concentration would not always reflect the actual concentration in the liquid, caused by limitations in liquid-to-gas mass transfer (Frigon and Guiot, 1995; Pauss et al., 1990).

At higher OLR, the process showed to be more sensitive to system disturbances. The changes in VFA concentration were not accurately reflected in pH. The increased amounts of VFA were demonstrated in a lower pH, because of the low buffering capacity of the process. Nevertheless, the pH was not presented as a reliable means of process monitoring, because of possible variation in buffering capacity as a result of variations in substrate composition. Therefore, a process imbalance causing significant accumulation of VFAs, could be unseen by this buffering effect. Therefore, relying on pH measurements for the process monitoring was not advised and the usage of pH measurements together with measurements of the PA or VFA was suggested by authors (Björnsson et al., 2000).

Different studies have been carried out to evaluate the impact of various effective factors on AnCoD processes. The main aim of the studies was to assess the influence of those parameters on biogas yield, biogas composition including biomethane or biohydrogen content. However, no comprehensive guidelines have been compiled so far to standardize the AnCoD systems. This would be firstly due to the complexity of the process caused by the variety of co-substrates and wastes composition, and secondly because it has not been broadly implemented in full scales.

A study for IEA Bioenergy has presented most important control parameters which regulate anaerobic co-digesters. The control parameters included the overall daily substrate flow (m^3/d or tones/d) and the amount of biogas produced daily (m^3/d). Based on the results obtained by this study, for appropriate control of the process, the determination of the CH_4 concentration was highly advised. In addition, a periodic calculation of the biogas yield referred to as daily biogas amount divided by daily substrate flow was recommended since it provides the efficiency of the digestion process. The analysis of the ammonia and the volatile fatty acids concentration were also suggested for large-scale operations. In addition, identifying the influences of co-substrates on the digester behavior was recommended. Particularly, detecting the formation of scum layers and bottom sediments was specified. It was suggested to maintain the record of the type and amount of separated contaminations in co-digestion. In the cases that sterilization is also involved, monitoring the type and the amount of waste streams and the treatment conditions such as time and temperature were also considered to be of necessary control parameters. The sampling frequency and methods for analysis required for quality assurance of the end product digestate or compost were also comprehensively defined in that study (Kim et al., 2006).

Although co-digestion of feedstocks such as poultry manure and kitchen waste with low C:N ratio with those of higher C:N ratio such as agricultural waste including rice and wheat straw is a solution to adjust its ratio at the optimum level, the existence of lignocellulosic material in the agricultural waste cause limitation during AnCoD as a result of long retention time and low biodegradability (Saha et al., 2011a).

Such problems still may demand for pretreatment techniques in order to speed up the hydrolysis which is the rate-limiting step in the anaerobic digestion process. The main purpose of the pre-treatment is to increase the solublization by the breakdown of the complex substrates such as lignin in lingocellulosic feedstocks or tough cell wall in seaweed biomass, in order to accelerate the hydrolysis rate (Elbeshbishy et al., 2011; Giordano, 2012; Noike et al., 1985).

C. Rodriguez et al, 2017 studied the effect of using co-substrate on methane production in co-digestion of waste paper (WP) with microalgal biomass (MA). Their study (Rodriguez et al., 2017) was carried out in batch mode and was intended to investigate the influence of the feedstocks mixing ratio (WP:MA) as well as feedstock to inoculum (F:I) ratio. They achieved the highest methane yield of 608 ml CH_4/ gVS at the F/I and WP:MA ratios of 0.2 and 50:50 respectively. At this mixing ratio of the feedstocks, the obtained methane yield was more than that of the feedstock's mono-digestion. The maximum 49.58% increase of the methane yield occurred at the same co-digestion ratio of 50:50 and F:I ratio of 0.4. Their study verified the synergetic effect at the feedstocks mixing ratio of 50:50 and all F:I ratios of 0.2, 0.3, and 0.4.

Pretreatment has proved successful at increasing the methane yield of numerous strains of microalgae in the digestion process. Most species of microalgae reduce the digestion rate due to their tough cell wall consisting of slowly biodegradable material (Uggetti et al., 2017).

With the increasing attention to anaerobic co-digestion, several researches have been allotted for co-digestion of various feedstocks and pre-treatment techniques from mechanical particle size reduction, thermal, chemical and ultrasonic treatment to enzymatic degradation and so on. For instance, mechanical pretreatment with Hollander beater in co-digestion of seaweed biomass with digester sludge increased biogas production by 20% at the ratio of 2:3 of algal pulp to sludge per reactor for 10 min beating time (Tedesco et al., 2013).

2.3.1. Microbial Diversity and Synergy in AnCoD

The selection of sludge inocula plays an important role in the effectiveness of biological anaerobic treatment of organic wastes. The analysis of microbial community dynamics has revealed that various waste streams and environmental factors can affect microbial community dynamics in an anaerobic co-digestion processes (Lin et al., 2012; Supaphol et al., 2011). Reportedly, mesophilic anaerobic co-digestion of mixed wastes allows for a better variety of substrates which in turn supports a wider diversity of bacteria and archaea. More diverse resource input results in more diverse communities and greater metabolic activity (Ike et al., 2010; Supaphol et al., 2011). However, there is limited awareness about the microbial consortia in anaerobic co-digestion process due to the lack of metabolic data on the microorganisms involved in the process. A comprehensive understanding of the microbial community is hindered by limitations of conventional molecular technology approaches that are restricted in terms of detecting sophisticated microbial diversity in the environment. Attempts for the analysis of the 16S rRNA gene sequencing have been carried out as an alternative to conventional culture techniques. This method is used to identify and compare microorganisms present within a given sample and it is a well-established method for studying complex microbial community or environments that are difficult or impossible to study. The method of 16S rRNA gene-based fingerprints could provide less biased and higher coverage information and can support many unknown details about the mechanism of microbial response to the digester enhancement. An improved understanding of the function and the metabolic role of microorganisms in the anaerobic co-digestion of various pollutants can be obtained b by the molecular inventories (Rivière et al., 2009; Zhang et al., 2011). Some of the results of these studies are presented in Table 2.2.

Organic matter in AD process, is decomposed synergistically by a bacterial consortium producing biogas including biomethane (Chandra et al., 2012; Liew et al., 2012). The process involves at least three functional groups of microorganisms that mainly regulate the mutual metabolic interactions under anaerobic conditions. The first microbial community hydrolyzes complex polymeric substances such as lipids, cellulose and protein to fundamental structural building blocks like glucose and amino acids. Subsequently, fermentation of these products to fatty acids, acetate and hydrogen is proceeded by the second community.

Among degradation processes involved in anaerobic digestion, the acidogenesis process has been shown to be the most important step. The third community develops methanogenesis process through which acetate and hydrogen are converted to methane and carbon dioxide. Therefore, microbial communities are vital to a stable and efficient transformation of organic matter to biogas (Zhang et al., 2011). General metabolism of microbial consortia involves extracellular polymeric substances (EPS) of sludge aggregates. It has been indicated that EPS is partly the result of the microbial metabolism that is affected by the microbial community structure and its activity. Growth conditions control the quantity of EPS which in turn affect the anaerobic digestibility and biogas production. It is not yet clear that how different microorganisms contribute to EPS secretion. A comparative study on the pathways of substrates degradation and the by-products of EPS sub-fractions could provide supplementary data on long-term impacts of microbial activity on anaerobic co-digestion reactors (Sheng et al., 2010; Yu et al., 2012). Monitoring qualitative and quantitative changes in a bacterial community structure, allows for the evaluation of the influence of the co-substrate on bacteria contributing to the biogas production.

However, there is not enough literature on this topic. Some attempts have been made to study microbial community structure and its influence on anaerobic co-digestion processes. Such studies as presented in Table 2.2 have been aimed to increase methane production by co-digestion of different organic-rich waste streams and they have been mostly developed with a view to the influencing parameters such as mixing ratio, organic loading rate (OLR), and carbon to nitrogen (C:N) ratio on the population of methanogenic archaea species (Benn and Zitomer, 2018; Gaby et al., 2017; A. J. Li et al., 2011).

It is reported that an even distribution of hydrogenotrophic and acetotrophic methanogen populations in a reactor is indicative of a stable operating condition (Demirel and Scherer, 2008; Kim et al., 2014; Yang et al., 2016). Yang et al. studied the performance of a co-digester for the treatment of sewage sludge with fat, oil and grease (FOG) using a mesophilic semi-continuous reactor and compared it to that of a mono-digester receiving only sewage sludge. It was indicated that the secretion of EPS increased by 40% in comparison with the mono-digester and that the improvement in co-digestion performance was stimulated due to the release of EPS. The analysis of microbial 16S rRNA gene showed the dynamic change of microbial community through the process. Both bacterial and archaeal community went through a progress with FOG addition, and a large amount of consortia such as Methanosaeta and N09 were involved in the process. As compared to sewer sludge mono-digestion, biogas production and TS removal efficiencies increased up to 35% and 26%, respectively. It was shown that FOG addition resulted in nutrition balance and regulating microbial composition. Also, metabolic activities were stimulated, and more EPS were obtained with the progressive addition of FOG.

Jihen et al. investigated microbial community's structures in anaerobic co-digestion of dairy wastewater and cattle manure. A maximum volatile solids (VS) reduction of 88.6% and biogas production of 0.87 L/g VS removed were obtained through their research corresponding to the C:N ratio of 24.7 at HRT of 20 days. The bacterial profile analysis showed a large quantity of Uncultured Bacteroidetes, Firmicutes and Synergistetes bacterium.

The Syntrophomonas strains associated with H_2-using bacteria comprising *Methanospirillum* sp., *Methanosphaera sp*., and *Methanobacterium formicicum* were observed as well. These syntrophic associations are necessary in anaerobic digestion reactors allowing for maintaining a low hydrogen partial pressure. On the other hand, high concentrations of volatile fatty acids (VFAs) resulted from dairy wastes acidogenesis allowed the growth of Methanosarcina species. It was indicated that high concentrations of VFAs would result preferentially in the growth of the acetoclastic Methanosarcina species (Jihen et al., 2015).

Table 2.2. Microbial consortia diversity in various AnCoD systems for biogas and methane improvement

Feedstocks	Microbial Consortia	Digester mode	HRT	Methane / Biogas increase%
Fruit vegetable waste +Food waste (1:1)	Methanoculleus, Methanosaeta, Methanosarcina	CSTR (mesophilic)	NA[1]	0.49 m^3 CH_4/kg VS
Food, fruit, vegetable + night soil waste	Methanosaeta (predominant methanogen) + hydrogenotrophs	Full scale wet fed-batch	18-20 d C:N 8.6	NA
Cow manure + grass silage	Clostridia, unclassified Bacteria, Bacteroidets	CSTR mesophilic	20 d	NA
Cow manure + oat straw	Clostridia, unclassified Bacteria, Bacteroidets, Deltaproteobacteria	CSTR (mesophilic)	20 d	NA
Cow manure + sugar beet tops	unclassified Bacteria,Clostridia, Bacteroidets, Bacilli	CSTR (mesophilic)	20 d	NA
STP-OGW + SC-OFMSW (1:6)	Methanobacterium, Methanoculleus, Methanothermobacter uncultured archaea	Batch (thermophilic)	14.4 d	52% biogas and 36% methane increase
biodiesel waste glycerin + municipal waste sludge (1.35:0.65)	Dominated by:Methanosaeta and Methanomicrobium	Two-stage CSTR (mesophilic)	20 d	100% biogas and 120 % methane increase
Sewage sludge +FOG	Dominantly Methanosaeta, and N09	Semi-continuous (mesophilic)	15 d	35% biogas increase
Dairy wastewater + Cattle manure	Uncultured Bacteroidetes, Firmicutes, Synergistetes, Syntrophomonas strains Methanosarcina species	ASBR (mesophilic)	20 d (C:N 24.7)	biogas produced: 0.87 L/g VS removed
Food wastewater +WAS (3:1)	Dominated by: Methanothermobacter and Methanosarcina	CSTR (thermophilic)	20 d	Max biogas: 316.11 mL CH_4/g COD removed

[1] Not available

Former studies verify that the AnCoD of cellulosic materials such as grass silage, oat straw, and sugar beet tops to methane to be mediated by bacteria and methanogenic archaea. The polymers which are hydrolyzed into soluble compounds under fermentative condition, are converted to acetate and one-carbon constituents by acidogens and acetogens and these intermediates in turn can be transformed directly by methanogenic archaea into methane and carbon dioxide (Nopharatana et al., 1998; Veeken and Hamelers, 1999).

Anaerobic digestion process of cellulosic material including grass silage, oat straw and sugar beet tops, is a multistep process mediated by Bacteria and methanogenic Archaea to produce methane. The process involves the hydrolysis of polymers into soluble compounds under fermentative condition. Subsequently, Acidogenic and acetogenic bacteria convert these soluble intermediates to acetate and mono-carbon compounds. These compounds subsequently can be converted to methane and carbon dioxide by methanogenic Archaea. It was found that in anaerobic digestion of cellulytic feedstocks, significant cellulolytic competences occurs due to the existence of species belonging to the order *Clostridiales* (Lynd et al., 2002).

Martín-González et al. found that thermophilic conditions was superior to mesophilic conditions for the enhancement of AnCoD along with the use of sewage treatment plant fat, oil and grease wastes (STP-FOGW) as co-substrates in co-digestion with organic fraction of municipal waste (OFMSW). Monitoring of microbial structure demonstrated that the bacterial profiles clustered in two separate groups, before and after the extended contact with STP-FOGW, whereas, archaeal community structure remained relatively constant throughout the operation. Bacterial population structure showed a dynamic change determined to be due to introducing FOG residues to the reactor.

2.3.2. Effect of Digester Mode on AnCoD

In general, anaerobic digesters can be configured as one-stage, two-stage, or multi-stage reactors in which the hydrolysis/acidogenesis and acetogenesis/methanogenesis steps occur in either the same or separated digesters (Figure 4). Separating the digesters makes the process easier to control, and makes it possible to separately optimize the operational and environmental conditions for hydrolysis/acidogenesis and methanogenesis processes in order to enhance the overall reaction rate and biogas yield (Lalman and Bagley, 2002; Nathao et al., 2013).

Fluctuations in organic loading rate, heterogeneity of wastes, and or the presence of excessive inhibitors can lead to instability of the process, and multi-stage systems have shown to be more stable as compared to single-stage ones. Two or multi-stage systems allow for the selection and enrichment of different types of microorganisms in each digester which results in extending the possibility of processing different biomass constituents, improving substrate conversion, enhancing the chemical oxygen demand (COD) reduction, and increasing energy recovery.

Although, multi-stage digesters are associated with greater construction and maintenance costs, multi-stage digesters provide higher performances as compared to single-stage systems (Azbar and Speece, 2001; Cuetos et al., 2007). Using two-stage digestion, controlled acidogenesis in the first digester, helps maintaining a high soluble feed to the second stage which subsequently enhances the biogas production (Dareioti and Kornaros, 2015).

In the two-stage anaerobic digestion systems, acid fermentation and methanogenesis are separated in two reactors in order to optimize reactor conditions for the different groups of microorganisms. The acidogenic stage is typically operated at a low HRT in the range of 2 to 3 days and a pH between 5 and 6. While the second stage, methanogenesis, is typically operated with a HRT of 20 to 30 days and a pH between 6 and 8 facilitating the development of slow-growing methanogenic archaea.

Moreover, acidogenesis phase allows for long chain fatty acids (LCFAs) saturation and degradation (Kangle et al., 2012; Kim et al., 2004). Owing to the bent molecular structure of unsaturated LCFAs because of the existence of double bonds, they have a greater cover area of cell wall per molecule as compared to saturated LCFAs. As a result, unsaturated LCFAs have demonstrated stronger inhibitory effects in comparison with saturated LCFAs (Demeyer and Henderickx, 1967; Thies et al., 1994). As such transformation of unsaturated to saturated LCFAs is beneficial. In addition, LCFA saturation would be necessary for the oxidative breakdown of fatty acid molecules and formation of acetic acid (Hanaki et al., 1981; Lalman and Bagley, 2002). The outcome of a study using a two-stage AD system treating a synthetic fat-containing wastewater comprised of glucose and LCFAs mixture revealed that, 19% of LCFAs were degraded and 12% of unsaturated LCFAs were saturated in the acidogenic phase (Kim et al., 2004). Acidogenic phase also can convert the unsaturated LCFAs to palmitic acid which reduces the lipid inhibition of methanogenesis in the second stage (Beccari et al., 1998). Food waste, which composes a large portion of OFMSW, contains a substantial amount of organic soluble compounds which can be simply converted to VFA.

Therefore, it can be an ideal substrate for biogas production. Nevertheless, formation of excessive amount of VFA at initial digestion stages can result in a remarkable pH reduction and subsequently methanogenesis inhibition. Utilization of two-stage anaerobic digestion systems for food waste has shown to be an effective solution for the pH inhibition of one stage systems (Dinsdale et al., 2000; Kinnunen et al., 2015; Klocke et al., 2008; Y. Li et al., 2011; Shen et al., 2013; Shin et al., 2010). Most of the studies on anaerobic co-digestion process have aimed to evaluate the digesters performance and optimal operating conditions for a particular type of waste.

Lafitte-Trouque et al. found two-stage thermophilic/mesophilic AnCoD systems to be effective for the co-digestion of sewage sludge and confectionery waste. The system with the second digester operating at a HRT of 12 days provided the best performance in terms of stability, VS reductions, and specific methane yield corresponding to an average 82% methane in the gas composition. However, a HRT of 8 days in the second stage digester was not able to assimilate high concentrations of volatile acid and low pH from the first digester. This was related to the insufficient retention time for maintaining a substantial methanogenic population. In a single-stage digester, a HRT of less than 20 days may cause methanogens to be washed out of the digester. Therefore, HRT is one of the important design parameters for the single-phase operations (Lafitte-Trouqué and Forster, 2000).

The study by Ratanatamskul et al. was conducted in pilot-scale on the development of an energy recovery system using a novel prototype single-stage anaerobic digester. Their system (Ratanatamskul et al., 2015) co-digested food waste with sewage sludge from a high-rise building for on-site biogas production.

The food waste to sewage sludge mixing ratio of 10:1 by weight was selected according to the result of lab experiments. Different HRTs of 27, 22, 19 days corresponding to 7.9, 10.8 and 14.0 $kgCOD/m^3$ d OLRs were applied and the optimal methane yield of 76.8% was achieved by their proposed single-stage reactor when the digester was operated at an HRT of 27 days. Although maximum biogas production occurred at the shortest HRT of 19 days. This indicated that the improvement of methane content of biogas could be attained by adequate operating HRT.

Although, the advantages of two-stage over single-stage digestion systems are addressed in the literature (Bertin et al., 2013; Shen et al., 2013; Yu et al., 2002) there exists a lack of adequate research available in terms of comparing the performances of single, two and or multi-phase co-digestion systems. Kim et al. developed a two-stage system comprised of a continuously stirred tank reactor for acidogenesis and a methanogenic up-flow bed reactor for the treatment of a high lipid wastewater from a milk and ice cream factory co-digested with slaughterhouse wastewater. They obtained 1.2 times increase in the COD removal, 1.9 times increase in lipids removal, and 1.4 times increase in the methane production compared to the single phase system (Kim and Shin, 2010).

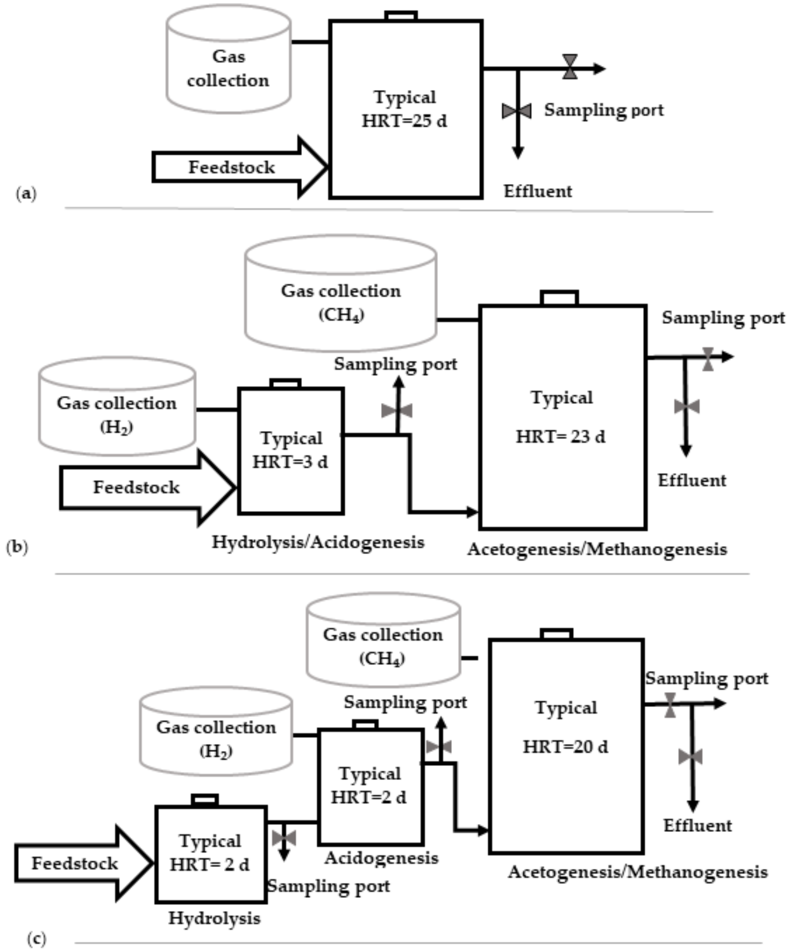


Figure 2.6. Types of digester configuration: (a) Single- stage; (b) Two- stage; and (c) Three- stage digester

Contrary to the result of that research (Kim and Shin, 2010), no significant increase in the overall energy recovery was attained by other study (Schievano et al., 2012) using a two-stage digester co-digesting swine manure and market biowaste in comparison with a single-stage one. Volumetric biogas productions were found to be 0.1 and 0.079 m^3 /L reactor for the single-stage and the two-stage systems, respectively. Even though, the average biogas methane content of the two-stage system showed 25% increase over that of the single-stage digester. The accumulation of un-degraded intermediate metabolites such as volatile fatty acids, ketones, amines, amino acids, and phenols was believed to be responsible for the reduced efficiency of the two-stage digester though. It was concluded that although the two-stage system could be capable of a higher bioenergy production, certain incompetent fermentative pathways may lead to formation of recalcitrant and toxic metabolites.

Hidalgo et al. compared a single-phase with a two-phase reactor for the co-digestion of residues from the used vegetable oil processing industry and pig manure. The maximum methane production of 1.06 m^3 /kg VS removed in the single-stage digestion corresponding to a methane production of 0.69 m^3 CH_4/kg VS removed (65 % CH_4) was obtained at the end of first 50-day operational period. The average biogas productions of 0.46 and 0.33 m^3 /kgVS $_{removed}$ were observed for the second and third operational period with methane productions of 0.30 (65.5 % CH_4) and 0.22 (66 % CH_4) m^3 CH_4/ kg VS $_{removed}$, respectively (Hidalgo et al., 2014).

The two-phase anaerobic digestion improved VS removal efficiencies and process stability in comparison with the single-phase reactor. Although the single-stage system produced more biogas, a higher methane content in produced biogas was obtained by the two-phase system and the latter was deemed to be more beneficial. Table 2.3 summarizes the results achieved by a number of studies on single and two-stage AnCoD systems.

The results obtained by applying a novel compact three-stage anaerobic digester in co-digestion of food waste and horse manure verified the advantages of the three-phase digester over single and two-stage ones as controls. By using three compartments in the three-stage anaerobic digester, three separated functional zones hydrolysis, acidogenesis and methanogenesis were created. This configuration significantly accelerated the solublization of solid organic matters and the formation of volatile fatty acids leading to an increase of 11 and 23% in methane yield in the two-stage and three-stage digesters in comparison with the single-stage one respectively. The analysis of 16 S rDNA showed that different microbial communities comprising hydrolyzing bacteria, acidogenic bacteria and methanogenic archaea were selectively enriched in the three separate reactors of the three-stage digester. It was also indicated that the abundance of the methanogenic archaea was increased by 0.8 and 1.28 times in the two-stage and the three-stage digesters compared to the single stage one correspondingly (Zhang et al., 2017b).

Despite its benefits, AnCoD implementation can be limited owing to long retention time and low biodegradability (Saha et al., 2011b). Therefore, certain techniques are required in order to overcome these obstacles. With the increasing interest in anaerobic co-digestion, a number of researches have been conducted during recent years with the purpose of improving co-digestion of various substrates. Part of those studies has been allotted for pre-treatment techniques from mechanical particle size reduction, thermal and ultrasonic treatment to enzymatic degradation and so on. The main purpose of the pre-treatment methods is to increase the solublization of the complex substrates by the breakdown of the complex substrates such as lignin in lingocellulosic feedstocks or tough cell wall in seaweed biomass, in order to accelerate the hydrolysis rate as hydrolysis is the limiting step in the anaerobic digestion process (Eastman and Ferguson, 1981; Esposito et al., 2012; Noike et al., 1985).

Table 2.3. Comparison of single-stage and two-stage digestion in AnCoD systems

Digester mode	Feedstocks	Mixing ratio	HRT	Biogas / Methane content
Single- stage (CSTR, mesophilic)	Sewage sludge + confectionery waste	NA[1]	20 d	Methane yield: 0.36-0.28 m3/kg VS applied (76- 82% methane)[2]
Two- stage (CSTR, thermophilic/mesophilic)			12 d	Methane yield: 0.3-0.34 m3/kg VS applied (66- 76% methane)[2]
Single stage (plug flow)	Food waste +sewage sludge	10:1 (weight)	27 d	Biogas production: 1045 ± 52.81 L/d
			19 d	1662.58 ± 37.32 L/d
Single- stage (UASB)	Slaughter house + milk wastewater	NA	2.14 d	40% Methane increase by two-stage reactor
Two- stage (CSTR/ UASB)			2.9 d	
Single- stage (CSTR, thermophilic)	Market biowaste + swine manure	1:4 (weight)	25 d	0.55 dm3/L digester d
Two- stage (CSTR, thermophilic)			3/22 d	0.54 dm3/L digester d
Single- stage (Batch)	Oil processing wastewater + pig manure	1:3 (weight)	20 d	Average biogas: 0.33 m3/kgVS removed, (0.66% methane)
Two-stage (Batch)			2/18 d	Average biogas: 0.4 m3/kgVS removed, (0.67% methane)
Single-stage	Food waste/ horse manure	NA	20 d	45.4 L cumulative methane production
Two-stage			4/16 d	50.7 L cumulative methane
Three-stage			2/2/16	55.7 L cumulative methane production

[1] Not Available,

[2] Numbers are mean values after 70- day period of phase 1 and phase 2, respectively

2.4. Pretreatment for Improving AnCoD

Pretreatment have been reported to improve the waste stabilization and the methane yield. However, given the additional costs of pretreatment, it mainly requires to be commercially viable (Esposito et al., 2012). Based on the existing literature, the pretreatment methods used for enhancing AnCoD fall into five main categories including mechanical, thermal/hydrothermal, chemical, biological, and hybrid (combined) pretreatment. As such, the different pretreatment methods that have been used for the enhancement of AnCoD of multi feedstocks are presented in the following sections.

2.4.1 Mechanical pretreatment

Mechanical pretreatment as a mean to improve the AnCoD process has been proposed by a number of researchers. The principal of the mechanical pretreatment technique is to breakdown and/or crushing the substrate particles reducing their particle size. The particle size reduction is proportional to the available specific surface area of the substrate constituents and therefore, it causes a more effective contact between the substrate and the anaerobic microorganisms during the AD process. As a result, the hydrolysis stage (as a known rate-limiting stage) is accelerated and the overall AD process is improved (Esposito et al., 2012).

In general, it has been observed that the smaller the size of the substrate particles is, the higher the methane production rate and yield are in the AD process. In addition to decreasing the particle size, the release of the intracellular components of the substrates has been reported by researchers specifically when the pretreatment was applied on waste activated sludge (WAS). Figure 2.4 illustrates different mechanical pretreatment techniques that have been practiced to enhance the AnCoD process.

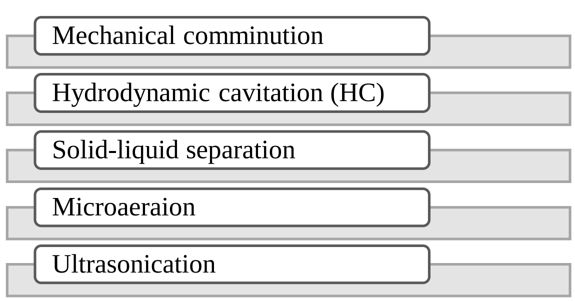


Figure 2.7. Mechanical methods of pretreatment used for AnCoD improvement

2.4.2. Thermal pretreatment

Thermal pretreatment has been commonly applied to both raw and digested substrate to help with dewatering, sludge solublization, viscosity, and pathogen reduction. During thermal pretreatment and as a result of the increase in thermal energy (heat), the structure of the particulate (insoluble) matter changes in a way that it becomes more susceptible to biodegradation (Bougrier et al., 2007). Thermal pretreatment can be applied via conventional heating (conductive heating) or microwave hydrolysis. Edström et al. studied the effect of thermal pretreatment for enhanced co-digestion of animal by-products, food waste from restaurants and food distributors, and sludge from a slaughterhouse wastewater treatment plant. The authors reported a significant increase in the biogas yield from 0.31 to 1.14 L /g VS-added when the animal by-product was exposed to conventional heating at a temperature of 70 ^{o}C for a duration of 1 h before the AnCoD process (Edström et al., 2003). In another study conducted by Paavola et al., thermal pretreatment via conductive heating was applied to a mixture of dairy manure and biowastes. An improvement in methane production at the extent of 14–18% was achieved compared to the process in the absence of thermal pretreatment (Paavola et al., 2006).

Despite these positive effect of thermal pretreatment on AD performance (including biogas production), the result of a study (Cuetos et al., 2010) indicated a 53% decrease in methane production in the co-digestion of a mixture of solid slaughterhouse waste and OFMSW that was thermally pretreated at a temperature of 133 ^{o}C. The reduction of methane production was due to a foaming problem and accumulation of fats in the reactor that most likely occurred due to the formation of recalcitrant compounds at the elevated temperature of 133 ^{o}C, exerting toxic effects on the AD process (Cuetos et al., 2010). Similarly, Guo et al. reported that during the co-digestion of food waste, fruit/vegetable residue, and thermally pretreated dewatered activated sludge, the methanogenesis process was inhibited by the accumulation of VFAs, which reduced the overall biogas production by 6% (Guo et al., 2014).

2.4.3. Chemical pretreatment

Chemical pretreatment can also lead to an improvement in the performance of AnCoD process through enhancement of the rate-limiting hydrolysis stage by facilitating the biodegradation of complex polymers via solubilizing the particulate fraction of the substrate. A variety of chemicals including acids, organic solvents, alkaline, and ionic liquids has shown a positive impact on disintegrating the structure of recalcitrant constituents of the substrates such as lignocellulosic compounds (Zhao et al., 2014).

Various types of alkaline compounds comprising NaOH, KOH, $Ca(OH)_2$, hydrazine, and anhydrous ammonia can increase the internal surface area of biomass resulting in a decreased degree of polymerization. As a result, the complex structures can be decomposed and the strong bounds between carbohydrate molecules can be disrupted, which results in a higher availability of carbohydrates in the hetero matrix and enhanced reactivity of residual carbohydrates polymers in the biochemical process. Pretreatment by alkaline agents is able to eliminate acetyl and other uronic acid substitutions of hemicellulose that obstructs the accessibility of enzymes to cellulose surface. Alkali pretreatment has shown to be most successful for biomasses with lower lignin contents such as agricultural residues (Anwar et al., 2014; Saini et al., 2015).

Acidic pretreatment including dilute solutions of sulfuric acid, hydro-chloric acid, phosphoric acid, and free nitrous acid can be also employed to disintegrate the substrate structure prior to AnCoD process. Compared to the alkaline pretreatment, the application of concentrated acids is limited due to their corrosive nature and elevated costs. Acidic pretreatment has been effective in the hydrolysis of hemicelluloses to its monomeric units and increasing the bioavailability of cellulose. Acidic pretreatment may also be required to neutralize the hydrolysate providing a favorable environment for microbial activities (Lin et al., 2009). Some of the researches have suggested alkaline pretreatment using an ammonia solution or NaOH because of their simplicity, ease of operation, and high methane production efficiency (Bali et al., 2015; Guo et al., 2015; Park and Kim, 2012).

Among different substrates utilized in the co-digestion process, the lignocellulosic biomass as a complex mixture of cellulose, hemicelluloses, and lignin was found to be one of the most difficult to digest by compounds as it contains unbalanced carbon to nitrogen ratio as well as recalcitrant lingocellulosic structure. The lignocellulosic biomass is typically found in plant dry matter such as rice straw. Chemical pretreatment of rice straw prior to AD has been suggested in the literature as an effective way to improve the biodegradability of its constituents (Himmel et al., 2007; Zhao et al., 2014). For instance, the results obtained by Zhao et al. showed that alkali pretreatment using NaOH in co-digestion of rice straw and municipal waste sludge increased biogas yield by 20%. In comparison, 40 and 45% increase in the removal rates of cellulose and hemicellulose were also reported, respectively due to the application of chemical pretreatment (Zhao et al., 2014).

Wei et al. studied the chemical pretreatment using ammonia and NaOH during AnCoD of corn stover and cattle manure, resulting in improved cumulative biomethane production and solids removal rate. In the co-digestion of cattle manure and ammonia solution-treated corn stover, the required time to reach 80% of the ultimate methane yield (T80) was found to be 28 ± 1 days, which was 20 % shorter than that of the control test with a T80 of 35 ± 1 days. Using sodium hydroxide, the T80 was determined to be 22 ± 1 days, 37% shorter as compared to that of the control digester (Wei et al., 2015).

2.4.4. Biological pretreatment

Biological pretreatment, which includes the addition of a particular strain, enzymes, or a consortium of microorganisms to the system, offers certain advantages over mechanical, thermal, and chemical pretreatment methods such as low energy requirements, avoiding toxic compounds generation, high yield of desired products, and the capability to target an specific compounds. Biological pretreatment methods such as bioaugmentation (stage 1) can result in the improvement of AD process by breaking complex polymers into simple monomers in hydrolysis stage which leads to increasing the rate of transformation of organic matter to biogas in the second stage. This process has shown to effectively increase both the methane yield and process stability (Song et al., 2014; Yuan et al., 2012; Zhong et al., 2011). Despite many researchers conducted on the application of biological pretreatment for improving conventional mono-digestion in AD systems, limited studies were performed on the application of these methods for enhancing biogas in AnCoD systems.

Table 2.4 summarizes the results of the studies conducted to enhance the AnCoD performance through biological pretreatment. In a study (Wei et al., 2015), biological pretreatment using liquid fraction of digestate (LFD) for AnCoD of cattle manure and corn stover was investigated. LFD obtained from anaerobic digester contains abundant microbes, inorganic substance as well as an organic substance such as amino acids, protein, a sugar that supplies nutrition substance for the process while acting as a microbial agent. As compared to untreated corn stover, pretreatment using LFD increased cumulative biomethane production (CBP) and VS removal rate by 25.40 and 30.12%, respectively. In addition, it reduced T80 and improved buffer capacity of the anaerobic digestion system (Wei et al., 2015).

Montusiewicz et al. studied the bioaugmentation pretreatment with a commercial product called Arkea® as a method for improving co-digestion of sewage sludge and mature landfill leachate. The co-digestion process was improved due to the enhanced activity of microorganisms involved in bioaugumenting system and their resistance to toxic elements (Montusiewicz, 2014). This was consistent with the results obtained by Duran et al. who used selected strains of Baccillus, Pseudomonas and Actinomycetes species for bioaugmentation (Duran et al., 2006).

In another study by Deng et al., the effect of enzymatic pretreatment on co-digestion of rice straw and soybean straw was evaluated. The research was conducted to investigate the effect of on-site generated cellulase produced by cultivation of Trichoderma reesei RUT C30 on lignocellulose to improve the hydrolysis of cellulose and hemicellulose content of the feedstock. They also investigated the influence of the pretreatment on biogas production via batch experiments. The authors reported more than 300% increase in the cumulative biogas yield compared to the untreated feedstock. They also achieved 40% shorter lag time in co-digestion of pretreated feedstock than the pretreated mono-digestion groups verifying a synergistic effect of rice and soybean straws co-digestion. This study suggested enzymatic pretreatment using Trichoderma reesei RUT C30 as an effective method for improving biogas production (Deng et al., 2018).

Zhang et al, also investigated the effects of biological co-pretreatment on biogas production in AnCoD of food waste (FW) and waste activated sludge (WAS). They used co-pretreatment of FW and WAS followed by anaerobic co-digestion to improve hydrolysis efficiency. Their method was established based on the fact that a biological solublization process by mixing FW, water and microorganisms was effective as a result of size reduction of substrate particles and increase of solublization. They hypothesized that using a mixture of WAS and FW for biological solublization pretreatment (biological co-pretreatment), would improve the hydrolysis of FW and WAS for the reason that: 1) generation of the alkalis from WAS could buffer VFAs and maintain optimum pH for hydrolysis stage, and 2) a lower pH would enhance solublization of WAS through accelerating the hydrolysis of proteins and carbohydrates. Their method of pretreatment included a 2 L glass reactor with a mechanical stirrer at a mixing speed of 150 rpm as a biological co-pretreatment reactor. They applied a blend ratio of 1:1 (weight) with different co-pretreatment time: 0 h, 15 h, 24 h and 35 h. The pretreated mixtures were used as the feed of the subsequent anaerobic digester. A mixture of fresh FW and WAS with 0 h co-pretreatment was used as the feed of the subsequent anaerobic digester as control. They achieved 24.6% higher methane production from co-digestion of co-pretreated substrates compared to control substrates without pretreatment. They also observed an increase of 10.1% in solids reduction under 24 h optimum pretreatment time (Zhang et al., 2017a).

Table 2.4. Biological pretreatment for enhanced AnCoD

Method of Pretreatment	Feedstock	Digester mode	Mixing ratio	Methane yield/ Biogas increase%
Liquid fraction of digestate	Corn stover+ cattle manure	Batch (mesophilic)	3:1 (weight)	25% methane increase
Bioaugmentation by Arkea®	sewage sludge* + mature landfill leachate + Arkea®	CSTR (mesophilic, HRT: 17.4 d)	87:4.3:8.7 (v/v)	5-8% biogas decrease
Biological (Enzymatic)	Rice straw + soybean straw	Batch (mesophilic)	1:1 TS ratio	318% biogas increase
biological co-pretreatment	Food waste and WAS	Semi-continuous	1:1 (weight)	24.6% methane increase

2.4.5. Hybrid pretreatment

Hybrid pretreatment is a combination two or more methods of mechanical, thermal, chemical, and biological pretreatment techniques. Of all the available hybrid pretreatment methods, thermo-alkaline, NaOH/ H_2O_2, and Ozone/NaOH have received more attention to enhance AnCoD. In a recent study performed by Benn et al. (2018), the usage of thermochemical bioplastic pretreatment was investigated in co-digestion with synthetic primary sludge in batch and continuous mode. The pretreatment experiments were conducted by applying different combinations of temperatures ranging from 35–90^{o}C with alkaline conditions in a pH range from 8 to 12 (Benn and Zitomer, 2018). Percent conversion values for bioplastics to biomethane were calculated as the proportion of BMP value divided by the theoretical maximum methane production value indicated by the bioplastic theoretical oxygen demand loading. According to the authors, the thermos-alkaline pretreatment led to an increase in average BMP values up to over 100 %. The batch system co-digesting synthetic primary sludge with bioplastic resulted in 80–100% conversion of bioplastics to biomethane and a 50% biomethane production increase over the non-pretreated substrate. In comparison with the findings of the batch study, less than 20% increase in methane production was obtained by continuous co-digestion of pretreated bioplastics with the synthetic primary sludge in a CSTR system compared to the system fed with non-pretreated feedstock (Benn and Zitomer, 2018).

Another study conducted by Naran et al. revealed that the cumulative methane yield increased from 116.7 to 177.3 mL/g VS added through the co-digestion of thermos-alkaline pretreated food waste from a food waste treatment plant and WAS generated at a municipal treatment compared to the non-pretreated sample (Naran et al., 2016). Similarly, the usage of thermo-alkaline pretreatment method to improve co-digestion of WAS and rice straw was studied and a biogas production of 409 L/kgVS$_{added}$ was obtained under the optimum condition equivalent to a 51% increase compared to the control test. The authors of that work also reported that the degree of WAS solublization was positively correlated with biogas production and VS removal. It was also observed that, following pretreatment, the cellulose and hemicellulose contents of rice straw decreased remarkably. According to the results of their research, the addition of NaOH caused 11%, 32%, and 22% reduction in hemicellulose, cellulose, and lignin contents, respectively. The improvement of digestion performance in this study was related to the increased solublization and reduced particle size of the organic matter (Abudi et al., 2016b).

Abudi et al., employed NaOH/H_2O_2 pretreatment on co-digestion of WAS and rice straw. The applied pretreatment resulted in a remarkable reduction in cellulose and hemicellulose contents of rice straw and hence improved the biogas production. NaOH/H_2O_2 pretreatment was able to decrease hemicellulose, cellulose and lignin contents of rice straw by 16%, 41%, and 7%, respectively. Pretreatment was more effective in the solublization of hemicellulose content than cellulose and lignin contents of rice straw. Consistent results were obtained by others for single digestion of rice and corn straws using NaOH/H_2O_2 pretreatment (He et al., 2009; Song et al., 2014, 2013).

Rajesh Banu et al. employed ozone/NaOH pretreatment for the co-digestion of cow manure and WAS. This resulted in increasing biogas production from 17.9 to 18.8 L/d. Despite the fact that ozone utilization is considered to be costly, the combination of alkali and ozone not only increased the sludge disintegration efficiency but also saved a considerable amount of energy (Rajesh Banu et al., 2015). The increase in biogas production was most likely due to the particles decomposition resulting from ozone reaction with the organic fraction of sludge and the availability of extra carbon source (Ahn et al., 2002).

In the study conducted by Ren et al., the effects of hot alkali pretreatment and mixing ratio on anaerobic co-digestion of duckweed and excess sludge were investigated. The result of their study primarily indicated that through co-digestion the delayed stage of gas generation reduced, and a cumulative gas yield of 2963 mL was obtained which was 11% higher than the calculated value. The methane content of the produced gas was 57%, which was 13% higher than that of the duckweed and 9% higher than that of the excess sludge single digestion. Additionally, pretreatment of the duckweed in the mixture, improved the methane yield by 8% (Ren et al., 2018).

In co-digestion of poultry manure with pig manure in a batch system, the effect of a combined thermochemical pretreatment and ammonia stripping on the digester performance was assessed. The result revealed that the optimal blend ratio of poultry manure to pig manure was 24:76 on volumetric basis which was corresponding to highest methane production. The combined pretreatment improved the co-digestion system achieving an OLR of 4 g COD/L d with a HRT of 20 days (Rodriguez-Verde et al., 2017). The results of these studies are summarized in table 2.5.

Table 2.5. Hybrid pretreatment for enhanced AnCoD

Method of Pretreatment	Feedstock	Digester mode	Mixing ratio	Methane yield/ Biogas increase%
Thermo-alkaline	Synthetic municipal primary sludge + bioplastics	Batch (mesophilic)	10:1 (v/v)	6% methane increase
Thermo-alkaline	WAS + food waste	Batch (mesophilic)	7:3 (v/v)	52% methane increase
Thermo-alkaline	TWAS+ rice straw	Batch (mesophilic)	1:1 (v/v)	51% biogas increase
Thermo-alkaline/H_2O_2	TWAS+ rice straw	Batch (mesophilic)	1:1 (v/v)	56% biogas increase
Ozone/NaOH	Cow manure +dairy wastewater	HUASB***	3:1 (v/v)	5% biogas increase
Thermo-alkaline	duckweed and waste activated sludge	Batch mesophilic	NA	8%
Thermo-alkaline	poultry manure and pig manure	Continuous mesophilic	24:76	37%

In summary, due to various advantages that co-digestion offers over conventional mono digestion, this area is attracted by several researchers and studies are still going on to better understand the system performance and to investigate different methods for improving the AnCoD systems. Such researches along with studies on control parameters, suitability of different feedstocks and their combinations and optimizing procedures would contribute in further improving this technology.

Chapter 3

Materials and methods

3. Materials and methods

3.1. Feedstocks and Inoculum

This research was designed to evaluate the effect of mixing ratio of the feedstocks and its relationship with the lipids: proteins: carbohydrates content on anaerobic co-digestion process. The experiment included two sections including BMP assay and hydrolysis/acidification. The BMP was designed to assess the influence of the mixing ratio on biomethane production. Hydrolysis/acidification experiment was carried out to evaluate the effect of mixing ratio on hydrolysis kinetics. Different feedstocks including dairy manure, TWAS and SSO in different combinations were used as digester feedstocks. TWAS (3.8 % TS) and inoculum were collected from Ashbridges Bay Wastewater Treatment Plant Toronto, Ontario. The inoculum was obtained from the effluent of the anaerobic digesters operating at mesophilic condition at a temperature range of 34-38°C, and receiving approximately 1600 m^3/d TWAS and 6500 m^3/d primary sludge. The average organic loading rate and SRT of the anaerobic digesters are 1.1 kg VS/m^3 and 18 d, respectively.

The Ashbridges Bay Wastewater Treatment Plant is the main wastewater treatment facility among the four treatment plants that service the city of Toronto. After Montreal's Jean-R. Marcotte facility, it is the second largest plant in Canada (Heffez, 2009) The plant treats the wastewater produced by approximately 1.4 million of the city of Toronto's residents and has a capacity of 818,000 m^3/d (City of Toronto, 2018).

The influent to the treatment plant comes from Mid-Toronto, high level, low level and Lakefront interceptor sewers in addition to Coxwell and Queen Street trunk sewers. Biosolids generated at the plant was approximately 149733 wet tones in 2016 with 28.1 % Total Solid (TS). The influent undergoes treatment processes which comprises preliminary treatment i.e. screening and grit removal, primary treatment, secondary treatment, nutrient removal, disinfection, Waste Activated Sludge (WAS) thickening, anaerobic digestion, biosolids dewatering and biosolids management. The influent Total Suspended Solids (TSS) and Biological Oxygen Demand (BOD) concentrations are 318.6 mg/L of 244.6 mg/L, respectively (City of Toronto, 2018; Razavi, 2019).

The unit processes of the plant is shown in figure 3.1. The activated sludge system consists of three main stages including aeration tank, settling tank and return activated sludge. In the aeration tank, the atmospheric air is introduced to the primary treated wastewater using air blowers and the biological mass that produces biological flocs is called waste activated sludge (WAS). The produced sludge then goes through thickening process using different mechanisms to produce thickened waste activated sludge (TWAS).

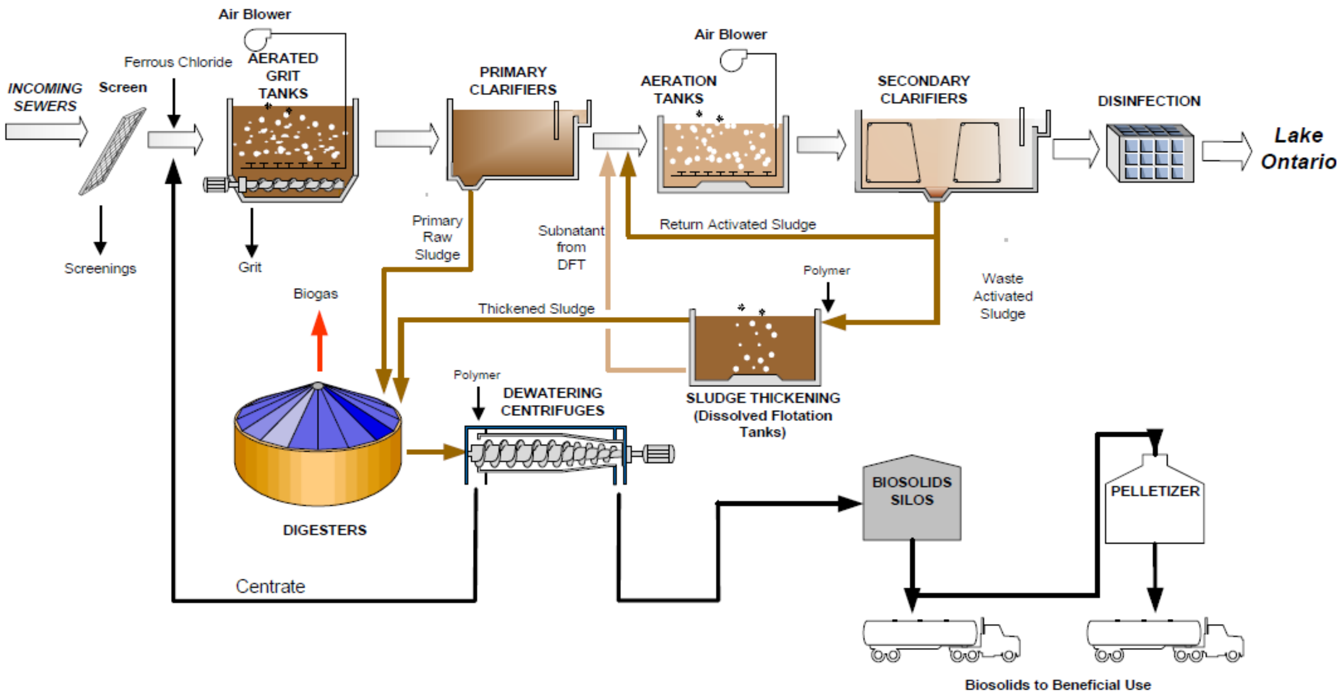


Figure 3.1. The flow diagram of the Ashbridges Bay Wastewater Treatment Plant (City of Toronto, 2018)

SSO was obtained from City of Toronto Disco Road Organics Processing Facility, Toronto, Ontario. The facility is located on 120 Disco Road and on a 1-hectare site and is the first full-scale plant in North America. It has been operated since 2014 for processing the source separated organics by anaerobic digestion. Being one of the municipality's diversion program, it receives almost half of the organics collected in Toronto and is capable of processing up to 75,000 tons of organic waste per year from homes and public buildings. The ultimate capacity of the facility is planned to rise to 130,000 tons in the near future. The acceptable materials to Disco Road facility includes food waste, paper food packaging, pet waste, diapers, houseplants, and biodegradable plastics. The organic processing at the facility is shown in figure 3.2. SSO first is delivered and stored and goes through a visual inspection to remove large unwanted items. At the next stage, the materials undergo the BTA® hydro-mechanical technology through which the organics will convert to a liquid (slurry) pulp. The BTA® consists of screens and hydropulpers for separating the unwanted materials including glass shards, plastic bags, metals and sand from the pulp (Razavi, 2019). In this study, the SSO samples were collected after the hydro-mechanical stage and transferred to the lab in the slurry form.

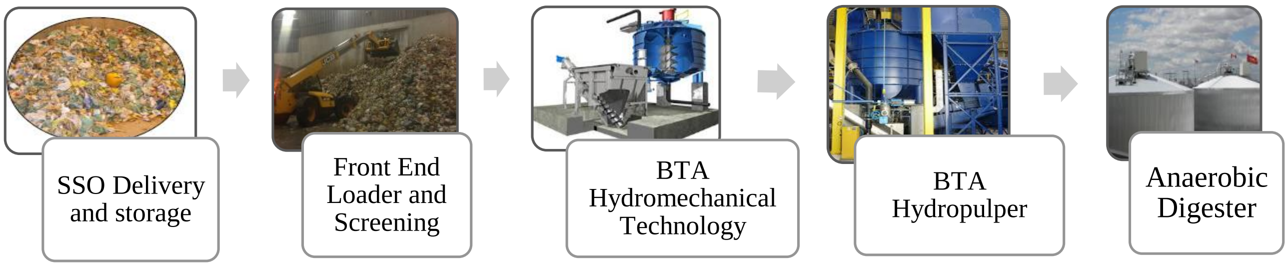


Figure 3.2. Source separated organics processing in Disco Road Facility

Cow manure was collected from a manure pit of a dairy farm located in Newmarket, Ontario. Manure slurry was prepared by addition and homogenization of cow manure with deionized distilled water using a blender followed by a VWR 400 DS bench top homogenizer. The reactors were fed with different combinations of the feedstocks.

The BMP included 3 binary co-digestion experiments using different feedstocks mixtures including different mixing ratios of TWAS/SSO, TWAS/manure, and Manure/SSO. The BMP of TWAS/SSO, TWAS/manure, and Manure/SSO were conducted individually in different periods. It also included a ternary co-digestion using the three feedstocks, TWAS/manure/SSO, at different mixing ratios. Each of the experiments continued until biogas production stopped or was negligible. A series of analysis for characterization of the inoculum, TWAS and SSO and manure was carried out primarily and presented in Table 3.1. Samples were transported and preserved according to Standard Method for Examination of Water and Wastewater (APHA, 2005). Feed of digesters as explained above were mixed at different mixture ratios on a volumetric basis. Batch reactors in working volume of 200 mL containing inoculum and feedstocks at different mixing ratios were prepared for the BMP essay. TWAS, manure, and SSO alone were also used as control reactor to assess the effect of co-digestion on the efficiency of the system in comparison with the single digestion of the feedstocks.

A series of analysis for characterization of the inoculum, TWAS and SSO was carried out primarily and are presented in table 3.1. The mean values are the average of four measurements on each of the parameters for the raw substrates and RSD is the ratio of standard deviation to the mean or relative standard deviation.

Table 3.1. Initial Characteristics of the feedstocks and inoculum used in this study

Parameters	Units	SSO		Manure		TWAS		Inoculum	
		MEAN	RSD	MEAN	RSD	MEAN	RSD	MEAN	RSD
TCOD	mg/L	110000	0.07	198833	0.03	40000	0.07	16400	0.03
SCOD	mg/L	44400	0.002	10933	0.04	360	0.07	362	0.05
TSS	mg/L	53833	0.12	56520	0.03	31450	0.08	17033	0.02
VSS	mg/L	38478	0.095	29998	0.02	25600	0.09	10900	0.02
TS	mg/L	62187	0.02	73727	0.04	38810	0.12	21450	0.03
VS	mg/L	43493	0.02	38647	0.02	31205	0.01	13140	0.02
Ammonia	mg/L	1738	0.003	22	0.07	255	0.12	1495	0.03
pH	-	5.6	0.001	6.4	0.01	6.3	0.005	7.2	0.001
Alkalinity	mg CaCO3/L	7700	0.07	11133	0.05	1953	0.07	3943	0.12
TN	mg/L	4167	0.18	2200	0.12	2900	0.14	2025	0.10
TSN	mg/L	1793	0.02	104	0.09	420	0.15	696	0.16
Total Carbs	mg/L	40360	0.09	7398	0.06	1288	0.08	961	0.08
Total Proteins	mg/L	2021	0.09	5199	0.08	1459	0.18	1448	0.09
Total Lipids	mg/L	18620	0.12	7241	0.09	551	0.08	1920	0.10

3.2. Experimental design and procedure

3.2.1. Co-digestion- BMP assay

The biochemical methane potential (BMP) assays were conducted according to the procedures described in the literature (Angelidaki et al., 2009a; Moody et al., 2009; Owen et al., 1979). The experiment was initiated by feeding the digesters with different mixing ratios of the feedstocks in triplicates. A 100% SSO and 100% TWAS, and 100% manure as control reactors were assessed in triplicates as well. In this research, a substrate-to-biomass ratio (S^0/X^0) of 2 g COD $_{substrate}$/g VSS $_{inoculum}$ was kept in all digesters which is within the range that has been suggested by the literature (Elbeshbishy et al., 2012). Substrate to inoculum ratio was selected for all of the batch reactors according to the procedure used in by Nasr et al., 2011 as following:

$$S^0/X^0 = \frac{g\ TCOD_{substrate}}{g\ VSS_{inoculum}} = \frac{V_{substrate} \times TCOD_{substrate}}{V_{inoculum} \times VSS_{substrate}} = 2 \quad \text{Eq.3.1}$$

The TCOD of the mixture used in Eq. 3.1 was calculated considering the TCOD of the feedstocks and the mixing ratios. Accordingly, the volumes of the feedstocks were calculated based on the mixing ratios of substrates. Because of the heterogeneous composition of the feedstocks, all of the combinations were prepared in triplicates. The specific amount of the substrates along with the mesophilic inoculum as described above was added to 250 mL glass bottles. The headspaces in the bottles were flushed with nitrogen gas for 3 minutes at 10 psi and subsequently the bottles were sealed to satisfy the anaerobic conditions. In addition, anaerobic systems require a pH within the range of 6.5–7.5 according to the literature (Cioabla et al., 2012; Droste, 1997). Therefore, the pH in each bottle were kept in a range of 7-7.4 using sulfuric acid and sodium hydroxide. The binary co-digestion of TWAS/SSO, TWAS/manure, and manure/SSO were conducted at the mixing ratios as presented in Table 3.2.

Table 3.2 Proportions of digesters' feed for binary co-digestion of TWAS/ SSO, TWAS/manure, and manure/ SSO in BMP experiment

Feedstock	TWAS	Manure	SSO
Proportion of the feedstocks in different runs of Binary co-digestion Volume: 200 mL	0	1	0
	1	0	0
	9	1	0
	7	3	0
	5	5	0
	3	7	0
	1	9	0
	0	2	4
	0	0	1
	0	9	1
	0	7	3
	0	5	5
	0	3	7
	0	1	9
	0	0	
	9	0	1
	7	0	3
	5	0	5
	3	0	7
	1	0	9

The ternary co-digestion of TWAS/manure/SSO was carried out at different mixing ratios of TWAS, manure and SSO as presented in Table 3.3. As demonstrated in Table 3.2 and 3.3 reactors containing only manure, only TWAS and only SSO were also used in triplicates as control in each run.

Table 3.3 Proportions of digesters' feed for ternary co-digestion of TWAS, SSO, and manure in BMP experiment

Feedstock	TWAS	Manure	SSO
Proportion of the feedstocks in different runs of ternary co-digestion Volume: 200 mL	8	1	1
	1	8	1
	1	1	8
	5	2.5	2.5
	2.5	5	2.5
	2.5	2.5	5
	4	4	2
	4	2	4
	2	4	4
	1	0	0
	0	1	0
	0	0	1

The sets of 21 bottles for each of the three binary co-digestion experiments, and the 36 bottles for the ternary co-digestion experiment were placed in the Thermo Scientific MAXQ 4000 shakers and a rotational speed of 150 RPM was applied during the entire process. The incubator temperature was set at 37 ^{o}c to satisfy mesophilic condition for the batch reactors.

Figure 3.3- Experimental set-up for biomethane potential experiment

3.2.2. Co-digestion- hydrolysis/acidification experiment

This experiment was conducted to assess the influence of the mixing ratio and its relationship with the lipids: proteins: carbohydrates in co-digestion on hydrolysis/acidification rate. Similar to BMP experiment, the binary co-digestion of TWAS/ SSO, TWAS/manure, and manure/SSO were conducted at different combinations as presented in Table 3.4. The ternary co-digestion of TWAS/manure/SSO was carried out at the mixing ratios that are presented in Table 3.5. For each of the experiment's rectors containing only TWAS, only manure and only SSO were also used in triplicates as control reactors.

The bottles for each of the three binary co-digestion experiments, and for the ternary co-digestion experiment were placed in the Polyscience WB28 water bathes. Bottles were equipped with a mixer which maintained a rotational speed of 150 RPM the entire process. The temperature in water bathes was set at 37 ^{o}c to satisfy mesophilic condition for the batch reactors.

Consistent with the BMP experiment, the substrate-to-biomass ratio (S^0/X^0) of to 2 g COD $_{substrate}$/g VSS $_{inoculum}$ was kept in all digesters. Feed of digesters as explained above were mixed at different mixture ratios on a volumetric basis. Batch reactors in working volume of 2000 mL containing inoculum and feedstocks at different mixing ratios were prepared for the BMP essay. In order to deactivate methanogens and to increase the accuracy of the hydrolysis/acidification experiment, the inoculum was heated to 70 °c for 30 min and pH was adjusted to a range between 5- 5.5 in all digesters. The experiment continued for a period of three days for each run of the binary co-digestions and the ternary co-digestion experiment. Samples were collected with time to evaluate the solublization and hydrolysis rate of the mixtures and to assess the influence of the mixing ratios on them. Consistent with the BMP assay, The TCOD of the mixture in Eq. 3.1 was calculated considering the TCOD of the feedstocks and the mixing ratios. Accordingly, the volumes of the feedstocks were calculated based on the mixing ratios of substrates. Because of the heterogeneous composition of the feedstocks, all of the combinations were prepared in triplicates. The specific amount of the substrates along with the mesophilic inoculum as described above was added to 2500 mL glass bottles.

Table 3.4 Proportions of digesters' feed for binary co-digestion of TWAS/ SSO, TWAS/manure, and manure/ SSO in hydrolysis/acidification experiment

Feedstock	TWAS	Manure	SSO
Proportion of the feedstocks in different runs of Binary co-digestion Volume: 2000 mL	0	1	0
	1	0	0
	9	1	0
	7	3	0
	5	5	0
	3	7	0
	1	9	0
	0	2	4
	0	0	1
	0	9	1
	0	7	3
	0	5	5
	0	3	7
	0	1	9
	0	0	
	9	0	1
	7	0	3
	5	0	5
	3	0	7
	1	0	9

Table 3.5 Proportions of digesters' feed for ternary co-digestion of TWAS, SSO, and manure in in hydrolysis/acidification experiment

Feedstock	TWAS	Manure	SSO
Proportion of the feedstocks in different runs of ternary co-digestion Volume: 200 mL	8	1	1
	1	8	1
	1	1	8
	5	2.5	2.5
	2.5	5	2.5
	2.5	2.5	5
	4	4	2
	4	2	4
	2	4	4
	1	0	0
	0	1	0
	0	0	1

The headspaces in the bottles were flushed with nitrogen gas for 3 minutes at 10 psi and subsequently the bottles were sealed to satisfy the anaerobic conditions. Gas production during the experiment was monitored using water displacement method and displayed real time. The percentage improvements in the soluble contents concentrations (degree of solubilization) (P) values was calculated using Eq 3.2.

$$P\ (\%) = SC_f - SC_i / PC_i * 100\% \qquad \text{Eq. 3.2}$$

Where SC_i and SC_f are the mass of soluble COD of the digester contents before and after the hydrolysis/acidification phase experiment (mg) and PC_i is the mass of initial particulate COD in the digesters (mg).

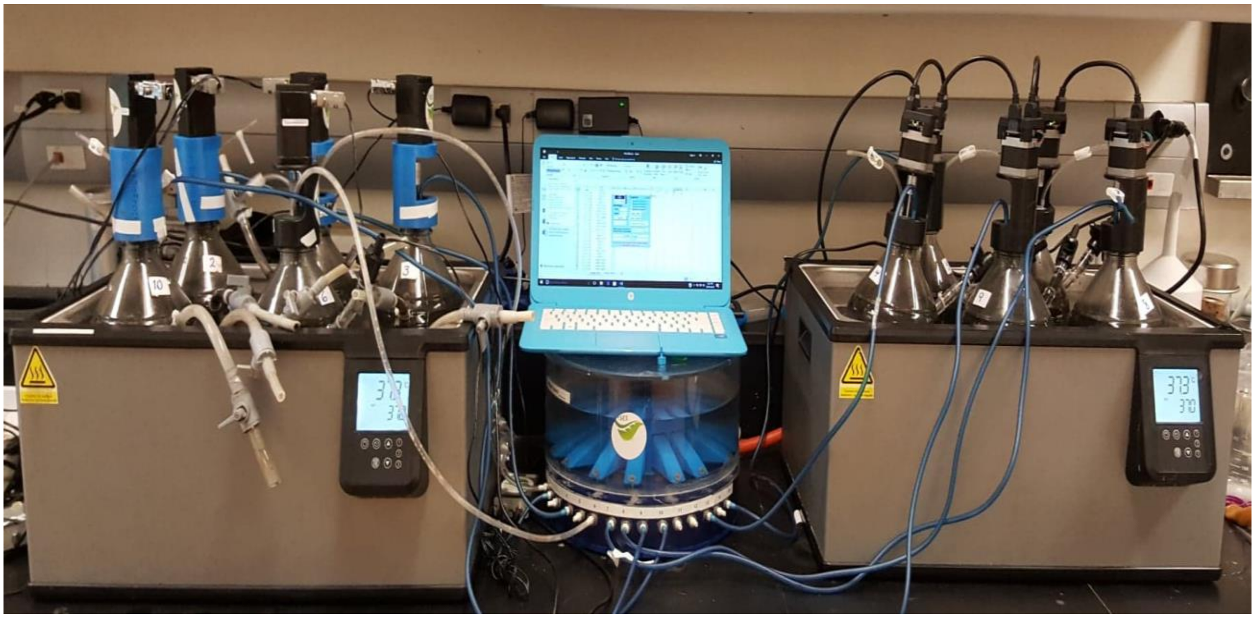


Figure 3.4. Experimental set-up for hydrolysis/acidification experiment

A first-order reaction model was applied using AquaSim 2.0 software to assess the effect of different mixing ratios on biodegradation rates of COD, proteins, lipids, and carbohydrates.

r_{su}= dC/dt= -kC   Eq. 3.3

Where C is the concentration (mg/L) of the parameters (TCOD, proteins, lipids, and carbohydrates) at time t, k is first-order specific biodegradation rate constant (1/d) and r_{su} is biodegradation rate (mg/L.d). Eq. 3.4 is derived by integration of Eq. (3.3).

$C_t= C_u e^{-kt}$ Eq. 3.4

Where t, C_t and C_u are time (d), concentration at time t (mg/L), ultimate particulate parameters (TCOD, proteins, lipids, and carbohydrates) in mg/L, respectively.

3.3. Analytical analysis

The analysis of solid contents of the feedstocks including total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) of the inoculum, TWAS, and SSO samples were determined according to the Standard Methods procedures (APHA, 2005). Chemical oxygen demand (TCOD) and (SCOD), ammonia, total nitrogen (TN) and total soluble nitrogen (TSN) were measured using a Hach spectrophotometer model 3900. For the measurement of the soluble content, samples were prepared by centrifuging at 9000 rpm for 45 min and then the supernatant was filtered using microfiber filters with a pore size of 0.45 µm. The absorbance was set at the wavelengths of 600, 560 and 650 nm for the analysis of COD, ammonia, and alkalinity, respectively.

Total lipids concentration was measured by solvatochromatic method that rely upon a dye or mixture of dyes which change optical properties upon a change in condition of the solvent in which they are dissolved. In this test, there is an increase in fluorescence when there is an increased amount of dissolved lipids that form micelles or other structures. Solvatochromatic analysis was carried out at the fluorescence 405 nm mode using Vernier SpectroVis spectrometer and Logger Pro software. The data then exported as CSV files for further analysis. Figure 3.5 shows the graph created by Logger Pro at 405 nm wavelength and the absorbance for different concentrations of total lipids. The peak values of the curves corresponding to the lipids concentration of different standard samples with known concentrations were used to plot the calibration curve and get the curve equation. The measured absorbance values for the actual samples with unknown lipids concentrations were substituted into the calibration equation and was solved for the true value.

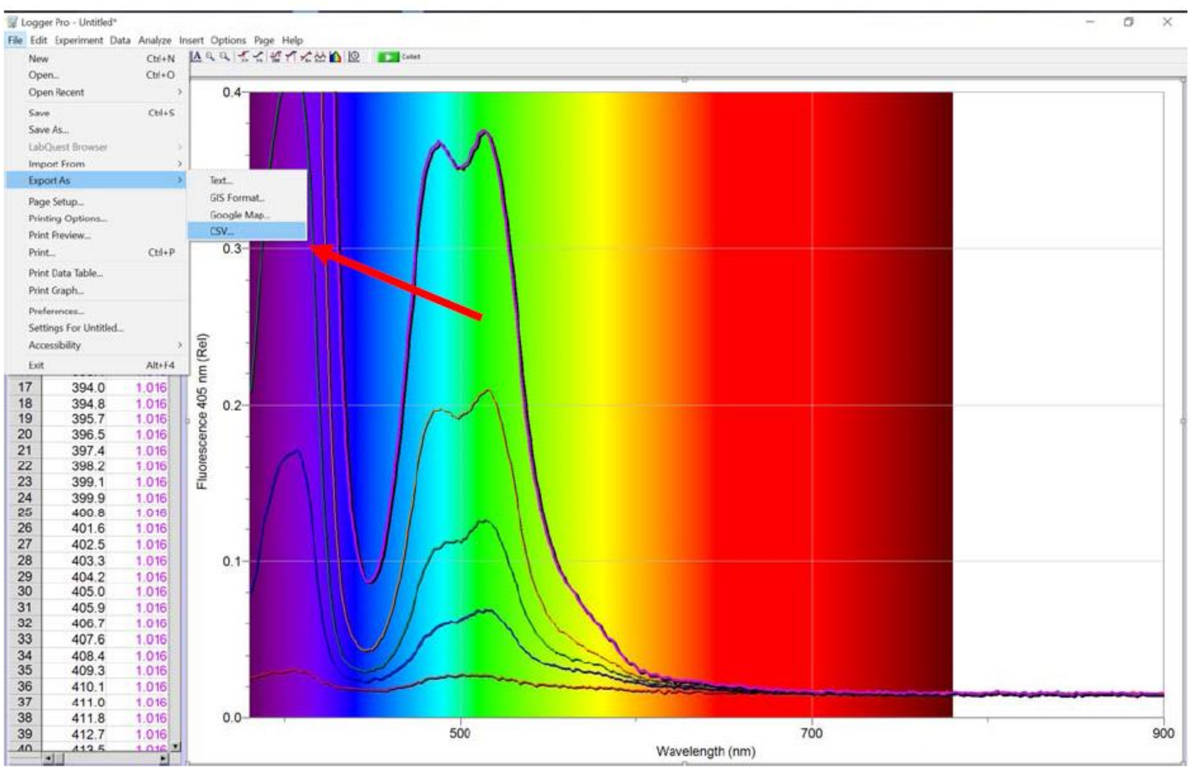


Figure 3.5. The spectra and flourometric data for different total lipids concentrations

The volume of the produced gas was measured manually using a 100-mL Gastight Luer-Lock glass syringe daily at the beginning of the digestion period. The gas measurement was continued every couple of days later on when the gas production rate slowed down over time. The amount of biomethane in the produced biogas during the anaerobic digestion process was measured using a Thermo Scientific Trace 1310 gas chromatograph (GC). The GC was equipped with a thermal conductivity detector and the temperature of the oven, detector, and filament were set to 80, 100, and 250 °C, respectively. The type of column used was a TG-Bond Msieve 5A model with a 30 m length and 0.53 mm diameter.

3.4. Statistical and Kinetics analysis

Statistical data including mean and standard deviation were calculated for the data obtained by the experiment. One way ANOVA for the analysis of variance was used to to find statistically significant differences between the group means and the results are presented in chapter 8.

Gompertz equations provides a wide range of applications in process kinetics of anaerobic digestion and the methane potential studies. In this work, Modified Gompertz model (Elbeshbishy and Nakhla, 2012; Lay et al., 1999) was used to predict the biogas yield and to assess the kinetic parameters and to describe the progress of cumulative methane production through the batch process (eq. 3.52) where CH_4 is the cumulative methane production (mL), P is the ultimate methane production (mL), R_m^e is the maximum methane production rate (mL/d), λ is the lag phase time (d), t is the digestion time (d).

$$CH_4 = p.\exp\left\{-\exp\left[\frac{R_m^e}{p}(\lambda - t) + 1\right]\right\} \qquad \text{eq. 3.5}$$

Chapter 4
Results and discussion

TWAS and SSO Co-digestion

4. Results and discussion- TWAS/SSO co-digestion

4.1. BMP of TWAS and SSO

This experiment was designed to evaluate the effect of mixing ratio of TWAS with SSO and its relationship with the lipids: proteins: carbohydrates on anaerobic co-digestion process using TWAS and SSO in different combinations as digester feedstocks. Co-digestion of SSO with TWAS was conducted as explained in Chapter 3. The characteristics of the feed in each digester having different mixing ratios of the substrates are summarized in Table 4.1. As presented in Table 4.1, the amount of TCOD of SSO is remarkably higher than that of TWAS. Increasing the fractions of SSO, increased the TCOD of the feed to digesters. The total lipids and total carbohydrates contents of SSO are also significantly more than that of TWAS and therefore, by increasing the proportion of SSO in the co-digesters the concentrations of lipids and carbohydrates increased. pH was kept at neutral level from 7.0- 7.3 in all the mixtures to satisfy the favorable condition for methanogenesis.

Table 4.1. Mean values of the feed characteristics in digesters with different mixing ratios of TWAS and SSO

		TWAS Only	SSO Only	TWAS:SSO 9:1	TWAS:SSO 7:3	TWAS:SSO 1:1	TWAS:SSO 3:7	TWAS:SSO 1:9
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)
TCOD	g/L	40	110	47	61	75	89	103
SCOD	g/L	1.4	44	5.7	14	23	32	40
TSS	g/L	32	54	34	38	43	47	52
VSS	g/L	26	39	27	30	32	35	37
TS	g/L	39	62	41	46	51	55	60
VS	g/L	35	44	35	37	39	41	43
Ammonia	g/L	0.3	1.1	0.3	0.5	0.7	0.9	1.0
pH		7.2	7.1	7.0	7.0	7.2	7.1	7.3
Alkalinity	g CaCO3/L	2.0	6.1	2.4	3.2	4.0	4.8	5.7
TN	g/L	2.9	3.3	2.9	3.0	3.1	3.2	3.2
TSN	g/L	0.4	0.9	0.5	0.6	0.7	0.8	0.9
T-Carbs	g/L	1.1	144	2.4	5.1	7.7	10.4	13.0
T-Proteins	g/L	3.8	2.3	3.6	3.3	3.0	2.7	2.5
T-Lipids	g/L	0.4	1.7	0.5	0.8	1.0	1.3	1.6

As shown in Fig. 4.1, no significant lag-phase occurred in the generation of biogas the reactors. Operation of the digesters continued until no significant biogas was produced. Fig. 4.1 shows the time-course profile of the cumulative biomethane production during the total operation period. As illustrated in the Figure 4.1, no significant lag time was observed for all the digesters. This no sign of significant inhibition would be due to the use of mesophilic inoculum acclimatized to municipal sludge streams similar to the one used in the experiment. The most lag-phase occurred at TWAS:SSO mixing ratios of 1:1, and 3:7, and 1:9 which could be due to the existence of particulate matters introduced by SSO to the mixture which could delay the hydrolysis phase and consequently affect methanogenesis.

The amount of biomethane produced by SSO was significantly higher than that of TWAS. Only 542 mL cumulative methane was produced by TWAS while the amount of cumulative methane obtained by SSO was 1101 mL. This verified the low biodegradability of TWAS compared to SSO and would be due to the composition of TWAS as it mostly consists of proteins and humic substances with some bacterial biomass and carbohydrates. Although proteins, DNA and carbohydrates are anaerobically biodegradable, their biodegradability decreases when they are combined into an organized structure similar to TWAS (Gonzalez et al., 2018; Stuckey and McCarty, 1984). Microbial cells are difficult to break down under anaerobic digestion (Foladori et al., 2015; Wett et al., 2010) and similarly, the presence of humic substances affects enzymatic activity by immobilizing enzymes and as a result, lowers biodegradability (Azman et al., 2015a, 2015b; Fernandes et al., 2015).

Another reason for the low biodegradability of TWAS was investigated by a study, in which low digestibility was attributed to the slow hydrolysis process for the exterior polymeric component of the microbial culture within the sample. Furthermore, the study also found that the ratio of SCOD to TCOD was 34.6% for untreated TWAS compared to 63.6% and 68.1% for thermally and alkaline pretreated samples, respectively. The COD ratio was a clear indicator of the expected biogas production by the TWAS feedstock samples where lower biogas yield was reported for the untreated raw TWAS as compared to pretreated samples (Abudi et al., 2016a).

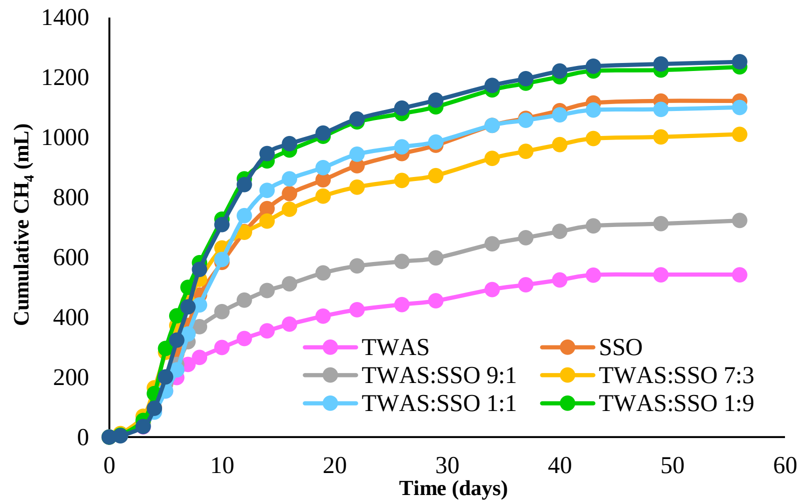


Figure 4.1. Cumulative methane production for different mixing ratios of TWAS and SSO

Addition of SSO to TWAS increased biodegradability and the methane yield compared to TWAS alone. However, as demonstrated in Figure 2, the trend showed an optimal mixing ratio of SSO with TWAS. The TWAS:SSO mixing ratio of 3:7 (V/V) delivered better results compared to TWAS:SSO mixing ratio of 1:9 (V/V) in terms of digestion process and methane enhancement.

Fig 4.2 shows the methane production rate in mL CH_4/d through the digestion period. Comparing TWAS and SSO, the methane production rate obtained by SSO was significantly higher than that of TWAS, although the maximum rate for the rectors digesting only TWAS occurred in earlier stage of the digestion process compared to the ones digested only SSO. Similar trend was observed in the digesters containing mixtures of TWAS and SSO so that increasing the fraction of SSO to the co-digesters, caused maximum methane production rate take place later than the co-digesters containing more fraction of TWAS. This could be as a result of abundant particulate matter that affects the hydrolysis rate and prolongs the entire process.

The biomethane data monitoring as presented in Figure 4.2, revealed that the highest portion of the biomethane was produced within the first month. The digesters generated 31–45%, 65–76% and 83–90% of their ultimate biogas productions during first 7, 14 and 30 days of operation, respectively. This would be due to the availability of sufficient nutrient right after the start of the operation which increases the metabolic activity of the microorganisms causing rapid conversion of substrate to biogas without inhibition in digesters (A. J. Li et al., 2011; Sung and Dague, 1995). The maximum methane production rate for mono and co-digestions is presented in Figure 4.3. The amount of maximum methane production rate of SSO mono digestion was 109 mL CH_4/d which was 60% higher that of TWAS mono digestion corresponding to 68 mL CH_4/d. Comparing to TWAS mono digestion, increasing the percentage of SSO in the co-digesters containing TWAS:SSO mixtures from the ratio of 1:9 ratio to 3:7, increased the maximum methane production rate by 25 % and 75%, respectively. Further increasing the percentage of SSO in the co digesters at TWAS:SSO mixing ratios of 1:1, 1:9 and 3:7, significantly increased the maximum methane production rate both compared to TWAS and SSO mono digestion, although the maximum rate values were almost the same for the three mixing ratios.

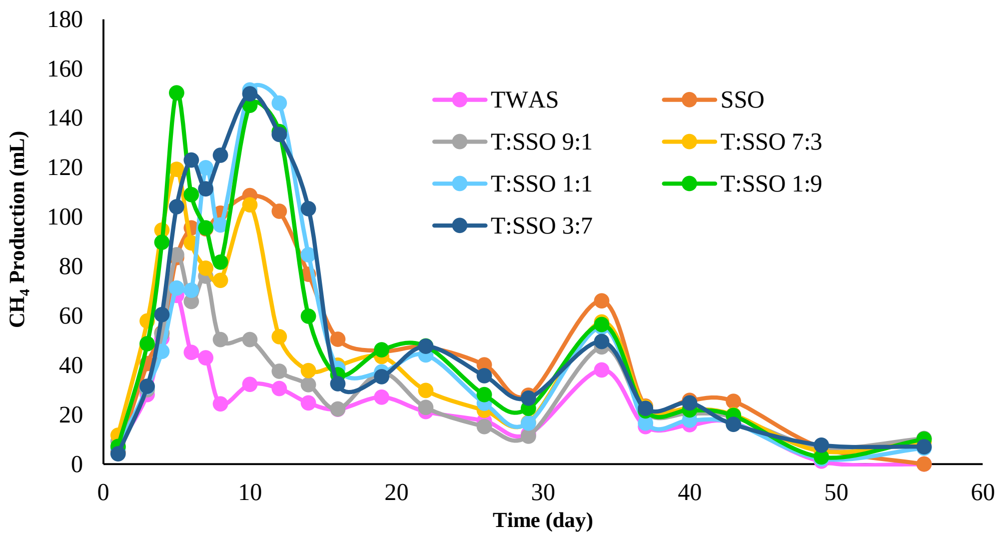


Figure 4.2. Methane production rate (mL/d) for different mixing ratios of TWAS and SSO

These findings as a matter of fact showed a close agreement to a previously conducted study (Abudi et al., 2016a), where the reported cumulative specific biogas yield (CSBY) for alternating ratios of organic fraction of municipal solid waste (OFMSW) and TWAS resulted in increasing biogas yield. As presented in Figure 4.3, the amount of maximum methane production rate was the highest for the TWAS:SSO mixing ratios of 1:1, 3:7, and 1:9 in a range between 150 to 151 mL/d. However, the maximum biomethane production rate for the TWAS:SSO mixing ratio of 3:7 occurred on the day 5 of the operational period while it was observed on the day 10 for the mixing ratios of and 1:1 and 1:9. This would be an evident to the advantage of co-digestion while emphasizing the necessity of a proper mixing ratio of the substrates which fulfills both enhancing the methane production and the process kinetics.

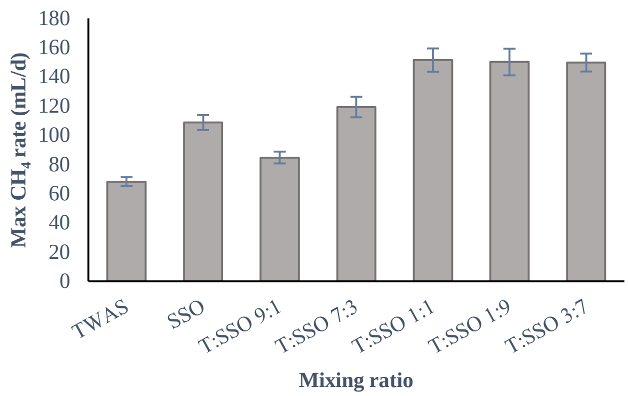


Figure 4.3. Maximum methane production rate (mL/d) for different mixing ratios of TWAS and SSO

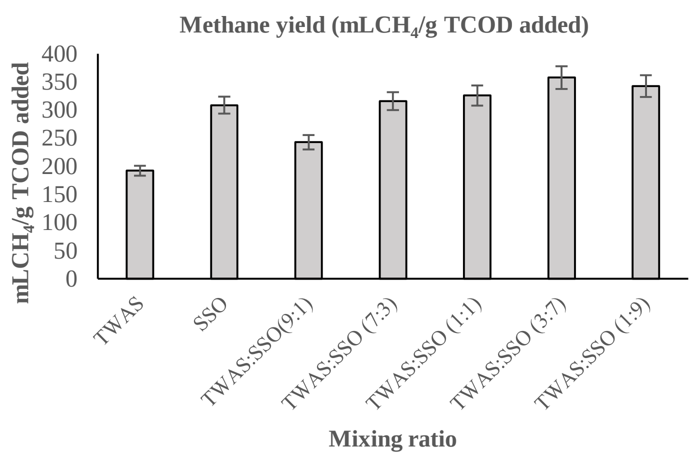
A COD mass balance was conducted for all of the digesters to assess the accuracy of the experiment. The mass balance was carried out with reference to the initial and the final TCOD concentrations of the digester contents, and the theoretical methane production per unit mass of TCOD removed. Comparison between the experimental methane production attained by this research and that of determined by TCOD mass balance, verified a deviation of less than 10% for all the digesters.

4.2. Cumulative methane yields

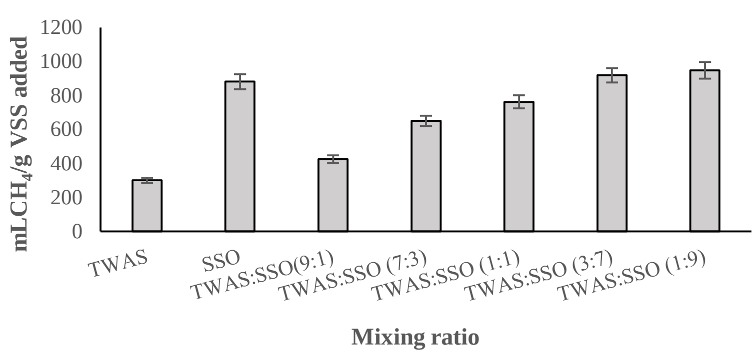
Cumulative methane yields were calculated and presented in Figures 4.4 a), b) and c. The cumulative methane yield was normalized per substrate unit mass COD added (mLCH_4/g TCOD added), per substrate unit mass VSS added (mLCH_4/g VSS added) and per unit volume of substrate added (mLCH_4/mL substrate added).

Figure 4.4 a) shows the result of cumulative methane yield per mass COD of substrate added for mono and co-digestions. Results showed that 192 mLCH_4/g TCOD added was obtained by TWAS mono digestion while a higher yield corresponding to 308 mLCH_4/g TCOD added was achieved by SSO alone. Mixing TWAS with SSO in co-digesters at all ratios excluding TWAS:SSO ratio of 9:1, increased the methane yield in comparison with both TWAS and SSO mono digestion. Even though, the ratio of 9:1 resulted in 27% increase of the CH_4 yield (mL) per mass of TCOD added compared to TWAS alone. The maximum cumulative methane yield was obtained by the 3:7 mixing ratio of TWAS:SSO corresponding to 358 mL CH_4/g TCOD added being 85% and 16% higher than that of TWAS and SSO alone. It was observed that addition of SSO to co-digesters significantly increased the methane yield compared to mono digestion of TWAS while it did not remarkably increased the yield in comparison with SSO mono digestion.

4.4. a)



4.4. b)



4.4. c)

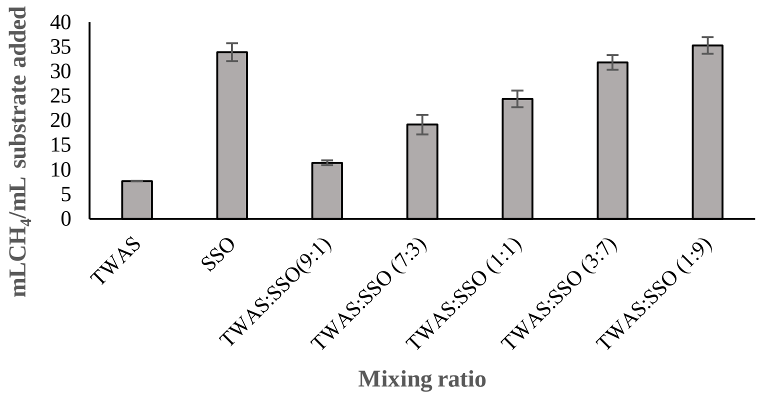


Figure 4.4. Methane yields: a) per unit mass TCOD added, b) per unit mass of VSS added and c) per unit volume of substrate added at different mixing ratio of TWAS and SSO

The yields of cumulative methane in terms of $mLCH_4$/g VSS added have been presented in Figure 4.4. b). Similarly, the methane yield per mass of VSS added by SSO mono digestion was significantly higher than that of TWAS. A 300 $mLCH_4$/g VSS added and 892 $mLCH_4$/g VSS added were attained by TWAS and SSO alone, respectively. All co-digesters produced higher methane yield per mass of VSS added compared to TWAS alone. However only the TWAS:SSO mixing ratios of 1:9 and 3:7 resulted in higher methane yields per mass of VSS added in comparison with both TWAS and SSO alone. The maximum methane yield of 975 $mLCH_4$/g VSS added occurred at the TWAS:SSO mixing ratio of 3:7.

It was revealed that addition of SSO to TWAS significantly increased biomethane production in the rectors co-digesting them compared to the control reactor digesting only TWAS. However, only the TWAS:SSO mixing ratio of 3:7 resulted in the increase of the methane yield compared to both TWAS and SSO mono digesters.

Eq. 4.1 was used for assessing the biodegradable fraction of the feedstocks for each of the rectors and the results are illustrated in Figure 4.5.

$$BF (\%) = (BM_{experimental} / BM_{theoretical}) \times 100 \qquad \text{Eq. 4.1}$$

Where BF is biodegradable fraction, $BM_{experimental}$ is the measured biomethane by the experiment and $BM_{theoretical}$ is the theoretical biomethane production in mL CH_4 per g TCOD of the substrate in mesophilic condition and standard pressure.

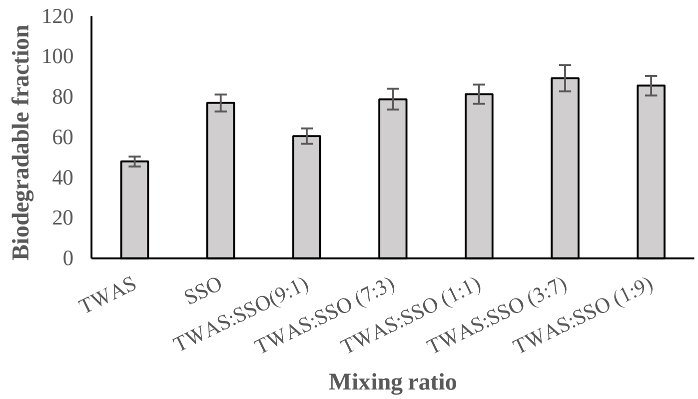


Figure 4.5. Biodegradable fraction of the feedstocks for different mixture ratios of TWAS and SSO

As shown in Figure 4.5, the TWAS:SSO mixing ratio of 3:7 corresponded to the maximum percentage of biodegradable fraction which complies with the maximum methane yield at the same mixing ratio. BF was 48% and 77% for TWAS and SSO, respectively. It was verified that addition of SSO as co-substrate increased biodegradability and enhanced the production of methane in the rectors co-digesting SSO with TWAS. All co-digesters had higher BF than TWAS alone. The reason would be the existence of readily biodegradable compounds in SSO that was introduced to the co-digesters. At the mixing ratio of 3:7, the BF was 89% which was 85% and 16% higher than that of TWAS and SSO alone respectively. The trend of BF changes in digesters complies with the methane yields obtained by the experimental results for the corresponding digesters.

4.3. Synergistic effect

In anaerobic digestion, production of biomethane develops through a syntrophic metabolism between both communities of methanogens including bacteria and archaea (Viotti et al., 2004). It is evident that both communities of bacteria and archaea are present in AnCoD systems. An improvement in the synergy and diverse microbial consortia is obtained when applying co-digestion of multiple feedstocks (Zamanzadeh et al., 2017). The synergistic effect of co-digestion can be estimated as an additional methane production (mL) for co-substrates over the weighted average of the methane production of individual substrates (Parra-Orobio et al., 2016). In this research, in order to investigate the synergetic effect of microbial populations on anaerobic co-digestion of TWAS and SSO at different mixing ratios, the weighted methane production (MP) of co-substrates were calculated using Eq. (4.2):

$$\text{Weighted MP} = MP_{SSO} * P_{SSO} + MP_{TWAS} * P_{TWAS} \qquad \text{Eq. (4.2)}$$

Where weighted MP is the weighted average of methane production for co-substrates ($mLCH_4$); MP_{SSO} and MP_{TWAS} are the experimental methane production ($mLCH_{4/}$ mL substrate added) for SSO and TWAS; and P_{SSO} and P_{TWAS} are the volume (mL) of SSO and TWAS in the substrate's mixture, respectively. When the percentage difference between experimental methane production for the mixtures and the calculated weighted average of methane production was positive, the synergistic effect could be concluded. Figure 4.6 shows the percentage of additional methane production for co-substrates over the weighted average of the methane production of individual substrates. As revealed in Fig 4.6, the maximum synergetic impact was observed in co-digestion of TWAS with SSO at the mixing ratio of 3:7. This is in good agreement with the maximum methane yield that occurred at the same mixing ratio of TWAS and SSO in their co-digestion.

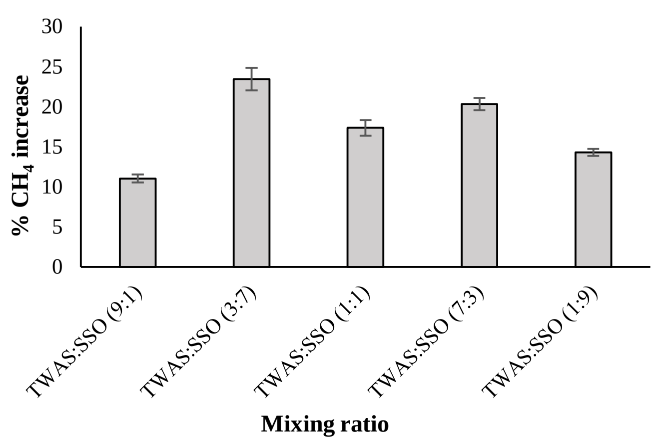


Figure 4.6. Synergetic effect of co-digestion at different mixing ratios of TWAS and SSO

Although all of the mixings demonstrated the synergetic impact of co-digestion on improving biomethane production, no specific trend for the change of the synergetic effect and methane increase corresponding to the fraction of SSO in the co-digestion mixtures was observed. The percentage of methane increase due to the synergetic impact varied from 11 to 23. The most percentage of biomethane increase of 23% as a result of the improved synergy occurred at the mixing ratio of 3:7. This is in good compliance with the results of maximum cumulative methane rate and the maximum methane yield which were achieved at the same mixing ratio. Although introducing fractions of SSO added more amounts of readily biodegradable materials to the co-digesters, the more fractions of SSO did not necessarily satisfied the optimum condition for the process improvement.

The improvement of the synergy and biogas yield could be as a result of diverse microbial consortia introduced by applying co-digestion of multiple feedstocks. Nevertheless, enhanced synergy would be dependent on some factors relying on the proper mixing ratios which satisfy the optimal nutrients balance and effective conditions for microbial syntrophy. This could be the reason for the optimum fraction of TWAS and SSO at the mixing ratio of 3:7 in the co-digestion rather than other ratios.

4.4. COD:N and lipids: proteins: carbohydrates ratios

The relationship between the COD:N ratios as well as lipids: proteins: carbohydrates ratios at different mixing ratios of TWAS and SSO with methane yield and ultimate methane production is presented in Table 4.2. The table also shows a comparison between the COD:N ratios with lipids: proteins: carbohydrates ratios for the digesters fed with different mixings of TWAS and SSO in volumetric basis. In co-digestion of TWAS and SSO, the COD:N ratio above 20 resulted in higher ultimate methane production, however, variation of the ratios from 28 to 34 caused a reduction of the amount of the produced methane. As shown in the table, the amounts of ultimate methane production were in a range between 542 to 1252 mL for different mixing ratios. The lowest ultimate methane production of 542 mL corresponded to a COD:N ratio of 14 and a lipids: proteins: carbohydrates ratio of 1:11:3 which occurred at TWAS:SSO mixing ratio of 1:0 in the reactors digesting TWAS alone. The maximum ultimate CH_4 corresponded to COD:N ratio of 28 associated with the lipids: proteins: carbohydrates ratio of 1:2:8 for the reactors co-digesting TWAS and SSO at the mixing ratio of 3:7 (v/v).

Considering the fact that lipids, proteins and carbohydrates are the main constituents of any organic material, considering the ratios of lipids: proteins: carbohydrates would be a good approach for optimizing the mixing ratios of the substrates. In addition, the optimal C:N or COD:N for some types of the feedstocks have shown to be very different from the generally observed the optimal values.

As presented in Table 4.2, the ratio of COD:N was 14 for TWAS alone while it was higher by 2.4 fold for SSO. The increase of the ratio of COD to N from 14 to 16, increased ultimate methane from 542 to 723 mL. This increase of methane production could be due to the sufficient amounts of nutrient for microbial activities which led to enhanced biomethane production. On the other hand, an optimal nutrient synergy is required to ensure synergetic interactions of the microbial communities as the increase of COD:N ratio from 28 to 34, decreased the amount of ultimate CH_4.

Table 4.2. Ultimate CH_4 at different ratios of the substrates, COD:N, and lipids:proteins:carbohydrates

Digester code	TWAS: SSO (V/V)	COD:N	Feedstock ratios code	Lipids: Proteins: Carbohydrates	Ultimate CH_4 (mL)	mLCH_4/g TCOD added
TWAS Only	1:0	14	AA	1:11:3	542	192
SSO Only	0:1	34	BB	1:1.3:8	1122	308
TWAS:SSO 9:1	9:1	16	A	1:7:5	723	243
TWAS:SSO 7:3	7:3	20	B	1:4:7	1010	316
TWAS:SSO 1:1	1:1	24	C	1:3:7	1100	326
TWAS:SSO 3:7	3:7	28	D	1:2:8	1252	358
TWAS:SSO 1:9	1:9	32	E	1:1.5:8	1235	343

The main effect plot for CH_4 yield data means in response to the feedstock ratios at different lipids:proteins:carbohydrates ratios is shown in Fig 4.7. As the trend shows, both type of the feedstock and their ratios have significant effect on the methane yield. Each of the feedstock ratios correspond to a different lipids:proteins:carbohydrates ratio. As shown in Figure 4.7, the minimum methane yield corresponds to the mono digestion of TWAS which occurred at the lipids:proteins:carbohydrates ratio of 1:11:3. The maximum CH_4 yield corresponded to TWAS:SSO ratio of 3:7 corresponding to the lipids: proteins: carbohydrates ratio of 1:2:8.

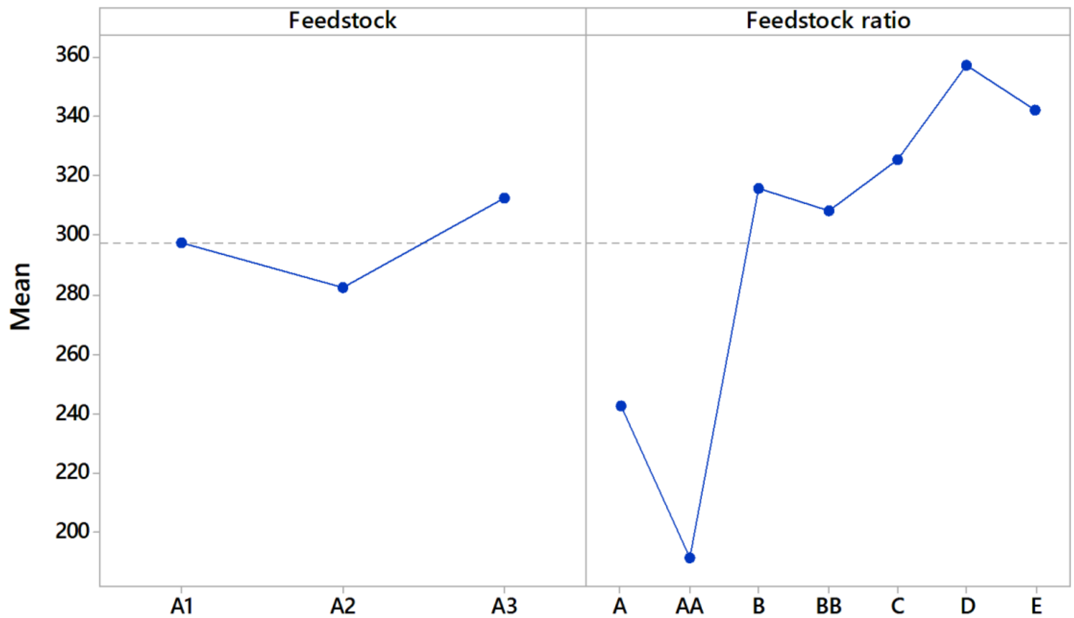
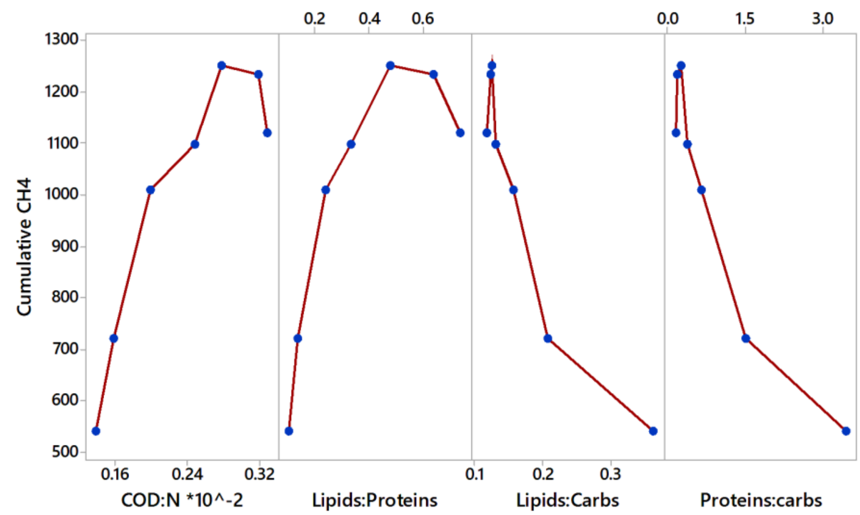
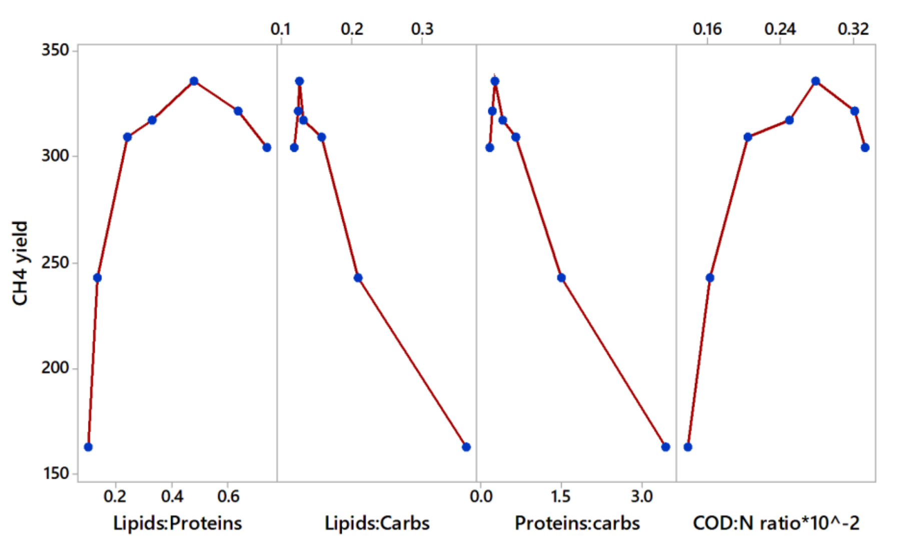


Figure 4.7. Main effects polot for CH4 yield data means in response to feedstock ratios at different lipids:proteins:carbohydrates ratios

A comparison between COD:N and the ratios of lipids: proteins, lipids: carbohydrates, and proteins:carbohydrates is presented in Figure 4.8. As illustrated in Figure 4.8 a) and 4.8 b), a same trend for the ultimate methane production in response to both COD:N ratios and lipids: proteins ratios was observed for the corresponding feedstocks mixing ratios. However, the COD:N ratio did not show the same trend as lipids: carbohydrates and proteins: carbohydrates ratios for the same feedstocks mixings, so that the minimum ultimate CH_4 (542 mL) corresponded to the minimum COD:N ratio of 14 while it corresponded to maximum lipids: carbohydrates of 0.32 as well as the maximum proteins: carbohydrates ratio of 3.48. Both lipids to carbohydrates and proteins to carbohydrates ratios showed the same trends at the corresponding feedstocks mixing ratios so that the maximum ultimate methane of 1252 mL occurred at the minimum lipids: carbohydrates of 0.12 and the minimum proteins: carbohydrates of 0.19. Similar trends were observed for the CH_4 yield (mLCH_4/g TCOD added) in response to the COD:N, lipids: proteins, lipids: carbohydrates and proteins: carbohydrates ratios.



4.8. a



4.8. b

Figure 4.8. Matrix effects plot for: a. ultimate CH_4 and b. CH_4 yield at different COD:N and Lipids: Proteins; lipids:carbohydrates and proteins:cabohydrates for the same corresponding mixing ratios of TWAS:SSO

4.5. Kinetic analysis results

The modified Gompertz model according to Eq. 3.5 was used to determine the coefficients for cumulated methane production and the results have been presented in Table 4.4. The modified Gompertz model could identify significant parameters related to anaerobic digestion including the maximum methane production rate, maximum methane production and lag phase, which underlines the time when the substrate is transformed and its correlation with the methane production phase. The volume data for the controls and for each mixing ratio collected during the experiment were used to apply the modified Gompertz model. The model corresponds to a sigmoid function expressing methane production in the reactor as a function of time (Lay et al., 1999; Parra-Orobio et al., 2016).

Applying the collected data for cumulative methane production (CH_4) per unit substrate in mL/g and the digestion time (t) in days to the Gompertz equation, the values of P, representing the maximum methane production per unit substrate (mL/g), R_m^e, the maximum methane production rate (ml/g h), and λ, the lag phase time (d) were calculated. The determination coefficient (R^2) as presented in Table 4 was used as criterion to evaluate the fitted models. The modified Gompertz model for mono- and co-digestions, respectively showed a good fit to the experimental results and the estimated parameters indicated that the co-digestion of SSO with TWAS improved the biogas production rate (Figure 1). The value of R^2= 0.9998 indicates that the proposed equations can accurately describe the variation of methane yield curves. Typically, S-shaped curves are obtained by the modified Gompertz model which shows a relatively slow upward trend related to the lag phase at the beginning of the curve. Therefore, the lag phase time for all of the TWAS/SSO co-digestion mixtures could verify the suitability of the modified Gompertz model in estimated performance of the process.

Table 4.3. Summary of results of kinetic study using modified Gompertz models.

	P (mL)	$\boldsymbol{R_m^e}$ (mL/d)	λ (d)	R^2
TWAS	510	28	0.1	0.999
SSO	1063	68	2.0	0.999
TWAS:SSO 9:1	661	44	1.0	0.999
TWAS:SSO 7:3	938	68	1.1	0.999
TWAS:SSO 1:1	1046	84	3.1	0.999
TWAS:SSO 3:7	1182	95	2.7	0.999
TWAS:SSO 1:9	1165	89	1.9	0.999

With reference to the adjustment to the Gompertz model, the lag phase varied from 0.1 to 3.1 days for different substrate mixing ratios. The shortest lag phase of 0.1 d corresponded to TWAS alone digestion. This result would be due to the existence of anaerobic inoculum that are easily assimilated by the microorganisms during this phase and cause rapid acclimatization to the substrate, which can be observed in the methane production. In theory, the inoculum activity, the amount of readily degradable constituent, and the initial pH of the feedstock affects the AD start-up time (Kafle and Chen, 2016).

The results showed that with the increase of the proportion of SSO in TWAS/SSO co-digestion, a longer lag phase time occurred. This trend stopped with further increasing the SSO proportion to the mixing ratio of 1:9. However, the mixing ratio of 3:7 resulted in slightly higher value of P which corresponds to the experimental results that showed the maximum methane production occurred at the mixing ratio of 3:7. Although, a long lag phase time is not favorable as it would increase the residence time and consequently, larger reactor volumes and higher costs during implementation and operation would be required.

The values of $P_,$ varied from 509.5 to 1182 mL corresponding to TWAS alone and TWAS:SSO mixing ratio of 3:7. The trend of changes in the P values complies with the experimental results as it showed an increasing trend in methane production by increasing the proportion of SSO. Similar to the experimental results, the trend changed from TWAS:SSO mixing ratio of 3:7 to 1:9 so that the ratio of 3:7 corresponded a higher P value for methane production than that of 1:9. The values obtained for R_{max}^{e} were within a range from 28 to 95 ml/d. The values of R_{max}^{e} and P_{max} obtained by Gompertz for the TWAS:SSO ratios of 3:7 were 95 mL/d and 1182 mL, respectively.

In co-digestion of TWAS and SSO, the COD:N ratio above 20 resulted in higher ultimate methane production, however, the difference in the ratios from 21 to 26 did not have significant effect on the amounts of the produced methane. Therefore, considering lipids: proteins: carbohydrates ratios would be a good approach for optimizing the mixing ratios of the substrates. A lipids: proteins: carbohydrates ratio of 7:1:15 corresponding to COD:N ratio of 26, resulted in an ultimate biomethane production of 1252 mL in TWAS and SSO co-digestion. It was verified that co-digestion of TWAS and SSO improved methane production in comparison with conventional single digestion of the feedstocks. It was concluded that cumulative methane production and methane yields varied at different mixing ratios of TWAS and SSO. Co- digestion of TWAS and SSO at the mixing ratio of 3:7 resulted in a biomethane yield of 353 mL CH_4/g TCOD $_{added}$ which is corresponding to an increase of methane yield by 84% and 15% compared to TWAS and SSO alone. A 23% increase in methane yield was observed as a result of synergetic effect of co-digestion at TWAS:SSO mixing ratio of 3:7 (v/v) equivalent to lipids: proteins: carbohydrates ratio of 1:2:8. This study verified the advantage of co-digestion over conventional single digestion of the feedstocks in terms of biomethane improvement and increased microbial synergy resulting higher methane production. Further studies utilizing a range of various feedstocks is required to promote the viability of lipids: proteins: carbohydrates ratio for optimizing the anaerobic digestion process and for optimizing the mixing ratios in anaerobic co-digestion of multiple feedstocks.

4. 6. Hydrolysis/acidification

This experiment was carried out to evaluate the hydrolysis/acidification stage in anaerobic co-digestion of TWAS with SSO. In hydrolysis which is known to be the rate limiting stage in the entire anaerobic digestion process, the large complex organic polymers including lipids, proteins, and carbohydrates break down to simple smaller molecules such as fatty acids, amino acids and sugars. Subsequently, acidogenic microorganisms break down the by-product of hydrolysis to further smaller molecules in acidification step (Gould, 2014). Therefore, this part of the present research was aimed to investigate the degradation of organic compounds in hydrolysis stage through the analysis of the degree of solublization, synergetic effect of co-digestion at different mixing ratios on hydrolysis and liquefaction, volatile fatty acids (VFAs) yield, and kinetics of lipids, proteins, and carbohydrates in co-digestion of TWAS and SSO.

A series of the analysis for the characterization of the feedstocks was primarily carried out in triplicates and the mean values are summarized in Table 4.4. As presented in Table 4.4, the COD concentration of SSO is higher than that of TWAS. Adding SSO to TWAS in the co-digesters, increased the COD concentrations compared to TWAS mono digestion. SSO also contains higher amounts of carbohydrates and lipids than TWAS while the proteins concentration of TWAS is more than SSO by 30%. The concentrations of total, soluble and particulate COD; total, soluble and particulate proteins; and total, soluble and particulate carbohydrates were monitored over time for calculating their hydrolysis rate coefficients.

Table 4.4. Characteristics of the feedstocks at different mixing ratios of TWAS and SSO

		TWAS Only	SSO Only	T/SSO 9:1	T/SSO 7:3	T/SSO 1:1	T/SSO 3:7	T/SSO 1:9
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)
TCOD	g/L	40	110	47	61	75	89	103
SCOD	mg/L	0.4	44.4	4.8	13.6	22.4	31.2	40.0
TSS	mg/L	31.5	53.8	33.7	38.2	42.6	47.1	51.6
VSS	mg/L	18.5	36.0	20.2	23.7	27.2	30.8	34.3
TS	mg/L	38.8	62.2	41.1	45.8	50.5	55.2	59.8
VS	mg/L	34.5	43.5	35.4	37.2	39.0	40.8	42.6
Ammonia	mg/L	0.3	1.1	0.3	0.5	0.7	0.9	1.0
pH	-	5.6	5.6	5.8	5.7	5.7	5.8	5.8
Alkalinity	mg CaCO3/L	2.0	6.8	2.4	3.4	4.4	5.3	6.3
TN	mg/L	2.9	4.0	3.0	3.2	3.4	3.6	3.9
TSN	mg/L	0.4	1.1	0.5	0.6	0.7	0.9	1.0
TotalCarbs	mg/L	0.9	13.5	2.2	4.7	7.2	9.7	12.2
TotalProteins	mg/L	2.8	2.1	2.7	2.6	2.4	2.3	2.2
TotalLipids	mg/L	0.3	1.1	0.4	0.5	0.7	0.9	1.0

The data form total and soluble COD (SCOD) concentrations monitoring with time was used to obtain the degree of COD solublization for each mixture and the results are as follows. The degree of solublization was calculated using Eq. 3.1 and the result is shown in Figure 4.8. The degree of the COD solublization varied from 14% to 30%. The maximum solublization of COD content was 30% for TWAS:SSO combination of 1:9 while the minimum value corresponded to the digester containing only TWAS. Except for TWAS:SSO mixing ratio of 9:1, other co-digesters achieved more than 25% improvement in solublization. The lower degree of solublization in TWAS mono digestion as well as TWAS:SSO mixing ratio of 9:1 with a high portion of TWAS would be due to the existence of slowly biodegradable content which slows down the hydrolysis and liquefaction. SSO contains sufficient amount of carbohydrates which decomposes more rapidly than proteins and lipids. This would be the reason for observing higher degree of solublization for SSO compared to TWAS which contains more proteins than carbohydrates.

However, by calculating the theoretical degree of solublization of each co-digester and comparing them to the ones obtained from the measured values based on the experimental data, it was revealed that co-digestion was effective for improving the solublization due to enhancing microbial synergy. As shown in Figure. 4.10, all co-digesters achieved an improvement in solublization due to synergistic effect of the microbial communities from 23 to 44% corresponding to TWAS:SSO mixing ratios of 3:7 and 7:3, respectively.

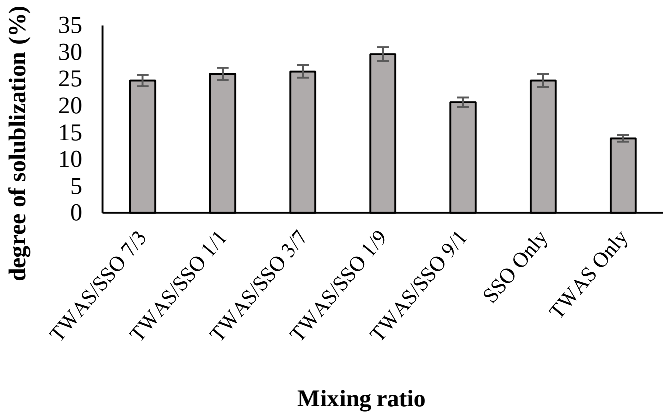


Figure 4.9. Degree of COD solublization at different mixing ratios of TWAS and SSO

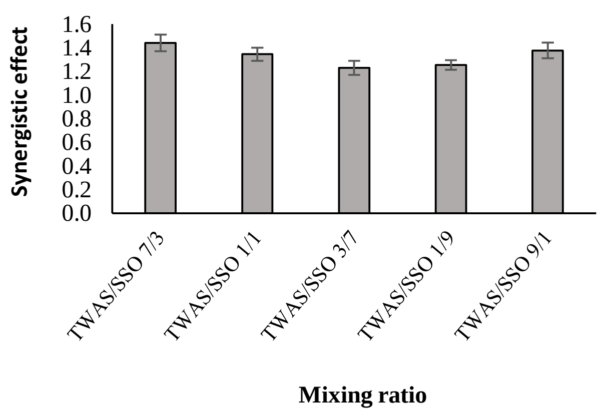


Figure 4.10. Synergistic effect on solublization at different mixing ratios of TWAS and SSO

As discussed earlier, the by-product of hydrolysis is subsequently broken down to smaller molecules by acidogenic bacterial communities. This leads to the formation of volatile fatty acids in acidogenesis. Therefore, monitoring VFAs concentration over time and calculating VFAs yield per mass of VSS added would be a good indicator of acidification progress. The VFAs concentrations monitoring showed an increasing trend during the 72-hr of the hydrolysis/acidification process. The total VFAs concentration was used for calculating the VFAs yield in terms of mass of VFAs produced in mg per mass of VSS added in g. As indicated in Figure

4.11, the VFAs yield decreased with the addition of TWAS in the digesters. This trend is similar to the trend of COD solublization as demonstrated in Figure 4.11. All of the reactors containing the mix of substrates had higher VFAs yield compared to the reactors containing only TWAS. The VFAs yields were 98 mg VFAs/g VSS added for TWAS mono digestion and 213 mg VFAs/g VSS added for TWAS:SSO ratio of 9:1, respectively. Other digesters resulted in higher amounts of VFAs production from 281 to 328 mg VFAs/g VSS added.

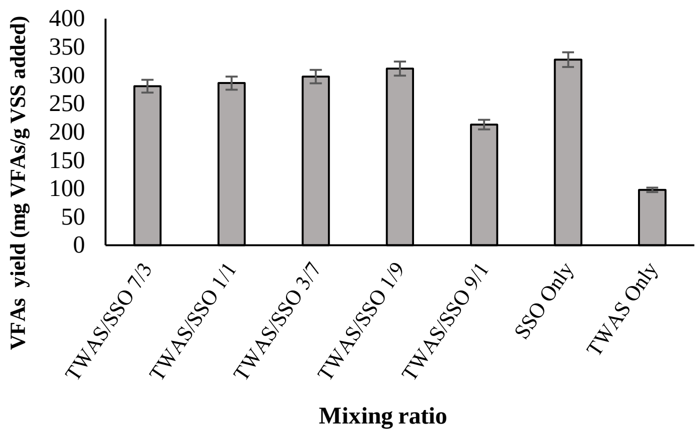


Figure 4.11. Total VFAs yield at different mixing ratios of TWAS/SSO

The analysis of soluble and particulate COD over time during the hydrolysis experimental period, showed an increasing trend in COD solublization and decreasing trend in particulate COD concentrations. Similarly, the analysis of soluble and particulate lipids, proteins, and carbohydrates over time demonstrated an increasing trend in solublization and particulate matter degradation. Although the trend is remarkably slower for lipids and proteins compared to carbohydrates. These results are summarized in the tables that are presented in the appendix. With applying the first order kinetics using equations 3.4., the hydrolysis rate coefficient (K_h) was calculated in AquaSim 2.0 for COD, lipids, proteins and carbohydrates based on the particulate matter degradation and the results are summarized in Table 4.5.

As presented in Table 4.4, the hydrolysis rate for COD content of SSO was higher than that of TWAS by 1.8 folds. The K_h values varied from 0.17 to 0.35 corresponding to TWAS alone and TWAS:SSO mixing ratio of 1:9, respectively. Hydrolysis rate coefficient increased in the reactors containing mixings of TWAS and SSO as compared to the reactors digesting only TWAS. The increase of COD hydrolysis rate could be the result of improved microbial synergy and good syntrophic interactions between the hydrolytic and acidogenic microbial communities through the process. The higher kinetic rate coefficient at the mixing ratio of 1:9 is in good agreement with the higher degree of solublization at the same mixing ratio compared to other co-digestion mixtures. Among lipids, proteins, and carbohydrates, lipids showed the lowest hydrolysis rate. In contrast, hydrolysis proceeded more rapidly for carbohydrates compared to lipids and proteins.

The higher hydrolysis rate of carbohydrates would be the result of more rapid biological metabolism of carbohydrates than lipids and proteins. K_h varied from 0.03 to 0.08 corresponding to hydrolysis of the lipid content of TWAS alone and SSO alone, respectively. All co-digesters had a higher hydrolysis rate of the lipid content in comparison with the digesters containing only TWAS.

As shown in Table 4.5, the hydrolysis rate of proteins content of the digesters was higher than that of lipids content, however it was still lower compared to the hydrolysis rate of carbohydrates content of the feedstocks. K_h for the proteins content was in a range between 0.19 to 0.34 corresponding to TWAS alone and TWAS:SSO mixings of 1:9, respectively. All of the reactors co-digesting TWAS with SSO demonstrated bigger hydrolysis rate coefficient than that of the TWAS mono digesters. The hydrolysis rate coefficient of the carbohydrates content was within a range between 0.32 to 0.68 corresponding to TWAS alone and the co-digestion of TWAS with SSO at the mixing ratio of 1:9. Contrary to expectation, the K_h values of carbohydrates did not increase with the addition of SSO portion in the mixtures.

The variation of hydrolysis rate for the carbohydrates content did not show the same trend as the lipids and proteins contents. This verifies that the hydrolysis of the lipids, proteins and carbohydrates of the feedstocks occurs independently during the hydrolysis/acidification stage. It was observed that co-digestion had an effect on the hydrolysis rate by mostly improving the hydrolysis of proteins and carbohydrates content rather than lipids.

Table 4.5. Hydrolysis rate coefficient for COD, lipids, proteins, and carbohydrate content in co-digesion of TWAS and SSO at different mixing ratios

K_h	TWAS:SSO 7:3	TWAS:SSO 5:5	TWAS:SSO 3:7	TWAS:SSO 9:1	TWAS:SSO 1:9	TWAS	SSO
Kh_{COD}	0.30	0.32	0.32	0.25	0.35	0.17	0.30
Kh_{Lipids}	0.06	0.07	0.07	0.03	0.05	0.03	0.08
$Kh_{Proteins}$	0.28	0.27	0.33	0.25	0.34	0.19	0.28
$K_{h\ Carbohydrates}$	0.56	0.57	0.54	0.46	0.68	0.32	0.55

Chapter 5

Results and discussion

TWAS and Manure Co-digestion

5. Results and discussion- TWAS and manure co-digestion

5.1. BMP of TWAS and manure

This experiment was designed to investigate the effect of mixing ratio of TWAS with manure and its correlation with the lipids: proteins: carbohydrates ratios on anaerobic co-digestion of TWAS and manure. The characteristics of the feed in each digester having different mixing ratios of the substrates are summarized in table 5.1. The COD concentration of manure slurry was significantly higher than that of TWAS. The average TCOD of manure was 122 g/L while it was 45 g/L for TWAS. Manure also contained significantly higher amount of total carbohydrates (27 g/L), proteins (5.1 g/L), and lipids (1.4 g/L) concentrations compared to TWAS. Mixtures of TWAS and manure as described in chapter 3 were prepared in different combinations and used as digester feedstocks. Addition of manure to TWAS increased the concentration of carbohydrates in co-digesters by a large extent as compared to TWAS mono digesters. It also increased lipids and proteins content of the co-digesters in comparison with the digester containing TWAS alone.

Table 5.1. Characteristics of feed to digesters with different mixing ratios

		TWAS	Manure	T*:M** 9:1	T:M 7:3	T:M 1:1	T:M 3:7	T:M 1:9
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)
TCOD	g/L	45	122	53	68	84	99	115
SCOD	g/L	2.0	8.9	2.7	4.1	5.5	6.7	8.2
TSS	g/L	36	80	40	49	58	67	75
VSS	g/L	27	77	38	42	52	62	72
TS	g/L	38	99	44	56	68	80	93
VS	g/L	28	86	33	45	57	68	80
Ammonia	g/L	0.32	0.02	0.29	0.23	0.17	0.11	0.05
pH	-	7.1	7.0	6.9	7.0	7.1	7.2	7.4
Alkalinity	g CaCO3/L	4.8	5.1	4.8	4.9	4.9	5.0	5.1
TN	g/L	2.9	2.2	2.8	2.7	2.5	2.4	2.3
TSN	g/L	0.4	0.1	0.4	0.3	0.3	0.2	0.1
T-Carbs	g/L	1.3	27.1	3.9	9.1	14.2	19.4	24.5
T-Proteins	g/L	3.9	5.1	4.1	4.3	4.5	4.8	5.0
T-Lipids	g/L	0.5	1.4	0.6	0.8	1.0	1.1	1.3

* T: TWAS
** M: Manure

The range of carbohydrates concentrations were within 3.9 to 24 g/L. Total proteins concentrations were 3.9 and 5.1 for TWAS and manure respectively and varied from 4.1 to 5 g/L in the co-digesters. The total lipids concentration of manure was 1.4 g/L and it was higher by 2.8 fold than TWAS. Adding manure to TWAS increased the lipids content. In the reactors containing the mixtures of the TWAS and manure, lipids concentrations varied from 0.6 to 1.3 g/ L.

Operation of the digesters continued until no significant biogas was produced. Fig. 5.1 shows the profile of the cumulative biomethane production versus time during the total digestion period. TWAS resulted in minimum cumulative methane production of 590 ml while manure alone produced 838 mL of cumulative methane during the operation period.

As shown in figure 5.1, the maximum cumulative methane production was obtained by co-digestion of TWAS:manure at the mixing ratio of 3:7 corresponding to the lipids: proteins: carbohydrates ratio of 1:4:17. Compared to mono digestion of manure, co-digestion at the mixing ratio of 9:1 did not increase methane production, although higher cumulative methane was obtained at that ratio in comparison with TWAS mono digestion. In fact TWAS alone with lipids: proteins: carbohydrates ratio of 1:7:2.5, produced the least amount of methane. Other co-digesters produced higher methane than that of TWAS and manure single digestion.

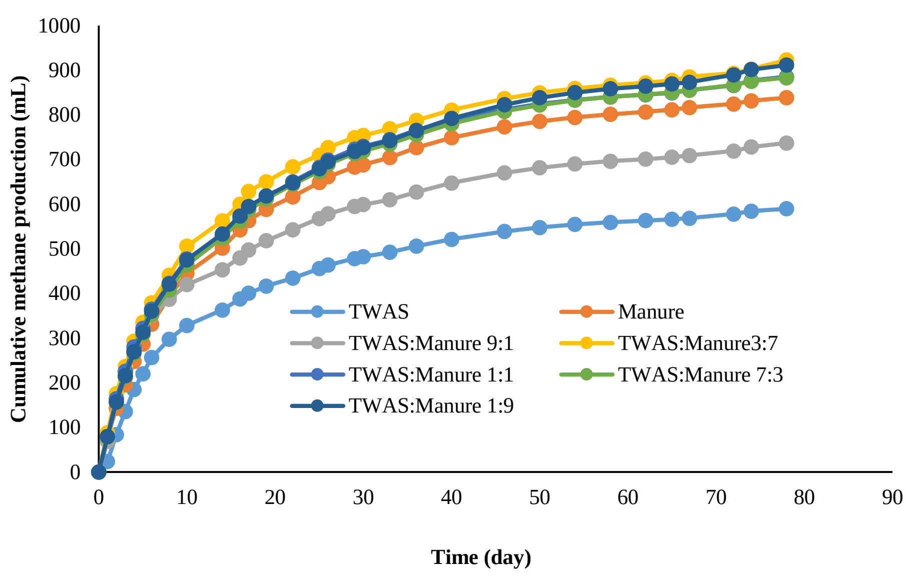


Figure 5.1. Cumulative methane production for different mixing ratios of TWAS and manure

As illustrated in Figure 5.2, for all of the digesters, the maximum methane rate occurred in the first week of the operation period. The amount of maximum methane production rate of the digesters differed from each other, although a similar trend was observed for all of the co-digestion mixtures. The reactors generated 47% to 52% of their ultimate methane production at the first week of the digestion period and 59% to 62% in two weeks operation. Compared to TWAS, manure alone resulted in higher methane rate. Addition of manure to TWAS increased partially the maximum methane production rate so that all of the co-digesters demonstrated slightly higher maximum methane rate compared to TWAS and manure mono digesters except for the reactor containing the TWAS and manure at the mixing ratios of 7:3 and 1:9 which had almost the same maximum methane rate as of manure alone. In comparison with TWAS alone, the maximum methane rate in co-digestion reactors were 30% to 47% higher.

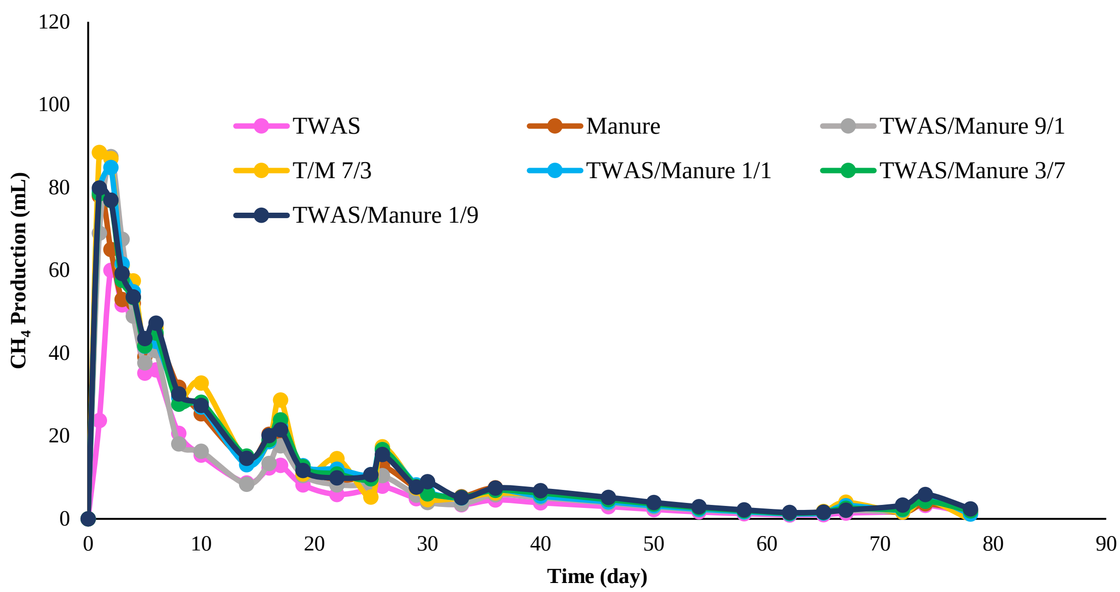


Figure 5.2. Methane production rate (mL/d) for different mixing ratios of TWAS and Manure

The maximum CH_4 production rate at different mixing ratios is presented in Figure 5.3. As shown in Figure 5.3, the maximum rate was 100 mL/day that occurred at the first day of the process for TWAS/manure mixing ratio of 1:1 corresponding to the lipids: proteins: carbohydrates ratio of 1:25:78 and the minimum rate corresponded to TWAS mono digestion at the lipids: proteins: carbohydrates ratio of 1:7:2.5.

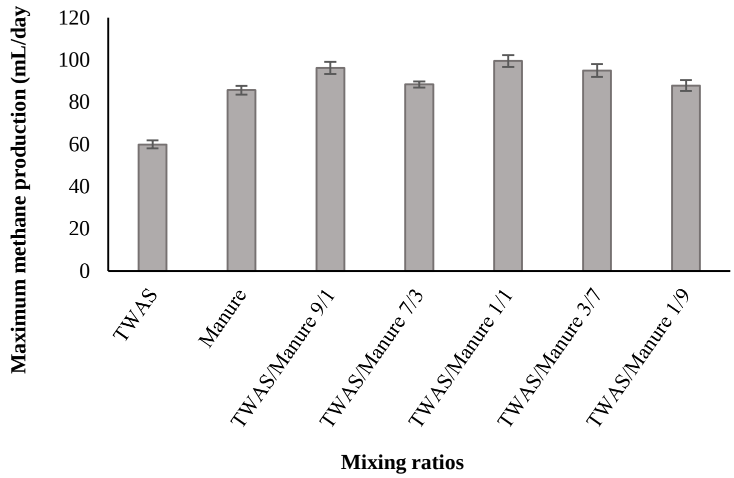


Figure 5.3. Maximum methane production rate (mL/d) for different mixing ratios of TWAS and manure

During the anaerobic process COD is only rearranged meaning that all COD that enters the system converts eventually to the end products methane minus the COD that incorporates in new microbial mass. Therefore, COD is generally taken as a useful control tool for the operation of anaerobic systems (Henze et al., 2015). A COD mass balance was conducted for all of the digesters to assess the accuracy of the experiment. The mass balance was carried out with reference to the initial and the final TCOD concentrations of the digester contents, and the theoretical methane production per unit mass of TCOD removed. Comparison between the experimental methane production attained by this research and that of determined by TCOD mass balance, as presented in figure 5.4 verified a deviation of less than 10% for all the digesters. The COD balance varied from 90 % to 98% in all mono digestion and co-digestion reactors.

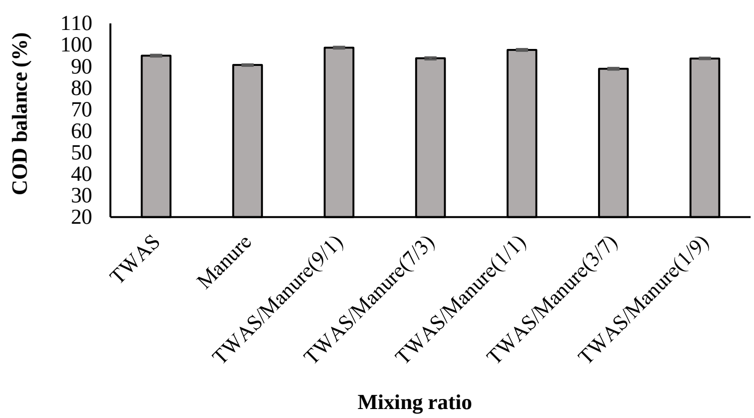


Figure 5.4. COD mass balance in co-digestion of TWAS and manure for different mixing ratios

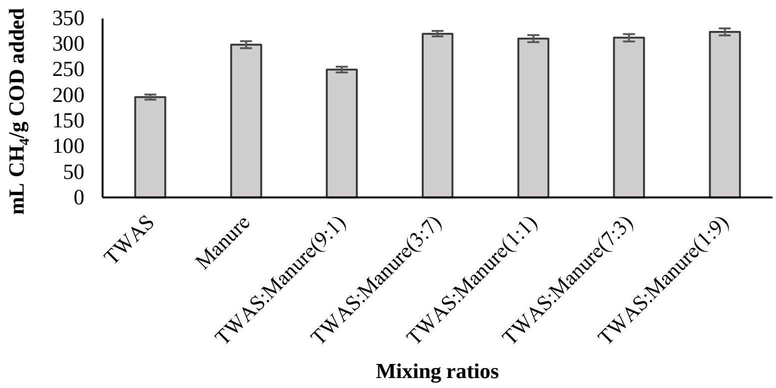
5.2. Cumulative methane yields

Cumulative methane yields including mLCH_4/g TCOD added, mLCH_4/g VSS added, and mLCH_4/mL substrate added are presented in Figure 5.5. Figure 5.5 a. shows cumulative methane yield per mass COD of substrate added for mono and co-digestions. Manure produced a higher amount of biomethane compared to TWAS. The methane yield was 196 mLCH_4/g TCOD added and 298 mLCH_4/g TCOD added for TWAS and manure mono digestion, respectively. The addition of manure to TWAS, only increased the methane yield by 15% in co-digesters compared to digestion of manure alone. Even though, it increased the methane yield by 65% in comparison with mono digestion of TWAS. Results showed that 320 and 324 mLCH_4/g TCOD added was obtained by co-digestion at the mixing ratios of 1:9 and 3:7. Mixing TWAS with manure in co-digesters except for TWAS:manure ratio of 9:1 and 7:3 enhanced the methane yield in comparison with both TWAS and manure mono digestion. Although, the ratios of 9:1 and 3:7, increased the methane yield per unit mass of COD added by 40 % and 59% compared to TWAS alone. The maximum cumulative methane yield of 324 mL CH_4/g TCOD added at TWAS/SSO ratio of 3:7 corresponded to 1:4:17 lipids: proteins: carbohydrates ratio.

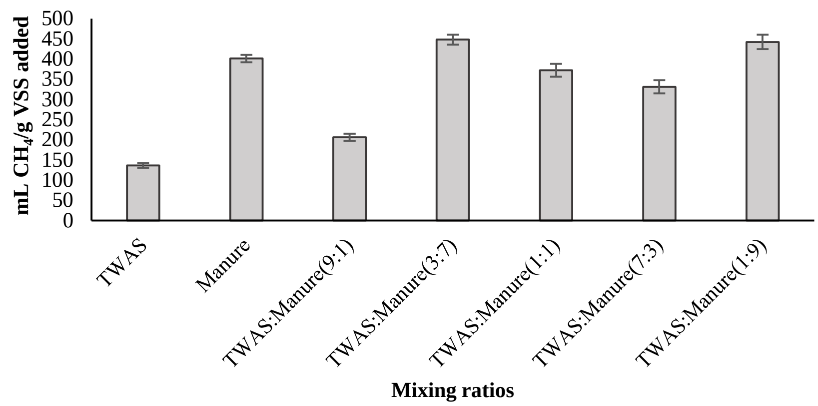
The methane yield per volume of substrate added in co-digesters were lower than that of manure alone. However, all of the co-digesters yielded higher methane per volume substrate added in comparison with TWAS mono digestion. Only 4 mL methane per unit volume of substrate added in single digestion of TWAS was obtained.

As shown in figure 5.5. b, cumulative methane yield in terms of mLCH_4/g VSS added significantly increased compared to TWAS alone due to addition of manure as co-substrate. Although the yield (mLCH_4/g VSS added) only increased by 12 % compared to manure alone. Co-digestion of manure with TWAS significantly increased the methane yield per unit mass of VSS added compared to TWAS alone. A 136 mLCH_4/g VSS added and 401 mLCH_4/g VSS added were attained by TWAS and manure alone, respectively. The amount of methane yield per unit mass of VSS added in co-digesters varied from 206 to 448 mLCH_4/g VSS added. The maximum CH_4 yield per unit mass of VSS added corresponded to lipids: proteins: carbohydrates ratio of 1:4:17 at TWAS:manure mixing ratio of 3:7 (v/v).

5.5. a)



5.5. b)



5.5. c)

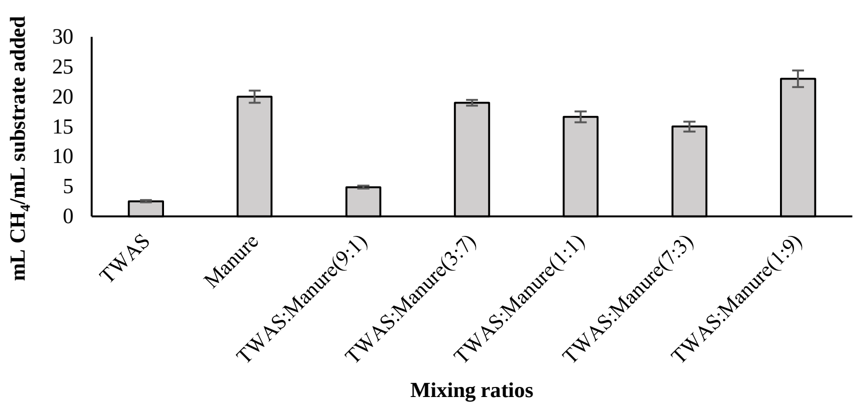


Figure 5.5. Methane yields a) per unit mass TCOD added, b) per unit mass of VSS added and c) per volume substrate added at different mixing ratio of TWAS and manure

Assessing biodegradable fraction of the feedstocks with reference to Eq. 4.1, confirmed a 53% more biodegradable fraction for manure than TWAS. This verifies the higher amount of biomethane obtained by manure compared to TWAS. Co- digestion enhanced biodegradability by 8% and 65% in comparison with the control reactors digesting only manure and TWAS, respectively.

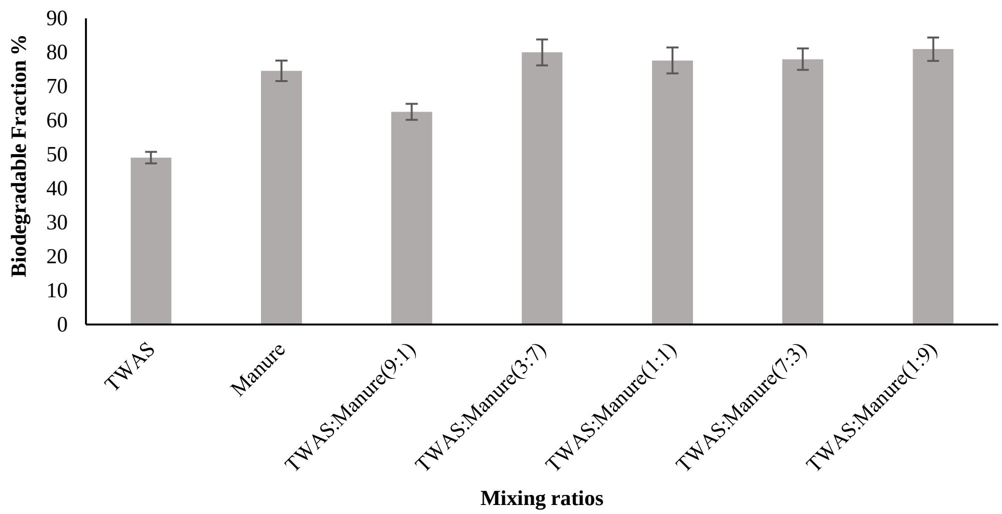


Figure 5.6. Biodegradable fraction of the feedstocks for different mixture ratios of TWAS and manure

As shown in Figure 5.6, biodegradable fraction was 49% for TWAS while it was 75 % for manure. The mixing ratios of 3:7 and 1:9 had the highest biodegradable fraction of almost 80% in TWAS/manure co-digestion. It was verified that addition of manure as co-substrate increased biodegradability and enhanced the production of methane in the rectors co-digesting manure with TWAS. All co-digesters had higher biodegradable fraction than TWAS alone. The reason would be the existence of readily biodegradable compounds in manure that was introduced to the co-digesters. At the mixing ratio of 3:7, the biodegradable fraction was almost 10 % and 0.53 % higher than that of manure and TWAS alone. The trend of biodegradable fraction changes in digesters complies with the methane yields obtained by the experimental results for the corresponding digesters.

5.3. Synergistic effect

Synergistic effect was evaluated with reference to Eq. 4.2 as described in chapter 4. Figure 5.7 shows the percentage of additional methane yield for co-substrates that was measured by the experiment, over the weighted average of the methane production of individual substrates per unit volume of substrate added. As shown in Fig 5.7, the synergistic effect varied from 10 to 24 % in co-digesters. The maximum synergetic impact was observed in co-digestion of TWAS/manure at the mixing ratio of 3:7. This is in compliance with the maximum methane yield that was obtained at the same mixing ratio of TWAS and manure in the reactors co-digesting them. In co-digestion of TWAS and manure, 24% improvement was achieved due to synergistic effect at the mixing ratios of 3:7.

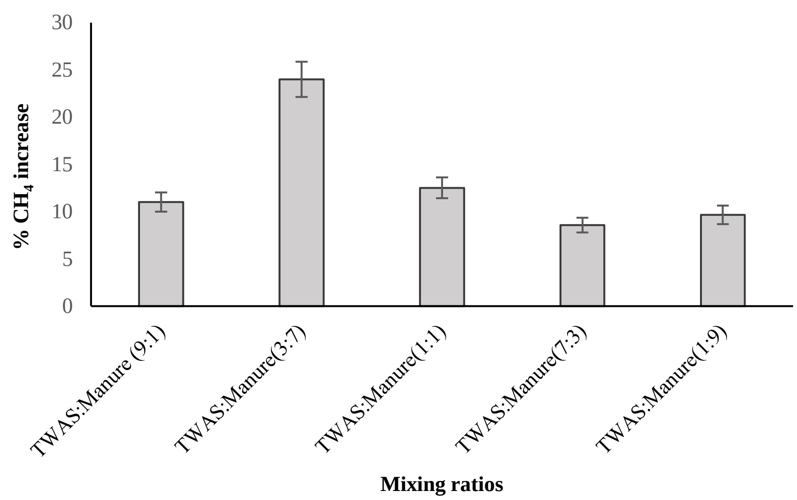


Figure 5.7. Synergetic effect of co-digestion at different mixing ratios of TWAS and SSO

Although all of the mixings demonstrated the synergetic impact of co-digestion on improving biomethane production, no specific trend for the change of the synergetic effect and methane increase corresponding to the fraction of manure in the co-digestion mixtures was observed. Similar to co-digestion of TWAS/SSO, adding fractions of manure as co-substrate enhanced the amounts of readily biodegradable materials to the co-digesters. The ratios with more fraction of carbohydrates produced more methane as the biodegradation of carbohydrate occurs more rapidly than lipids and proteins content. However, increasing the fractions of manure did not essentially resulted in the process improvement.

The improvement of the synergy and biogas yield in TWAS/manure co-digestion could be due to the abundance of methanogenic populations and diversity of archaeal communities present in manure. Nevertheless, improved synergy would also depend on the optimal nutrients balance and effective conditions for microbial growth. This could explain the optimum fraction of TWAS and manure at the mixing ratio of 3:7 in the co-digestion rather than other ratios.

5.4. COD:N and Lipids: Proteins: Carbohydrates ratios

Table 5.2 presents the ultimate methane production and the methane yield per unit mass of COD added, the COD:N and lipids: proteins: carbohydrates ratios of the digesters. Table 5.2 provides a comparison between the COD:N ratios and the lipids: proteins: carbohydrates ratios of the digesters fed with different mixings of TWAS and manure. In co-digestion of TWAS and manure, the COD:N ratios between 33 and 56 resulted in higher ultimate methane production, however, the ratios below 20 resulted in lower methane yields. The ultimate methane production and methane yield ranged between 590 to 1069 mL and 196 to 324 mL/g TCOD added for different mixing ratios, respectively.

As presented in Table 5.2, the ratios of COD:N and lipids: proteins: carbohydrates were 16 and 1:7:2.5 for TWAS alone while they were 56 and 1:4:20 for manure, respectively. The methane yield obtained by manure was 52% more than that of TWAS. The lowest ultimate methane production and methane yield of 590 mL and 196 mL/g TCOD added, corresponded to the TWAS/manure mixing ratio of 1:0 in the control reactors digesting only TWAS. The maximum ultimate CH_4 and CH_4 yield corresponded to the COD:N ratio of 41 and the lipids: proteins: carbohydrates ratio of 1:4:17 for the reactors co-digesting TWAS and manure at the mixing ratio of 3:7 (v/v).

Figure 5.8. shows the main effect plot for CH_4 yield data means in response to feedstock and lipids:proteins:carbohydrates ratios at different feedstock mixing ratios in co-digestion of TWAS/manure. As illustrated in Figure 5.8, both feedstock and lipids:proteins:carbohydrates ratios has significant effect on the methane yield. The minimum methane yield corresponded to TWAS alone with lipids:proteins:carbohydrate ratio of 1:7:2.5 while the maximum yield occurred at the mixing ratio of 3:7 and lipids:proteins:carbohydrate ratio of 1:4:17 in co-digestion of TWAS with SSO. With reference to the results of the ANOVA test, the both COD:N and Lipids: Proteins:

Carbohydrates ratios had statistically significant effects on the ultimate methane production ($P < 0.05$).

Table 5.2. Ultimate CH_4 and yield at different ratios of the substrates, COD:N, and lipids:proteins:carbohydrates

Digester code	TWAS: Manure (V/V)	COD:N	Feedstock ratios code	Lipids: Proteins: Carbohydrates	Ultimate CH_4 (mL)	$mLCH_4$/g TCOD added
TWAS Only	1:0	16	AA	1:7:2.5	590	196
Manure Only	0:1	56	CC	1:4:20	922	298
TWAS/Manure 9/1	9:1	19	A	1:7:6	811	250
TWAS/Manure 7/3	7:3	26	B	1:5.5:12	902	320
TWAS/Manure 1/1	1:1	33	C	1:25:78	1015	310
TWAS/Manure 3/7	3:7	41	D	1:4:17	1069	324
TWAS/Manure 1/9	1:9	51	E	1:4:19	1002	312

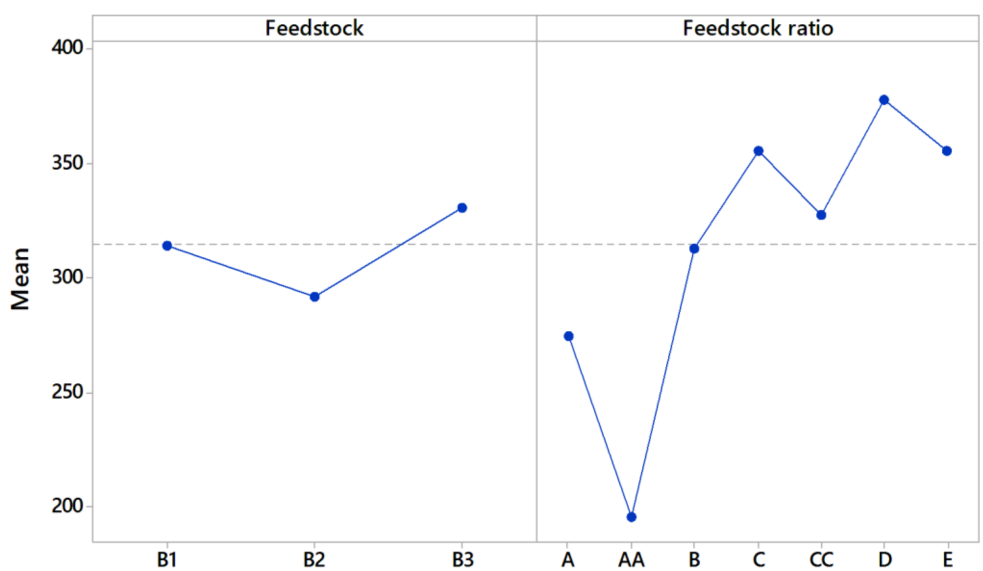
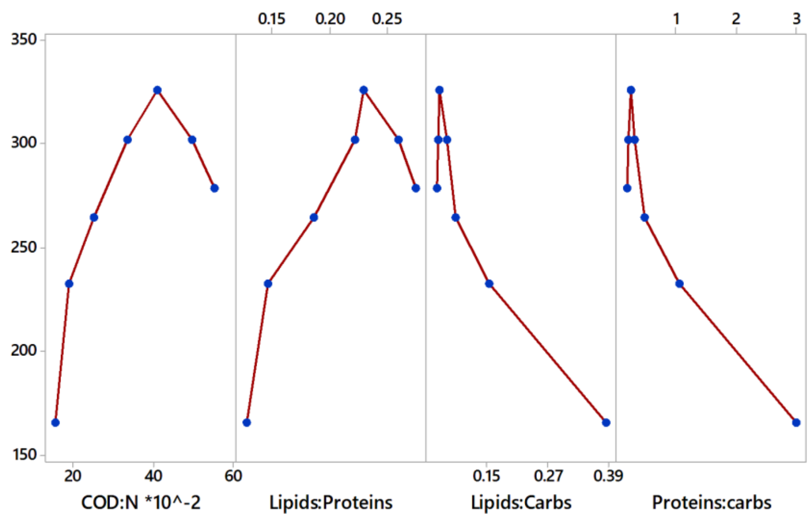
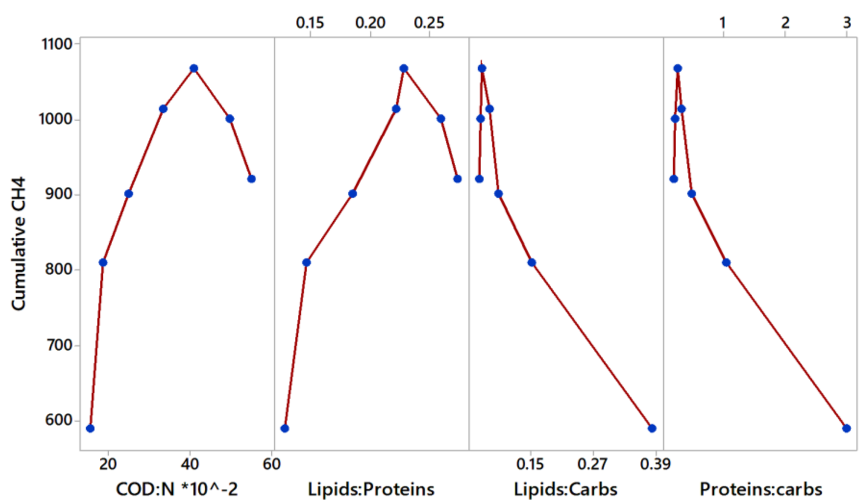


Figure 5.8. Main effect plot for CH_4 yield data means in response to feedstock and lipids:proteins:carbohydrates ratios at different feedstock mixing ratios in AnCoD of TWAS/manure

Figures 5.9. a and 5.9. b show the variation of ultimate methane and the methane yield versus COD:N ratio, lipids:proteins ratio, and proteins:carbohydrates ratios. As illustrated in Figure 5.9, different trends for the variations of methane yield and the ultimate methane versus COD:N, lipids: proteins, lipids: carbohydrates, and proteins: carbohydrates ratios were observed. For the corresponding feedstocks mixing ratios, a similar trend for the variations of methane versus COD:N and lipids: proteins ratios was observed. However, the COD:N ratio did not show the same trend as lipids: carbohydrates and proteins: carbohydrates ratios for the same feedstocks mixings.



5.9. a



5.9 b.

Figure 5.10. Matrix plot for: a. ultimate CH_4 and b. CH_4 yield at different COD/N and lipids: proteins, lipids: carbohydrates, and proteins: carbohydrates ratios

5.5. Kinetic analysis results

Table 5.3 presents the results of analyzing biomethane production according to the modified Gompertz model using Eq. 3.5. The data collected by the experiment from the control reactors and from each co-digester was applied to the model. The experimental data for cumulative methane production (CH_4) per unit substrate in mL/g and the digestion time (t) in days were applied to the Gompertz equation to calculate the values of P, representing the maximum methane production per unit substrate (mL/g), R_m^e, the maximum methane production rate (mL/g h), and λ, the lag phase time (d). The modified Gompertz model for mono- and co-digestions of TWAS and manure, showed a good fit to the experimental results with less than 5% diversion from the experimental values. The estimated parameters indicated that the co-digestion of manure with TWAS enhanced the biogas production rate. Such performance (R^2= 0.9998) shows that the proposed equations can accurately describe the variation of methane yield curves.

Table 5.3. Summary of results of kinetic study using modified Gompertz model

	P (mL)	**R_m^e (mL/d)**	**λ (d)**	**R^2**
TWAS	554	21	0.09	0.999
Manure	880	31	0.38	0.999
TWAS/Manure 9/1	763	27	0.06	0.999
TWAS/Manure 7/3	861	32	0.04	0.999
TWAS/Manure 5/5	969	36	0.04	0.999
TWAS/Manure 3/7	1016	35	0.04	0.999
TWAS/Manure 1/9	947	31	0.05	0.999

With reference to the adjustment to the nonlinear regression Gompertz model, the lag phase varied from 0.04 to 0.06 days for the co-digesters with different substrate mixing ratios. By increasing the proportion of manure higher values of P was estimated. The mixing ratio of 7:3 and 1:9 corresponded to the higher P values. These findings were in good agreement with the data collected from the experiment. As verified by the experiment, a longer lag phase was observed in co-digestion of TWAS:manure compared to co-digestion of TWAS:SSO. As mentioned before, a long lag phase time is unfavorable as it demands for a higher residence time and consequently, larger reactor volumes which increases the operational costs of the anaerobic system. The values of P, varied from 554 to 1016 mL corresponding to TWAS alone and TWAS:manure mixing ratio of 3:7. The trend of changes in the P values complies with the experimental data as it showed an increasing trend in methane production by increasing the proportion of manure. The values of R^{e}_{max} were within a range from 21.3 to 36.2 mL/d. The values of R^{e}_{max} and P_{max} obtained by Gompertz for the TWAS/manure ratios of 3:7, were 35.1 mL/d and 1016 mL, respectively.

5. 6. Hydrolysis/acidification

This experiment was conducted to investigate the hydrolysis/acidification phase in anaerobic co-digestion of TWAS and manure. The degradation of organic compounds in hydrolysis stage was evaluated using a series of analysis such as degree of solublization, synergetic effect of co-digestion at different mixing ratios on hydrolysis and liquefaction, volatile fatty acids (VFAs) yield, and hydrolysis kinetics of lipids, proteins, and carbohydrates .

Characterization of the feedstocks was initially carried out in triplicates and the mean values are summarized in table 5.4. As presented in the table, the amount of COD concentration is remarkably higher in manure than TWAS. Adding manure to TWAS in the co-digesters, increased the COD concentrations compared to the reactor digesting only TWAS. Manure also contains higher amounts of carbohydrates, lipids, and proteins than TWAS. The concentrations of total, soluble and particulate COD; total, soluble and particulate proteins; and total, soluble and particulate carbohydrates were monitored over time to obtain their hydrolysis rate coefficients.

The data form total and soluble COD (SCOD) concentrations monitoring over time during a 72- h experimental period was used to obtain the degree of COD solublization for each mixture. The degree of solublization was calculated using Eq. 3.1 and the result is summarized in Figure 5.10. The degree of the COD solublization varied from 21% to 34%. The maximum solublization 34% occurred at TWAS/manure combination of 3:7 while the minimum value corresponded to the digesters containing only TWAS. Except for TWAS/manure mixing ratio of 9:1, other co-digesters demonstrated an increase of solublization compared to both TWAS and manure alone. A 31% and 62% improvement was achieved by TWAS/Manure co-digestion at the mixing ratio of 3:7 compared to manure and TWAS single digestion, respectively. Manure contains sufficient amount of rapidly biodegradable materials than TWAS. The lower degree of solublization in TWAS mono digestion as well as TWAS:manure mixing ratio of 9:1 with a high portion of TWAS could be due to the existence of slowly biodegradable materials which slows down the hydrolysis and liquefaction process and decreases the degree of solublization.

Table 5.4. Characteristics of the feedstocks at different mixing ratios of TWAS and manure

		TWAS	Manure	T*:M** 9:1	T:M 7:3	T:M 1:1	T:M 3:7	T:M 1:9
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)
TCOD	g/L	41	110	48	62	76	90	104
SCOD	g/L	2.2	6.6	2.6	3.5	4.4	5.2	6.1
TSS	g/L	37.3	52.6	38.8	41.9	44.9	48.0	51.1
VSS	g/L	26.5	42.8	28.1	31.4	34.7	37.9	41.2
TS	g/L	39.2	68.0	42.1	47.9	53.6	59.4	65.2
VS	g/L	28.6	58.5	31.6	37.5	43.5	49.5	55.5
Ammonia	g/L	0.3	0.0	0.3	0.2	0.2	0.1	0.0
pH	-	5.8	5.8	5.6	5.7	5.8	5.8	5.8
Alkalinity	g $CaCO_3$/L	5.0	7.7	5.2	5.8	6.3	6.9	7.4
TN	g/L	3.0	1.4	2.8	2.5	2.2	1.9	1.6
TSN	g/L	0.4	0.1	0.4	0.3	0.2	0.2	0.1
T-Carbs	g/L	1.3	27.1	3.9	9.1	14.2	19.4	24.5
T-Proteins	g/L	4.0	5.1	4.1	4.3	4.6	4.8	5.0
T-Lipids	g/L	0.5	1.4	0.6	0.8	0.9	1.1	1.3

* T: TWAS
** M: Manure

Manure contains a large portion of carbohydrates which decomposes more rapidly than proteins and lipids. This would lead to a higher degree of solublization of manure than TWAS which contains more proteins than carbohydrates.

Comparing the theoretical degree of solublization of each co-digester to the ones obtained from the experimental data, showed that co-digestion improved solublization due to enhancing microbial synergy. As shown in Figure. 5.11, all co-digesters demonstrated an improvement in solublization due to synergistic effect of the microbial communities from 11 to 38% corresponding to TWAS:manure mixing ratios of 1:1 and 3:7, respectively.

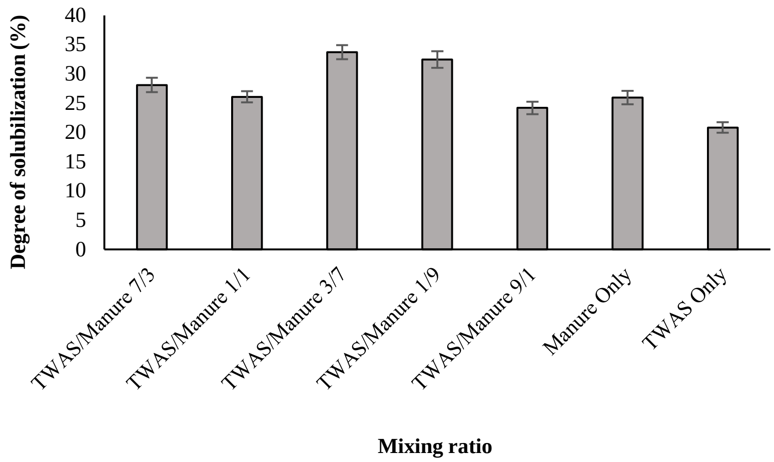


Figure 5.10. Degree of COD solublization at different mixing ratios of TWAS:Manure

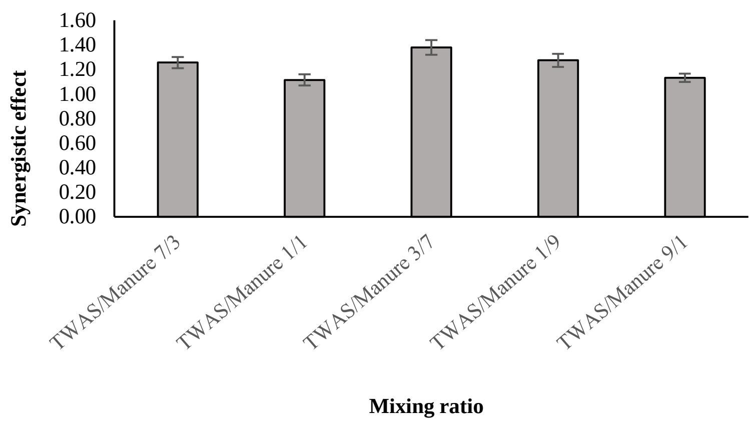


Figure 5.11. Synergistic effect on solublization at different mixing ratios of TWAS:Manure

The VFAs concentrations monitoring showed an increasing trend over the 72-hr of the hydrolysis/acidification experimental period. The total VFAs yield is presented in terms of mass of VFAs produced in mg per mass of VSS added in g. As indicated in Figure 5.12, manure alone had a significantly higher VFAs yields than TWAS alone. The VFAs yield in the co-digester was correlated to the mixing ratios of the feedstocks. The trend of VFAs yield did not conform to the trend of COD solublization of the corresponding mixing ratios (Figure 5.10). Therefore, hydrolysis and liquefaction showed a different trend from acidification. All of the reactors containing the mix of substrates had higher VFAs yield compared to the reactors containing only TWAS. The VFAs yields were 95 mg VFAs/g VSS added and 260 mg VFAs/g VSS added for TWAS and manure mono digestions, respectively. A VFAs yield of 307 mg VFAs/g VSS added was achieved for TWAS:manure ratio of 3:7.

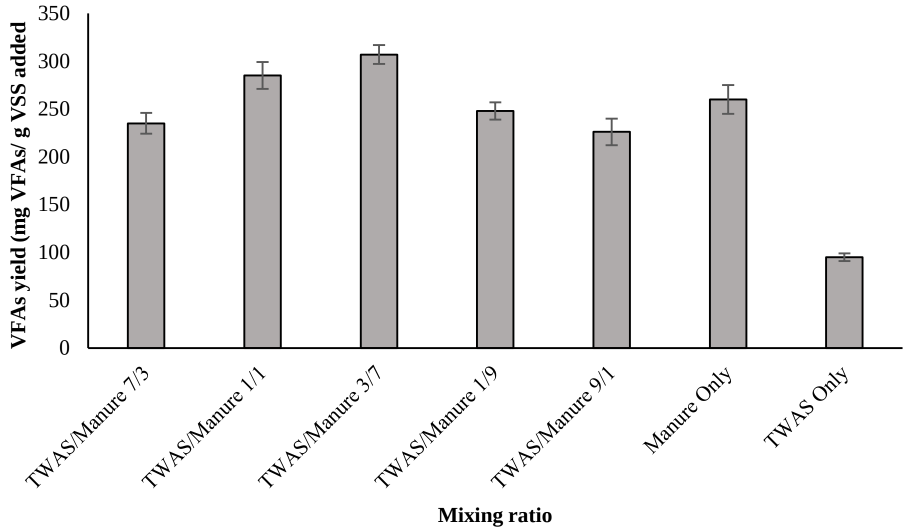


Figure 5.12. Total VFAs yield at different mixing ratios of TWAS/Manure

Monitoring COD over time, showed an increasing trend in solublization of COD and decreasing particulate COD concentrations. In addition, the analysis of soluble and particulate lipids, proteins, and carbohydrates over time showed an increasing trend in solublization and particulate matter degradation. For lipids and proteins, the hydrolysis rate was slower than carbohydrate. These results are summarized in the tables in the appendix. The hydrolysis rate coefficient (K_h) was calculated by applying first order kinetics using AquaSim 2.0 for COD, lipids, proteins and carbohydrates based on the particulate degradation and results are summarized in Table 5.5.

As presented in Table 5.5, the hydrolysis rate for COD content of TWAS was higher than that of manure by 35%. The K_h values in the co-digesters varied from 0.21 to 0.33 corresponding to TWAS and manure mixing ratios of 9:1 and 3:7, respectively. The maximum hydrolysis rate coefficient corresponded to the reactors containing mixings of TWAS and manure at the ratio of 3:7.

Lipids showed the lowest hydrolysis rate compared to proteins, and carbohydrates. On the contrary, the most rapid hydrolysis rate was observed for carbohydrates as a result of more rapid biological metabolism of carbohydrates than lipids and proteins. K_h varied from 0.4 to 0.09 in the reactors co-digesting TWAS with manure. The maximum hydrolysis rate coefficient of the lipids contents also corresponded to TWAS:manure mixing of 7:3. TWAS alone has the minimum K_h for the lipids. TWAS alone and manure alone had K_h values of 0.03 and 0.07, respectively. As presented in Table 5.5, the hydrolysis rate of proteins was slightly higher than that of lipids content of the digesters, although it was still lower than the hydrolysis rate of carbohydrates. K_h for the proteins content of the feedstocks was within a range between 0.22 to 0.27 corresponding to TWAS:manure mixings of 1:9 and 3:7, respectively. The hydrolysis rate coefficient of the carbohydrates varied from 0.38 to 0.59 corresponding to the digestion of TWAS:manure with the mixing ratios of 9:1 and 3:7, respectively. The carbohydrates content of the manure showed more rapid biodegradability than TWAS. The hydrolysis rate variation of lipids did not show the same trend as the proteins and carbohydrates of the feedstocks. This revealed that the hydrolysis of the lipids, proteins and carbohydrates of the feedstocks developed independently during the hydrolysis/acidification stage. This independent hydrolysis of the lipids, proteins, and carbohydrates was observed in co-digestion of TWAS and SSO as well.

Table 5.5. Hydrolysis rate coefficients for COD, lipids, proteins, and carbohydrate content in co-digestion of TWAS and manure at different mixing ratios

K_h	T/M 7/3	T/M 5/5	T/M 3/7	T/M 1/9	T/M 9/1	TWAS	Manure
Kh_{COD}	0.26	0.24	0.33	0.28	0.21	0.17	0.23
Kh_{Lipids}	0.04	0.05	0.09	0.07	0.04	0.03	0.07
$Kh_{Proteins}$	0.23	0.22	0.27	0.25	0.22	0.19	0.21
$K_{h\ Carbohydrates}$	0.49	0.44	0.59	0.48	0.38	0.32	0.43

* T: TWAS
** M: Manure

Chapter 6

Results and discussion

Manure and SSO Co-digestion

6. Results and discussion- manure and SSO co-digestion

6.1. BMP of manure and SSO

In this experiment co-digestion of manure and SSO was investigated. Manure slurry was prepared as discussed in chapter 3 and was fed to the reactors in different combinations with SSO. The influence of the feedstocks mixing ratios and their correlation with the lipids: proteins: carbohydrates ratios on biomethane production in anaerobic co-digestion of manure and SSO was evaluated. The characteristics of the feed in each digester containing different mixing ratios of the substrates are summarized in table 6.1. The values are the average of each parameter that was measured in triplicate. As presented in Table 6.1, both manure and SSO have high amount of COD concentrations and as a result, the amount of COD in the digesters are high and exceed 100 g/L. Both VSS and COD values did not vary significantly (less than 8%) in the digesters. The amount of carbohydrates and proteins of manure is significantly higher than that of SSO. Therefore, addition of manure increased the carbohydrates and proteins content of the co-digesters compared to the rectors digesting only SSO.

Table 6.1. Characteristics of feed to digesters with different mixing ratios of manure and SSO

		Manure Only	SSO Only	M/SSO 9/1	M/SSO 7/3	M/SSO 5/5	M/SSO 3/7	M/SSO 1/9
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)
TCOD	g/L	105	115	106	108	110	112	114
SCOD	g/L	44	43	44	44	44	43	43
TSS	g/L	54	62	55	57	58	60	61
VSS	g/L	46.4	45.6	46.3	46.2	46.0	45.8	45.7
TS	g/L	70	68	70	69	69	69	68
VS	g/L	59	49	58	56	54	52	50
Ammonia	g/L	0.02	1.07	0.13	0.3	0.5	0.8	1.0
pH	-	6.6	5.8	6.9	7.0	7.2	7.1	7.0
Alkalinity	g CaCO3/L	4.9	6.2	5.0	5.3	5.6	5.8	6.1
TN	g/L	2.5	3.5	2.6	2.8	3.0	3.2	3.4
TSN	g/L	0.1	1.0	0.2	0.4	0.6	0.8	1.0
T-Carbs	g/L	29	13	27	24	21	18	15
T-Proteins	g/L	5.9	2.1	5.5	4.8	4.0	3.2	2.5
T-Lipids	g/L	1.4	1.1	1.4	1.3	1.3	1.2	1.1

* M: Manure

Carbohydrates concentrations were within a range between 13 to 29 g/L in the rectors. Total proteins concentrations of manure and SSO were 2.1 and 5.9, respectively and varied from 2.5 to 5.5 g/L in the co-digesters. The total lipids concentrations varied from 1.1 to 1.4 g/L in the digesters including the controls. Adding manure slightly increased the lipids content in the reactors containing the combination of manure and SSO.

Operation of the digesters proceeded until no significant amount of biogas was generated. Fig. 6.1 shows the profile of the cumulative biomethane production versus time during the digestion period of manure with SSO including the controls. The cumulative methane production generated by SSO was higher than that of manure in the control reactors. All co-digesters produced more biomethane than the control reactors containing only manure and only SSO.

SSO alone produced 15% more methane than manure alone. The amount of ultimate CH_4 obtained by single digestion of SSO and manure was 1063 and 919 mL, corresponding to the lipids: proteins: carbohydrates ratios of 1:2:12 and 1:4.2:21, respectively. As shown in figure 6.1, the maximum cumulative methane production of 1186 mL corresponded to the manure:SSO mixing ratio of 7:3 and lipids: proteins: carbohydrates ratio of 1:3.5:18.5.

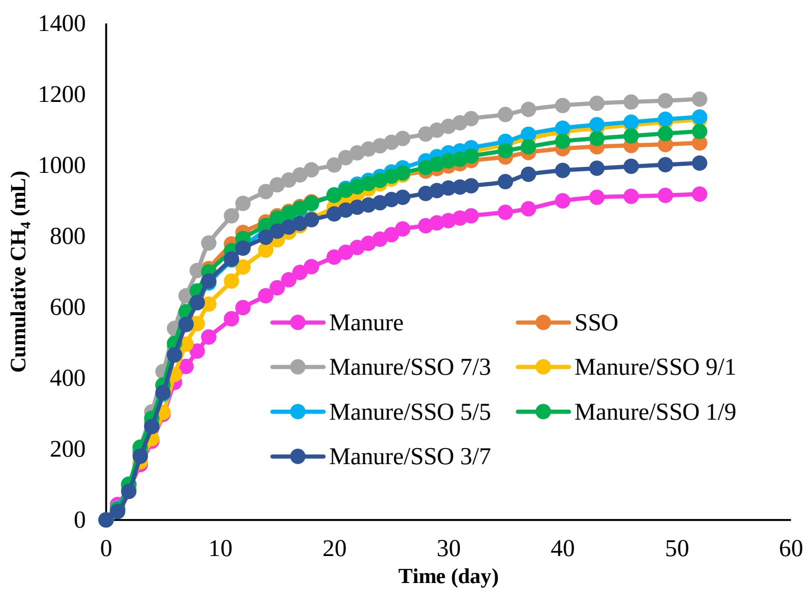


Figure 6.1. Cumulative methane production for different mixing ratios of manure and SSO

Figure 6.2, shows the methane rate in mL/day for all of the feedstocks combinations including the control reactors. For all of the digesters, the maximum methane rate occurred in the first week of the operation period. The maximum methane production rate of the digesters containing the mixings of manure and SSO was higher compared to the control reactors. The reactors generated 44% to 55% of their ultimate methane production at the first week of the digestion period and 69% to 79% of it in two weeks of operation. Compared to SSO alone, a higher CH_4 rate was observed for single digestion of manure. Addition of manure to SSO increased the maximum methane production rate so that all of the co-digester demonstrated a higher maximum methane rate compared to the control rectors. The manure/SSO mixing ratio of 7:3 achieved the highest maximum biomethane rate in comparison with other combinations and the lowest value for the

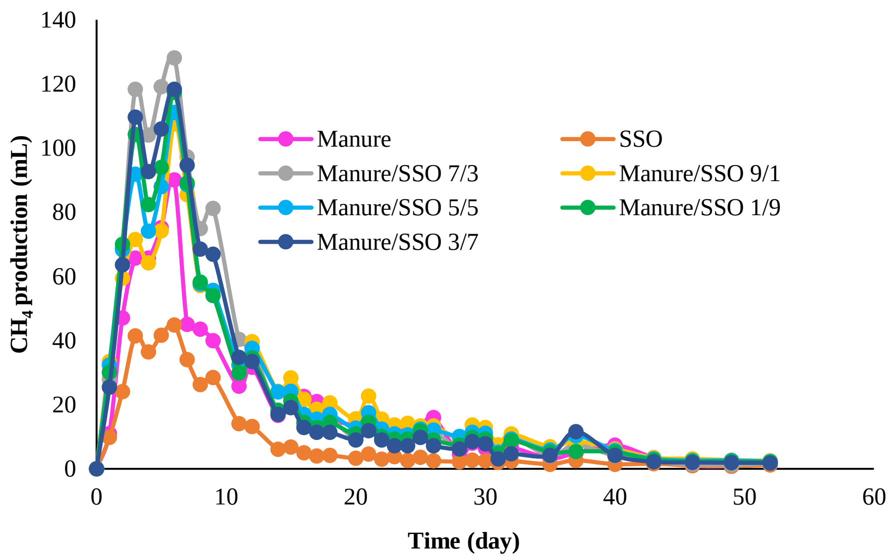


Figure 6.2. Methane production rate (mL/d) for different mixing ratios of Manure and SSO

maximum biomethane rate corresponded to single digestion of SSO. The maximum CH_4 production rate at different mixing ratios is shown in Figure 6.3. As presented in the figure, the most maximum rate was 128 mL/day corresponding to the mixing ratio of 7:3. The lowest value of 45 mL/day corresponded to single digestion of SSO. Manure alone had the maximum CH_4 rate of 90 mL/day which it was higher than that of SSO by 2 fold. The maximum CH_4 rate varied from 108 to 128 mL/day for the digesters containing combinations of manure and SSO.

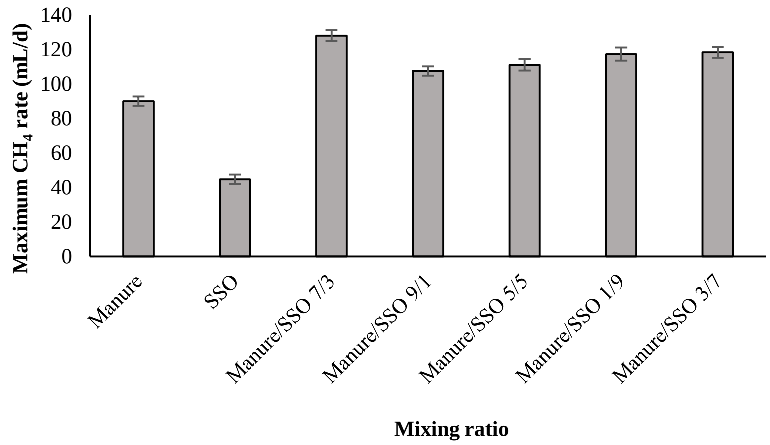


Figure 6.3. Maximum methane production rate (mL/d) for different mixing ratios manure of and SSO

The COD mass balance was conducted for all of the digesters with reference to the initial and the final TCOD concentrations of the digester contents, and the theoretical methane production per unit mass of TCOD removed. Comparison between the experimental methane production data and that of obtained by TCOD mass balance, showed a deviation of less than 8% for all the digesters. As shown in Figure 6.4, the COD balance varied from almost 90% to 97% in all mono and co-digesters.

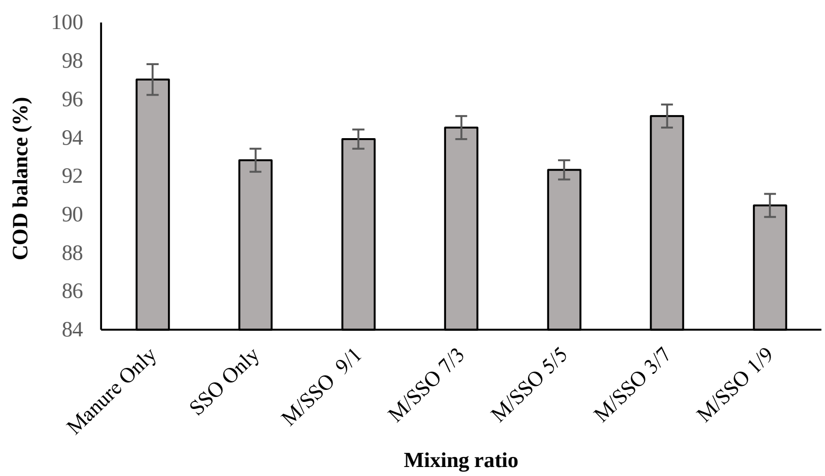
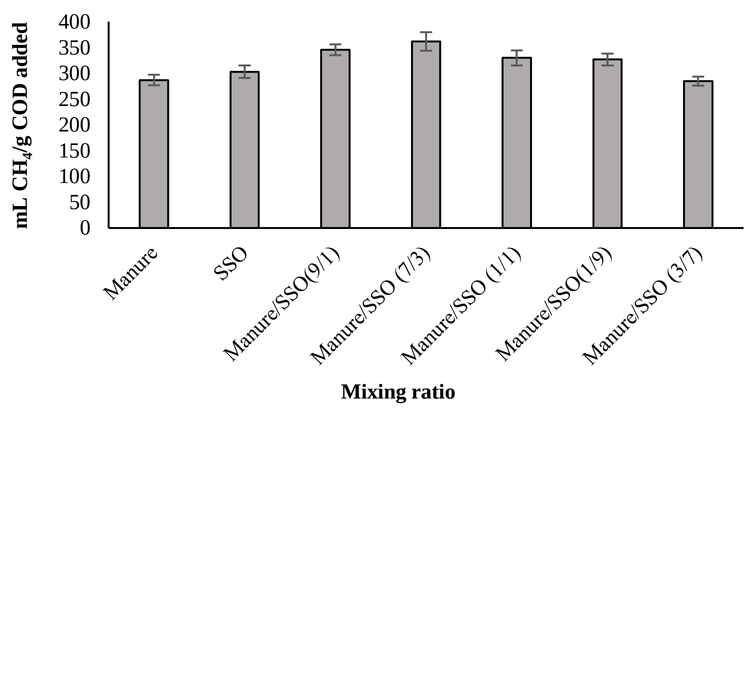


Figure 6.4. COD mass balance in co-digestion of manure and SSO for different mixing ratios

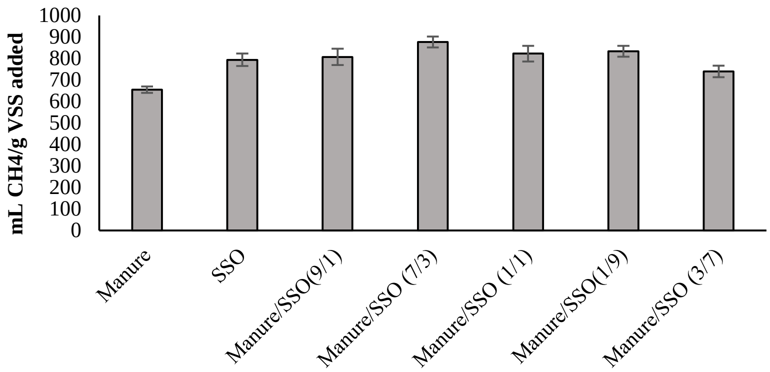
6.2. Cumulative methane yields

Cumulative methane yields including mLCH_4/g TCOD added, mLCH_4/g VSS added, and mLCH_4/mL substrate added are presented in Figure 6.5. It was observed that cumulative methane yield per mass COD of substrate added increased in co-digesters in comparison with the control reactors. As shown in Figure 6.5. a), SSO and manure alone produced 303 mLCH_4/g TCOD added and 287 mLCH_4/g TCOD added, respectively. The addition of manure with SSO, increased the methane yield in the co-digesters. The amounts of the biomethane yields were within a range between 316 and 362 mLCH_4/g TCOD added in the co-digesters. The highest yield of 362 mLCH_4/g TCOD added occurred at the manure/SSO mixing ratio of 7:3. CH_4 yield increased by 26% and 20% compared to single digestion of manure and SSO, respectively.

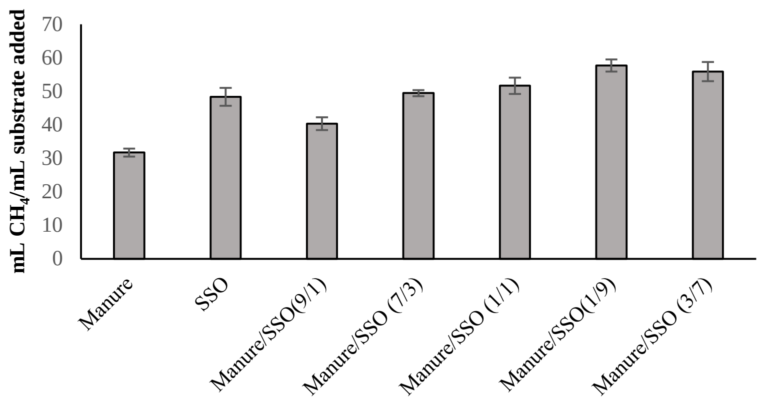
Figure 6.5. b), shows the methane yields in mLCH_4/g VSS added. Manure produced 654 mLCH_4/g VSS added. The yield was higher for SSO corresponding to 793 mLCH_4/g VSS added. The highest yield occurred at the manure/SSO mixing ratio of 7:3 corresponding to 876 mLCH_4/g VSS added. The CH_4 yield improved by 30% compared to the digestion of manure alone. All of the reactors with mixings of manure and SSO resulted in higher amounts of methane yields per unit mass of VSS added. The methane yields of from 819 to 847 mLCH_4/g VSS added were obtained in co-digestion of manure and SSO at different mixing ratios.



6.5. a)



6.5. b)



6.5. c)

Figure 6.5. Methane yields a) per unit mass TCOD added, b) per unit mass of VSS added and c) per volume substrate added at different mixing ratio of TWAS and manure

Figure 6.5. c), shows the methane yield per unit volume of substrate added in mLCH_4/ mL substrate added. Manure and SSO individually produced 32 mLCH_4/mL substrate added and 48 mLCH_4/ mL substrate added, respectively. The reactors with the combinations of manure and SSO resulted in methane yields ranging from 40 mLCH_4/mL substrate added to 58 mLCH_4/mL substrate added. The most values of the CH_4 yield in terms of unit volume of substrate added corresponded to manure/SSO co-digestion at the mixing ratios of 1:9 and 3:7.

Biodegradable fraction of the feedstocks was obtained using the Eq. 4.1 and the result is summarized in Figure 6.6. Manure and SSO individually had biodegradable fractions of 72% and 76 %, respectively. Co- digestion increased biodegradable fraction of the feedstocks by 20% and 26 % in comparison with the control reactors digesting only SSO and manure, respectively. The most percentage of biodegradable fraction occurred at the reactor co-digesting manure with SSO at the mixing ratio of 7:3. This in good compliance with the maximum methane production at the same mixing ratio.

As shown in Figure 6.6, the trend of biodegradable fraction variations in the digesters conforms to the trend of the methane yields obtained by the experimental results for the corresponding digesters. It was verified that addition of manure to SSO as co-substrate increased biodegradability and enhanced methane production in the rectors co-digesting manure and SSO. The reason would be the existence of abundant of methanogenic populations in manure that was introduced to the co-digesters and enhanced degradation of organic matters.

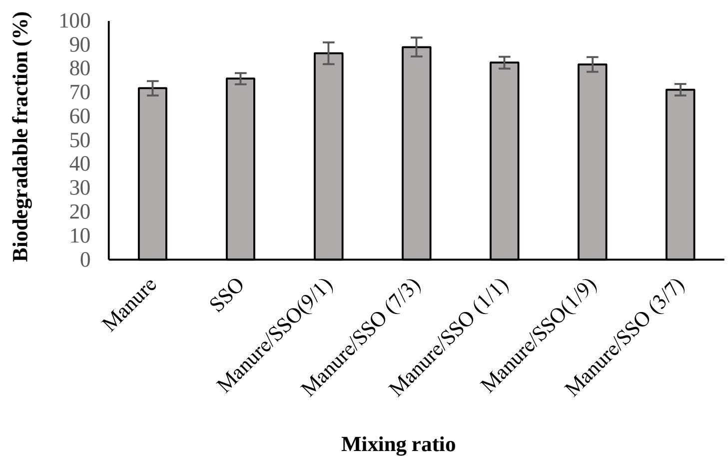


Figure 6.6. Biodegradable fraction of the feedstocks for different mixture ratios of TWAS and manure

6.3. Synergistic effect

Synergistic effect was assessed using Eq. 4.2 as presented in chapter 4 and the result is summarized in Figure 6.7. Synergistic effect represents the percentage of additional methane yield for co-substrates that was measured by the experiment, over the weighted average of the methane yield of individual substrates per unit volume of substrate added. As demonstrated in Fig 6.7, the most synergetic impact corresponds to the co-digestion of manure/SSO at the mixing ratio of 7:3. In co-digestion of manure with SSO, the increase of CH_4 yield due to synergistic effect ranged from 22% to 36 % corresponding to the reactor co-digesting manure and SSO at the mixing ratio of 9:1 and 7:3, respectively. It was revealed that only adding the fraction of manure in co-digesters, did not lead to increasing synergy. Although increasing the fraction of manure would introduce more populations of methanogenic archaea and bacteria, a balance between the microbial populations and nutrient is necessary for the effective microbial growth and enhanced methanogenesis.

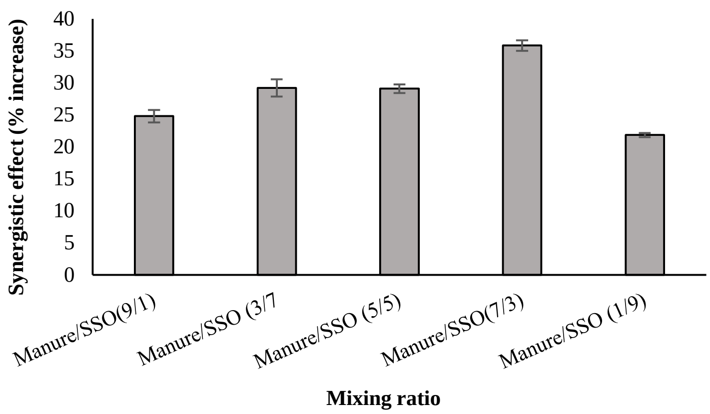


Figure 6.7. Synergetic effect of co-digestion at different mixing ratios of manure and SSO

6.4. COD:N and lipids: proteins: carbohydrates ratios

Table 6.2 presents the COD:N ratios, lipids: proteins: carbohydrates ratios, the ultimate methane production, and the methane yield per unit mass of COD added of the digesters with different mixing ratios. The COD:N ratios were 33 and 42 corresponding to SSO and manure alone, respectively. The values of COD:N varied from 34 to 41 in the co-digesters. The ultimate methane production and methane yield ranged from 919 to 1186 mL, and 287 to 363 mL/g TCOD added for different mixing ratios, respectively.

As shown in Table 6.2 The minimum ultimate methane and methane yield occurred at mono digestion of manure corresponding to the COD:N ratio of 42 and lipids: proteins: carbohydrates ratio of 1:4.2:21. However, the maximum ultimate methane and methane yield occurred at the mixing ratio of 7:3 corresponding to the COD:N ratio of 41 and lipids: proteins: carbohydrates ratio of 1:3.5:18.5. SSO alone with a lipids: proteins: carbohydrates ratio of 1:2:12 produced 15% more ultimate methane than manure alone with the lipids: proteins: carbohydrates ratio of 1:4.2:21. Although, it only resulted in 6% more methane yield per unit mass of COD added than manure. On the other side, the 1:4.2:21 lipids: proteins: carbohydrates ratios for manure alone and 1:4:20 for manure/SSO co-digestion at the mixing ratio of 9:1 had only a minor variation while the ultimate methane and the methane yield were 23% and 20% higher for the latter.

Table 6.2. Ultimate CH_4 and yield at different ratios of the substrates, COD:N, and Lipids:Proteins:Carbohydrates

Digester code	TWAS: Manure (V/V)	COD:N	Feedstock ratio codes	Lipids: Proteins: Carbohydrates	Ultimate CH_4 (mL)	mLCH_4/g TCOD added
Manure	1:0	42	CC	1:4.2:21	919	287
SSO	0:1	33	BB	1:2:12	1063	303
Manure/SSO 7:3	7:3	41	A	1:3.5:18.5	1186	363
Manure/SSO 9:1	9:1	39	B	1:4:20	1129	344
Manure/SSO 5:5	1:1	37	C	1:3:17	1136	330
Manure/SSO 3:7	3:7	35	D	1:2.7:15	1095	327
Manure/SSO 1:9	1:9	34	E	1:2:13	1115	316

Manure alone with lipids:proteins:carbohydrates ratio of 1:4.2:21 corresponded the minimum methane yield while the maximum yield occurred at manure:SSO mixing ratio of 7:3 and lipids:proteins:carbohydrates ratio of 1:3.5:18.5. This increase of the methane would be the result of microbial population diversity introduced by manure to the co-digesters and the synergetic impact of co-digestion rather than the ratio of lipids: proteins: carbohydrates.

Figure 6.8. shows the main effect plot for CH_4 yield data mean in response to feedstock and lipids:proteins:carbohydrates ratios in AnCoD of manure/SSO. As shown in figure 6.8, the different lipids:proteins:carbohydrates ratios for the different mixing ratios of the feedstocks have significant effect on the methane yield.

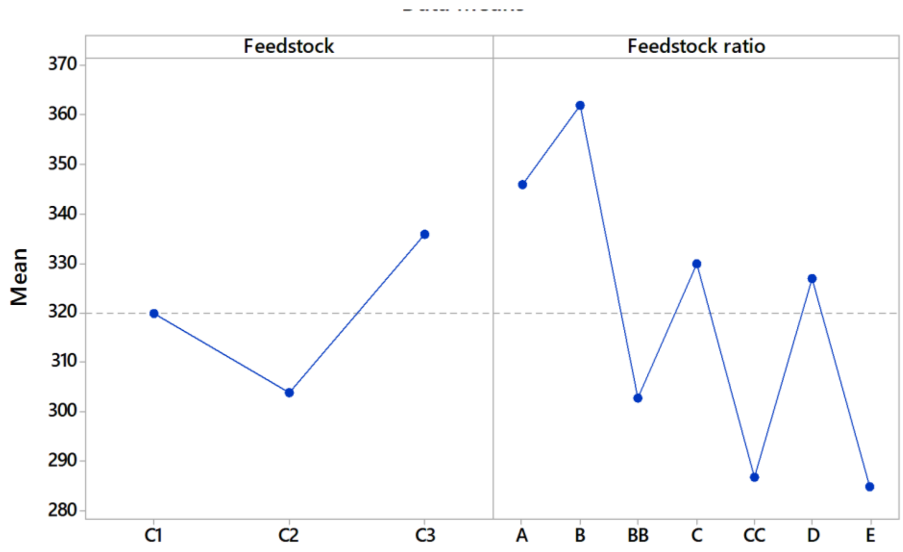
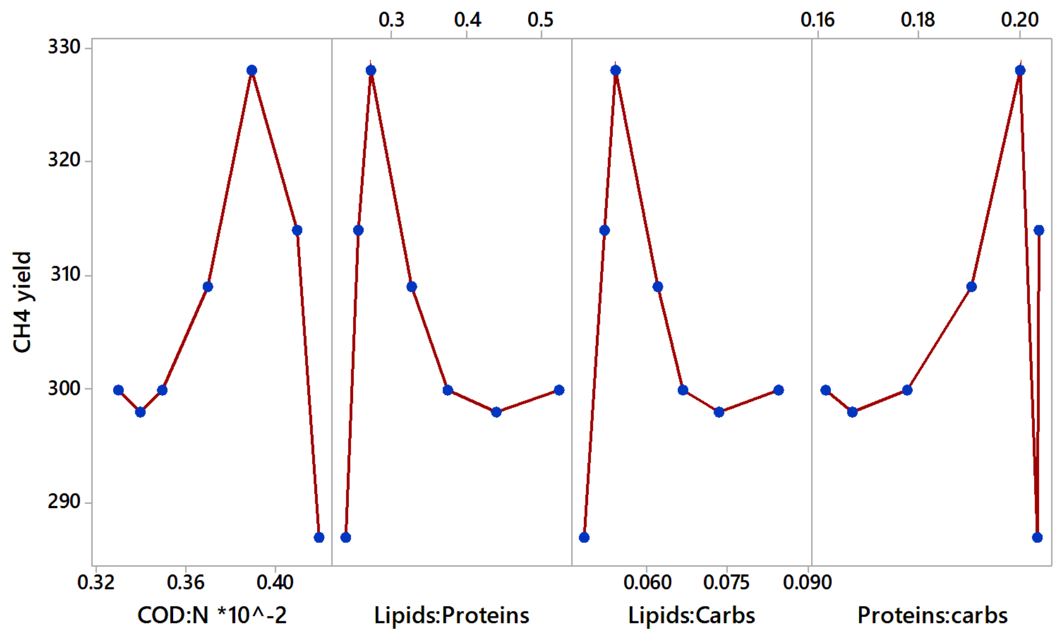
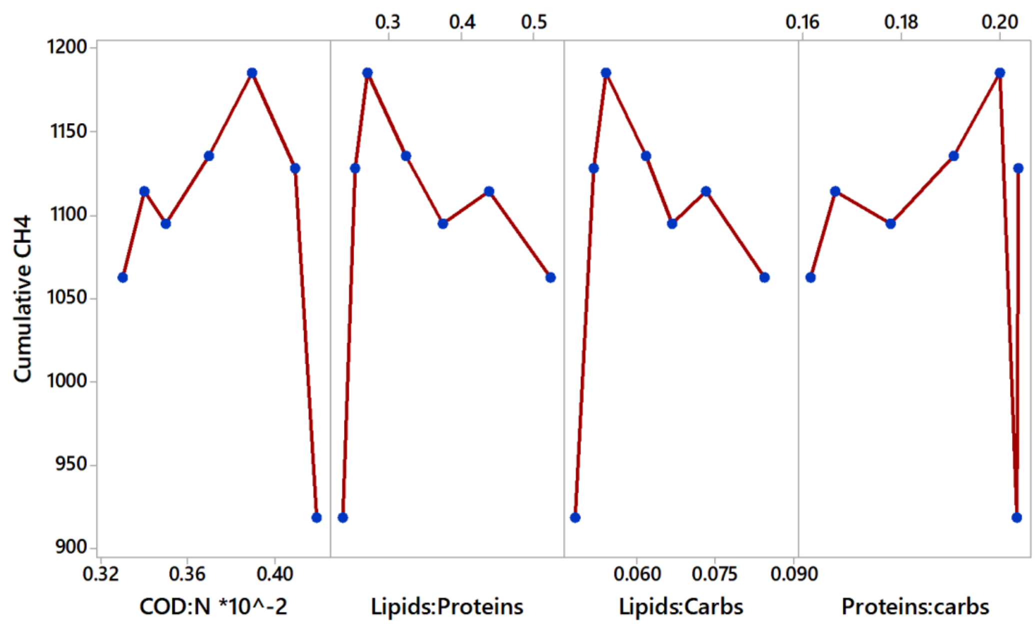


Figure 6.8. Main effect plot for CH_4 yield data mean in response to feedstock and lipids:proteins:carbohydrates ratios in AnCoD of manure/SSO

Figure 6.9 shows the matrix plot for the variations of the methane yield and the ultimate methane versus COD:N ratio, proteins: lipids, carbohydrates: lipids, and carbohydrates: proteins ratios. As illustrated in Figure 6.9. a, the trend of variations of the methane yields versus COD:N and versus proteins: carbohydrates were similar. lipids: proteins and lipids: carbohydrates also showed a similar trend but for both of them the trend was the mirror image of COD:N and proteins: carbohydrates. The ultimate methane as illustrated in Figure 6.9. b also demonstrated a similar response to those ratios. These observations were contrary to the results of TWAS:SSO and TWAS/Manure co-digestion. The reason would be the minor variations of proteins: lipids ratios at the different combinations of manure and SSO. As mentioned earlier, in co-digestion of manure/SSO the methane yield in the co-digesters could more depend on the microbial diversity than the ratio of the lipids: proteins: carbohydrates.



6.9. a



6.9. b

Figure 6.10. Matrix plot for: a. ultimate CH_4 and b. CH_4 yield at different COD:N and Lipids: Proteins, Lipids: Carbohydrates, and Proteins: Carbohydrates Ratios

6.5. Kinetic analysis results

The results of kinetic study by modified Gompertz model using Eq. 3.5 is summarized in Table 6.3. The model was applied to the experimental data from mono and co-digestion of manure and

SSO. The values of P for the maximum methane production per unit substrate (mL/g), R_m^e, the maximum methane production rate (mL/g h), and λ, the lag phase time (d) were calculated and the results values are summarized in Table 6.3. The modified Gompertz model for mono- and co-digestions of manure and SSO, rather showed a good fit to the experimental results with less than 10 % diversion from the measured values. The estimated values and their correlation with the mixing ratio of the feedstock were in good compliance with the data obtained by the experiment. An increasing in P values was observed in co-digestion of manure with SSO which conformed to the experimental results.

Table 6.3. Summary of results of kinetic study using modified Gompertz model

	P (mL)	R_m^e $(\frac{mL}{D})$	**λ (d)**	R^2
Manure	875	48	0.4	0.999
SSO	998	83	1.6	0.999
Manure/SSO 7/3	1112	89	0.9	0.999
Manure/SSO 9/1	1065	57	0.7	0.999
Manure/SSO 5/5	1060	66	0.9	0.999
Manure/SSO 1/9	1018	76	1.1	0.999
Manure/SSO 3/7	936	81	0.8	0.999

The trend of P variations complied with the experimental data as it showed the same trend in response to the corresponding mixing ratios. For instance, the highest P value of 1112 mL corresponded to the mixing ratio of 7:3. The lag phase varied from 0.01 to 0.7 days for different substrate mixing ratios. The lag phase time was quite short and less than 1 day for all the digesters. A short lag phase is advantageous as it does not demand for a long residence time and therefore, it does not require a large reactor volume which reduces the operational costs of the system. The values of R_{max}^e ranged from 48 to 89 mL/d corresponding to manure mono digestion and manure/SSO mixing ratio of 7:3, respectively.

6. 6. Hydrolysis/acidification

This experiment was carried out for evaluating the hydrolysis/acidification phase in anaerobic co-digestion of manure and SSO. A series of analysis such as degree of solublization, synergetic effect of co-digestion at different mixing ratios on hydrolysis and liquefaction, volatile fatty acids (VFAs) yield, and hydrolysis kinetics of lipids, proteins, and carbohydrates in co-digestion of manure and SSO was carried out and the results are summarized below.

Initially, characterization of the feedstocks in triplicates was conducted and the mean values are presented in Table 6.4. As shown in Table 6.4, both manure and SSO have high amount of COD concentration which is above 100 g/L. Therefore, COD concentrations of the feed for all of the reactors containing the combinations of manure and SSO were above 100 g/L. Manure contains high amount of carbohydrates and proteins. Adding manure to SSO increased carbohydrates and proteins concentrations in the co-digesters compared to the reactor digesting SSO alone. In order to obtain the hydrolysis rate coefficients of COD, lipids, proteins, and manure, the concentrations of total, soluble and particulate COD; total, soluble and particulate proteins; and total, soluble and particulate carbohydrates were monitored over time.

The data form total and soluble COD (SCOD) concentrations monitoring in all the digesters over time during a 72-h hydrolysis/acidification period was used to calculate the degree of COD solublization for each digester. As explained earlier, the degree of solublization was calculated using Eq. 3.1 and the result is summarized in Figure 6.10.

As illustrated in figure 6.10, the degree of the COD solublization ranged from 24% to 35%. The most solublization of the COD content was 35% which corresponded to manure:SSO combination of 3:7 and 1:9. The COD solublization in mono digestion of manure and SSO were 24% and 28%, respectively. The reactors containing only manure had the lowest degree of solublization. Manure resulted in a relatively lower solublization than SSO which could be due to the presence of some recalcitrant contents such as fibers and cellulosic compounds in manure that delay its hydrolysis and liquefaction.

Table 6.4. Characteristics of the feedstocks at different mixing ratios of manure and SSO

		Manure	SSO	M*/SSO 9/1	M/SSO 7/3	M/SSO 5/5	M/SSO 3/7	M/SSO 1/9
Parameters	Units	Mixture (1) Ave.	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)
TCOD	g/L	101	115	103	105	108	111	114
SCOD	g/L	10	43	14	20	27	33	40
TSS	g/L	55	47	54	52	51	49	48
VSS	g/L	32.1	39.9	32.9	34.5	36.0	37.6	39.2
TS	g/L	69	78	69	71	73	75	77
VS	g/L	59	64	59	60	61	62	63
Ammonia	g/L	0.02	1.40	0.16	0.4	0.7	1.0	1.3
pH		5.6	5.8	5.9	5.7	5.7	5.6	5.8
Alkalinity	g CaCO3/L	7.2	6.7	7.2	7.1	7.0	6.9	6.8
TN	g/L	1.7	4.2	2.0	2.5	3.0	3.5	4.0
TSN	g/L	0.1	1.1	0.2	0.4	0.6	0.8	1.0
T-Carbs	g/L	26.8	14	25.5	23.0	20.5	17.9	15.4
T-Proteins	g/L	5.4	2	5.1	4.4	3.8	3.1	2.5
T-Lipids	g/L	1.7	1.5	1.7	1.6	1.6	1.5	1.5

* M: Manure

It was observed that co-digestion increased solublization as all of the reactors containing the mixings of manure and SSO achieved a higher degree of solublization than the control reactors.
A 46% and 25% improvement in solublization was achieved in the co-digestion of manure and SSO in comparison with the control reactors digesting only manure and only SSO. Although SSO demonstrated more solublization than manure, no correlation between the portion of SSO and the degree of solublization at different mixing ratio was observed. This could verify that a proper mixing ratio is requires to enhance the microbial synergy for the process improvement.
The synergistic effect on the solublization of the feedstocks was evaluated by comparing the theoretical degree of solublization of each co-digester to the measured data from the experiment. The results indicated that co-digestion improved solublization by improving microbial synergy. As shown in Figure 6.11, all co-digesters achieved an improvement in solublization due to the synergistic effect of the microbial communities. The synergistic effect varied from 18 to 34% corresponding to manure/SSO mixing ratios of 5:5 and 9:1, respectively.

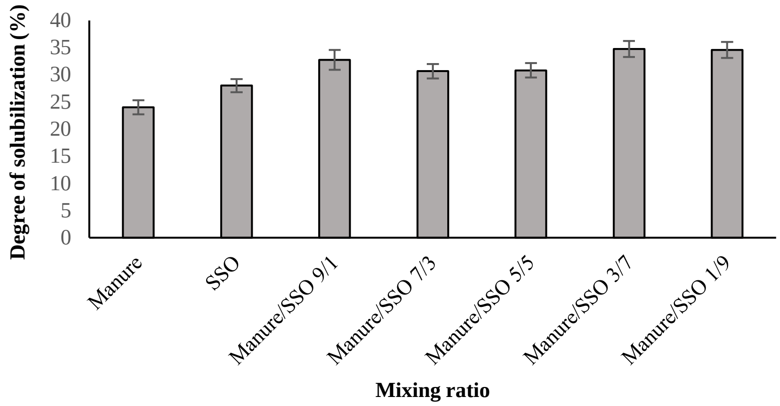


Figure 6.10. Degree of COD solublization at different mixing ratios of Manure/SSO

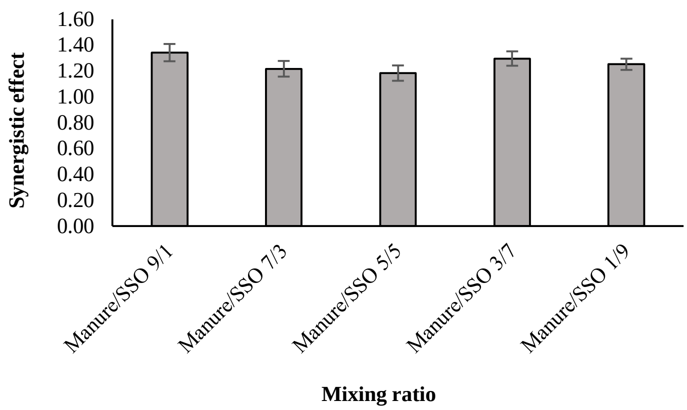


Figure 6.11. Synergistic effect on solublization at different mixing ratios of Manure/SSO

The monitoring of VFAs concentrations over time showed an increasing trend during the 72-hr of the hydrolysis/acidification process. The total VFAs yields were calculated in terms of mass of produced VFAs per mass of VSS added (mg VFAs/g VSS added). As illustrated in Figure 6.12, SSO alone had more VFAs yields than manure alone. The VFAs yield in the co-digester was correlated to the mixing ratios of the feedstocks so that the VFAs yield increased by increasing the fraction of SSO in the co-digesters. The trend of VFAs yield did not comply with the trend of COD solublization of the corresponding mixing ratios (Figure 6.10). Hence, hydrolysis/liquefaction occurred independently of acidification and followed a different trend from acidification. All of the reactors containing the mix of manure and SSO had higher VFAs yield than the control reactors. The VFAs yields were 231 mg VFAs/g VSS added and 325 mg VFAs/g VSS added for the manure and SSO mono digestions, respectively. The maximum VFAs yield of 400 mg VFAs/g VSS added was achieved by manure/SSO ratio of 1:9.

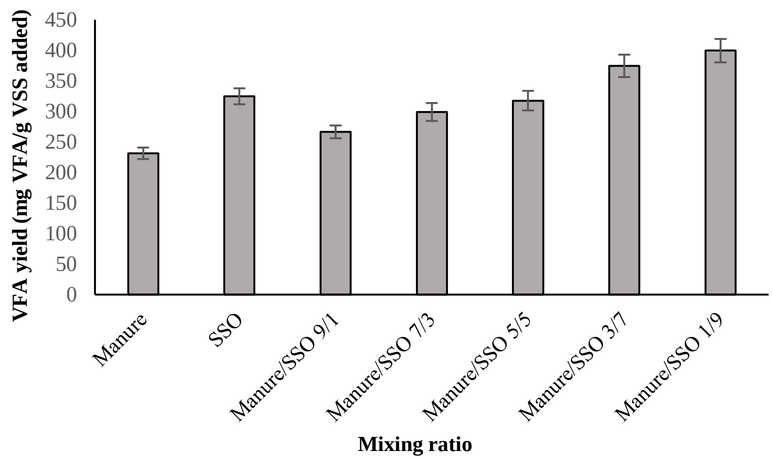


Figure 6.12. Total VFAs yield at different mixing ratios of Manure/SSO

Monitoring soluble and particulate COD over time, indicated an increasing trend in solublization of COD and particulate COD degradation. Furthermore, the monitoring of soluble and particulate lipids, proteins, and carbohydrates over time also demonstrated an increasing trend in solublization and particulate matter degradation. Similar to the previous experiments, in codigestion of manure and SSO, the hydrolysis and solublization rates of the lipids and proteins, were slower than carbohydrate. These results are summarized in the tables presented in the appendix. The hydrolysis rate coefficient (K_h) based on first order kinetics (equations 3.3 and 3.4) was calculated using AquaSim 2.0 for COD, lipids, proteins and carbohydrates based on the particulate degradation and results are summarized in Table 6.5.

In manure and SSO co-digestion, hydrolysis did not show the same trend as methanogenesis. The hydrolysis kinetics showed a higher rate in the manure:SSO mixing ratio of 3:7 while the most methane yield occurred at the mixing ratio of 7:3 with more fraction of manure. This would be due to the more microbial population of methanogens which was introduced by more fraction of manure in the co-digester. Similar to the co-digestion of TWAS with SSO and TWAS with manure, the improvement of the hydrolysis in co-digestion of manure with SSO resulted from the improvement of proteins and carbohydrates hydrolysis than that of lipids contents of the feedstocks. Unlike TWAS/manure and TWAS/SSO co-digestion, the fluctuations of hydrolysis rate coefficient of lipids and carbohydrates at different feedstocks mixing ratios showed similar trend in manure/SSO co-digestion. Nevertheless, in co-digestion of manure with SSO the variations of K_h for proteins demonstrated a different trend from that of lipids and carbohydrates. The maximum hydrolysis rate of proteins corresponded to the manure:SSO mixing ratio of 1:9. However, the maximum K_h values for lipids and carbohydrates were 0.11 and 0.68 corresponding to the mixing ratio of 3:7.

Table 6.5. Hydrolysis rate coefficients for COD, lipids, proteins, and carbohydrate content in co-digestion of SSO and manure at different mixing ratios

K_h	M*/SSO 1/9	M/SSO 3/7	M/SSO 5/5	M/SSO 7/3	M/SSO 9/1	Manure	SSO
$K_{h\ COD}$	0.37	0.38	0.31	0.32	0.35	0.23	0.30
$K_{h\ Lipids}$	0.09	0.11	0.08	0.07	0.08	0.07	0.08
$K_{h\ Proteins}$	0.35	0.33	0.25	0.29	0.32	0.21	0.28
$K_{h\ Carbohydrates}$	0.63	0.68	0.57	0.55	0.61	0.43	0.55

* M: Manure

Chapter 7

Results and discussion

TWAS, Manure, SSO Co-digestion

7. Results and discussion- TWAS, manure, SSO co-digestion

7.1. BMP of TWAS, manure, SSO

This experiment was conducted on ternary co-digestion of TWAS, manure, and SSO. Manure slurry was prepared as discussed in chapter 3 and was fed to the reactors in different combinations with SSO and TWAS in triplicates. Control reactors containing TWAS, manure, and SSO individually were also used in triplicates. The influence of feedstocks mixing ratios and their correlation with the lipids: proteins: carbohydrates ratios on biomethane production in anaerobic co-digestion of TWAS/manure/SSO was evaluated. The characteristics of the feed in each digester with different mixing ratios of the substrates for the average of three measurement of each parameter are summarized in Table 7.1.

As presented in Table 7.1, both manure and SSO have high amount of COD concentrations compared to TWAS. The COD concentrations of the feedstocks were 100, 109, and 101 g/L for the digesters fed with manure, SSO and mixture of TWAS/manure/SSO at 1:1:8 ratio, respectively. Other reactors which contained the combinations of TWAS, manure, and SSO had a COD concentration ranging from 53 to 95 g/L. VSS concentrations were 26.5, 45.4, and 47.0 for TWAS, manure, and SSO respectively and ranged from 30.4 to 44.8 for the reactors containing the combinations of them. The amount of carbohydrates and lipids of manure and SSO were significantly more than that of TWAS. Therefore, addition of manure and SSO to TWAS increased the carbohydrates and lipids content of the co-digesters compared to the rectors digesting only TWAS.

Carbohydrates concentrations were within a range between 5.4 to 23.8 g/L in the co-digesters. Total proteins concentrations of TWAS, manure, and SSO were 3.9, 5.8, and 2.4 respectively and varied from 2.9 to 4.5 g/L in the co-digesters. The total lipids concentrations of TWAS was 0.4 g/L while manure and SSO had lipids concentrations of 1.4 and 1.5 g/L respectively. Adding manure and SSO increased the lipids content in the reactors containing the combination of TWAS, manure, and SSO compared to the reactors containing only TWAS. The total lipids concentrations varied from 0.6 to 1.3 g/L in the co-digesters containing the mix of the three feedstocks. pH was around neutral point varying from 7 to 7.2 for all of the reactors.

Table 7.1. Characteristics of feed to digesters with different mixing ratios of TWAS/ manure/SSO

		TWAS	Manure	SSO	T*:M**:SSO 8:1:1	T:M:SSO 1:8:1	T/M/SSO 1:1:8	T/M/SSO 5:2.5:2.5	T/M/SSO 2.5:5:2.5	T/M/SSO 2.5:2.5:5	T/M/SSO 4:4:2	T/M/SSO 2:4:4	T/M/SSO 4:2:4
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)	Mixture (8)	Mixture (9)	Mixture (10)	Mixture (11)	Mixture (12)
TCOD	g/L	40	100	109	53	95	101	72	87	89	78	71	79
SCOD	g/L	1.4	42	41	9	38	37	21	32	31	26	25	25
TSS	g/L	31	52	62	37	51	58	44	50	52	46	42	48
VSS	g/L	26.5	45.4	47.0	30.4	43.7	44.8	36.3	41.1	41.5	38.2	33.2	38.5
TS	g/L	38.9	67.8	67.0	44.6	64.8	64.3	53.2	60.4	60.2	56.1	48.1	55.9
VS	g/L	35.2	55.6	49.6	38.6	52.9	48.7	43.9	49.0	47.5	46.2	38.0	45.0
Ammonia	g/L	0.2	0.0	1.3	0.3	0.2	1.1	0.4	0.4	0.7	0.4	0.6	0.6
pH	-	7.0	7.0	7.2	7.1	7.0	7.0	7.1	7.1	7.1	7.0	7.0	7.1
Alkalinity	g $CaCO_3$/L	1.9	5.2	6.2	2.7	5.0	5.7	3.8	4.6	4.9	4.1	3.9	4.3
TN	g/L	2.8	2.1	4.0	2.8	2.4	3.7	2.9	2.7	3.2	2.7	2.6	3.1
TSN	g/L	0.4	0.1	1.0	0.4	0.2	0.8	0.5	0.4	0.6	0.4	0.5	0.6
Total carbs	g/L	1.5	27.8	14.1	5.4	23.8	14.2	11.3	17.8	14.4	14.6	11.5	11.8
Total proteins	g/L	3.9	5.8	2.4	3.9	5.2	2.9	4.0	4.5	3.6	4.3	2.9	3.7
Total lipids	g/L	0.4	1.4	1.5	0.6	1.3	1.4	0.9	1.2	1.2	1.0	0.9	1.0

* T: TWAS

** M: Manure

Operation of the digesters carried on until no significant amount of biogas was produced. Fig. 7.1 shows the profile of the cumulative biomethane production versus time during the co-digestion of TWAS, manure and SSO including the control rectors. SSO alone produced more cumulative methane than TWAS in the control reactors. The amount of methane produced by SSO alone was 13% more methane than manure alone.

All co-digesters produced more biomethane than the control reactors containing only TWAS. The amounts of ultimate CH_4 obtained by single digestion of SSO and manure were higher than that of TWAS alone by 3.2 and 2.9 fold. As illustrated in figure 7.1, all of the co-digesters generated higher amounts of CH_4 than the control reactors containing only manure. The reactors with the mix of the three feedstocks at the ratios of 8:1:1 and 5:2.5:2.5 produced more methane than TWAS alone however, they did not show any improvement in comparison with single digestion of manure and SSO. Other combinations produced more methane than TWAS and SSO alone, although only the three of them with the mixing ratios of 2.5:2.5:5, 4:2:2, and 4:2:4 resulted in higher cumulative CH_4 production comparing to all of the control reactors. The maximum cumulative methane production of 1424 mL corresponded to the TWAS/manure/SSO mixing ratio of 2:4:4 and lipids: proteins: carbohydrates ratio of 1:3:12.

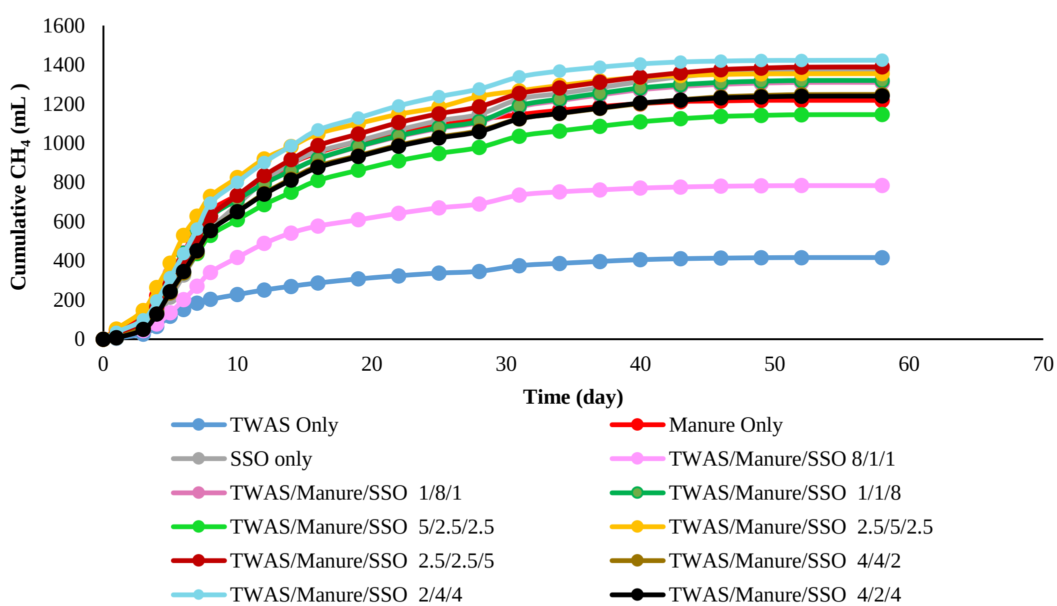


Figure 7.1. Cumulative methane production for different mixing ratios of TWAS/manure/SSO

Figure 7.2, illustrates the methane rate in mL/day for all of the feedstocks combinations including the control reactors. The lowest maximum CH_4 rate corresponded to TWAS mono digestion. Among control reactors, manure had the highest maximum CH_4 production rate. All of the digesters, reached their maximum methane rate in less than 10 days of the process operation. The digesters containing the mixings of TWAS/manure/SSO at the ratio of 1:8:1 achieved the highest maximum methane production rate at the first week of the operational period. The digesters produced 32% to 47% of their ultimate CH_4 production during the first week and 65% to 73% of it during the two weeks of the digestion period.

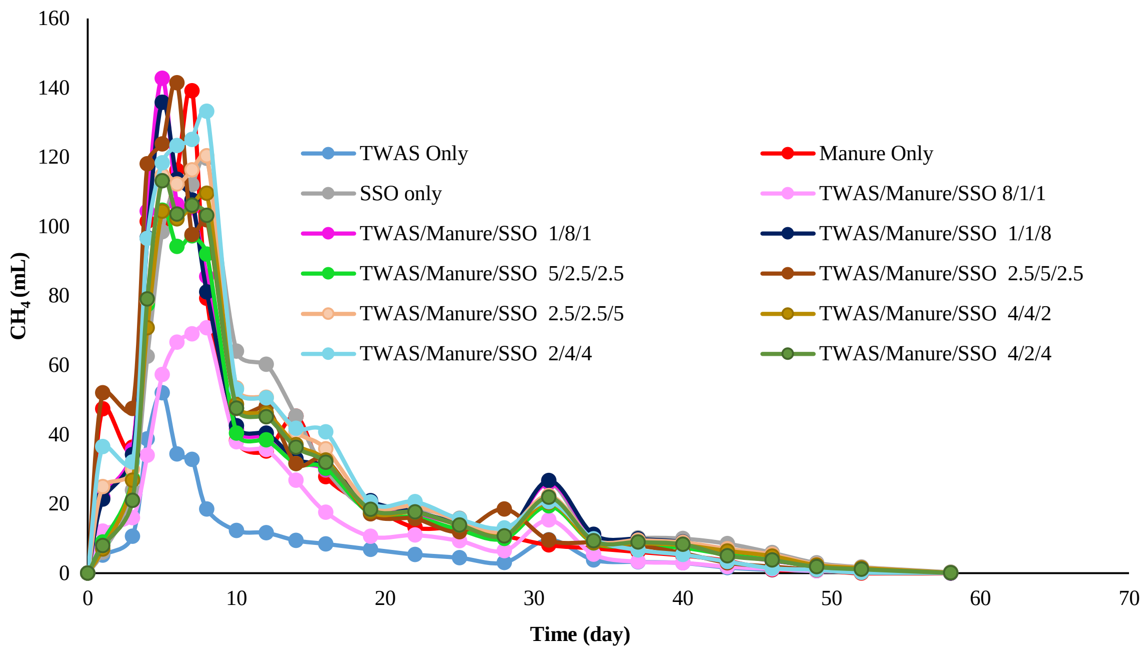


Figure 7.2. Methane production rate (mL/d) for different mixing ratios of TWAS/Manure/SSO

The maximum CH_4 production rates at different mixing ratios including the controls are shown in Figure 7.3. The lowest maximum rate of 52 mL/day corresponded to single digestion of TWAS. The maximum CH_4 rates of manure and SSO alone were 139 mL/day and 120 mL/day, respectively. The maximum CH_4 rates of the co-digesters including the mix of the three feedstocks varied from 71 to 143 mL/day. As presented in the figure, the highest maximum rate of 143 mL/day corresponded to the TWAS/manure/SSO mixing ratio of 1:8:1.

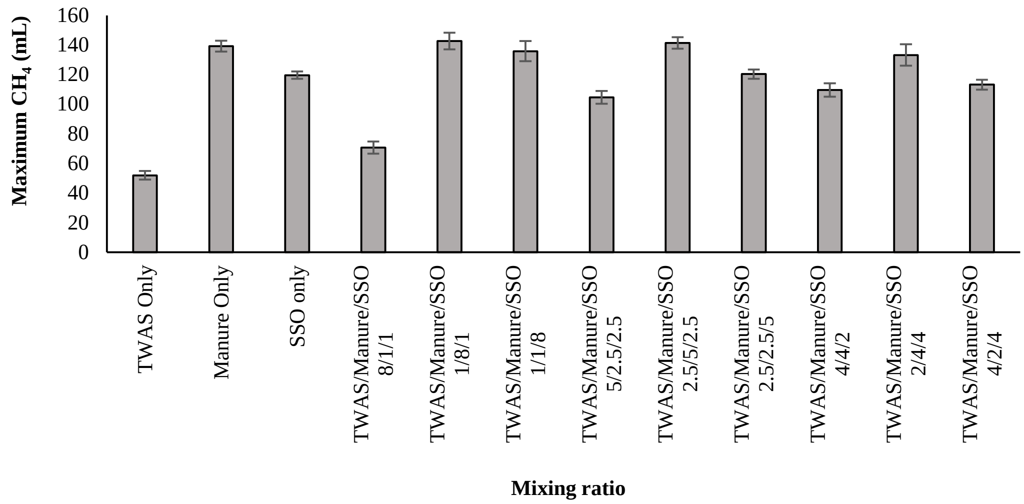


Figure 7.3. Maximum methane production rate (mL/d) for different mixing ratios TWAS/manure/SSO

The COD mass balance was conducted for all of the digesters and the result is summarized in Figure 7.4. The mass balance was calculated considering the initial and the final TCOD concentrations of the reactors' contents, and the theoretical methane production per unit mass of TCOD removed. Comparing the experimental methane production data with the ones obtained by the calculations, showed a deviation of less than 5% for all of the digesters.

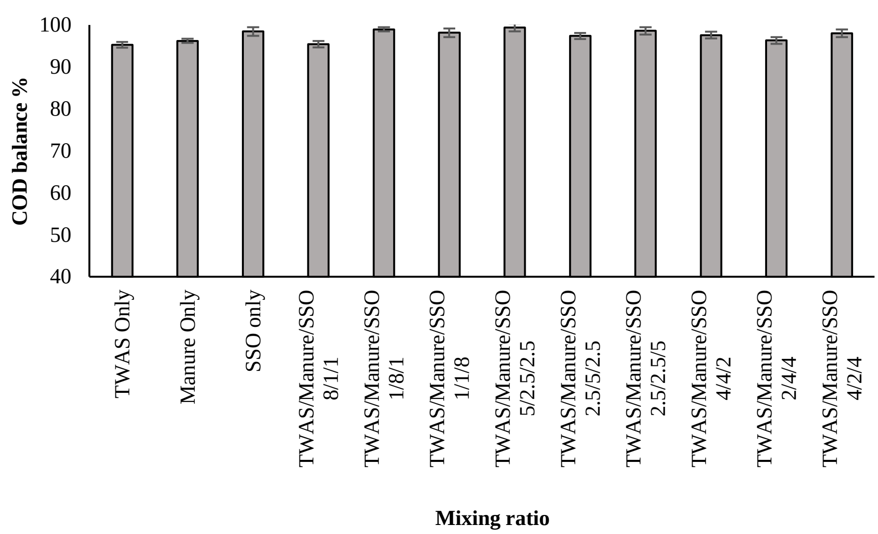


Figure 7.4. COD mass balance in co-digestion of manure and SSO for different mixing ratios 4

7.2. Cumulative methane yields

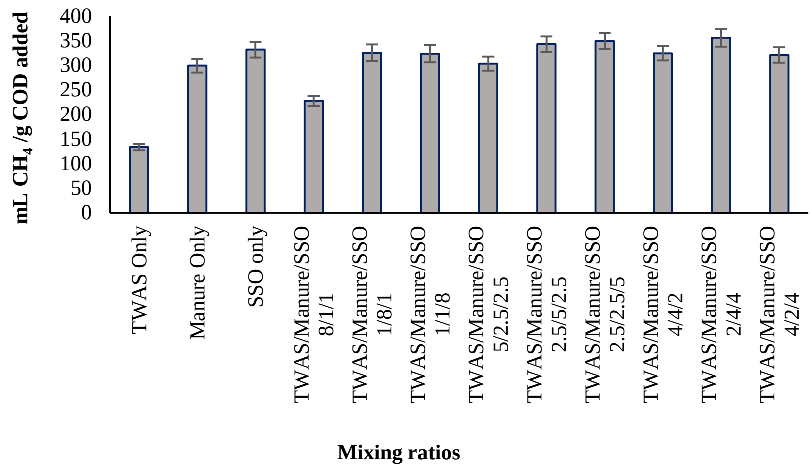
Figure 7.5 shows the cumulative methane yields of the digesters including $mLCH_4/g$ TCOD added, $mLCH_4/g$ VSS added, and $mLCH_4/mL$ substrate added. The cumulative methane yield per mass COD of substrate added were 134, 299, and 332 $mLCH_4/g$ TCOD added for the control reactors digesting only TWAS, manure, and SSO, respectively. As shown in Figure 7.5. a), the minimum and the maximum yield corresponded to mono digestion of TWAS and TWAS/manure/SSO mixing ratio of 2:4:4, respectively. The biomethane yields ranged from 228 to 356 $mLCH_4/g$ TCOD added in the co-digesters. CH_4 yield increased by 165%, 19% and 7% compared to single digestion of TWAS, manure, and SSO, respectively.

The methane yields in $mLCH_4/g$ VSS added are presented in Figure 7.5. b). The CH_4 yields of 202, 659, and 766 $mLCH_4/g$ VSS added corresponded TWAS, manure, and SSO for the control reactors, respectively. The CH_4 yield varied from 395 to 982 $mLCH_4/g$ VSS added in the reactors containing the combinations of the three feedstocks. The maximum CH_4 yield of 395 $mLCH_4/g$ VSS added was achieved by the co-digestion of TWAS/manure/SSO at the mixing ratio of 2:4:4.

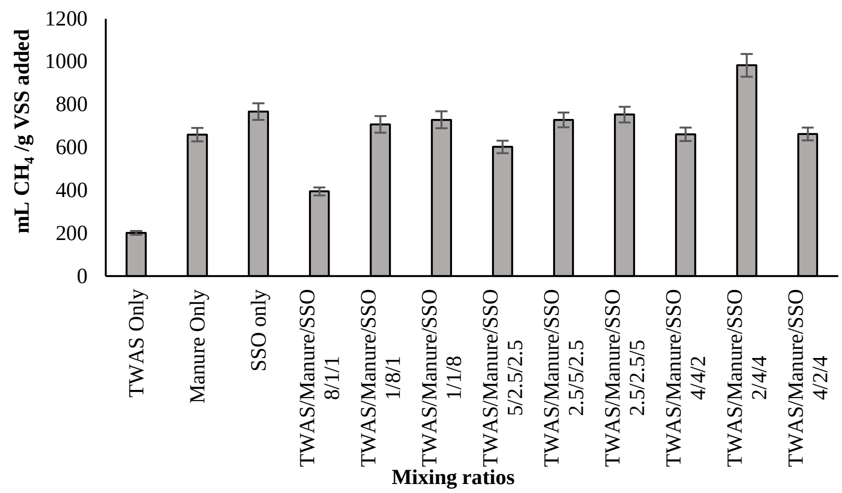
Figure 7.5. c), illustrates the methane yield per unit volume of substrate added. TWAS, Manure and SSO individually resulted in 5.3, 30 and 36 $mLCH_4/mL$ substrate added, respectively. The reactors with the combinations of manure and SSO resulted in methane yields ranging from 12 $mLCH_4/mL$ substrate added to 32.6 $mLCH_4/mL$ substrate added. The most CH_4 yield per unit volume of substrate added corresponded to single digestion of SSO and TWAS/manure/SSO co-digestion at the mixing ratios of 2:4:4.

Biodegradable fraction as mentioned earlier was calculated using the Eq. 4.1 and the result is summarized in Figure 7.6. The biodegradable fraction of the digesters ranged from 33% to 89%. TWAS, manure and SSO alone, corresponded to biodegradable fractions of 33%, 75 % and 83%, respectively. The most percentage of biodegradable fraction occurred at the reactor co-digesting TWAS, manure and SSO at the mixing ratio of 2:4:4. This in good compliance with the maximum methane production that occurred at the same mixing ratio. The trend of variation in the methane yields also conforms to the trend that was observed for the variations of biodegradable fractions at the different mixing ratios. All of the co-digesters demonstrated a more biodegradable fraction than TWAS alone, nevertheless not all of the combinations resulted in more biodegradable fraction than manure and SSO alone.

7.5. a)



7.5. b)



7.5. c)

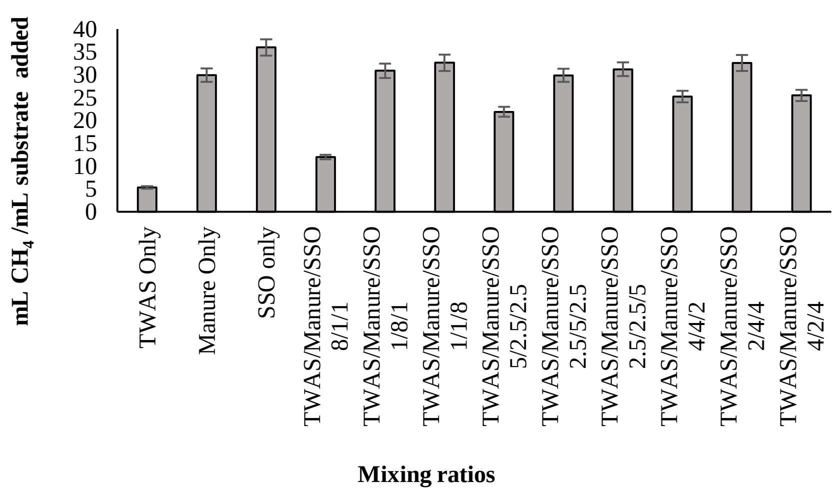


Figure 7.5. Methane yields a) per unit mass TCOD added, b) per unit mass of VSS added and c) per volume substrate added at different mixing ratio of TWAS and manure

The reason would be that the balance of the nutrient with the microbial communities was not necessarily ideal for methanogenic populations in the reactors containing the mix of the three feedstocks at all of the mixing ratios.

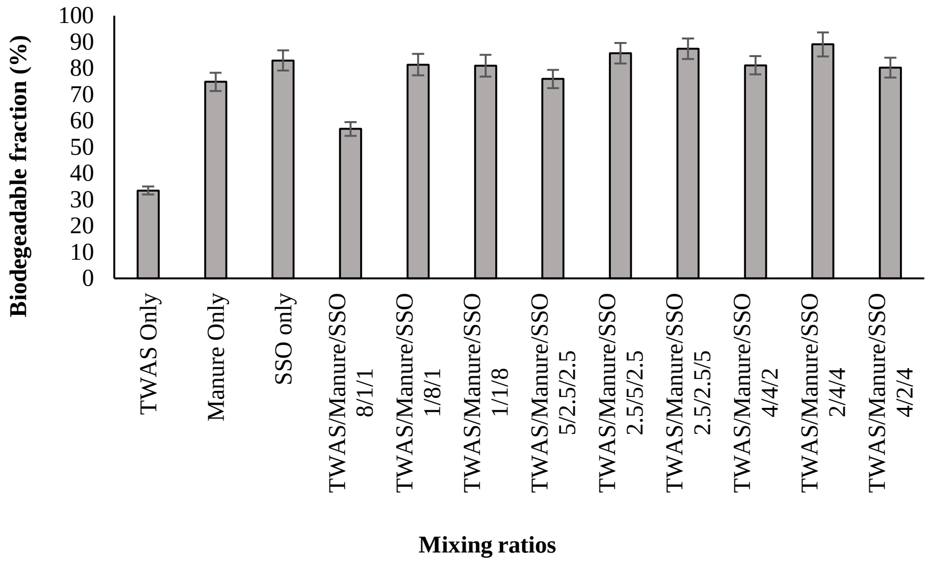


Figure 6.6. Biodegradable fraction of the feedstocks for different mixture ratios of TWAS and manure

7.3. Synergistic effect

Synergistic effect was obtained by calculating the percentage of additional methane yield achieved in co-digestion. This was done by dividing the measured yields, over the weighted average of the methane yield of individual substrates per unit volume of substrate added. Figure 7.7 shows the percentage improvement of biomethane production due to the synergistic impact. As demonstrated in Fig 7.7, in the ternary co-digestion of TWAS/manure/SSO, the most synergetic impact corresponds to the mixing ratio of 2:4:4. No significant improvement due to synergy was observed at the mixing ratio of 1:1:8. The maximum synergistic effect that was achieved by this experiment was 19 % which was slightly lower than the result obtained by some of digesters in the binary co-digestion.

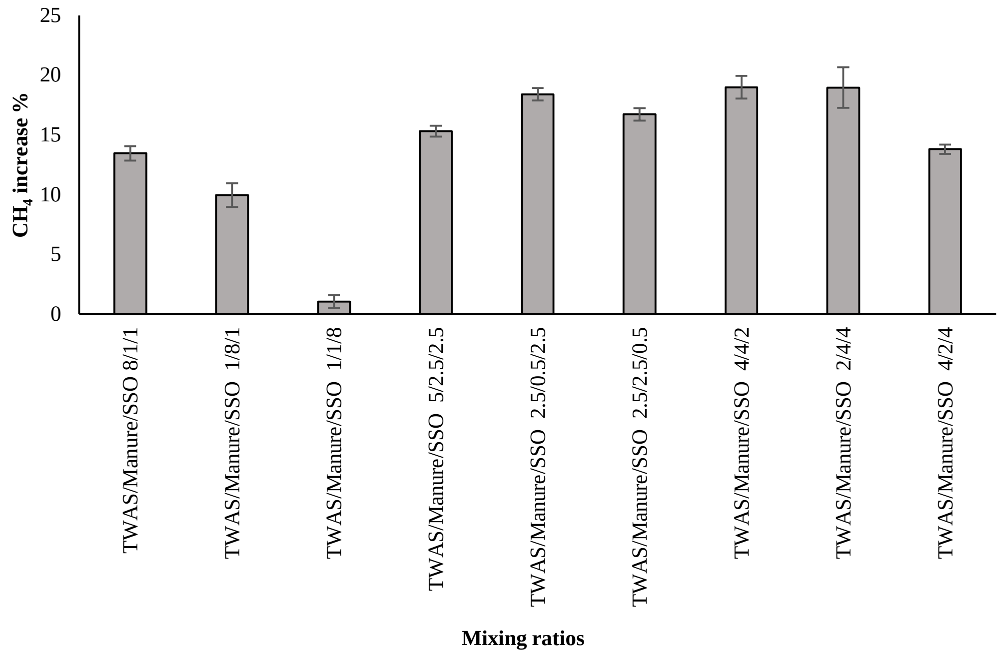


Figure 7.7. Synergetic effect of co-digestion at different mixing ratios of manure and SSO

7.4. COD:N and Lipids: Proteins: Carbohydrates ratios

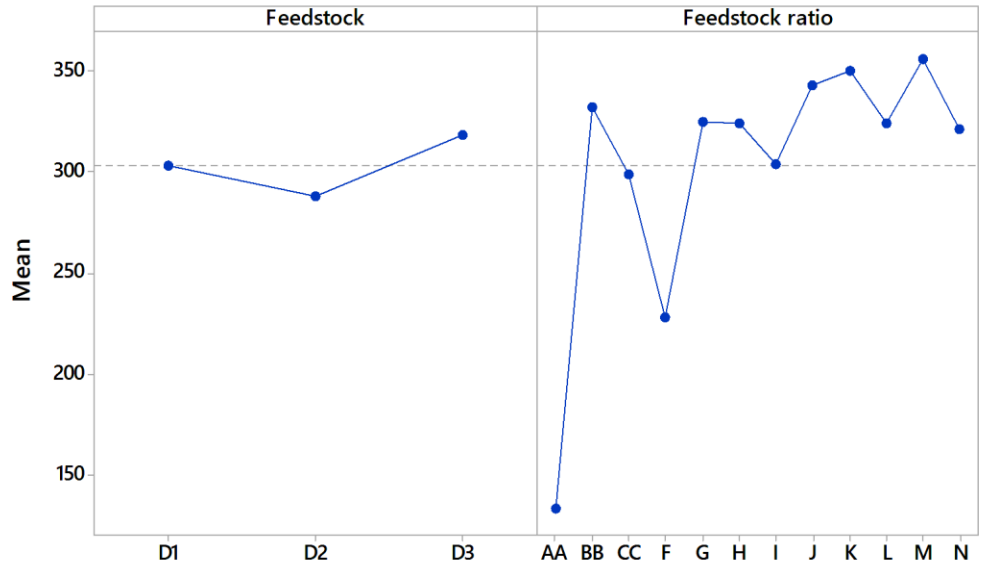
The values of COD:N ratios, lipids: proteins: carbohydrates ratios, the ultimate methane production and the methane yield per unit mass of COD added at different mixing ratios are presented in Table 7.2. The COD:N ratios of TWAS, manure and SSO were 15, 47, and 27 respectively. For the co-digesters, COD:N varied from 19 to 40. The lipids: proteins: carbohydrates ratios were 1:10:4, 1:4:20, and 1:1.6:9 for TWAS, manure, and SSO respectively. Among them SSO had the most ultimate methane production and methane yield corresponding to 1373 mL and 332 mL CH_4/g COD added. As shown in Table 7.2, the minimum ultimate methane production and the methane yield occurred at TWAS mono digestion corresponding to the COD:N ratio of 15 and lipids: proteins: carbohydrates ratio of 1:10:4. On the other side, the maximum ultimate methane production and yields occurred at the mixing ratios of 2:4:4 corresponding to the COD:N ratio of 28 and lipids: proteins: carbohydrates ratio of 1:3:12.

Table 7.2. Ultimate CH_4 and yield at different ratios of the substrates, COD:N, and lipids:proteins:carbohydrates

Digester code	TWAS: Manure: SSO (V/V)	COD:N	Feedstock ratio codes	Lipids: Proteins: Carbohydrates	Ultimate CH_4 (mL)	mLCH_4/g TCOD added
TWAS Only	1:0:0	15	AA	1:10:4	417	134
Manure Only	0:1:0	47	CC	1:4:20	1218	299
SSO only	0:0:1	27	BB	1:1.6:9	1373	332
T/M/SSO 8/1/1	8:1:1	19	F	1:6.5:9	784	228
T/M/SSO 1/8/1	1:8:1	40	G	1:4:19	1311	325
T/M/SSO 1/1/8	1:1:8	28	H	1:2:10	1320	324
T/M/SSO 5/2.5/2.5	5:2.5:2.5	25	I	1:4:12	1146	304
T/M/SSO 2.5/5/2.5	2.5:5:2.5	32	J	1:3.8:15.5	1355	343
T/M/SSO 2.5/2.5/5	2.5:2.5:5	28	K	1:3:12	1390	350
T/M/SSO 4/4/2	4:4:2	28	L	1:4:15	1248	324
T/M/SSO 2/4/4	2:4:4	28	M	1:3:12	1424	356
T/M/SSO 4/2/4	4:2:4	26	N	1:3.7:11.8	1241	321

Figure 7.8. illustrates the main effect plot for CH_4 yield data mean in response to feedstock and lipids:proteins:carbohydrates ratios in AnCoD of TWAS/manure/SSO. As shown in Figure 7.8, feedstocks and lipids:proteins:carbohydrates ratios have significant effect on CH_4 yield. TWAS only with lipids:proteins:carbohydrates ratio of 1:10:4 has the minimum methane yield. TWAS/manure/SSO co-digestion at the mixing ratio of 2:4:4 and lipids:proteins:carbohydrates ratio of 1:3:12 corresponded to the maximum methane yield.

The matrix plot in Figure 7.9 shows the relationship of COD:N, proteins: lipids, carbohydrates: lipids, and carbohydrates: proteins ratios with the ultimate methane production (mL) and with the yield (mg CH_4/g TCOD added). The responses of the ultimate methane production and the methane yield to the different ratios is illustrated in figures 7.9. a) and 7.9. b), respectively.



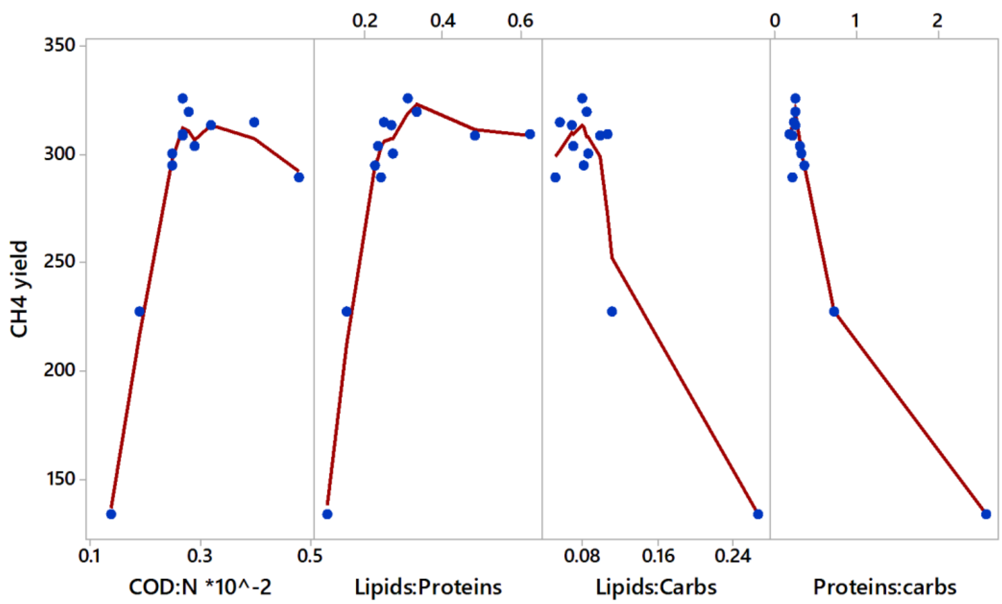
Figure

Figure 7.8. Main effect plot for CH_4 yield data mean in response to feedstock and lipids:proteins:carbohydrates ratio in AnCoD of TWAS/manure/SSO

As shown in Figures 7.9. a) and 7.9. b) the trends of variations of methane versus COD:N, and lipids: proteins were similar. However, lipids: carbohydrates and proteins: carbohydrates did not conform to COD:N ratio. As illustrated in the Figures 7.8. a), the minimum ultimate methane production corresponds to the minimum COD:N ratio and the minimum lipids: proteins ratio while it corresponded to the maximum lipids: carbohydrates and maximum protein: carbohydrates ratios. The trend of the ultimate methane production variations in response to the COD:N and to the lipids: proteins ratios relatively conform to each other excluding some of the ratios. The ultimate methane production was higher at the COD:N ratios between 26 and 47 and at the lipids: proteins ratios between 0.23 and 0.62. On the contrary, the increase of the lipids: carbohydrates and proteins: carbohydrates ratios, reduced the ultimate methane production. The most ultimate methane production occurred at the minimum lipids: carbohydrates and proteins: carbohydrates ratios of 0.05 and 0.17, respectively. In contrast the minimum ultimate methane production corresponded to the maximum lipids: carbohydrates and proteins: carbohydrates ratios of 0.26 and 2.55, respectively. The lipids: carbohydrates ratios above 0.1 and proteins: carbohydrates ratios above

0.3 resulted in significant decrease in the ultimate methane production. Similar trend was observed for the methane yield in response to the COD:N, lipids: proteins and lipids: carbohydrates, and proteins: carbohydrates ratios.

7.9. a)



7.9. b)

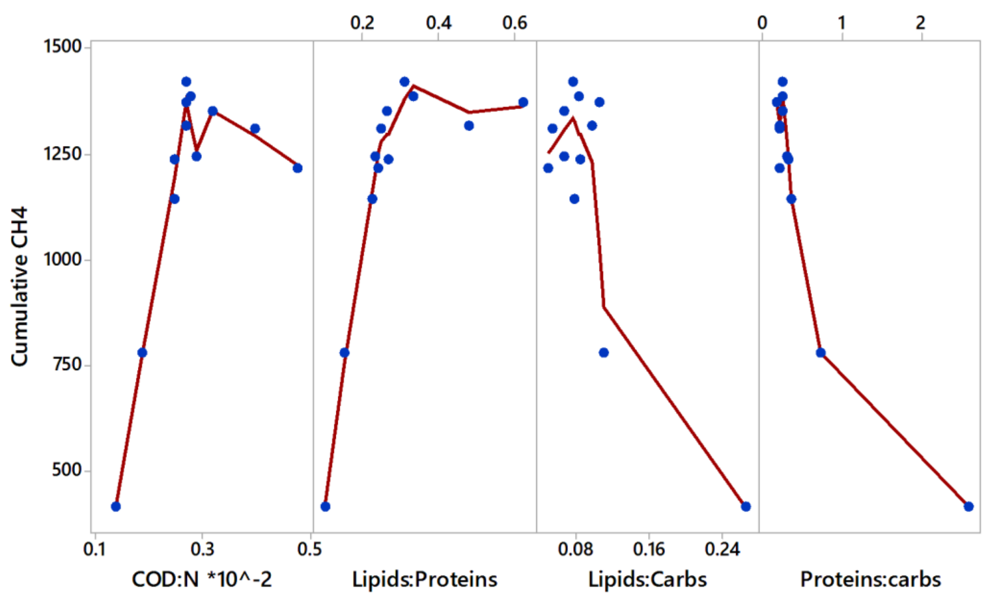


Figure 7.9. Matrix plot for: a. ultimate CH_4 and b. CH_4 yield at different COD/N and Lipids: Proteins, Lipids: Carbohydrates, and Proteins: Carbohydrates Ratios

7.5. Kinetic analysis results

The results of kinetic by modified Gompertz model using Eq. 3.5 is summarized in Table 7.3. The model was applied to the experimental data from mono and co-digestion of manure and SSO. The values of P for the maximum methane production (mL), R_m^e, the maximum methane production rate (mL/g day), and λ, the lag phase time (d) were calculated and the estimated values are presented in Table 7.3. The modified Gompertz model for mono- and co-digestions of TWAS, manure and SSO, showed a good fit to the experimental results with less than 10 % diversion from the measured values. The estimated values and their correlation with the mixing ratio of the feedstock showed compliance with the data obtained by the experiment. The trend of P variations showed the same trend as the trend observed by the experimental data in response to the corresponding mixing ratios.

Table 7.3. Summary of results of kinetic study using modified Gompertz model for TWAS/manure/SSO co-digestion

Digester codes	P (mL)	R_m^e (mL/d)	λ (d)	R^2
TWAS Only	400	20.3	0.02	0.999
Manure Only	1180	78.4	0.7	0.999
SSO only	1307	73.5	1.76	0.999
T*:M**:SSO 8:1:1	760	46.6	1.7	0.999
T:M:SSO 1:8:1	1257	66.9	0.2	0.999
T:M:SSO 1:1:8	1267	66.1	0.2	0.999
T:M:SSO 5:2.5:2.5	1099	60.7	0.8	0.999
T:M:SSO 2.5:5:2.5	1308	84.9	0.5	0.999
T:M:SSO 2.5:2.5:5	1330	74.4	1.0	0.999
T:M:SSO 4:4:2	1193	67.2	1.2	0.999
T:M:SSO 2:4:4	1382	84.6	1.1	0.999
T:M:SSO 4:2:4	1191	66.8	1.2	0.999

* T: TWAS
** M: Manure

The Maximum P value for the predicted cumulative methane production was 1382 mL and corresponded to the mixing ratio of 2:4:4. The lag phase varied from 0.0 to 1.7 days for different substrate mixing ratios. The lag phase time was less than 1 day for half of the digesters. The other half had a lag phase time from 1 to 1.7 days. The values of R^{e}_{max} ranged from 20 to 84.9 mL/g d corresponding to TWAS mono digestion and TWAS/manure/SSO mixing ratio of 2:4:4, respectively.

7.6. Hydrolysis/acidification

This experiment was carried out for evaluating the hydrolysis/acidification phase in anaerobic co-digestion of TWAS, manure, and SSO. The analysis of degree of solublization, synergistic effect of co-digestion at different mixing ratios, volatile fatty acids (VFAs) yield, and hydrolysis kinetics of lipids, proteins, and carbohydrates was conducted by monitoring the soluble and particulate contents during a 72-hr period. Initially, characterization of the feedstocks in triplicates was carried out and the mean values are presented in Table 7.4. As shown in the table, both manure and SSO have higher amount of COD concentration than TWAS. Manure contains high amount of carbohydrates and proteins. The lipids and carbohydrates content of manure and SSO are also remarkably higher than that of TWAS. pH values of the digesters were adjusted to satisfy the required condition for hydrolysis/acidification and ranged from 5.6 to 5.8 in this experiment.

The data form total and soluble COD (SCOD) concentrations monitoring in all of the digesters over time during a 72-h hydrolysis/acidification period was used to obtain the degree of COD solublization for each digester. As explained earlier, the degree of solublization was calculated using Eq. 3.1 and the result is summarized in Figure 7.10.

As illustrated in figure 7.10, the degree of the COD solublization varied from 15% to 30%. The most solublization of the COD content of 30% corresponded to TWAS/manure/SSO combinations of 1:8:1 and 2:4:4. For the control reactors, the COD solublization in mono digestion of TWAS, manure and SSO were 15% and 23%, and 26% respectively. The reactors containing only TWAS had the lowest degree of solublization. Similar to the results of previous experiments, manure resulted in a relatively lower solublization than SSO which would be due to the presence of some recalcitrant contents such as fibers and cellulosic compounds that delay hydrolysis and liquefaction.

Table 7.4. Characteristics of the feedstocks at different mixing ratios of TWAS/manure/SSO

		TWAS	Manure	SSO	T*:M**:SSO 8:1:1	T:M:SSO 1:8:1	T:M:SSO 1:1:8	T:M:SSO 5:2.5:2.5	T:M:SSO 2.5:5:2.5	T:M:SSO 2.5:2.5:5	T:M:SSO 4:4:2	T:M:SSO 2:4:4	T:M:SSO 4:2:4
Parameters	Units	Mixture (1)	Mixture (2)	Mixture (3)	Mixture (4)	Mixture (5)	Mixture (6)	Mixture (7)	Mixture (8)	Mixture (9)	Mixture (10)	Mixture (11)	Mixture (12)
TCOD	g/L	37	100	102	50	94	95	69	85	85	75	68	76
SCOD	g/L	1.9	10	37	6	12	31	13	15	22	12	17	17.7
TSS	g/L	29	54	50	34	51	48	40	47	46	43	36	42
VSS	g/L	23	45	44	27	43	42	34	39	39	36	31	36
TS	g/L	36	68	77	43	65	72	54	62	64	57	51	59
VS	g/L	32	58	63	37	56	59	46	53	54	48	43	49
Ammonia	g/L	0.2	0.0	1.3	0.3	0.2	1.1	0.5	0.4	0.7	0.4	0.6	0.6
pH	-	5.6	5.7	5.8	5.6	5.7	5.8	5.7	5.7	5.7	5.7	5.8	5.7
Alkalinity	g CaCO3/L	1.8	7.0	6.4	2.8	6.4	6.0	4.2	5.5	5.4	4.8	4.3	4.7
TN	g/L	2.7	1.7	4.1	2.7	2.0	3.7	2.8	2.5	3.1	2.6	2.5	3.0
TSN	g/L	0.4	0.1	1.1	0.4	0.2	0.9	0.5	0.4	0.7	0.4	0.5	0.6
T-Carbs	g/L	0.8	26.0	13.6	4.6	22.2	13.5	10.3	16.6	13.5	13.4	10.8	11.0
T-Proteins	g/L	2.5	5.1	2.1	2.8	4.6	2.4	3.1	3.7	3.0	3.5	2.4	2.9
T-Lipids	g/L	0.3	1.7	1.4	0.5	1.5	1.3	0.9	1.2	1.2	1.0	0.9	1.0

*T: TWAS

**M: Manure

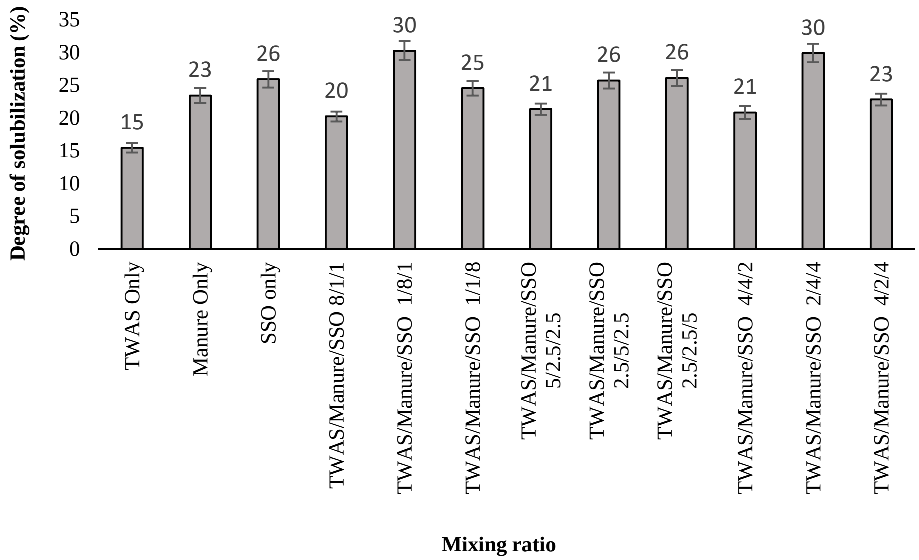


Figure 7.10. Degree of COD solublization at different mixing ratios of TWAS/Manure/SSO

It was observed that all of the reactors containing the mixings of TWAS, manure and SSO achieved a higher degree of solublization than the control reactors containing only TWAS. Solublization increased by 100%, 30% and 15% in the co-digestion of TWAS/manure/SSO in comparison with the control reactors digesting only TWAS, manure and SSO, respectively. Although SSO demonstrated more solublization than manure, no correlation between the portion of SSO and the degree of solublization at different mixing ratio was observed. This could verify that a proper mixing ratio is required to enhance the microbial synergy of the hydrolytic and acidogenic microbial communities for the process improvement.

The synergistic effect on the solublization of the feedstocks was evaluated by comparing the theoretical degree of solublization of each co-digester to the measured data from the experiment and the result is summarized in Figure 7.11. Although the improvement of synergy was not significant at some combinations, most of the co-digesters resulted in an increase of solublization due to the synergistic effect of the microbial communities. The synergistic effect varied from 2% to 35% in hydrolysis/acidification of TWAS/manure/SSO at different mixing ratios.

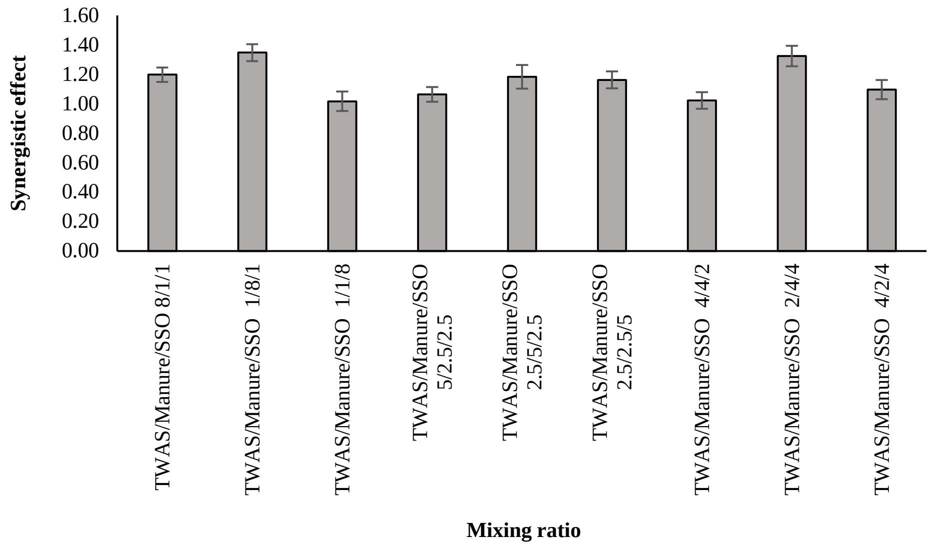


Figure 7.11. Synergistic effect on solublization at different mixing ratios of TWAS/Manure/SSO

The monitoring of VFAs concentrations over time indicated an increasing trend during the 72-hr of the hydrolysis/acidification period. The total VFAs yields were calculated in terms of mass of produced VFAs per mass of VSS added (mg VFAs/g VSS added). As illustrated in Figure 7.12, SSO alone had more VFAs yields than manure alone. Both manure and SSO had a higher VFAs yield than TWAS. The VFAs yield in the co-digester was correlated to the mixing ratios of the feedstocks so that the VFAs yield increased by increasing the fractions of manure and SSO in the co-digesters. Similar to the result of the previous experiments, the trend of VFAs yield did not comply with the trend of COD solublization of the corresponding mixing ratios (Figure 7.10). Therefore, it was assumed that hydrolysis/liquefaction would occur independently of acidification. All of the reactors containing the mix of manure and SSO had higher VFAs yield than the control reactors containing TWAS alone. The VFAs yields were 231 mg VFAs/g VSS added and 325 mg VFAs/g VSS added for the manure and SSO mono digestions, respectively. The maximum VFAs yield of 400 mg VFAs/g VSS added was achieved by manure:SSO ratio of 1:9.

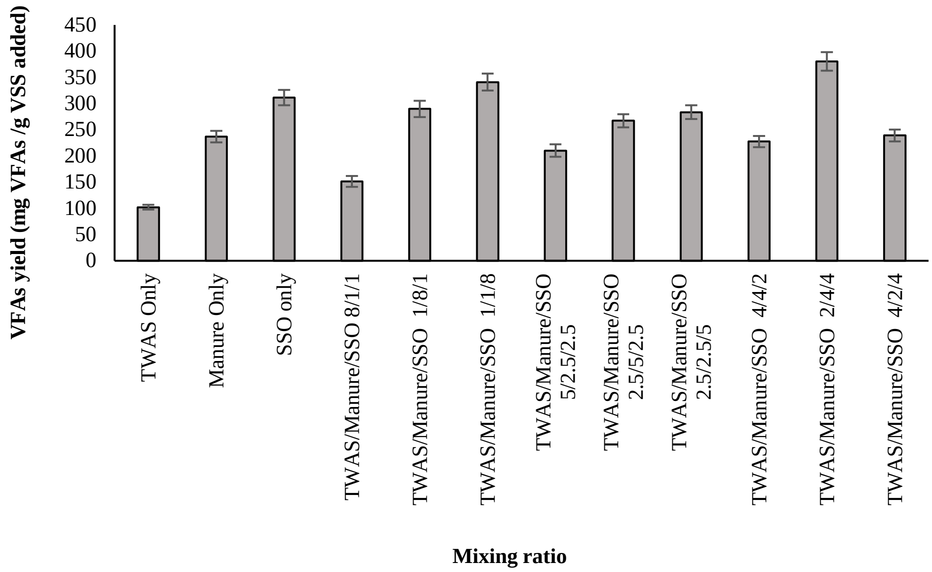


Figure 7.12. Total VFAs yield at different mixing ratios of TWAS/Manure/SSO

In co-digestion of TWAS/manure/SSO, by monitoring soluble and particulate COD over time, an increasing trend in solublization of COD and decreasing particulate COD concentrations was observed. Furthermore, monitoring soluble and particulate lipids, proteins, and carbohydrates over time also showed an increasing trend in solublization and particulate matter degradation of the feedstocks. Similar to the previous experiments, the hydrolysis rates of the lipids and proteins, were slower than carbohydrate. These results are summarized in the tables presented in the appendix. The hydrolysis rate coefficient (K_h) was calculated according to first order kinetic (equations 3.3 and 3.4) for COD, lipids, proteins and carbohydrates based on the particulate degradation and results are summarized in Table 7.5.

Although the first order kinetics was compatible with the trend of particulate matters degradation, the hydrolysis kinetics coefficient did not show the same trend as solublization and acidification as well as methanogenesis. Hydrolysis rate coefficients of the co-digesters increased in the co-digesters although, it did not change significantly in all of them compared to the single digestion of SSO. In some co-digesters the K_h values were even lower than that of SSO alone. The maximum VFAs yield corresponded to TWAS:manure:SSO 2:4:4 and 1:1:8. However, the Kh for the mixing ratio of 1:8:1 was higher than that of 1:1:8. The maximum solublization corresponded to

TWAS:manure:SSO mixing ratios of 2:4:4 and 1:8:1. However, the maximum methane yield occurred at the mixing ratios of 2:4:4 and 2.5:2.5:5 in the reactors co-digesting TWAS, manure, and SSO. Only the mixing ratio of 2:4:4 resulted in improving both methanogenesis and hydrolysis which would be due to the balance of the nutrients that favors both methanogenic and hydrolytic microbial consortia.

Table 7.5. Hydrolysis rate coefficients for COD, lipids, proteins, and carbohydrate content in co-digestion of TWAS, Manure, and SSO at different mixing ratios

K_h	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 5/2.5/2.5	T/M/SSO 2.5/5/2.5	T/M/SSO 2.5/2.5/5	T/M/SSO 4/4/2	T/M/SSO 2/4/4	T/M/SSO 4/2/4
Kh_{COD}	0.17	0.23	0.3	0.21	0.37	0.27	0.24	0.31	0.3	0.24	0.39	0.26
Kh_{Lipids}	0.03	0.07	0.08	0.05	0.12	0.06	0.05	0.09	0.08	0.09	0.14	0.05
$Kh_{Proteins}$	0.19	0.21	0.28	0.23	0.31	0.24	0.2	0.23	0.24	0.21	0.31	0.21
$K_{h\ Carbs}$	0.32	0.43	0.55	0.36	0.66	0.51	0.46	0.63	0.58	0.26	0.69	0.52

[*] T: TWAS
[**] M: Manure

Chapter 8

Correlation of CH_4 with lipids, proteins, and carbohydrates

8.1 Correlation of organic compositions and biomethane yield

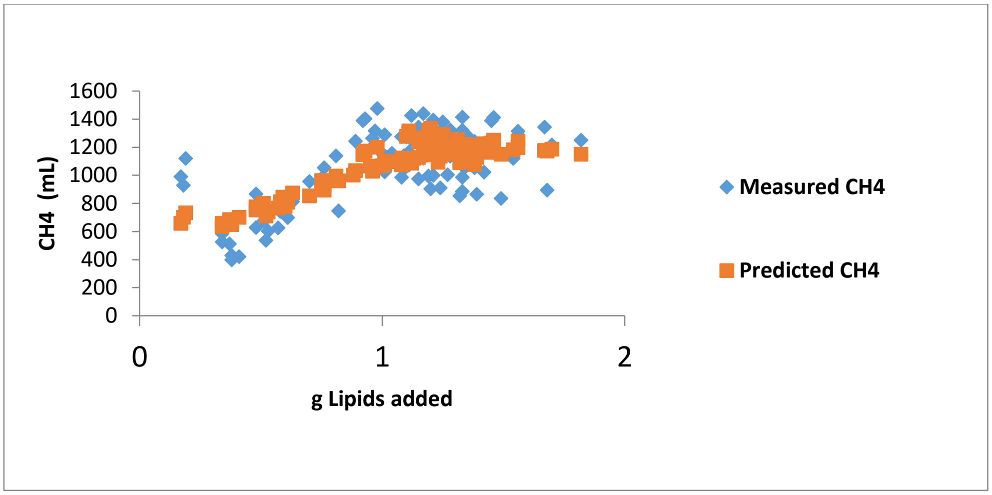
Lipids, proteins and carbohydrates make up the principal constituents of organic waste. Currently, there is limited information on how the three main components of organics impacts the AD performance. Therefore, assessing the correlation between the performance of anaerobic digestion system and the three main organic composition is required in order to enhance the efficiency of the AD process and to provide a tool for the prediction of the system performance.

In addition, in designing a co-digestion system this would provide a tool to optimize the system based on lipids, proteins, and carbohydrates fractions along with C:N ratio as they are both important design parameters. In this work the influence of lipids, proteins and carbohydrates content on the anaerobic digestion of TWAS, SSO and manure was investigated. The feedstocks and their mixtures with different ratios as presented in Table 3.2- Table 3.5, were analysed to determine their total lipids, proteins, and carbohydrates contents. The results of biogas monitoring and the methane in biogas using GC from the sets of BMP essay were used to determine an empirical model based on the relationship between lipids, proteins, carbohydrates components and the system efficiency in terms of methane production and yield. The functional relationship between responses (CH_4) and the factors (Lp, Pr, and Cr) were described by estimating the coefficients of the second-order polynomial model based on the experimental data according to Eq. 8.1 where B_0 is a constant, B_1, B_3, and B_4 are linear coefficients and B_2 is quadratic coefficient. The empirical model was used to predict the methane yield ($mLCH_4$/ $gCOD_{added}$) in terms of lipids, proteins, and carbohydrates components and the results are depicted by fit plots and residual plots as presented in figures 8.1 and 8.2.

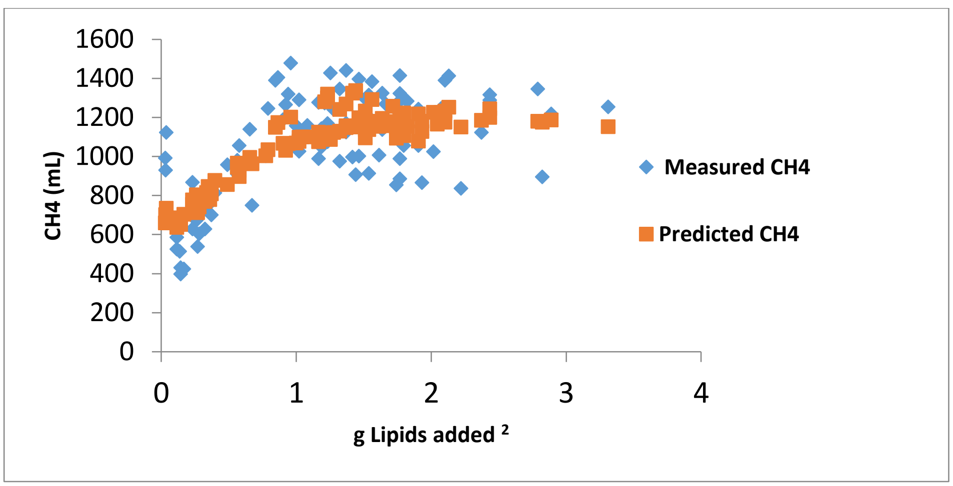
$$CH_4 = B_0 + B_1 Lp + B_2 Lp^2 + B_3 Pr + B_4 Cr \qquad \text{Eq. 8.1}$$

Each of the fit plots as shown in Figure 8.1 contains adequate number of observations throughout the entire range of the predictor values. Although for the lipids a few points on the top left corner of the plot seems to be outlier, the multiple R value of 0.7 indicates the model properly fits in the data.

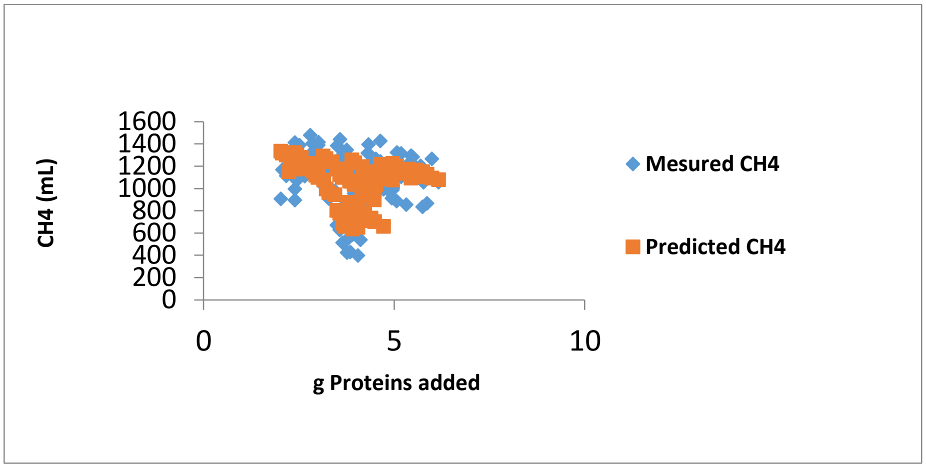
8.1. a)


1600
1400
1200
1000
800
600
400
200
0
CH4 (mL)
0
1
2
g Lipids added
Measured CH4
Predicted CH4

8.1.b)


1600
1400
1200
1000
800
600
400
200
0
CH4 (mL)
0
1
2
3
4
g Lipids added ²
Measured CH4
Predicted CH4

8.1.c)



8.1. d)

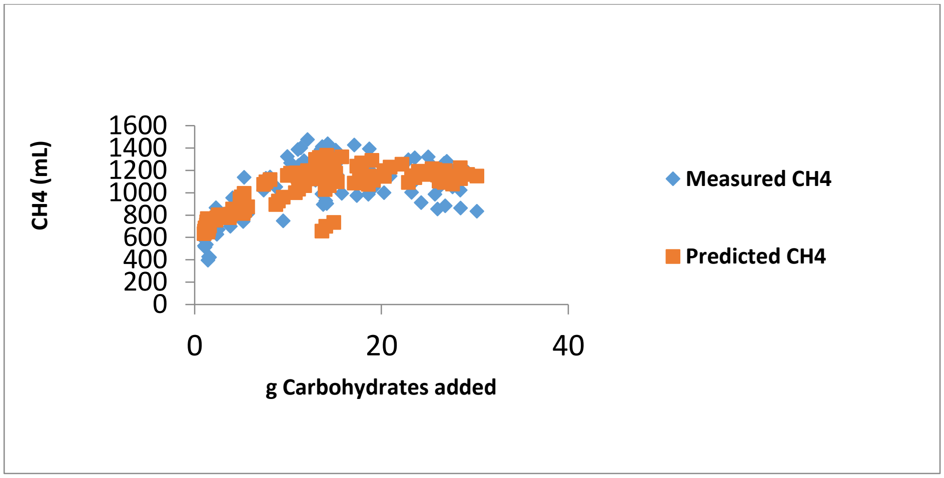
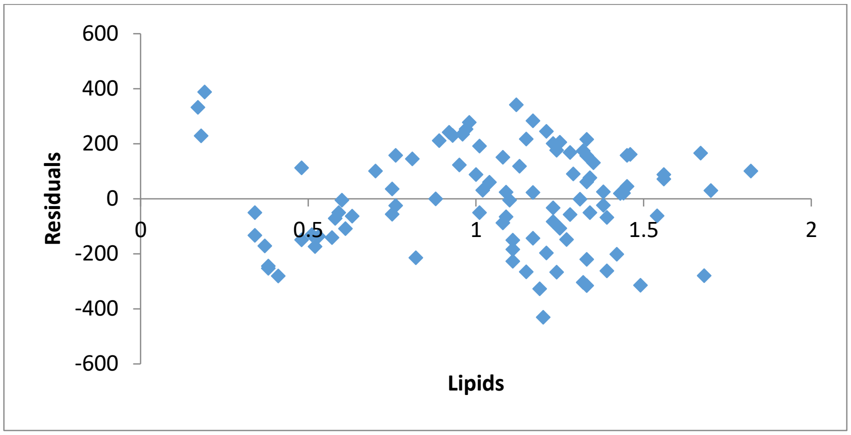


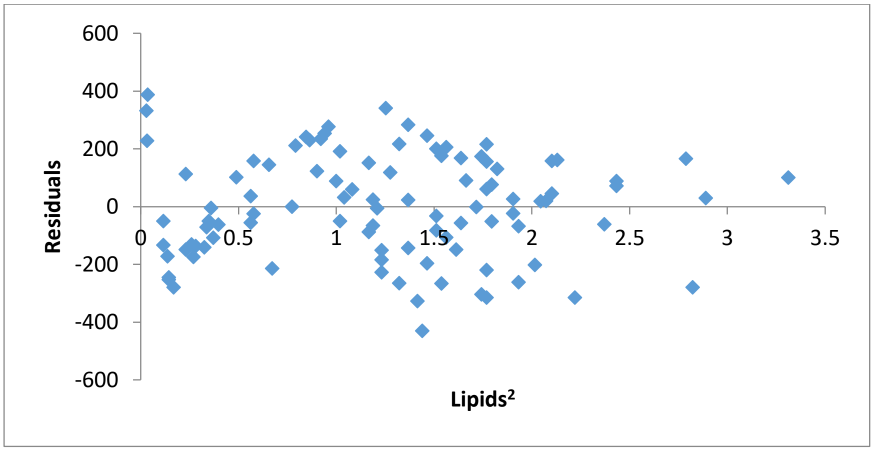
Figure 8.1. Fitted plot of response variable to lipids, Lipids^2, proteins, and carbohydrate

The residual plot was used to determine whether the model adequately meets the assumptions of the model. As the patterns in the points show, the points on the residual plots are randomly on both side of 0 which indicate that the model meets the model assumptions.

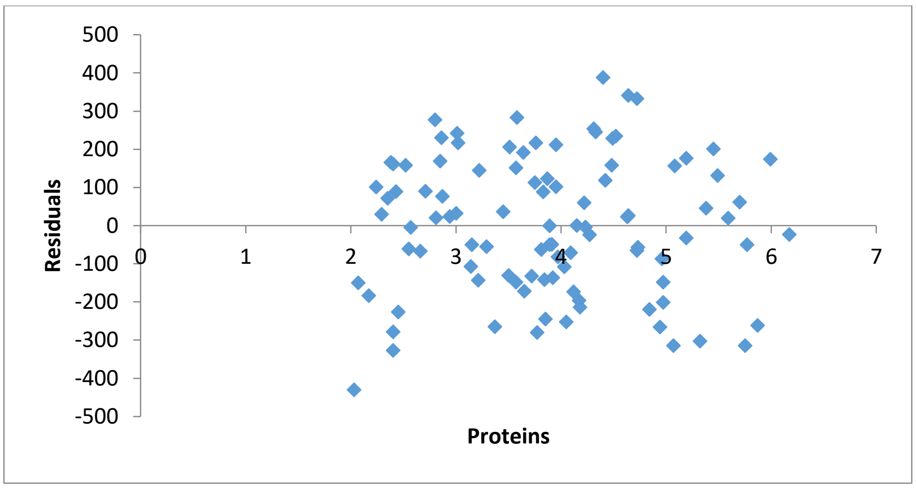
8.2. a)


600
400
200
0
-200
-400
-600
Residuals
0
0.5
1
1.5
2
Lipids

8.2. b)


600
400
200
0
-200
-400
-600
Residuals
0
0.5
1
1.5
2
2.5
3
3.5
Lipids²

8.2. c)


500
400
300
200
100
0
-100
-200
-300
-400
-500
Residuals
0
1
2
3
4
5
6
7
Proteins

8.2.d.)

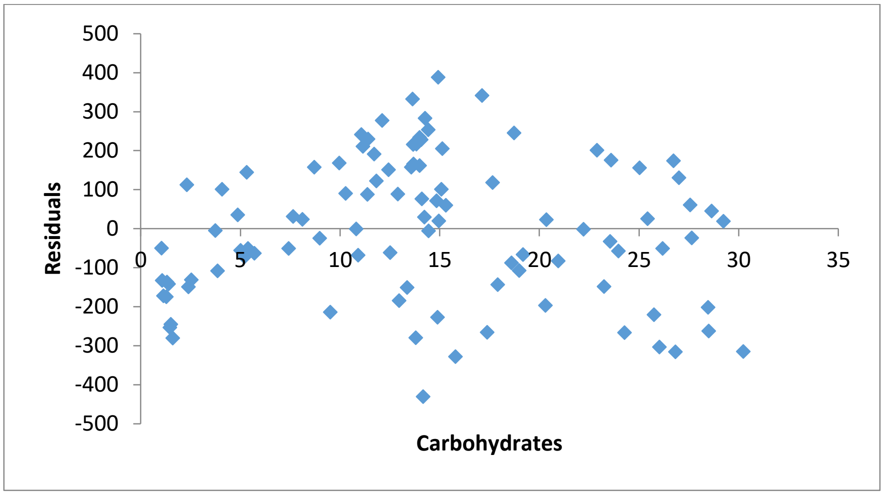


Figure 8.2. Residuals versus fits plot (residuals versus estimated response)

Table 8.1 includes the intercept, linear and quadratic coefficients of the Eq. 8.1 and summarizes some of the results of ANOVA analysis. The P-values < 0.05 as shown in the table verifies that the correlation between the response and the variables is statistically significant. The findings suggest that the amount of lipids in substrate is directly proportional to the methane yield and its impact is significantly more than carbohydrates. While the lower fraction of proteins than lipids and carbohydrates increase the methane yield. Y. Li et al, 2017 also used a second-order polynomial model as a quick estimation of AD parameters, however their model only included proteins and lipids as variables. Their findings though verified that the more fraction of lipids significantly increases the methane production compared to proteins.

Table 8.1.Coefficients of variables in the second-order polynomial model and the results of ANOVA analysis

	Coefficients	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	825.279203	5.12631511	1.576E-06	505.632211	1144.926	505.6322	1144.926
Lipids	862.890011	3.63241266	0.0004573	391.223242	1334.557	391.2232	1334.557
Lipids^2	-380.17074	-3.2738253	0.0014856	-610.738303	-149.603	-610.738	-149.603
Proteins	-117.12258	-3.7852707	0.0002703	-178.557985	-55.6872	-178.558	-55.6872
Carbs	18.4329527	3.36910525	0.0010949	7.56981336	29.29609	7.569813	29.29609

8.2- Statistical Analysis

Table 8.2 shows the experimental set-ups and the corresponding feedstock ratios and lipids: proteins: carbohydrates ratios. ANOVA was carried out for the CH_4 yields in response to the feedstock ratios and lipids: proteins: carbohydrates ratios. The main effect plot for CH_4 yield data mean is presented in Figure 8.3. The main effect plot shows the mean response of each level factors connected by the line. The steeper slope in the line explains the greater scale of the main effect. An effect is the variation in the methane yield when the factors i.e. the feedstock and lipids: proteins: carbohydrates ratios change from one level to another. The P value from the ANOVA results showed that the feedstock and lipids: proteins: carbohydrates ratios have significant effects on the CH_4 yield.

Table 8.2. Experimental set-ups and the corresponding ratios of the feedstock and lipids: proteins: carbohydrates

Experiment code	Feedstock ratios	Feedstock ratios code	Lipids: Proteins: Carbs
A	TWAS Only	AA	1:11:3
A	SSO Only	BB	1:1.3:8
A	TWAS/SSO 9/1	A	1:7:5
A	TWAS /SSO 7/3	B	1:4:7
A	TWAS /SSO 1/1	C	1:3:7
A	TWAS /SSO 3/7	D	1:2:8
A	TWAS /SSO 1/9	E	1:1.5:8
B	TWAS	AA	1:7:2.5
B	Manure	CC	1:4:20
B	TWAS / Manure 9/1	A	1:7:6
B	TWAS / Manure 7/3	B	1:5.5:12
B	TWAS / Manure 1/1	C	1:25:78
B	TWAS / Manure 3/7	D	1:4:17
B	TWAS / Manure 1/9	E	1:4:19
C	Manure	CC	1:4.2:21
C	SSO	BB	1:2:12
C	Manure/SSO 9/1	A	1:3.5:18.5
C	Manure /SSO 7/3	B	1:4:20
C	Manure /SSO 5/5	C	1:3:17
C	Manure /SSO 3/7	D	1:2.7:15
C	Manure /SSO 1/9	E	1:2:13

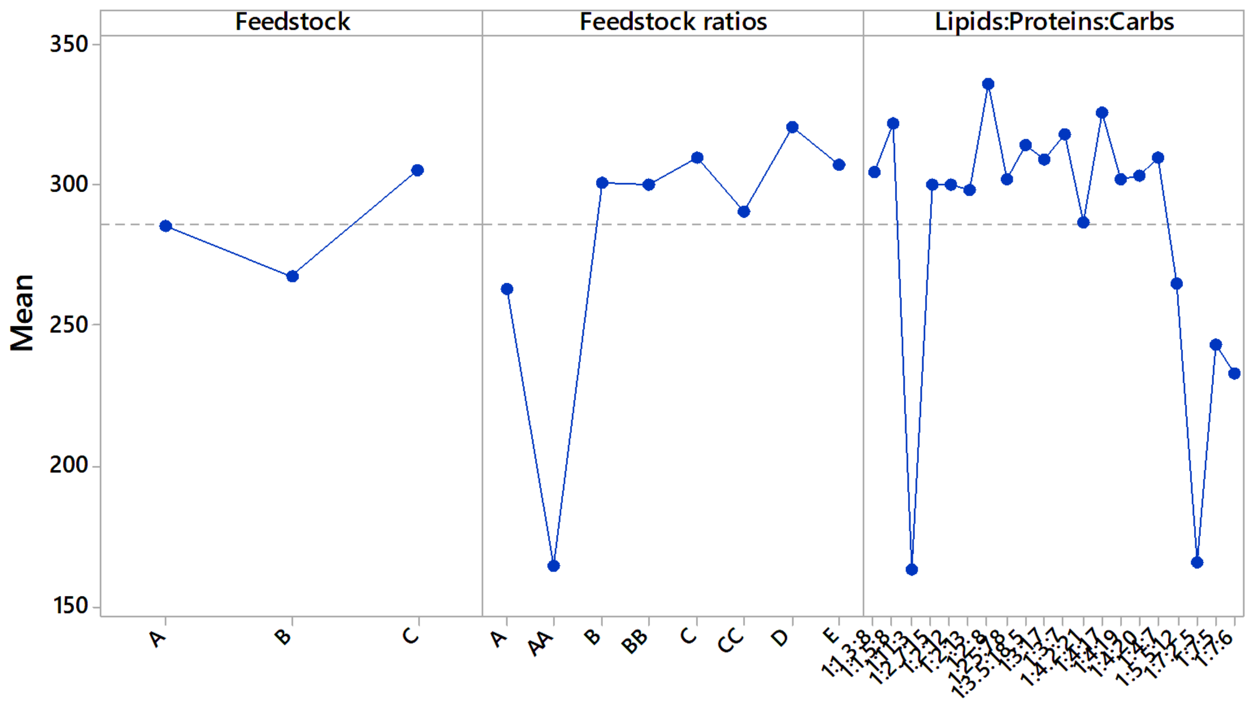


Figure 8.3. a. Main effect plot for CH_4 yield data mean

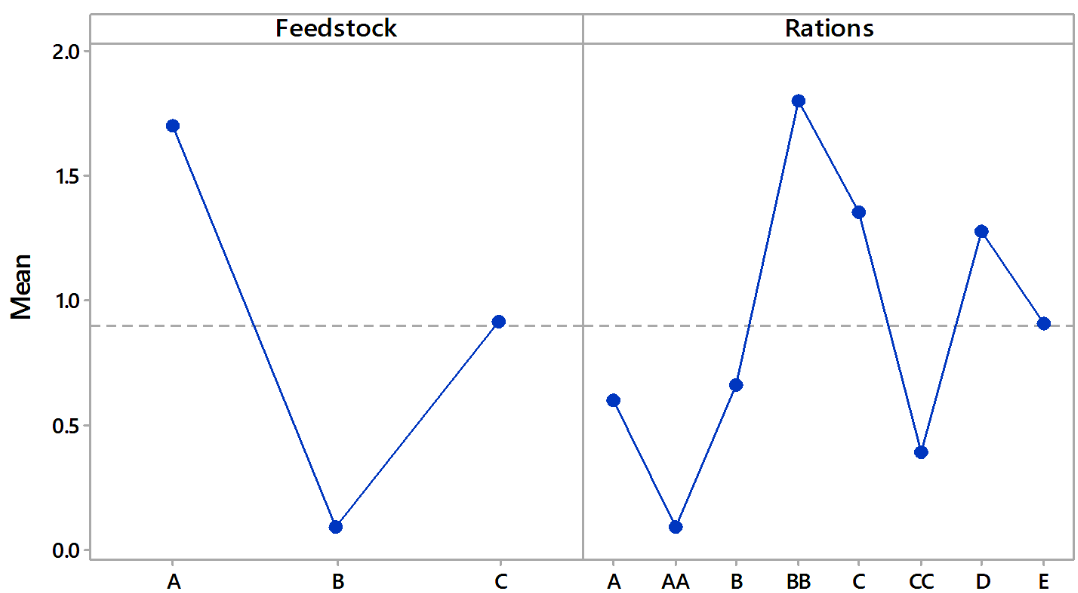


Figure 8.3. b. Main effect plot for CH_4 yield data mean for the lag phase data mean

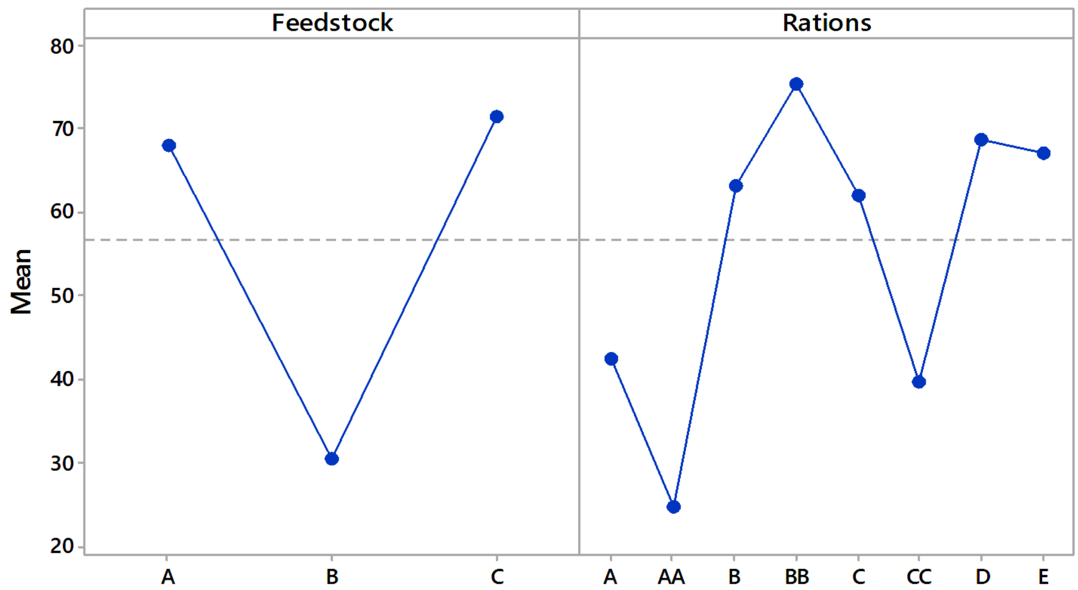


Figure 8.3. c. Main effect plot for CH_4 yield data mean for the CH_4 maximum rate data mean

The interval plot of CH_4 yield with 95% confidence interval for the mean using individual standard deviations to calculate the intervals is shown in Figure 8.4. The width of the interval plot represents the extent of variation in the data. A small interval would indicate more consistent data and less variation. As shown in Figure 8.4 the variation is more for some ratios than the other ones. The variation in the methane yield for the feedstock ratios of 1:9 and 7:3 were more than the other feedstock mixing ratios. The data was quite consistent with that of SSO alone. As shown in Figure 8.5.a, all of the intervals include 0 which means that the corresponding CH_4 yield data means are not significantly different in response to the feedstocks. However, according to Figure 8.5.b., for some of the feedstock mixing ratios, the corresponding means are significantly different.

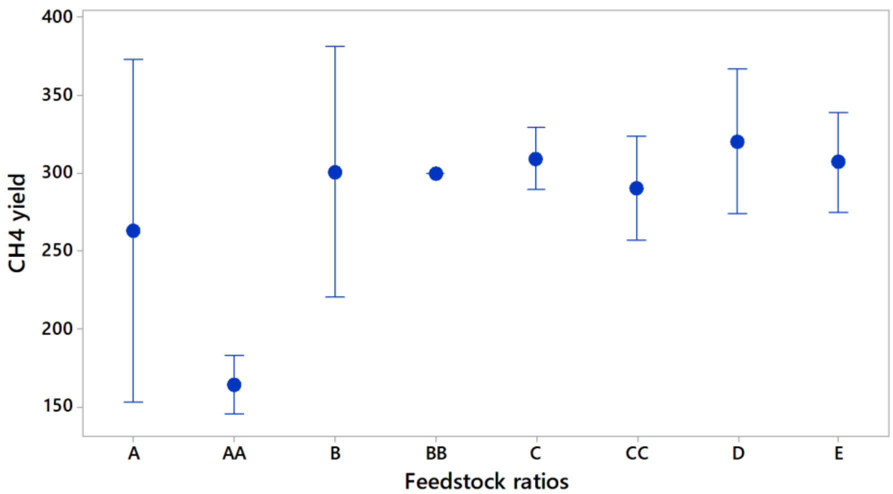


Figure 8.4. Interval plot of CH_4 yield with 95% confidence interval for the mean

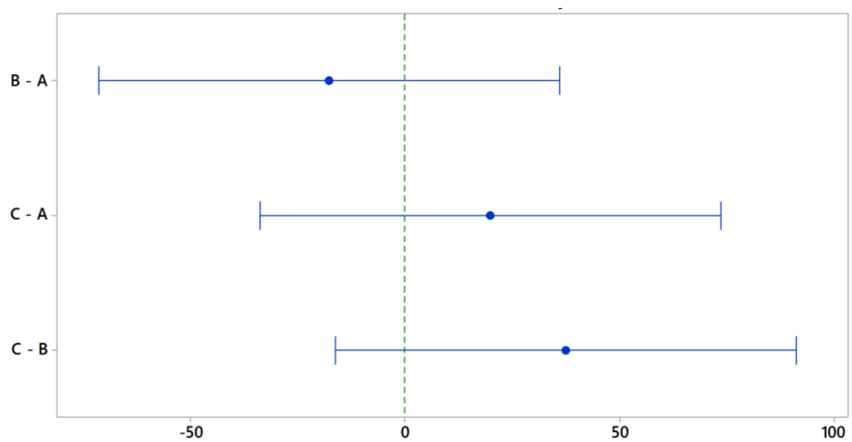


Figure 8.5. a. Fisher individual 95% confidence interval differences of means for CH_4 yields in response to the feedstocks

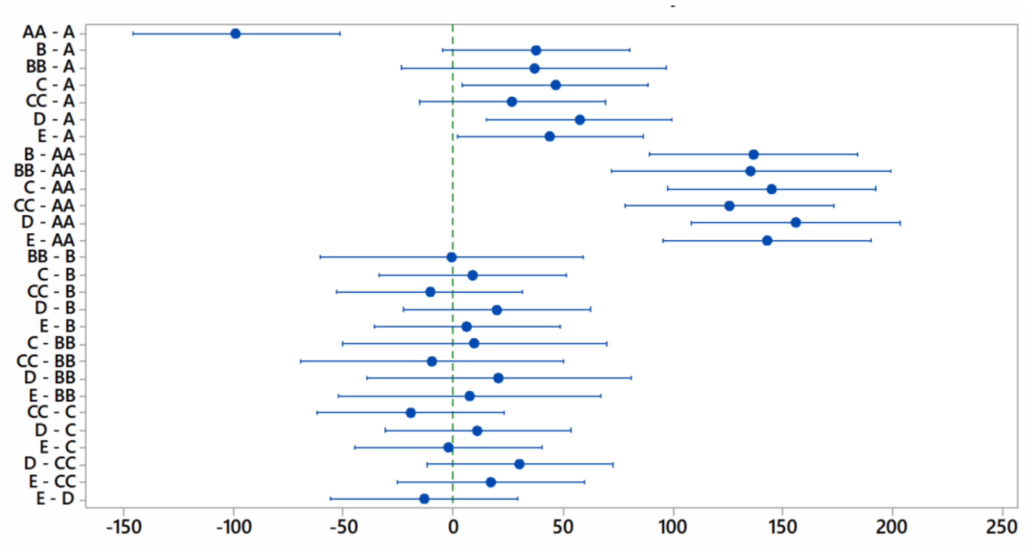


Figure 8.5. b. Fisher individual 95% confidence interval differences of means for CH_4 yields in response to the ratios

Chapter 9

Conclusions

9. Conclusions

9.1. Biomethane potential

A series of binary and ternary anaerobic co-digestion was designed to evaluate the biomethane potential and to investigate the correlation between the biomethane production and the mixing ratios as well as lipids: proteins: carbohydrates ratios of the feedstock. Gompertz model was used to assess the compatibility of the model to the data obtained by the experiment. A first order kinetic model was applied using AquaSim 2.0 to assess the hydrolysis kinetics and its influence on the process. A simple empirical model was derived based on the correlation of biomethane production to the lipids, proteins and carbohydrates content of the feedstocks which included TWAS, manure and SSO.

9.1.1. Binary co-digestion of TWAS, manure, and SSO

- The three sets of the binary co-digestion primarily verified the advantages of co-digestion over mono-digestion of TWAS, manure and SSO.
- Among the three binary co-digestion including TWAS/SSO, TWAS/manure, and Manure/SSO, the most amount of the methane yield was achieved by co-digestion of Manure/SSO at the mixing ratio of 7:3 corresponding to 363 mL CH_4/g COD added.
- The modified Gompertz model showed a good fit to the experimental data by a diversion ranging from 4% to 6%.
- The optimum ratios of the feedstocks as well as the lipids: proteins: carbohydrates varied by the different feedstocks.
- In co-digestion of TWAS/SSO the maximum ultimate CH_4 of 1252 mL and CH_4 yield of 357 mL CH_4/g COD added corresponded to the mixing ratio of 3:7 and lipids: proteins: carbohydrates ratio of 1:2:8.
- TWAS/manure co-digestion resulted in 1069 mL ultimate CH_4 production and 324 mL CH_4/g COD added biomethane yield at the mixing ratio of 3:7 and lipids: proteins: carbohydrates ratio of 1:4:17.
- For Manure/SSO co-digestion an ultimate CH_4 of 1186 mL and a CH_4 yield of 363 mL CH_4/g COD added was achieved corresponding to the mixing ratio of 7:3 and lipids: proteins: carbohydrate ratio of 1: 3.5: 18.5.

9.1.2. Ternary co-digestion of TWAS, manure and SSO

- Among co-digestion of the three feedstocks at different ternary combinations, the most ultimate methane production and yield occurred at TWAS/manure/SSO mixing ratio of 2:4:4 and lipids: proteins: carbohydrate ratio of 1:3:12.
- The maximum methane production and yield in co-digestion of TWAS/manure/SSO were 1424 mL 356 mL CH_4/g COD added, respectively.
- The modified Gompertz model showed a good fit to the results obtained by the experiment with less than 5% diversion.
- A higher maximum ultimate methane and methane yield was achieved by ternary co-digestion of TWAS/manure and SSO, however at some ratios the ternary co-digestion did not produce higher methane than some of the combinations in the binary co-digestion.
- Synergistic effect did not demonstrate further improvement in the ternary co-digestion of TWAS, manure, and SSO compared to their combinations in the binary co-digestion.
- The most synergistic effect in co-digestion of TWAS/manure/SSO was 19% while the maximum synergistic effect was 36 % in manure/SSO co-digestion.

9.2. Hydrolysis/acidification

Along with the biomethane potential assay, a series of hydrolysis/ acidification of the binary and ternary combinations of the feedstocks was designed to evaluate the hydrolysis and acidification process in co-digestion of TWAS, manure, and SSO. The binary and ternary anaerobic co-digestion were set up with the same combination as the BMP experiments for investigating the correlation between the solublization, VFAs yield, and hydrolysis rate, with the mixing ratios as well as lipids: proteins: carbohydrates ratios of the feedstock. First-order kinetic model was used to assess the compatibility of the model to the data obtained by the experiment. The model was used to obtain the kinetic rare coefficients of COD, lipids, proteins and carbohydrates of the feedstocks at different mixing ratios.

9.2.1. Co-digestion of TWAS/SSO

- An improvement of hydrolysis/acidification was observed in co-digestion of the feedstocks at different mixing ratios
- In TWAS/SSO co-digestion the maximum degree of solublization was 30% at the mixing ratio of 1:9.
- The synergistic effect was the highest at TWAS/SSO mixing ratio of 7:3 corresponding to 44% improvement as a result of using co-substrate and improving synergy.
- A maximum VFAs yield of 312 mg/ g VSS added was attained by TWAS/SSO co-digestion at the mixing ratio of 1:9.
- The hydrolysis kinetic rate coefficient was compatible to the first order kinetic model with a diversion less than 10%.
- The maximum hydrolysis rate coefficient of COD, proteins, and carbohydrates in co-digestion of TWAS/SSO were 0.35, 0.07, 0.34 and 0.68 d^{-1}, corresponding to the mixing ratios of 1:9, 3:7, 1:9, respectively.
- The maximum hydrolysis rate coefficient of lipids was 0.07 which corresponded to the TWAS:SSO mixing ratios of 5:5 and 3:7.

9.2.2. Co-digestion of TWAS/manure

- The percentage improvement of hydrolysis/acidification varied by different mixing ratios of TWAS and manure.
- In co-digestion of TWAS/manure, the maximum degree of solublization was 34% corresponding to the TWAS/manure mixing ratio of 3:7.
- A synergistic effect of 38% was achieved by TWAS/manure co-digestion at the mixing ratio of 3:7.
- The maximum VFAs yield of 507 mg/ g VSS added was achieved by TWAS/manure mixing ratio of 3:7.
- The hydrolysis kinetic rate coefficient was compatible to the first order kinetic model with a diversion less than 10%.

- The maximum hydrolysis rate coefficient of COD, lipids, proteins, and carbohydrates in co-digestion of TWAS/manure were 0.33, 0.09, 0.27, and 0.59 d^{-1}, corresponding to the mixing ratio of 3:7.

9.2.3. Co-digestion of manure /SSO

- The hydrolysis/acidification improved to different extents in response to the different mixing ratios in co-digestion of TWAS and manure.
- The most degree of solublization was 35% corresponding to manure/SSO mixing ratios of 3:7 and 1:9.
- The highest synergistic effect of 34% in co-digestion of manure and SSO occurred at the mixing ratio of 9:1.
- In manure/SSO co-digestion, the maximum VFAs yield of 400 mg/ g VSS added was achieved at the mixing ratio of 1:9.
- The hydrolysis kinetic rate coefficient was compatible to the first order kinetic model with a diversion less than 10%
- The maximum hydrolysis rate coefficient of COD, lipids, proteins, and carbohydrates in co-digestion of manure/SSO were 0.38, 0.11, 0.33, and 0.68 d -1, corresponding to the mixing ratio of 3:7.

9.2.4. Co-digestion of TWAS/manure /SSO

- In the ternary co-digestion of TWAS, manure, and SSO the maximum solublization was 30% corresponding to TWAS/manure/SSO mixing ratio of 2:4:4 and 1:8:1.
- The maximum synergistic effect of 35% corresponded to TWAS/manure/SSO mixing ratio of 1:8:1.
- In co-digestion of TWAS/manure/SSO the highest VFAs yield of 380 mg VFAs/g VSS added was achieved at the mixing ratio of 2:4:4.
- The hydrolysis kinetic rate coefficient was compatible to the first order kinetic model with a diversion less than 10%
- The hydrolysis rate coefficient of COD were within the range of 0.21 to 0.39 for all of the co-digesters.

- The maximum hydrolysis rate coefficients of COD, lipids, and carbohydrates in co-digestion of TWAS/manure/SSO were 0.39, 0.14, and 0.69 d^{-1} corresponding to the mixing ratio of 2:4:4.
- In co-digestion of TWAS/manure/SSO, the maximum hydrolysis rate coefficient for the proteins content was 0.23 which corresponded to the mixing ratios of 8:1:1 and 2.5:5:2.5.

9.3 Correlation of biomethane production with the organic content

9.3.1. COD:N and lipids:proteinscarbohydrates ratios

- In TWAS/SSO co-digestion the maximum ultimate CH_4 corresponded to COD:N ratio of 28 corresponding to the lipids: proteins: carbohydrates ratio of 1:2:8. The trend of variations of the methane yield versus COD:N was similar to the trend that was observed for its variation vursus lipids:proteins. The changes of methane yield versus lipids:carbohydrates and proteins:carbohydrates showed a similar trend while it did not comply with that of COD:N ratio.
- In TWAS/manure co-digestion, the maximum CH_4 yield occurred at COD:N ratio of 41 corresponding to the lipids: proteins: carbohydrates ratio of 1:4:17. Similar to the co-digestion of TWAS/SSO, in TWAS/manure co-digestion the trend of variations of the methane yield versus COD:N was similar to the trend that was observed for its variation versus lipids:proteins. The changes of methane yield versus lipids:carbohydrates and proteins:carbohydrates did not comply with that of COD:N ratio although they both showed a simiar trend.
- In Manure/SSO co-digestion, the maximum methane yield corresponded to the COD:N ratio of 41 and lipids: proteins: carbohydrates ratio of 1:3.5:18.5. The variations of methane yield versus COD:N and lipids: proteins did not show a similar trend. Although, in terms of the changes of the CH_4 yield, both lipids: carbohydrates and proteins: carbohydrates ratios demonstrated similar trend.
- In TWAS/manure/SSO co-digestion the maximum methane yield corresponded to COD:N tatio of 28 and the lipids:proteins:carbohydrates ratio of 1:3:12. The trend of variations of the methane yield vursus COD:N was similar to the trend that was observed for its variation versus lipids:proteins. The changes of methane yield vursus lipids:carbohydrates and

proteins:carbohydrates showed a similar trend while it did not comply with that of COD:N. TWAS/manure/SSO co-digestion.

9.3.2. Anaerobic co-digestion model

An empirical model would be a useful approach to predict the methane yield ($mLCH_4$/ $gCOD_{added}$) based on the lipids, proteins, and carbohydrates components of the feedstock in anaerobic co-digestion of multi substrate. As the three main components of any type of feedstocks in biowaste are lipids, proteins, and carbohydrates, an empirical model was presented that correlates the CH_4 yields with lipids, proteins and carbohydrates content of the feedstock. The functional relationship between responses which was CH_4 yield and the factors including lipids, proteins and carbohydrates (Lp, Pr, and Cr) were described by estimating the coefficients of the second-order polynomial model based on the experimental data. The fit plots together with the residual plots for lipids, proteins and carbohydrates showed that the model adequately fits to the data and meet the model assumption.

9.4. Suggested future works

- Validating the batch study results for AnCoD of Manure/SSO and TWAS/Manure/SSO at 7:3 and 2:4:4 mixing ratios in CSTR mode
- Investigating the validity of the proposed empirical model for a variety of the feedstocks including the industrial wastes
- Investigating the validity of the proposed empirical model using CSTR mode.
- Implementing a cost-benefit analysis for applying AnCoD of Manure/SSO and TWAS/Manure/SSO for GTA versus individual AD of manure and SSO.
- Analyzing carbon footprint reduction by AnCoD of manure with TWAS as compared to conventional AD and composting

Appendices

A. Analytical results for AnCoD of TWAS and SSO

Table A. 1. Characteristics of raw feedstocks in AnCoD of TWAS and SSO

Parameters	Units	SSO		TWAS		Seed	
		MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	110000	7795	40000	2053	16400	444
SCOD	mg/L	44400	100	1360	26	762	19
TSS	mg/L	53833	6252	31450	1006	15033	400
VSS	mg/L	38478	3649	25600	2216	10900	200
TS	mg/L	62187	1025	38810	1859	17450	582
VS	mg/L	43493	761	34477	1027	13140	328
Ammonia	mg/L	1138	6	255	9	795	40
pH	-	5.6	0.2	6.3	0.2	7.2	0.2
Alkalinity	mg CaCO3/L	6080	522	1953	148	4943	465
TN	mg/L	3267	751	2900	400	1425	203
TSN	mg/L	910	38	420	64	696	112
Total Carbs	mg/L	14360	3580	1080	112	549	82
Total Proteins	mg/L	2308	198	3759	298	1648	56
Total Lipids	mg/L	1731	2420	351	27	163	52

Table A. 2. Average CH_4 production in AnCoD of TWAS and SSO

Average CH_4 Measurement (mL)														
Time (day)	TWAS	SD	SSO	SD	T/SSO 9/1	SD	T/SSO 7/3	SD	T/SSO 1/1	SD	T/SSO 1/9	SD	T/SSO 3/7
1	6	0.3	3	0.1	6	0.3	7	0.3	3	0.1	4	0.1	3
3	24	1.2	20	1.0	20	1.0	33	1.7	17	0.7	27	1.0	20
4	43	2.3	27	1.3	35	1.7	54	2.8	25	1.1	49	1.9	38
5	58	3.0	42	2.0	57	2.8	68	3.5	39	1.7	82	3.1	65
6	39	2.0	48	2.3	44	2.2	51	2.6	39	1.6	60	2.3	76
7	37	1.9	48	2.3	51	2.5	45	2.3	66	2.8	52	2.0	69
8	21	1.1	51	2.5	34	1.7	42	2.2	53	2.2	45	1.7	78
10	28	1.4	55	2.7	34	1.7	60	3.0	84	3.5	80	3.0	93
12	26	1.4	51	2.5	25	1.2	29	1.5	81	3.4	74	2.8	83
14	21	1.1	39	1.9	22	1.1	22	1.1	47	2.0	33	1.2	64
16	19	1.0	25	1.2	15	0.7	23	1.2	21	0.9	20	0.8	20
19	23	1.2	23	1.1	25	1.2	25	1.3	21	0.9	25	1.0	22
22	18	0.9	24	1.2	15	0.8	17	0.9	24	1.0	26	1.0	30
26	15	0.8	20	1.0	10	0.5	12	0.6	14	0.6	15	0.6	22
29	10	0.5	14	0.7	8	0.4	10	0.5	9	0.4	12	0.5	17
34	33	1.7	33	1.6	32	1.6	33	1.7	31	1.3	31	1.2	31
37	13	0.7	12	0.6	14	0.7	13	0.7	9	0.4	12	0.4	14
40	14	0.7	13	0.6	14	0.7	13	0.7	10	0.4	12	0.5	15
43	14	0.7	13	0.6	13	0.6	11	0.6	9	0.4	11	0.4	10
49	1	0.1	3	0.2	5	0.2	3	0.2	1	0.1	2	0.1	5
56	0	0.0	0	0.0	7	0.3	5	0.3	4	0.2	6	0.2	4

Table A. 3. Cumulative CH_4 production in AnCoD of TWAS and SSO

	Cumulative CH_4 Production (mL)													
Time (day)	TWAS	SD	SSO	SD	T/SSO 9/1	SD	T/SSO 7/3	SD	T/SSO 1/1	SD	T/SSO 1/9	SD	T/SSO 3/7	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
1	7	0	5	0	9	0	12	1	6	0	7	0	4	0.2
3	35	2	46	2	39	2	70	3	37	2	56	3	36	1.4
4	86	4	99	5	92	5	164	8	83	4	146	8	96	3.8
5	154	8	182	8	177	8	284	14	154	8	296	16	200	8.0
6	199	10	278	13	243	13	373	18	225	11	405	22	323	12.9
7	242	12	373	17	319	17	452	22	345	18	500	27	435	17.4
8	267	13	475	22	369	22	527	26	441	23	582	31	560	22.3
10	299	15	583	27	419	27	631	31	593	30	727	39	710	28.3
12	330	16	685	31	457	31	683	34	739	38	862	46	843	34.5
14	354	17	762	35	489	35	721	35	823	42	922	49	946	37.8
16	377	18	813	37	511	37	761	37	862	44	958	51	979	39.1
19	404	20	859	39	548	39	804	40	899	46	1004	54	1014	40.5
22	425	21	906	42	571	42	834	41	944	48	1052	56	1062	42.4
26	443	22	946	43	586	43	856	42	968	49	1080	58	1098	43.8
29	455	22	974	45	598	45	873	43	985	50	1102	59	1124	44.9
34	493	24	1040	48	645	48	930	46	1040	53	1159	62	1174	46.8
37	508	25	1064	49	666	49	953	47	1057	54	1180	63	1196	47.7
40	524	26	1089	50	686	50	976	48	1075	55	1202	64	1221	48.7
43	541	26	1115	51	705	51	996	45	1091	56	1222	65	1237	45.4
49	542	26	1122	48	712	49	1001	49	1094	55	1225	62	1245	49.7
56	542	26	1122	47	723	50	1010	50	1100	57	1235	66	1252	50.0

Table A. 4. Cumulative methane yield per unit mass of COD added in AnCoD of TWAS and SSO

							Cumulative CH_4 yield (mLCH$_4$/COD added)							
Time (day)	TWAS	SD	SSO	SD	T/SSO 9/1	SD	T/SSO 7/3	SD	T/SSO 1/1	SD	T/SSO 3/7	SD	T/SSO 1/9	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	2	0.1	1	0.1	3	0.2	4	0.2	2	0.1	2	0.1	1	0.1
3	12	0.6	13	0.7	13	0.6	22	1.1	11	0.5	16	0.9	10	0.4
4	30	1.6	27	1.5	31	1.5	51	2.5	25	1.1	42	2.3	27	1.2
5	55	2.8	50	2.7	59	2.9	88	4.3	46	2.1	84	4.6	56	2.4
6	71	3.7	76	4.1	81	4.0	116	5.7	66	3.0	116	6.4	90	3.9
7	86	4.5	103	5.5	107	5.2	141	6.9	102	4.6	143	7.9	121	5.2
8	94	4.9	130	7.0	124	6.1	164	8.0	131	5.9	166	9.1	156	6.7
10	106	5.5	160	8.7	141	6.9	197	9.6	175	7.9	208	11.4	197	8.5
12	117	6.1	188	10.2	153	7.5	213	10.4	219	9.8	246	13.5	234	10.1
14	126	6.5	209	11.3	164	8.0	224	11.0	244	11.0	263	14.5	263	10.5
16	133	6.9	223	12.1	172	8.4	237	11.6	255	11.5	273	15.0	272	11.7
19	143	7.4	236	12.7	184	9.0	250	12.3	266	12.0	287	15.8	282	12.1
22	151	7.8	249	13.4	192	9.4	260	12.7	279	12.6	300	16.5	295	11.3
26	157	8.2	260	13.8	197	9.6	266	13.1	287	12.9	308	17.0	305	13.1
29	161	8.4	268	14.5	201	9.8	272	13.3	292	13.1	315	17.3	312	13.7
34	175	9.1	286	15.4	217	10.6	290	14.2	308	13.9	331	18.2	326	14.0
37	180	9.4	292	15.8	223	11.0	297	14.5	313	14.1	337	18.5	332	14.3
40	186	9.7	299	14.8	230	10.3	304	14.9	318	12.7	343	18.9	339	13.5
43	192	10.2	306	16.5	237	12.2	310	16.1	323	14.5	349	17.2	344	14.8
49	192	9.8	308	15.5	239	11.7	312	15.3	324	13.2	350	16.3	346	15.2
56	192	9.5	308	16.2	243	10.6	315	14.4	326	14.7	353	15.8	348	14.4

Table A. 5. Cumulative methane yield per unit mass of VSS added in AnCoD of TWAS and SSO

Cumulative CH_4 yield ($mLCH_4$/VSS added)														
Time (day)	TWAS	SD	SSO	SD	T/SSO 9/1	SD	T/SSO 7/3	SD	T/SSO 1/1	SD	T/SSO 3/7	SD	T/SSO 1/9	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	4	0.2	1	0.0	5	0.3	8	0.4	4	0.2	5	0.3	3	0.2
3	19	0.9	10	0.4	23	1.2	45	2.2	26	1.3	41	2.0	28	1.3
4	47	2.3	21	0.8	54	2.8	106	5.2	58	2.8	107	5.2	74	3.6
5	85	4.1	39	1.6	104	5.4	183	9.0	107	5.2	217	10.6	154	7.6
6	110	5.4	60	2.4	142	7.4	240	11.9	156	7.6	297	14.6	249	12.2
7	134	6.5	81	3.2	187	9.7	291	14.4	239	11.6	367	18.0	335	16.4
8	148	7.2	102	4.1	216	11.3	339	16.8	306	14.9	427	20.9	431	21.1
10	165	8.0	126	5.0	246	12.8	407	20.1	411	20.0	534	26.2	546	26.8
12	182	8.9	148	5.9	268	14.0	440	21.8	512	24.9	633	31.0	649	31.8
14	196	9.5	165	6.6	287	15.0	465	23.0	571	27.8	677	33.2	728	35.7
16	208	10.1	175	7.0	300	15.6	490	24.3	597	29.1	703	34.5	753	36.9
19	224	10.9	185	7.4	322	16.8	518	25.7	623	30.4	737	36.1	781	38.2
22	235	11.4	196	7.8	335	17.5	538	26.6	654	31.8	772	37.8	817	40.0
26	245	11.9	204	8.1	344	17.9	552	26.5	671	32.7	793	38.8	845	41.4
29	252	12.2	210	8.4	351	18.3	563	27.8	683	33.2	809	39.7	865	42.4
34	273	13.3	225	8.9	379	19.7	600	29.7	721	35.1	851	41.7	903	44.3
37	281	13.7	230	9.1	391	20.4	615	30.4	732	35.7	867	42.5	921	45.1
40	290	15.4	235	9.4	403	21.0	629	32.3	745	36.3	883	43.3	940	46.1
43	299	14.6	241	10.0	414	20.2	642	31.8	756	36.8	897	43.2	952	46.7
49	300	13.8	242	9.9	418	19.8	645	30.5	758	35.4	899	45.4	958	45.1
56	300	15.1	242	9.2	424	21.6	651	32.2	762	37.1	907	44.4	964	47.2

Table A. 6. Cumulative methane yield per unit volume of VSS added in AnCoD of TWAS and SSO

Cumulative CH_4 yield ($mLCH_4$/mL substrate added)														
Time (day)	TWAS	SD	SSO	SD	T/SSO 9/1	SD	T/SSO 7/3	SD	T/SSO 1/1	SD	T/SSO 3/7	SD	T/SSO 1/9	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.10	0.00	0.15	0.01	0.14	0.01	0.22	0.01	0.14	0.01	0.18	0.01	0.12	0.00
3	0.49	0.02	1.38	0.07	0.62	0.03	1.32	0.06	0.83	0.04	1.42	0.07	1.02	0.04
4	1.22	0.05	2.99	0.14	1.45	0.08	3.12	0.15	1.84	0.09	3.70	0.18	2.76	0.10
5	2.18	0.09	5.51	0.27	2.79	0.15	5.38	0.26	3.43	0.17	7.52	0.36	5.74	0.22
6	2.82	0.12	8.40	0.41	3.83	0.21	7.08	0.34	4.99	0.25	10.29	0.49	9.26	0.35
7	3.43	0.14	11.28	0.55	5.03	0.27	8.59	0.41	7.65	0.39	12.72	0.61	12.45	0.47
8	3.78	0.16	14.34	0.70	5.82	0.32	10.00	0.48	9.80	0.50	14.79	0.71	16.02	0.61
10	4.24	0.18	17.63	0.85	6.62	0.36	11.99	0.58	13.16	0.67	18.48	0.88	20.31	0.77
12	4.67	0.20	20.72	1.00	7.21	0.39	12.97	0.62	16.40	0.84	21.90	1.05	24.12	0.92
14	5.02	0.21	23.04	1.12	7.72	0.42	13.69	0.66	18.28	0.93	23.42	1.12	27.08	1.03
16	5.34	0.22	24.57	1.19	8.07	0.44	14.45	0.69	19.14	0.98	24.34	1.16	28.01	1.06
19	5.72	0.24	25.95	1.26	8.65	0.47	15.27	0.73	19.97	1.02	25.51	1.22	29.03	1.10
22	6.02	0.25	27.39	1.33	9.01	0.49	15.84	0.76	20.95	1.07	26.73	1.28	30.39	1.15
26	6.27	0.26	28.60	1.39	9.25	0.51	16.25	0.78	21.50	1.10	27.44	1.31	31.42	1.19
29	6.45	0.27	29.44	1.43	9.43	0.51	16.57	0.80	21.87	1.12	28.02	1.34	32.18	1.22
34	6.99	0.29	31.44	1.52	10.18	0.56	17.67	0.85	23.10	1.18	29.45	1.41	33.60	1.28
37	7.20	0.30	32.15	1.56	10.50	0.57	18.11	0.87	23.47	1.20	30.00	1.48	34.24	1.30
40	7.43	0.31	32.93	1.60	10.83	0.59	18.53	0.89	23.86	1.22	30.56	1.46	34.95	1.33
43	7.67	0.29	33.70	1.63	11.13	0.61	18.91	0.78	24.23	1.24	31.06	1.48	35.42	1.45
49	7.68	0.22	33.91	1.56	11.24	0.52	19.01	0.88	24.28	1.35	31.13	1.39	35.64	1.25
56	7.68	0.32	33.91	1.70	11.40	0.65	19.18	0.92	24.42	1.25	31.39	1.50	35.84	1.36

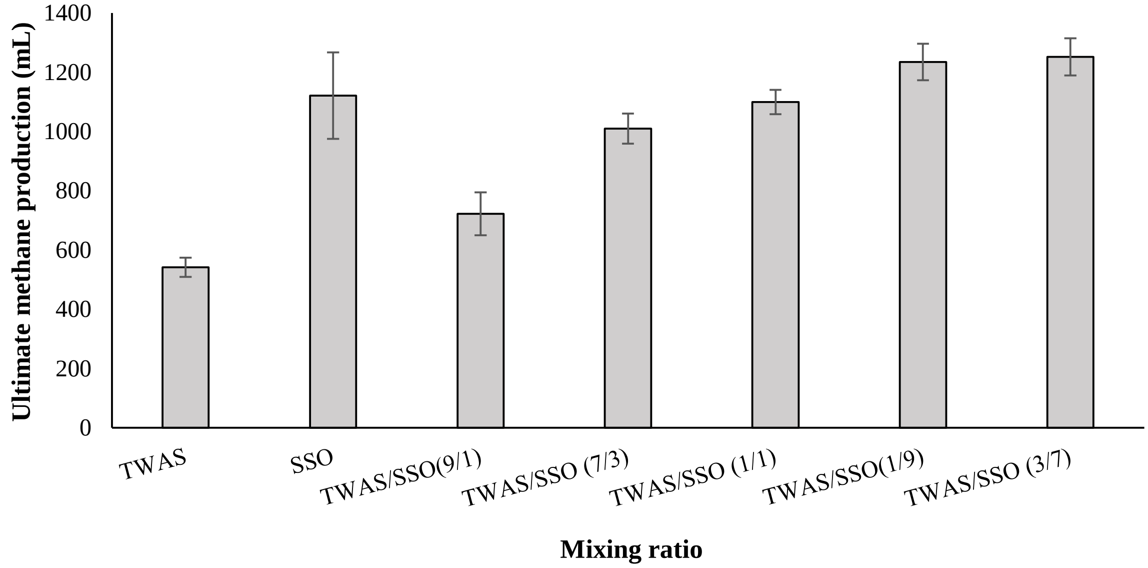


Figure A.1. Ultimate CH_4 production in AnCoD of TWAS and SSO

Table A. 7. Characteristics of raw feedstocks for hydrolysis/acidification in AnCoD of TWAS/SSO

Parameters	Units	SSO		TWAS		Seed	
		MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	110000	7795	40000	2853	16400	444
SCOD	mg/L	44400	100	2360	26	960	19
TSS	mg/L	53833	6252	31450	1006	17033	400
VSS	mg/L	36030	3649	18460	2216	10900	200
TS	mg/L	62187	1025	38810	6859	21450	582
VS	mg/L	43493	761	34477	27	13140	328
Ammonia	mg/L	1138	6	255	30	1495	40
pH	-	5.6	0.006	6.3	0.03	7.2	0.1
Alkalinity	mg CaCO3/L	6800	522	1953	148	3943	465
TN	mg/L	3967	751	2900	400	2025	203
TSN	mg/L	1055	38	420	64	696	112
Total Carbs	mg/L	13495	3580	923	112	545	82
Total Proteins	mg/L	2087	198	2769	298	1635	56
Total Lipids	mg/L	1103	2420	289	27	164	52

Table A. 8. Soluble and particulate COD concentrations (mg/L) over time

Soluble COD concentrations (mg/L) over time							
Time	T/SSO 7/3	T/SSO 1/1	T/SSO 3/7	T/SSO 1/9	T/SSO 9/1	SSO Only	TWAS Only
0	4590	5527	6525	7318	2281	7630	1905
2	4698	6087	6959	7857	2529	8123	2254
4	5036	6527	7233	8216	2727	8416	2377
6	5284	6767	7557	8655	3345	8689	2524
8	5802	7017	8011	8994	3503	9082	2548
10	5990	7487	8275	9353	3961	9355	2597
12	6198	7787	8599	9712	4209	9858	2769
24	6616	8047	8933	10221	4397	10261	2988
48	7534	8427	9337	10540	4715	11094	3087
72	7732	9067	9981	11079	5435	10817	3120

Particulate COD concentrations (mg/L) over time								
Time	T/SSO 7/3	T/SSO 1/1	T/SSO 3/7	T/SSO 1/9	T/SSO 9/1	SSO Only	TWAS Only	
0	24029	24473	24182	24237	23558	13979	23635	
2	23817	24256	23905	24105	23416	13717	22604	1
4	23681	23968	23495	23987	23203	13527	22518	
6	23432	22832	22843	23602	22825	13256	22418	
8	22948	22683	22747	23360	22635	12967	22325	
10	22629	22410	22684	23020	22438	12392	22223	
12	22379	22112	21558	22844	22289	12228	22125	
24	21256	21110	21222	21163	20870	11287	21897	
48	20684	20371	21021	20815	20376	10951	21581	
72	20484	20233	20574	20471	20154	10623	21508	

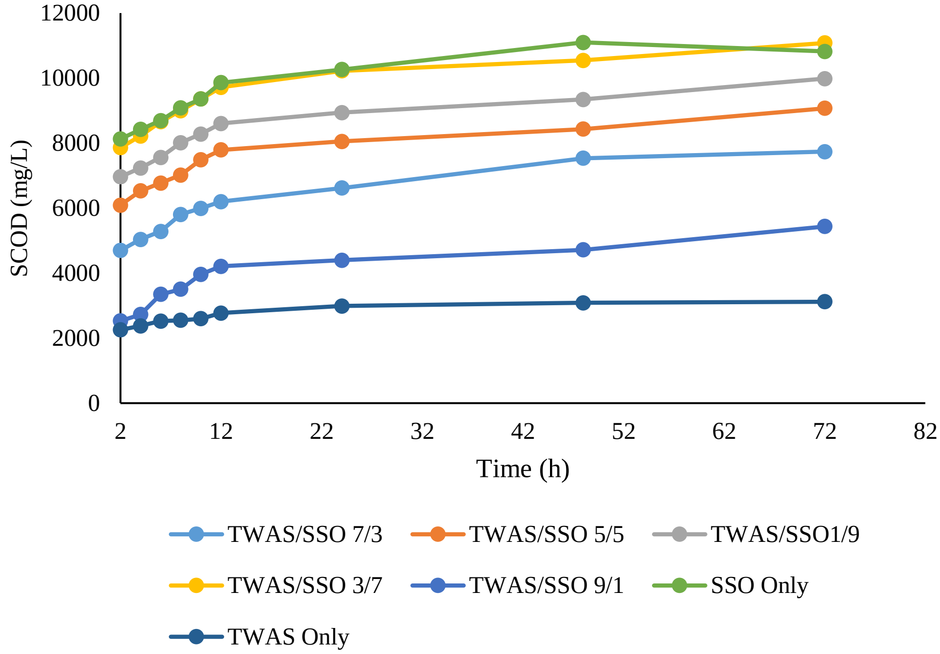


Figure A.2. Concentration of soluble COD over time for different mixing ratios

B. Analytical results for AnCoD of TWAS and manure

Table B.1 Characteristics of raw feedstocks in AnCoD of TWAS and manure

Parameters	Units	Manure		TWAS		Inoculum	
		MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	122833	19553	45600	500	17167	306
SCOD	mg/L	8933	503	2020	265	1250	173
TSS	mg/L	79920	19060	36150	538	15580	459
VSS	mg/L	76998	1789	26710	349	10313	284
TS	mg/L	98727	1035	38053	1832	16590	302
VS	mg/L	85647	1123	27730	1092	10180	286
Ammonia	mg/L	22	3	317	20	497	68
pH	-	6.4	0.1	6.8	0.1	7	0.1
Alkalinity	mg CaCO3/L	5133	902	4820	452	5517	306
Total N	mg/L	2200	100	2883	278	2050	278
Total Soluble N	mg/L	104	15	410	22	747	15
Total Carbs	mg/L	27100	958	1322	104	508	23
Total Proteins	mg/L	5115	244	3958	276	1498	50
Total Lipids	mg/L	1355	54	536	44	205	8

Table B. 2. Cumulative CH_4 production in AnCoD of TWAS and manure

Cumulative methane production (mL)														
time (day)	TWAS	SD	Manure	SD	T/M 9/1	SD	T/M 7/3	SD	T/M 1/1	SD	T/M 3/7	SD	T/M 1/9	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	24	1	86	5	76	3	88	5	100	5	95	5	88	4.39
2	84	4	157	9	172	7	175	9	197	7	188	9	172	8.62
3	135	7	215	12	246	11	237	12	266	12	258	13	238	11.9
4	185	9	273	15	300	13	294	15	331	15	322	16	297	14.8
5	220	11	316	17	341	15	336	17	378	17	373	18	344	17.2
6	256	12	364	20	386	17	382	20	430	20	427	21	396	19.8
8	298	14	434	24	426	19	441	23	496	23	494	24	463	23.1
10	328	16	490	27	462	20	506	26	570	26	562	28	523	26.1
14	363	18	552	30	498	22	562	29	633	29	635	31	587	29.4
16	388	19	597	33	528	23	600	31	675	31	681	33	631	31.6
17	400	19	620	34	547	24	629	33	708	33	710	35	655	32.7
19	417	20	648	36	570	25	650	34	732	34	740	36	680	34
22	434	21	678	37	597	26	694	36	781	36	780	38	713	35.6
25	456	22	713	39	625	27	710	37	799	37	815	40	748	37.4
26	464	22	728	40	636	28	727	38	818	38	835	41	765	38.2
29	478	23	752	41	655	28	749	39	843	39	863	42	790	39.5
30	482	23	757	42	659	29	754	39	848	39	870	43	800	39
33	492	24	775	43	671	29	769	40	865	40	889	44	817	40.8
36	506	24	799	44	690	30	788	41	886	41	914	45	841	42.1
40	521	25	824	45	712	31	811	42	912	42	944	46	871	43.5
46	539	26	851	47	737	32	836	43	941	43	978	48	905	45.2
50	548	27	864	48	750	33	850	44	956	44	995	49	922	46.1
54	555	27	874	48	759	33	859	45	967	42	1007	49	935	46.7
58	559	27	881	48	766	33	866	42	975	45	1017	48	944	47.2
62	563	27	887	49	771	34	872	44	981	41	1023	51	951	45
65	566	27	893	49	776	34	877	46	987	43	1029	50	956	47.8
67	569	28	898	49	780	34	885	45	996	44	1035	52	961	42
72	578	28	907	50	791	34	893	46	1005	42	1049	48	979	48.9
74	584	28	915	50	801	33	903	47	1016	45	1059	49	992	43
78	590	29	922	51	811	35	902	43	1015	47	1069	51	1002	50.1

Table B. 3. Cumulative methane yield per unit mass of COD added in AnCoD of TWAS and manure

Cumulative methane yield (mL CH_4/g COD added)														
time (day)	TWAS	SD	Manure	SD	T/M 9/1	SD	T/M 7/3	SD	T/M 1/1	SD	T/M 3/7	SD	T/M 1/9	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	8	0.3	30	1.6	26	1.2	31	1.4	35	1.3	34	1.8	34	1.2
2	28	1.2	56	2.9	58	2.7	61	2.9	69	2.6	66	3.7	67	2.5
3	45	1.9	77	4.0	84	3.8	82	3.9	93	3.5	91	5.0	91	3.4
4	62	2.7	97	5.0	102	4.7	102	4.8	116	4.4	114	6.3	114	4.2
5	73	3.2	112	5.8	116	5.3	116	5.5	133	5.0	132	7.2	132	4.9
6	85	3.7	130	6.7	131	6.0	132	6.2	151	5.7	151	8.3	152	5.6
8	99	4.3	154	8.0	144	6.6	153	7.2	174	6.6	175	9.6	175	6.5
10	109	4.7	174	9.1	157	7.2	175	8.2	200	7.6	199	10.9	200	7.4
14	121	5.2	196	10.2	169	7.8	195	9.2	222	8.4	224	12.3	225	8.3
16	129	5.5	212	11.0	179	8.2	208	9.8	237	9.0	241	13.2	242	8.9
17	133	5.7	221	11.5	186	8.5	218	10.2	248	9.4	251	13.8	252	9.3
19	139	6.0	230	12.0	193	8.9	225	10.6	257	9.8	262	14.4	263	9.7
22	145	6.2	241	12.6	202	9.3	241	11.3	274	10.4	275	15.2	277	10.2
25	152	6.5	254	13.2	212	9.7	246	11.6	280	10.6	288	15.8	289	10.7
26	154	6.6	259	13.5	216	9.9	252	11.8	287	10.9	295	16.2	296	11.0
29	159	6.8	267	13.9	222	10.2	260	12.2	296	11.2	305	16.8	306	11.3
30	160	6.9	269	14.0	224	10.3	261	12.3	298	10.2	307	16.9	309	11.4
33	164	7.0	276	14.3	227	10.5	267	12.5	303	11.5	314	17.3	316	11.7
36	168	7.2	284	14.8	234	10.8	273	12.8	311	11.8	323	17.8	325	13.3
40	173	7.5	293	15.2	242	11.1	281	13.2	320	12.2	334	18.3	335	12.4
46	179	7.7	303	15.7	250	11.5	290	13.6	330	12.5	345	17.6	347	12.8
50	182	7.8	307	16.0	254	11.7	294	13.8	335	10.7	351	19.3	353	14.1
54	184	7.9	311	16.2	257	11.8	298	14.0	339	12.9	356	17.5	358	13.2
58	186	8.0	314	16.3	260	11.9	300	13.5	342	13.5	359	19.8	361	14.2
62	187	7.5	316	15.7	261	12.0	302	14.2	344	12.9	362	19.3	363	13.4
65	188	8.1	318	16.5	263	11.8	304	14.3	346	13.2	364	20.0	365	12.8
67	189	7.9	320	15.5	265	12.2	307	13.8	349	14.1	366	19.2	368	14.5
72	192	8.3	323	16.8	268	10.9	310	14.6	353	12.8	371	20.4	372	11.7
74	194	7.6	326	15.3	272	11.5	313	13.5	356	13.5	374	18.9	376	13.9
78	196	8.4	328	17.1	275	12.6	313	14.7	356	11.6	378	20.8	379	14.0

Table B. 4. Cumulative methane yield per unit mass of VSS added in AnCoD of TWAS and manure

Cumulative methane yield (mL CH4/g VSS added)														
time (day)	TWAS	SD	Manure	SD	T/M 9/1	SD	T/M 7/3	SD	T/M 1/1	SD	T/M 3/7	SD	T/M 1/9	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	16	0.7	127	5.8	62	2.4	92	4.2	119	5.1	136	4.8	117	4.208
2	58	2.6	233	10.7	142	5.5	182	8.4	237	10.2	270	9.4	229	8.258
3	94	4.2	320	14.7	203	7.9	245	11.3	319	13.7	370	13.0	316	11.38
4	128	5.8	404	18.6	247	9.6	304	14.0	397	17.1	463	16.2	394	14.2
5	153	6.9	468	21.5	281	11.0	348	16.0	453	19.5	536	18.7	458	16.49
6	178	8.0	540	24.9	318	12.4	396	18.2	516	22.2	613	21.5	527	18.98
8	206	9.3	644	29.6	351	13.7	456	21.0	595	25.6	710	24.8	615	22.15
10	227	10.2	727	33.4	380	14.8	524	24.1	683	29.4	807	28.3	695	25.03
14	251	11.3	819	37.7	410	15.6	582	26.8	759	32.6	912	31.9	781	28.11
16	268	12.1	885	40.7	435	16.9	621	28.6	810	34.8	979	34.3	839	30.22
17	277	12.5	919	42.3	451	17.6	651	29.9	849	36.5	1020	35.7	871	31.35
19	289	13.0	961	44.2	469	18.3	673	31.0	878	37.7	1064	37.2	905	32.58
22	301	13.5	1006	45.3	492	19.2	719	33.1	937	40.3	1120	39.2	948	34.13
25	316	14.2	1058	48.7	514	20.1	735	33.8	958	39.1	1170	41.0	995	35.81
26	321	14.5	1079	46.7	524	18.7	753	34.6	981	42.2	1199	42.0	1017	36.63
29	331	14.9	1115	51.3	539	21.0	776	35.7	1011	43.5	1240	43.4	1051	39.2
30	334	13.8	1123	49.5	543	22.4	781	34.2	1017	42.8	1250	43.8	1064	38.3
33	341	15.4	1149	52.9	552	21.5	796	36.6	1038	44.6	1277	44.7	1086	39.1
36	351	15.8	1186	54.5	568	20.3	815	37.5	1063	45.7	1313	46.0	1119	38.6
40	361	16.3	1222	50.6	586	22.9	840	38.6	1094	47.1	1356	44.8	1158	41.7
46	373	14.5	1261	58.0	607	23.7	866	39.8	1129	48.5	1404	49.2	1204	43.33
50	380	17.1	1281	54.5	617	24.1	880	40.5	1147	45.2	1429	50.0	1226	44.15
54	384	16.9	1296	59.6	625	23.8	890	39.3	1160	49.9	1447	48.7	1243	44.76
58	387	15.2	1307	58.3	631	24.6	897	41.3	1169	50.3	1461	51.1	1256	46.3
62	390	17.6	1316	60.5	635	25.2	903	41.5	1177	48.6	1470	52.6	1265	45.52
65	392	16.8	1324	56.4	639	24.9	908	39.7	1184	50.9	1478	49.8	1272	45.78
67	394	17.7	1332	61.3	642	23.5	917	40.9	1195	49.7	1487	51.3	1278	46
72	400	16.7	1345	59.2	651	25.4	925	42.6	1206	51.8	1507	52.7	1302	45.2
74	405	17.5	1357	58.1	660	24.8	935	43.0	1218	47.4	1522	50.2	1319	47.48
78	408	18.4	1368	60.8	667	26.7	934	41.5	1217	50.2	1535	53.7	1333	47.98

Table B. 5. Cumulative methane yield per unit volume of substrate added in AnCoD of TWAS and manure

	Cumulative methane yield (mL CH_4/mL substrate added)													
time (day)	TWAS		Manure		T/M 9/1		T/M 7/3		T/M 1/1		T/M 3/7		T/M 1/9	
0	0	0	0	0	0	0	0	0.00	0	0.00	0	0.00	0	0.00
1	0.3	0.01	2.2	0.10	0.7	0.03	1.1	0.04	1.4	0.08	1.8	0.10	2.0	0.10
2	1.0	0.04	4.0	0.18	1.6	0.10	2.2	0.08	2.9	0.16	3.5	0.19	4.0	0.19
3	1.6	0.06	5.5	0.24	2.2	0.09	3.0	0.11	3.9	0.21	4.8	0.26	5.5	0.26
4	2.2	0.08	7.0	0.31	2.7	0.11	3.7	0.14	4.8	0.26	6.0	0.33	6.9	0.32
5	2.6	0.09	8.1	0.36	3.1	0.12	4.2	0.16	5.5	0.30	6.9	0.38	8.0	0.38
6	3.1	0.11	9.3	0.41	3.5	0.14	4.8	0.18	6.2	0.34	7.9	0.43	9.2	0.43
8	3.5	0.13	11.1	0.49	3.9	0.13	5.5	0.21	7.2	0.40	9.1	0.50	10.8	0.51
10	3.9	0.14	12.6	0.55	4.2	0.16	6.3	0.24	8.3	0.45	10.4	0.57	12.2	0.57
14	4.3	0.16	14.2	0.62	4.5	0.18	7.0	0.27	9.2	0.50	11.8	0.65	13.7	0.64
16	4.6	0.17	15.3	0.67	4.8	0.19	7.5	0.29	9.8	0.54	12.6	0.69	14.7	0.69
17	4.8	0.15	15.9	0.70	5.0	0.15	7.9	0.30	10.3	0.56	13.1	0.72	15.2	0.72
19	5.0	0.18	16.6	0.73	5.2	0.20	8.1	0.31	10.6	0.58	13.7	0.75	15.8	0.74
22	5.2	0.19	17.4	0.77	5.4	0.21	8.7	0.33	11.3	0.62	14.4	0.79	16.6	0.78
25	5.4	0.20	18.3	0.80	5.7	0.22	8.9	0.34	11.6	0.64	15.1	0.83	17.4	0.82
26	5.5	0.19	18.7	0.82	5.8	0.23	9.1	0.35	11.9	0.65	15.5	0.85	17.8	0.84
29	5.7	0.20	19.3	0.85	6.0	0.23	9.4	0.36	12.2	0.67	16.0	0.88	18.4	0.86
30	5.7	0.21	19.4	0.85	6.0	0.27	9.4	0.36	12.3	0.68	16.1	0.89	18.6	0.87
33	5.9	0.21	19.9	0.87	6.1	0.24	9.6	0.41	12.5	0.69	16.5	0.91	19.0	0.89
36	6.0	0.20	20.5	0.90	6.3	0.20	9.8	0.37	12.8	0.71	16.9	0.93	19.6	0.92
40	6.2	0.22	21.1	0.93	6.5	0.25	10.1	0.39	13.2	0.73	17.5	0.96	20.3	0.95
46	6.4	0.23	21.8	0.96	6.7	0.26	10.5	0.40	13.6	0.75	18.1	1.00	21.0	0.99
50	6.5	0.21	22.2	0.97	6.8	0.29	10.6	0.36	13.9	0.76	18.4	1.01	21.4	1.01
54	6.6	0.24	22.4	0.99	6.9	0.27	10.7	0.39	14.0	0.77	18.7	1.03	21.7	0.99
58	6.7	0.33	22.6	0.88	7.0	0.25	10.8	0.41	14.1	0.78	18.8	1.04	22.0	1.03
62	6.7	0.24	22.7	1.22	7.0	0.27	10.9	0.45	14.2	0.78	19.0	1.04	22.1	1.04
65	6.7	0.31	22.9	1.01	7.1	0.22	11.0	0.42	14.3	0.79	19.1	1.05	22.2	1.05
67	6.8	0.24	23.0	1.35	7.1	0.24	11.1	0.36	14.4	0.79	19.2	0.98	22.3	0.97
72	6.9	0.25	23.3	0.98	7.2	0.28	11.2	0.42	14.6	0.80	19.4	1.07	22.8	1.07
74	7.0	0.29	23.5	1.23	7.3	0.25	11.3	0.39	14.7	0.81	19.6	1.08	23.1	1.08
78	7.0	0.25	23.6	1.04	7.4	0.29	11.3	0.43	14.7	0.81	19.8	1.09	23.3	1.10

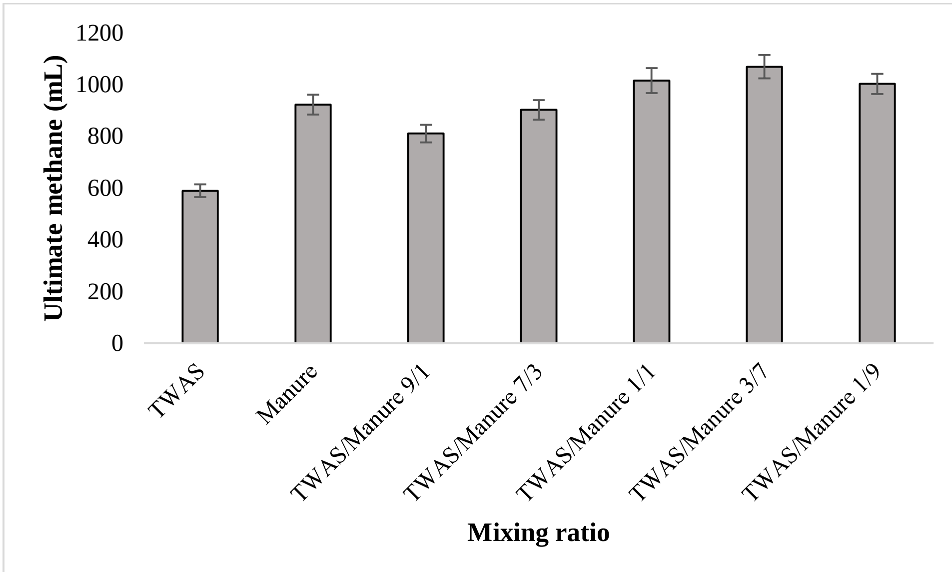


Figure B. 1. Ultimate methane production in AnCoD of TWAS and manure

Table B. 6. Characteristics of the raw feedstocks for hydrolysis/acidification in AnCoD of TWAS and manure

Parameters	Units	Manure		TWAS		Seed	
		MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	110433	4422	41200	1236	16652	552
SCOD	mg/L	6560	228	2161	65	1255	50
TSS	mg/L	52600	2530	37254	1118	15112	604
VSS	mg/L	42800	2040	26521	796	10067	403
TS	mg/L	68040	3202	39198	1176	36882	1475
VS	mg/L	58520	2526	28562	857	26874	1075
Ammonia	mg/L	13	1	327	10	307	12
pH	-	6.5	0.2	6.8	0.204	6	0.2
Alkalinity	mg CaCO3/L	7689	384	4964	149	4671	187
TN	mg/L	1435	72	2969	89	2794	112
TSN	mg/L	68	3	422	13	397	16
Total Carbs	mg/L	27116	1355.8	1327	40	503	20
Total Proteins	mg/L	5123	256.15	3978	119	1510	60
Total Lipids	mg/L	1360	68	530	16	201	8

Table B. 7. Measured and theoretical VFAs concentrations over time

Measured VFAs over time							
	Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5	Mixture 6	Mixture 7
Time (hr)	TWAS/Manure 7/3	TWAS/Manure 1/1	TWAS/Manure 3/7	TWAS/Manure 1/9	TWAS/Manure 9/1	Manure Only	TWAS Only
0	584	686	718	790	581	810	432
6	596	719	793	831	593	849	458
12	786	892	827	958	640	974	504
24	912	1105	971	1230	681	1141	514
48	942	1131	1299	1378	735	1366	528
72	965	1175	1530	1491	775	1396	542
Theoretical VFAs over time							
Time (hr)	TWAS/Manure 7/3	TWAS/Manure 1/1	TWAS/Manure 3/7	TWAS/Manure 1/9	TWAS/Manure 9/1	Manure Only	TWAS Only
0	545	621	697	772	470	810	432
6	575	654	732	810	497	849	458
12	645	739	833	927	551	974	504
24	702	827	953	1078	576	1141	514
48	780	947	1115	1283	612	1366	528
72	798	969	1140	1311	627	1396	542

Table B. 8. Soluble and particulate COD concentrations over time

Soluble COD concentrations (mg/L) over time							
Time (hr)	TWAS/Manure 7/3	TWAS/Manure 5/5	TWAS/Manure 3/7	TWAS/Manure 1/9	TWAS/Manure 9/1	Manure only	TWAS only
0	1880	1920	1611	1940	1929	1628	2181
2	1920	1960	1817	2020	1972	1661	2254
4	2040	2100	1868	2060	2471	1744	2377
6	2260	2260	2005	2180	2536	1861	2524
8	2380	2360	2159	2300	2644	1960	2548
10	2420	2480	2228	2360	2904	1977	2597
12	2540	2560	2451	2400	2969	2010	2769
24	2780	2580	2622	2540	3099	2126	2988
48	2920	2940	2896	2780	3208	2193	3087
72	3990	3690	4190	4020	3860	3530	3120
Particulate COD concentrations (mg/L) over time							
Time (hr)	TWAS/Manure 7/3	TWAS/Manure 5/5	TWAS/Manure 3/7	TWAS/Manure 1/9	TWAS/Manure 9/1	Manure only	TWAS only
0	23155	22556	22486	21637	24388	21836	23635
2	23015	22326	22467	21427	24545	21582	23115
4	22895	22156	22219	21257	24186	21489	22696
6	22675	22215	22081	21227	23712	21223	22468
8	22555	22050	21527	20787	23893	21010	22276
10	22515	21690	21399	20297	23543	20527	22083
12	22485	21310	21016	20127	23228	20324	21945
24	21155	20496	19691	19787	22801	20117	21697
48	20615	20336	19300	19187	22203	19856	21563
72	20445	20186	19056	19107	22177	19434	21508

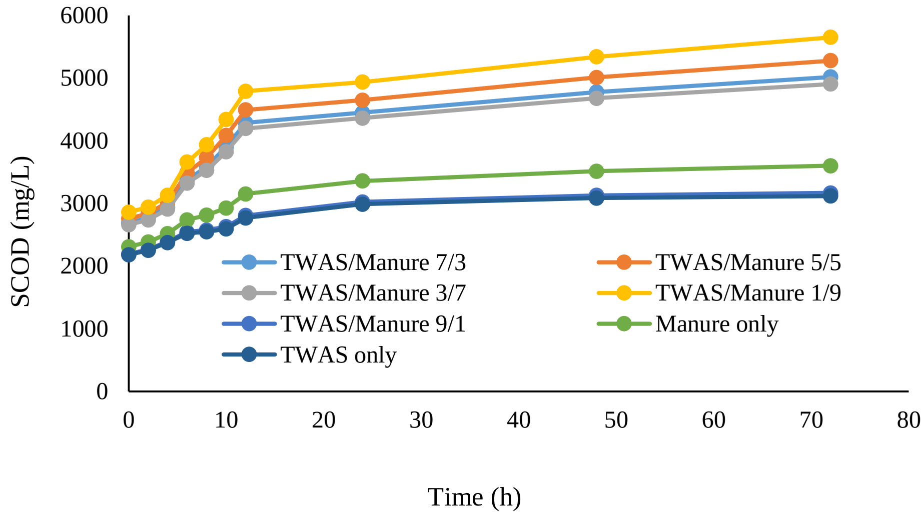


Figure B.2. Concentration of soluble COD over time at different mixing ratios of TWAS and manure

C. Analytical results for AnCoD of manure and SSO

Table C. 1. Characteristics of raw feedstocks in AnCoD of manure and SSO

Parameters	Units	Manure		SSO		Inoculum	
		MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	105120	4795	115163	6090	17980	400
SCOD	mg/L	44116	489	42932	92	722	16
TSS	mg/L	54380	2252	62125	3063	15128	330
VSS	mg/L	46420	2649	45584	2283	11687	160
TS	mg/L	69785	3025	67987	1011	16160	503
VS	mg/L	58590	2761	48535	682	12380	308
Ammonia	mg/L	20	68.0	1073	5.87	745	36
pH		6.8	0.030	5.8	0.050	7.2	0.05
Alkalinity	mg CaCO3/L	4924	522	6176	339	4186	460
TN	mg/L	2510	751	3498	202	1940	186
TSN	mg/L	105.00	38	1046	21	716	93
Total Carbs	mg/L	28796	3580	13495	687	453	68
Total Proteins	mg/L	5930	198	2087	98	2598	38
Total Lipids	mg/L	1400	2420	1103	92	225	43

Table C. 2. Average CH_4 measurements in AnCoD of manure and SSO

Average CH_4 Measurement (ml)														
Time (day)	Manure	SD	SSO	SD	M/SSO 9/1	SD	M/SSO 7/3	SD	M/SSO 5/5	SD	M/SSO 1/9	SD	M/SSO 3/7	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	44	1.7	24	1.0	28	1.2	33	1.5	32	1.7	30	1.6	25	1.1
2	47	1.8	59	2.5	69	3.0	59	2.7	68	3.7	70	3.6	64	2.7
3	66	2.5	102	4.3	118	5.1	72	3.3	92	5.0	104	5.4	110	4.7
4	66	2.5	90	3.8	104	4.5	64	3.0	74	4.0	82	4.3	93	4.0
5	75	2.9	103	4.3	119	5.1	74	3.4	88	4.7	94	4.9	106	4.6
6	90	3.4	111	4.6	128	5.5	108	4.9	111	6.0	117	6.1	118	5.1
7	45	1.7	84	3.5	97	4.2	85	3.9	89	4.8	89	4.6	95	4.1
8	44	1.7	65	2.7	75	3.2	57	2.6	58	3.1	58	3.0	68	2.9
9	40	1.5	70	2.9	81	3.5	56	2.6	56	3.0	54	2.8	67	2.9
11	51	2.0	70	2.9	81	3.5	64	3.0	65	3.5	60	3.1	69	3.0
12	32	1.2	33	1.4	38	1.6	40	1.8	38	2.0	34	1.8	33	1.4
14	33	1.3	30	1.3	35	1.5	48	2.2	48	2.6	37	1.9	34	1.5
15	23	0.9	17	0.7	19	0.8	28	1.3	24	1.3	21	1.1	19	0.8
16	23	0.9	12	0.5	14	0.6	22	1.0	17	0.9	14	0.7	13	0.6
17	21	0.8	14	0.6	16	0.7	19	0.9	15	0.8	13	0.7	11	0.5
18	16	0.6	14	0.6	16	0.7	21	0.9	17	0.9	14	0.7	11	0.5
20	27	1.0	16	0.7	19	0.8	31	1.4	25	1.4	22	1.1	18	0.8
21	14	0.5	11	0.5	13	0.6	23	1.0	18	0.9	14	0.7	12	0.5
22	13	0.5	11	0.5	13	0.6	15	0.7	12	0.7	10	0.5	9	0.4
23	12	0.5	9	0.4	11	0.5	14	0.6	11	0.6	9	0.5	7	0.3
24	12	0.5	9	0.4	10	0.4	14	0.7	11	0.6	9	0.5	7	0.3
25	13	0.5	9	0.4	10	0.4	13	0.6	12	0.7	12	0.6	10	0.4
26	16	0.6	10	0.4	11	0.5	13	0.6	12	0.7	9	0.5	7	0.3
28	9	0.3	11	0.5	13	0.5	19	0.9	20	1.1	15	0.8	12	0.5
29	8	0.3	7	0.3	9	0.4	14	0.6	11	0.6	10	0.4	8	0.4
30	6	0.2	7	0.3	9	0.4	13	0.6	11	0.6	9	0.5	8	0.3
31	7	0.3	6	0.3	7	0.3	7	0.3	5	0.3	5	0.2	3	0.1
32	7	0.3	9	0.4	10	0.4	11	0.5	9	0.5	9	0.5	5	0.2
35	10	0.4	10	0.4	12	0.5	21	0.9	18	0.9	15	0.8	12	0.5
37	10	0.4	13	0.5	15	0.6	17	0.8	21	1.1	11	0.6	23	1.0
40	22	0.8	10	0.4	12	0.5	19	0.9	17	0.9	15	0.8	12	0.5
43	10	0.4	6	0.2	7	0.3	10	0.5	9	0.5	8	0.4	6	0.3
46	3	0.1	3	0.1	4	0.2	9	0.3	8	0.4	7	0.4	6	0.2
49	2	0.1	3	0.1	3	0.1	8	0.4	8	0.4	7	0.3	6	0.1
52	4	0.2	4	0.2	5	0.2	7	0.3	7	0.4	6	0.3	5	0.2

*M: manure

Table C. 3. Cumulative CH_4 production in AnCoD of manure and SSO

Cumulative CH_4 production (mL)														
Time (day)	Manure	SD	SSO	SD	M*/SSO 9/1	SD	M/SSO 7/3	SD	M/SSO 5/5	SD	M/SSO 1/9	SD	M/SSO 3/7	SD
0	0	0	0	0	0	0	0	0	0	0.0	0	0.0	0	0.0
1	44	2.0	24	1.3	28	1.3	33	1.8	32	1.5	30	1.3	25	1.0
2	90	4.1	83	4.6	93	4.5	93	5.0	101	4.5	100	4.2	89	3.4
3	156	7.0	186	10.2	206	9.9	164	8.9	193	8.7	204	8.6	199	7.6
4	222	10.0	276	15.2	305	14.6	229	12.3	267	12.0	287	12.0	291	11.1
5	297	13.4	379	20.8	418	20.1	303	16.4	355	16.0	381	16.0	397	15.1
6	387	17.4	489	26.9	540	25.9	410	22.2	466	21.0	498	20.9	516	19.6
7	432	19.4	573	31.5	632	30.3	496	26.8	554	24.9	587	24.7	611	23.2
8	476	21.4	638	35.1	703	33.7	553	29.9	612	27.5	645	27.1	679	25.8
9	516	23.2	708	38.9	780	37.5	609	32.9	668	30.0	699	29.4	746	28.3
11	567	25.5	778	42.8	857	41.1	673	36.3	732	33.0	759	31.9	815	31.0
12	599	26.9	810	44.6	893	42.9	713	38.5	770	34.7	793	33.3	849	32.3
14	632	28.4	840	46.2	926	44.4	761	41.1	818	36.8	830	34.9	883	33.5
15	654	29.5	857	47.1	944	45.3	789	42.6	842	37.9	851	35.7	902	34.3
16	677	30.5	870	47.8	958	46.0	811	43.8	859	38.7	866	36.4	915	34.8
17	698	31.4	883	48.6	973	46.7	829	44.8	875	39.4	878	36.9	926	35.2
18	714	32.1	897	49.3	987	47.4	850	45.9	892	40.1	893	37.5	937	35.6
20	741	33.3	913	50.2	1001	48.0	881	47.6	917	41.3	914	38.4	955	36.3
21	755	34.0	924	50.8	1021	49.0	904	48.8	934	42.0	929	39.0	967	36.8
22	768	34.5	936	51.5	1035	49.7	919	49.6	947	42.6	939	39.4	976	37.1
23	780	35.1	945	52.0	1045	50.2	933	50.4	957	43.1	948	39.8	984	36.3
24	792	35.6	954	52.4	1055	50.6	947	51.1	968	43.6	957	40.2	991	37.6
25	804	36.2	962	52.9	1065	51.1	960	51.9	980	44.1	969	40.7	1001	38.0
26	820	36.9	972	53.5	1076	51.6	974	52.6	992	44.7	978	41.1	1008	35.4
28	829	37.3	983	54.1	1088	52.2	993	53.6	1013	45.6	993	41.7	1020	38.8
29	837	37.7	991	54.5	1099	52.7	1007	54.4	1024	46.1	1003	42.1	1029	39.1
30	844	38.0	998	54.9	1110	53.3	1019	55.0	1035	46.6	1012	42.5	1036	40.6
31	851	38.3	1004	55.2	1120	53.7	1027	55.5	1040	46.8	1017	42.7	1039	39.5
32	858	38.6	1013	55.7	1132	54.3	1038	56.0	1049	47.2	1026	43.1	1044	38.4
35	867	39.0	1023	56.3	1143	54.9	1058	57.1	1067	48.0	1041	43.7	1056	39.7
37	877	39.5	1036	57.0	1157	55.5	1075	58.1	1087	48.9	1052	44.2	1080	41.0
40	899	40.5	1046	57.6	1168	56.1	1094	59.1	1104	49.7	1067	44.8	1092	40.2
43	910	40.9	1052	57.9	1175	56.4	1104	59.6	1114	50.1	1076	43.8	1098	41.7
46	912	41.1	1056	58.1	1179	56.6	1113	60.1	1121	49.2	1082	45.5	1104	42.0
49	915	41.2	1059	58.2	1182	56.7	1121	58.3	1129	50.8	1089	44.3	1110	43.5
52	919	41.3	1063	58.5	1186	56.9	1129	61.0	1136	51.1	1095	46.0	1115	42.4

*M: manure

Table C. 4. Cumulative CH_4 production per mass of COD added in AnCoD of manure and SSO

Cumulative CH_4 yield (mL CH4/g COD added)														
Time (day)	Manure	SD	SSO	SD	M*/SSO 9/1	SD	M/SSO 7/3	SD	M/SSO 5/5	SD	M/SSO 1/9	SD	M/SSO 3/7	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	14	0.6	7	0.3	9	0.3	10	0.4	9	0.3	9	0.5	7	0.3
2	28	1.2	24	0.9	29	1.1	28	1.0	29	1.0	30	1.5	25	0.9
3	49	2.0	53	2.0	63	2.5	50	1.8	56	2.0	61	3.1	56	2.0
4	69	2.9	79	3.0	93	3.7	70	2.4	77	2.7	86	4.4	82	3.0
5	93	3.9	108	4.1	128	5.1	92	3.2	103	3.6	114	5.8	112	4.0
6	121	5.1	140	5.3	165	6.6	125	4.4	135	4.7	149	7.6	146	5.3
7	135	5.7	163	6.2	193	7.7	151	5.3	161	5.6	175	8.9	173	6.2
8	149	6.2	182	6.9	215	8.6	169	5.9	178	6.2	193	9.8	192	6.9
9	161	6.8	202	7.7	239	9.6	186	6.5	194	5.8	209	10.6	211	7.6
11	177	7.4	222	8.4	262	10.5	205	7.2	213	7.4	226	11.5	231	8.3
12	187	7.9	231	8.8	273	10.6	217	7.6	224	7.8	237	12.1	240	8.6
14	197	8.3	240	9.1	283	11.3	232	8.1	238	8.3	248	11.6	250	9.0
15	204	7.5	244	9.3	289	10.4	241	8.6	245	7.6	254	13.0	255	8.2
16	211	8.9	248	9.4	293	11.7	247	7.7	250	8.7	258	12.2	259	9.3
17	218	9.2	252	10.1	298	10.3	253	8.9	254	8.9	262	13.4	262	9.8
18	223	9.4	256	9.7	302	11.2	259	9.1	259	9.1	266	12.3	265	8.6
20	231	8.6	260	9.9	306	12.3	269	9.4	266	8.3	273	13.9	270	7.7
21	236	9.9	264	10.0	312	12.5	276	8.7	271	9.5	277	14.1	274	8.9
22	240	10.1	267	10.1	317	12.7	280	9.8	275	8.6	280	13.2	276	9.9
23	244	9.7	269	9.4	320	11.6	285	10.0	278	9.7	283	14.4	278	9.3
24	247	10.4	272	10.3	323	12.9	289	10.6	281	9.8	286	13.6	280	9.1
25	251	10.6	274	9.7	326	13.0	293	10.3	285	10.0	289	14.7	283	10.5
26	256	10.8	277	10.5	329	13.2	297	9.8	288	9.1	292	12.9	285	9.3
28	259	10.9	280	9.8	333	13.3	303	10.6	294	10.3	296	15.1	289	10.4
29	262	11.0	283	10.7	336	13.4	307	10.7	297	9.4	299	14.3	291	9.5
30	264	10.4	285	9.3	340	13.6	311	10.9	301	10.5	302	15.4	293	10.6
31	266	11.2	286	10.9	343	12.7	313	11.0	302	9.6	303	13.5	294	9.6
32	268	10.7	289	12.3	346	13.9	317	9.7	305	10.7	306	15.6	295	10.6
35	271	11.4	292	11.1	350	14.0	323	11.3	310	9.8	311	13.8	299	9.8
37	274	11.5	295	10.5	354	13.8	328	9.8	316	11.1	314	14.9	306	11.0
40	281	11.8	298	11.3	358	14.5	334	11.7	321	9.8	318	16.2	309	10.1
43	284	11.9	300	10.2	360	13.2	337	10.2	323	11.3	321	15.1	311	11.2
46	285	11.6	301	11.4	361	14.4	340	11.9	326	10.5	323	16.5	312	10.4
49	286	10.2	302	10.6	362	13.1	342	10.9	328	9.2	325	14.6	314	9.7
52	287	12.1	303	11.5	363	14.5	344	11.6	330	11.5	327	16.7	316	10.6

*M: manure

Table C. 5. Cumulative CH_4 production per mass of VSS added in AnCoD of manure and SSO

	Cumulative CH_4 yield (mL CH4/g VSS added)													
Time (day)	Manure	SD	SSO	SD	M*/SSO 9/1	SD	M/SSO 7/3	SD	M/SSO 5/5	SD	M/SSO 1/9	SD	M/SSO 3/7	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
1	31	1.4	18	0.6	20	0.8	25	0.9	23	0.8	23	0.9	19	0.9
2	64	2.9	62	2.2	67	2.8	69	2.5	73	2.5	76	2.9	65	3.1
3	111	5.0	139	4.9	147	6.2	121	4.5	139	4.9	155	5.9	146	6.9
4	158	7.1	206	7.2	218	9.1	169	6.2	193	6.8	218	8.3	214	10.1
5	212	9.5	282	9.9	298	12.5	224	8.3	257	9.0	289	11.0	292	13.7
6	276	12.4	365	12.8	385	16.2	303	11.2	337	11.8	379	14.4	379	17.8
7	308	13.9	428	15.0	451	19.0	366	13.6	401	14.0	446	17.0	448	21.1
8	339	15.2	476	16.7	502	21.1	408	15.1	443	15.5	491	18.6	499	23.4
9	367	16.5	528	18.5	557	23.4	450	16.6	483	16.9	532	20.2	548	25.8
11	404	18.2	580	20.3	612	25.7	497	18.4	530	18.5	577	21.9	599	28.2
12	426	19.2	605	21.2	638	26.8	526	19.5	557	19.5	603	22.9	624	29.3
14	450	20.3	627	21.9	661	27.8	562	20.8	592	20.7	631	24.0	648	30.5
15	466	21.0	640	22.4	674	28.3	583	21.6	609	21.3	647	24.6	662	31.1
16	482	21.7	649	22.7	684	28.7	599	22.2	622	21.8	658	23.7	672	31.6
17	497	20.5	659	22.1	695	28.2	613	23.7	633	19.3	668	25.4	680	29.5
18	508	22.9	669	23.4	705	29.6	628	23.2	645	22.6	679	25.8	689	32.4
20	528	23.7	681	23.8	715	30.0	651	24.1	663	21.5	695	26.4	702	33.0
21	538	24.2	690	24.1	729	29.6	668	24.7	676	23.7	706	26.8	710	31.9
22	547	24.6	698	24.4	739	31.1	679	25.1	685	24.0	714	27.1	717	33.7
23	555	25.0	705	23.5	747	30.4	689	25.5	693	23.2	721	25.8	722	34.0
24	564	24.4	711	24.9	753	31.6	700	24.2	700	24.5	728	27.7	728	34.2
25	573	25.8	718	25.1	760	30.9	709	26.2	709	24.8	737	28.0	735	33.5
26	584	26.3	725	25.4	768	32.3	719	25.6	718	25.1	744	26.8	740	34.8
28	591	24.6	734	24.7	777	32.6	733	27.1	733	24.6	755	28.7	749	35.2
29	596	26.8	739	25.9	785	33.0	743	26.5	741	25.9	763	29.0	755	34.5
30	601	25.5	745	25.1	792	33.3	753	27.9	749	26.2	770	29.2	761	35.8
31	606	27.3	749	26.2	800	33.6	758	28.1	753	25.7	773	27.9	763	35.9
32	611	26.5	756	24.4	808	32.9	766	27.4	759	26.6	780	29.6	767	34.9
35	618	27.8	763	26.7	816	34.3	782	28.9	772	25.3	792	30.1	776	36.5
37	625	28.1	773	27.1	826	34.7	794	28.4	787	27.5	800	28.4	793	37.3
40	641	28.8	781	27.3	835	35.1	808	29.9	799	28.0	811	30.8	802	36.8
43	648	29.2	785	255.0	839	34.2	815	30.2	806	26.2	818	29.3	807	37.9
46	650	27.9	788	27.6	842	35.4	822	30.4	811	28.4	823	31.3	811	38.1
49	652	28.3	790	25.6	844	34.5	828	29.7	817	27.6	828	30.5	815	37.3
52	654	29.4	793	27.8	847	35.6	834	30.8	822	28.8	833	31.6	819	38.5

*M: manure

Table C. 6. Cumulative CH_4 production per volume of substrate added in AnCoD of manure and SSO

	Cumulative CH_4 yield (mL CH4/mL substrate added)													
Time (day)	Manure	SD	SSO	SD	M*/SSO 9/1	SD	M/SSO 7/3	SD	M/SSO 5/5	SD	M/SSO 1/9	SD	M/SSO 3/7	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1.45	0.06	1.4	0.05	1.0	0.04	1.4	0.06	1.5	0.07	1.6	0.07	1.4	0.08
2	3.02	0.12	4.9	0.16	3.3	0.14	3.9	0.18	4.6	0.21	5.3	0.22	5.0	0.27
3	5.21	0.20	10.9	0.35	7.3	0.31	6.8	0.32	8.8	0.40	10.8	0.45	11.0	0.61
4	7.40	0.29	16.2	0.52	10.9	0.46	9.5	0.44	12.1	0.56	15.1	0.63	16.2	0.89
5	9.90	0.39	22.3	0.71	14.9	0.63	12.6	0.58	16.1	0.74	20.0	0.84	22.1	1.21
6	12.91	0.50	28.8	0.92	19.3	0.81	17.1	0.79	21.2	0.97	26.2	1.10	28.7	1.58
7	14.41	0.56	33.7	1.08	22.6	0.95	20.7	0.95	25.2	1.16	30.9	1.30	33.9	1.87
8	15.86	0.62	37.5	1.20	25.1	1.05	23.0	1.06	27.8	1.28	34.0	1.43	37.7	2.07
9	17.19	0.67	41.7	1.33	27.9	1.17	25.4	1.17	30.3	1.40	36.8	1.55	41.4	2.28
11	18.90	0.74	45.7	1.46	30.6	1.29	28.0	1.29	33.3	1.53	39.9	1.88	45.3	1.94
12	19.96	0.78	47.7	1.53	31.9	1.34	29.7	1.37	35.0	1.61	41.8	1.75	47.2	2.59
14	21.06	0.82	49.4	1.58	33.1	1.39	31.7	1.46	37.2	1.71	43.7	2.03	49.0	1.97
15	21.81	0.95	50.4	1.61	33.7	1.42	32.9	1.51	38.3	1.76	44.8	1.88	50.1	2.76
16	22.57	0.88	51.2	1.64	34.2	1.44	33.8	1.55	39.1	1.80	45.6	1.91	50.8	1.80
17	23.27	0.91	52.0	1.66	34.7	1.46	34.6	1.59	39.8	1.83	46.2	1.94	51.5	2.83
18	23.79	0.83	52.8	1.69	35.2	1.48	35.4	1.63	40.5	1.86	47.0	1.97	52.1	1.86
20	24.69	0.96	53.7	1.72	35.7	1.50	36.7	1.69	41.7	1.92	48.1	2.02	53.1	2.92
21	25.16	0.98	54.4	1.74	36.5	1.53	37.7	1.73	42.5	1.95	48.9	2.05	53.7	1.96
22	25.59	1.00	55.0	1.76	37.0	1.55	38.3	1.76	43.0	1.98	49.4	2.08	54.2	2.98
23	25.99	1.01	55.6	1.78	37.3	1.57	38.9	1.79	43.5	2.00	49.9	2.75	54.6	3.01
24	26.39	9.83	56.1	1.79	37.7	1.58	39.5	1.82	44.0	2.02	50.4	2.12	55.0	2.93
25	26.81	1.05	56.6	1.81	38.0	1.60	40.0	1.84	44.6	2.05	51.0	2.53	55.6	3.06
26	27.35	9.87	57.2	1.83	38.4	1.61	40.6	1.87	45.1	2.08	51.5	2.16	56.0	2.98
28	27.65	1.08	57.8	1.85	38.8	1.63	41.4	1.90	46.0	2.12	52.3	1.92	56.7	3.12
29	27.91	1.09	58.3	1.86	39.2	1.65	41.9	1.93	46.5	2.14	52.8	2.22	57.1	2.94
30	28.13	1.10	58.7	1.88	39.6	1.66	42.5	1.95	47.0	1.98	53.3	1.94	57.6	3.17
31	28.36	1.11	59.1	1.89	40.0	1.68	42.8	1.97	47.3	2.17	53.5	2.25	57.7	2.98
32	28.58	1.11	59.6	1.91	40.4	1.70	43.2	1.99	47.7	1.92	54.0	1.27	58.0	3.19
35	28.92	9.83	60.2	1.93	40.8	1.71	44.1	2.03	48.5	2.23	54.8	2.30	58.7	3.23
37	29.25	1.14	60.9	1.95	41.3	1.74	44.8	2.06	49.4	1.72	55.4	1.33	60.0	2.30
40	29.98	1.17	61.6	1.97	41.7	1.75	45.6	2.10	50.2	2.31	56.2	2.36	60.7	3.34
43	30.32	1.18	61.9	1.98	42.0	1.76	46.0	2.12	50.6	1.63	56.6	1.83	61.0	2.36
46	30.41	1.19	62.1	1.99	42.1	1.77	46.4	2.13	51.0	2.34	57.0	2.39	61.3	3.37
49	30.49	1.23	62.3	1.99	42.2	1.77	46.7	2.15	51.3	1.36	57.3	1.94	61.6	3.39
52	30.63	1.19	62.5	2.00	42.4	1.78	47.0	2.16	51.6	2.37	57.6	2.42	61.9	2.41

*M: manure

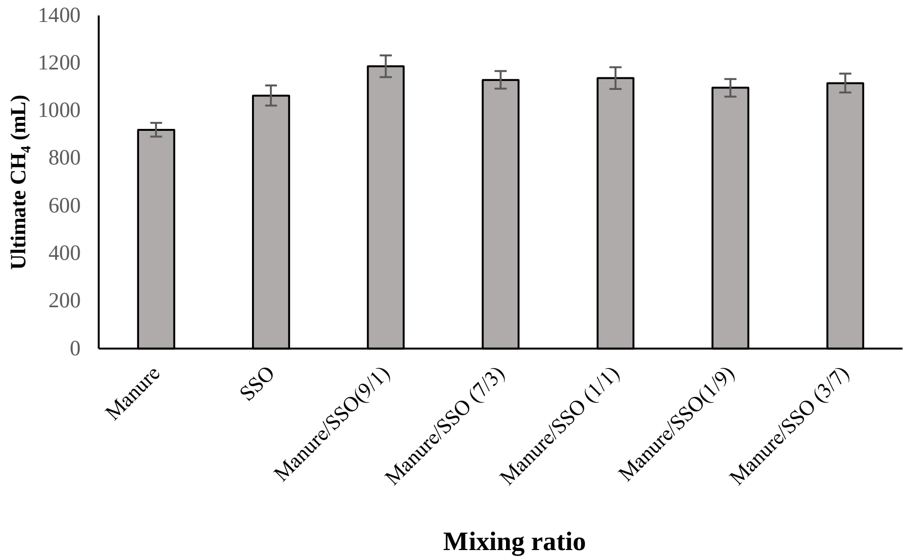


Figure C. 1. Ultimate methane production in AnCoD of TWAS and manure

Table C. 7. Characteristics of the raw feedstocks for hydrolysis/acidification of manure and SSO

Parameters	Units	Manure		SSO		Inoculum	
		MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	101125	4551	115163	4607	17100	400
SCOD	mg/L	10480	472	42932	1717	986	16
TSS	mg/L	54600	2457	47112	1884	17143	330
VSS	mg/L	32115	1445	39940	1598	11200	160
TS	mg/L	68540	3084	77987	3119	21870	503
VS	mg/L	58520	2633	63537	2541	13380	308
Ammonia	mg/L	24.3	1	1396	55.84	1505	36
pH	-	6.7	0.302	5.8	0.2	7.2	0.1
Alkalinity	mg CaCO3/L	7224	325	6720	269	3986	460
TN	mg/L	1740	78	4198	167.92	2048	186
TSN	mg/L	107	5	1146	46	716	93
Total Carbs	mg/L	26796	1206	14125	565	595	68
Total Proteins	mg/L	5408	243	2156	86.24	1680	38
Total Lipids	mg/L	1703	77	1468	59	168	93

Table C. 8. Measured and theoretical VFAs over time in hydrolysis/acidification of manure and SSO

Measured VFAs concentrations (mg/L)over time							
	Mixture 1	Mixture 2	Mixture 3	Mixture 4	Mixture 5	Mixture 6	Mixture 7
Time (hr)	Manure	SSO	Manure/SSO 9/1	Manure/SSO 7/3	Manure/SSO 5/5	Manure/SSO 3/7	Manure/SSO 1/9
0	668	635	646	649	666	740	722
6	783	790	661	688	749	861	803
12	803	1000	864	72	855	917	973
24	1119	1178	983	843	1092	1054	1178
48	1204	1365	1025	975	1143	1299	2756
72	1114	1102	1227	1237	1258	1338	1408
Theoretical VFAs concentrations (mg/L)over time							
Time (hr)	Manure	SSO	Manure/SSO 9/1	Manure/SSO 7/3	Manure/SSO 5/5	Manure/SSO 3/7	Manure/SSO 1/9
0	668	635	665	658	651	645	638
6	783	790	784	785	786	788	789
12	803	1000	823	862	902	941	980
24	1119	1178	1125	1137	1148	1160	1172
48	1204	1365	1220	1252	1284	1317	1349
72	1114	1102	1113	1110	1108	1106	1103

Table C. 9. Concentration of soluble and particulate COD over time in hydrolysis/acidification of manure and SSO

Soluble COD concentrations (mg/L) ocer time							
Time (hr)	Manure/SSO 7/3	Manure/SSO 5/5	Manure/SSO 3/7	Manure/SSO 1/9	Manure/SSO 9/1	Manure only	SSO only
0	3986	4710	5624	6555	3460	2860	6980
2	4580	5159	5941	6771	3860	2940	6992
4	5336	6273	6273	6887	4440	3131	7009
6	5559	6379	6379	7004	4725	3659	7015
8	5751	6469	6469	7105	4927	3936	7026
10	5974	6500	6469	7105	5280	4338	7040
12	6127	6530	6509	7149	5525	4790	7043
24	6326	6547	6547	7193	6268	4935	7052
48	6543	6639	6639	7295	6675	5340	7061
72	7189	7120	6934	7418	7355	5650	7080
Particulate COD concentrations (mg/L) over time							
Time (hr)	Manure/SSO 7/3	Manure/SSO 5/5	Manure/SSO 3/7	Manure/SSO 1/9	Manure/SSO 9/1	Manure only	SSO only
0	23605	22489	20787	19466	25746	26535	18565
2	23298	21940	20539	19250	25160	26155	18446
4	22780	21426	20176	19085	24726	25940	18279
6	22489	21270	20099	18957	24141	25766	18165
8	22385	21130	19887	18895	23749	25665	18050
10	22205	21045	19830	18796	23470	25560	17955
12	21935	20989	19695	18752	23255	25340	17915
24	21325	20542	19280	18508	22410	24795	17755
48	20895	20255	18965	18430	21978	24535	17646
72	20809	20195	18925	18405	21860	24400	17614

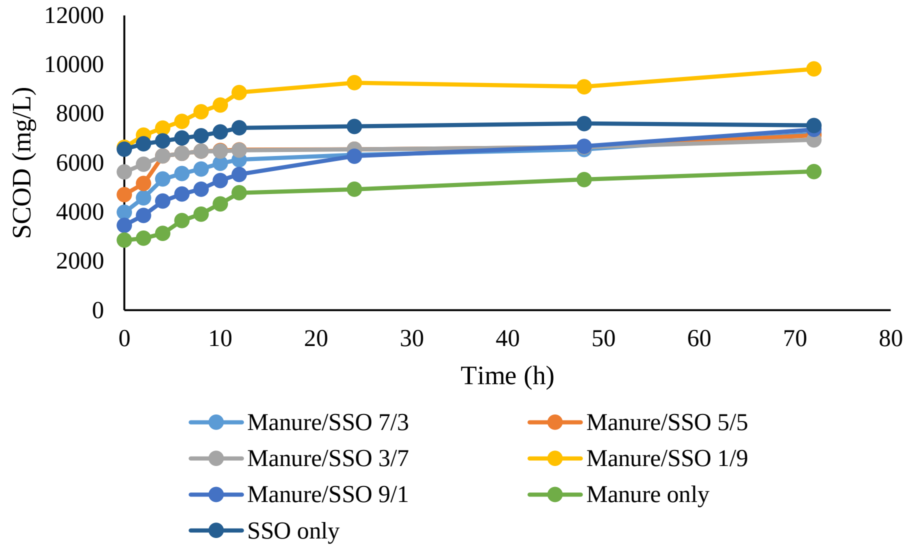


Figure C.2. Concentration of soluble COD over time at different mixing ratios

D. Analytical results for AnCoD of TWAS, manure and SSO

Table D. 1. Characteristics of raw feedstocks in AnCoD of TWAS, Manure, and SSO

Parameters	Units	TWAS		Manure		SSO		Seed	
		MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	39977	1199	100126	3004	108560	3257	17105	513
SCOD	mg/L	1354	41	42116	1263	40855	1225.65	761	23
TSS	mg/L	31445	943	52380	1571	62136	1864	16055	482
VSS	mg/L	26480	794	45420	1363	46990	1410	12781	383
TS	mg/L	38944	1168	67785	2034	66985	2010	17125	514
VS	mg/L	35155	1055	55590	1668	49585	1488	14380	431
Ammonia	mg/L	218	7	18	1	1289	39	800	24
pH		6.5	0.2	6.7	0.2	5.7	0.17	7.2	0.2
Alkalinity	mg CaCO3/L	1888	57	5224	157	6227	187	4215	126
TN	mg/L	2754	83	2110	63	3970	119	1975	59
TSN	mg/L	376	11	125	4	965	29	716	21
Total Carbs	mg/L	1524	46	27842	835	14126	424	839	25
Total Proteins	mg/L	3892	117	5762	173	2420	73	1917	58
Total Lipids	mg/L	396	12	1356	41	1494	45	191	6

Table D. 2. Average CH_4 measurements (mL) in AnCoD of TWAS/Mnaure/SSO (T: TWAS, M: Manure)

Time (day)	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 0.5/0.25/0.25	T/M/SSO 0.25/0.5/0.25	T/M/SSO 0.25/0.25/0.5	T/M/SSO 0.4/0.4/0.2	T/M/SSO 0.2/0.4/0.4	T/M/SSO 0.4/0.2/0.4
0	0	0	0	0	0	0	0	0	0	0	0	0
1	5	47	6	12	22	21	9	52	25	7	37	8
3	21	73	48	32	71	68	55	95	60	54	64	42
4	39	101	63	34	104	97	77	118	79	71	97	79
5	52	101	98	57	143	136	105	124	115	104	118	113
6	34	116	113	67	106	114	94	141	112	102	123	104
7	33	139	112	69	105	108	97	98	116	106	125	106
8	19	79	120	71	86	81	92	102	120	110	133	103
10	25	77	128	76	83	85	81	95	107	97	106	95
12	23	70	121	71	79	81	77	96	101	92	101	90
14	19	91	90	54	65	66	63	63	81	74	84	73
16	17	56	60	35	60	61	61	65	72	65	81	64
19	21	58	54	32	61	63	52	51	59	53	61	55
22	16	40	56	33	52	53	49	47	58	53	62	53
25	13	40	47	28	42	43	38	36	46	41	47	42
28	9	32	33	19	31	32	30	56	36	33	39	32
31	29	24	78	46	78	80	58	29	67	61	62	66
34	12	22	28	16	33	34	26	27	29	27	30	28
37	10	19	30	10	29	30	24	24	29	26	20	27
40	9	15	30	9	26	27	22	18	27	24	16	25
43	5	11	26	6	17	17	16	9	21	19	10	15
46	3	3	18	4	11	11	12	6	16	15	4	11
49	2	3	9	2	6	6	6	3	8	7	3	6
52	1	0	5	1	3	4	4	1	5	4	1	3
58	0	0	2	0	0	0	1	1	1	1	1	1

*T: TWAS

* M: Manure

Table D. 3. Cumulative CH_4 production (mL) in AnCoD of TWAS/Mnaure/SSO (T: TWAS, M:Manure)

Time (day)	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 0.5/0.25/0.25	T/M/SSO 0.25/0.5/0.25	T/M/SSO 0.25/0.25/0.5	T/M/SSO 0.4/0.4/0.2	T/M/SSO 0.2/0.4/0.4	T/M/SSO 0.4/0.2/0.4
0	0	0	0	0	0	0	0	0	0	0	0	0
1	5	48	6	12	18	24	9	52	25	8	35	8
3	27	120	54	44	89	93	64	147	85	62	99	50
4	65	222	116	78	194	189	141	265	164	132	196	129
5	117	323	215	135	336	325	246	389	278	237	314	242
6	152	440	327	202	442	439	340	530	391	339	437	346
7	185	579	440	271	548	547	437	628	507	445	562	452
8	203	658	559	342	633	628	529	730	627	554	696	555
10	228	735	687	417	717	713	610	825	734	652	802	650
12	251	805	808	489	796	793	687	921	836	744	903	741
14	270	895	898	542	860	859	750	984	917	818	986	813
16	287	951	958	577	920	921	811	1049	989	883	1068	877
19	307	1010	1012	609	981	983	862	1101	1047	937	1129	932
22	324	1049	1067	642	1034	1037	911	1148	1105	989	1191	985
25	337	1089	1115	670	1075	1079	948	1184	1151	1031	1238	1027
28	346	1121	1148	690	1107	1111	978	1239	1187	1063	1277	1059
31	375	1146	1225	736	1185	1191	1037	1268	1254	1125	1339	1125
34	387	1167	1253	752	1218	1225	1063	1295	1283	1151	1369	1153
37	397	1186	1284	762	1247	1255	1087	1319	1312	1177	1389	1180
40	406	1201	1314	771	1273	1281	1109	1336	1338	1202	1405	1205
43	411	1212	1339	777	1290	1298	1125	1345	1360	1221	1415	1220
46	414	1215	1357	781	1301	1310	1137	1351	1376	1236	1419	1232
49	416	1218	1366	783	1307	1316	1142	1354	1384	1243	1422	1237
52	417	1218	1371	784	1310	1320	1146	1355	1388	1247	1423	1241
58	417	1218	1373	784	1311	1320	1146	1355	1390	1248	1424	1241

*T: TWAS

* M: Manure

Table D. 4. Cumulative CH_4 production per mass of COD added in AnCoD of TWAS/Mnaure/SSO (T: TWAS, M: Manure)

Time (day)	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 0.5/0.25/0.25	T/M/SSO 0.25/0.5/0.25	T/M/SSO 0.25/0.25/0.5	T/M/SSO 0.4/0.4/0.2	T/M/SSO 0.2/0.4/0.4	T/M/SSO 0.4/0.2/0.4
0	0	0	0	0	0	0	0	0	0	0	0	0
1	2	12	1	4	4	6	2	13	6	2	9	2
3	9	30	13	13	22	23	17	37	21	16	25	13
4	21	54	28	23	48	46	37	67	41	34	49	33
5	38	79	52	39	83	80	65	98	70	62	79	63
6	49	108	79	59	110	108	90	134	98	88	109	89
7	59	142	106	79	136	134	116	159	128	116	141	117
8	65	162	135	99	157	154	140	185	158	144	174	144
10	73	180	166	121	178	175	162	209	185	169	201	168
12	80	198	195	142	198	195	182	233	210	193	226	191
14	87	220	217	157	214	211	199	249	231	213	247	210
16	92	234	231	168	228	226	215	265	249	230	267	227
19	99	248	244	177	244	241	228	278	263	243	283	241
22	104	258	258	186	257	254	241	290	278	257	298	255
25	108	267	269	195	267	265	251	299	290	268	310	266
28	111	275	277	200	275	272	259	314	299	276	320	274
31	120	281	296	214	294	292	275	321	315	292	335	291
34	124	287	303	218	302	300	282	328	323	299	343	298
37	127	291	310	221	310	308	288	334	330	306	348	305
40	130	295	317	224	316	314	294	338	337	312	352	312
43	132	298	324	225	320	318	298	340	342	317	354	315
46	133	298	328	227	323	321	301	342	346	321	355	318
49	133	299	330	227	324	323	303	343	348	323	356	320
52	134	299	331	228	325	324	303	343	349	324	356	321
58	134	299	332	228	325	324	304	343	350	324	356	321

*T: TWAS

* M: Manure

Table D. 5. Cumulative CH_4 production per mass of VSS added in AnCoD of TWAS/Mnaure/SSO (T: TWAS, M: Manure)

Time (day)	TWAS Only	Manure Only	SSO only	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 0.5/0.25/0.25	T/M/SSO 0.25/0.5/0.25	T/M/SSO 0.25/0.25/0.5	T/M/SSO 0.4/0.4/0.2	T/M/SSO 0.2/0.4/0.4	T/M/SSO 0.4/0.2/0.4
0	0	0	0	0	0	0	0	0	0	0	0	0
1	3	26	27	6	10	13	5	28	14	4	24	4
3	13	65	67	22	48	51	34	79	46	33	68	27
4	32	120	124	39	104	105	74	142	89	70	135	69
5	57	175	181	68	181	180	129	209	151	125	217	129
6	73	238	245	102	239	242	179	285	212	179	302	185
7	89	313	323	137	296	302	230	337	275	235	388	241
8	98	356	367	172	342	347	278	392	340	294	480	296
10	110	398	410	210	387	394	321	443	398	345	553	347
12	122	436	449	246	429	438	361	494	453	394	623	395
14	131	485	500	273	464	475	395	528	497	433	681	434
16	139	515	531	291	496	508	426	563	536	468	737	468
19	149	546	564	307	530	543	454	591	567	496	779	498
22	157	568	586	324	558	572	479	616	599	524	822	526
25	163	589	608	338	580	596	499	636	623	546	854	548
28	168	607	626	348	597	614	515	665	643	563	881	566
31	182	620	640	371	639	658	545	681	679	596	924	601
34	187	632	652	379	657	676	559	695	695	610	944	616
37	192	642	662	384	673	693	572	708	711	623	958	630
40	196	650	671	389	687	708	583	718	725	636	969	643
43	199	656	677	392	696	717	592	722	737	647	976	651
46	200	658	678	394	702	723	598	726	745	654	979	657
49	201	659	680	395	705	727	601	727	750	658	981	660
52	202	659	680	395	707	729	603	727	752	661	982	662
58	202	659	680	395	707	729	603	728	753	661	982	663

*T: TWAS

* M: Manure

Table D. 6. Cumulative CH_4 production per volume of substrate added in AnCoD of TWAS/Mnaure/SSO (T: TWAS, M: Manure)

Time (day)	TWAS Only	Manure Only	SSO only	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 0.5/0.25/0.25	T/M/SSO 0.25/0.5/0.25	T/M/SSO 0.25/0.25/0.5	T/M/SSO 0.4/0.4/0.2	T/M/SSO 0.2/0.4/0.4	T/M/SSO 0.4/0.2/0.4
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	0	1	0	1	1	0	1	0
3	0	3	1	1	2	2	1	3	2	1	2	1
4	1	5	3	1	5	5	1	6	4	3	4	3
5	2	8	6	2	8	8	2	9	6	5	7	5
6	2	11	9	3	10	11	3	12	9	7	10	7
7	2	14	12	4	13	14	4	14	11	9	13	9
8	3	16	15	5	15	16	5	16	14	11	16	11
10	3	18	18	6	17	18	6	18	16	13	18	13
12	3	20	21	7	19	20	7	20	19	15	21	15
14	3	22	24	8	20	21	8	22	21	17	23	17
16	4	23	25	9	22	23	8	23	22	18	24	18
19	4	25	27	9	23	24	9	24	24	19	26	19
22	4	26	28	10	24	26	9	25	25	20	27	20
25	4	27	29	10	25	27	10	26	26	21	28	21
28	4	28	30	11	26	27	10	27	27	21	29	22
31	5	28	32	11	28	29	10	28	28	23	31	23
34	5	29	33	12	29	30	11	29	29	23	31	24
37	5	29	34	12	29	31	11	29	29	24	32	24
40	5	30	34	12	30	32	11	29	30	24	32	25
43	5	30	35	12	30	32	11	30	31	25	32	25
46	5	30	36	12	31	32	11	30	31	25	32	25
49	5	30	36	12	31	33	11	30	31	25	33	25
52	5	30	36	12	31	33	12	30	31	25	33	25
58	5	30	36	12	31	33	12	30	31	25	33	25

*T: TWAS

* M: Manure

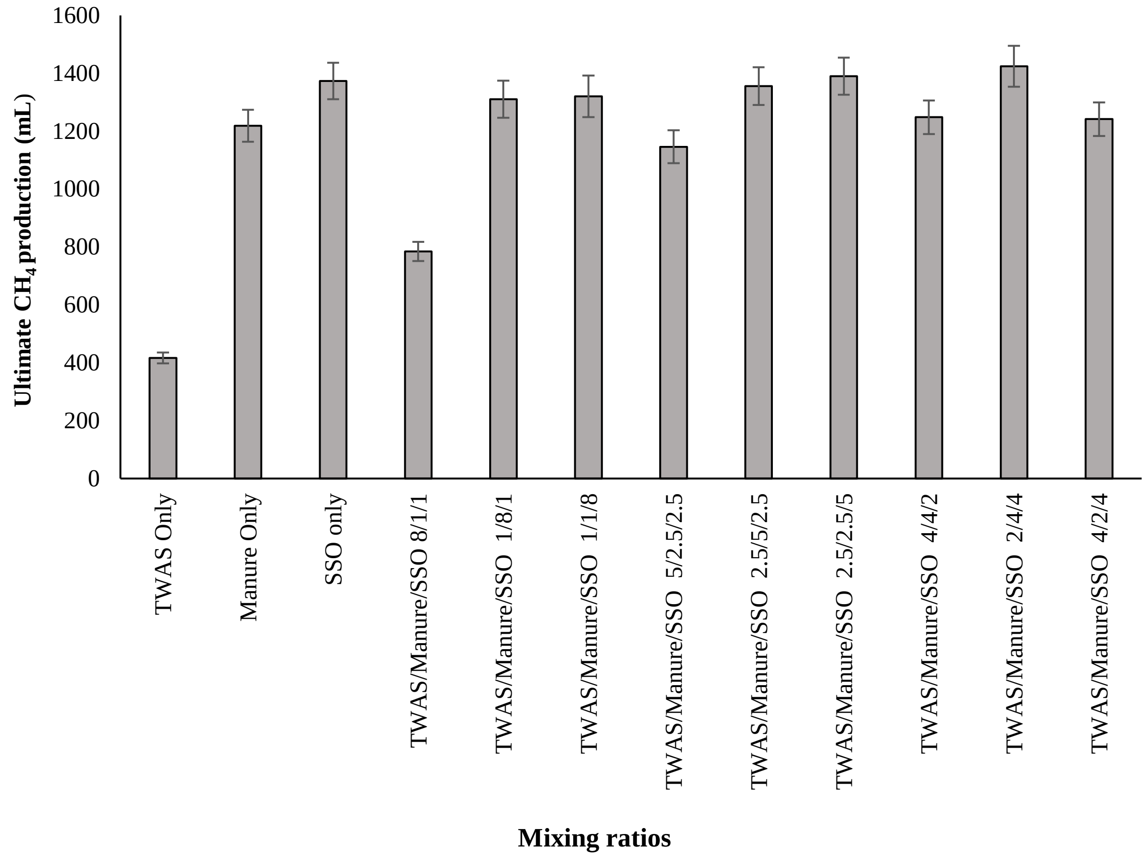


Figure D. 1. Ultimate CH4 production in AnCoD of TWAS/Manure/SSO

Table D. 7. Characteristics of raw feedstocks for hydrolysis/acidification in AnCoD of TWAS/Manure/SSO

Parameters	Units	TWAS		Manure		SSO		Inoculum	
		MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
TCOD	mg/L	36875	2853	100240	4014	102180	4120	16854	389
SCOD	mg/L	1931	26	10145	405	37350	1861	786	22
TSS	mg/L	28934	1006	54100	2164	49510	2093	15022	326
VSS	mg/L	22983	2216	45216	1289	44130	1743	10175	298
TS	mg/L	35705	6859	67680	2707	76878	3460	20650	425
VS	mg/L	31719	27	57830	2313	62948	2833	12988	396
Ammonia	mg/L	235	30	22.3	1	1326	60	1478	32
pH	-	6	0.1	6.8	0.2	5.8	0.2	7.1	0.2
Alkalinity	mg $CaCO_3$/L	1797	148	6984	279	6415	289	3867	135
TN	mg/L	2668	400	1690	68	4086	184	1983	69
TSN	mg/L	386	64	119	4	1096	49	679	24
Total Carbs	mg/L	849	112	25968	1039	13575	611	548	19
Total Proteins	mg/L	2547	298	5126	205	2087	94	1596	56
Total Lipids	mg/L	266	27	1652	66	1395	63	153	5

Table D. 8. Measured and theoretical VFAs concentrations (mg/L) over time in hydrolysis/acidification of TWAS/Manure/SSO

	Measured VFAs concentrations (mg/L) over time											
	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 5/2.5/2.5	T/M/SSO 2.5/5/2.5	T/M/SSO 2.5/2.5/5	T/M/SSO 4/4/2	T/M/SSO 2/4/4	T/M/SSO 4/2/4
Time (hr)	1	2	3	4	5	6	7	8	9	10	11	12
0	428	1096	1538	673	1353	1660	977	1258	1357	1055	1402	1127
6	340	1286	1914	657	1580	2032	1086	1459	1609	1188	1658	1297
12	441	1319	2423	807	1690	2537	1295	1664	1949	1367	1949	1578
24	672	1837	2854	1117	2296	3041	1690	2178	2424	1811	2474	1991
48	744	1976	3309	1247	2503	3503	1897	2422	2755	2012	2783	2258
72	925	2196	2671	1361	2667	2939	1881	2417	2497	2050	2622	2103
	Theoretical VFAs concentrations (mg/L) over time											
time (hr)	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/SSO 1/8/1	T/SSO 1/1/8	T/SSO 5/2.5/2.5	T/SSO 2.5/5/2.5	T/SSO 2.5/2.5/5	T/SSO 4/4/2	T/SSO 2/4/4	T/SSO 4/2/4
0	428	1096	1538	606	1074	1383	873	1040	1150	917	1140	1006
6	340	1286	1914	592	1254	1694	970	1206	1363	1033	1348	1158
12	441	1319	2423	727	1341	2114	1156	1375	1651	1188	1585	1409
24	672	1837	2854	1007	1823	2534	1509	1800	2054	1575	2011	1778
48	744	1976	3309	1124	1986	2919	1693	2001	2334	1750	2263	2016
72	925	2196	2671	1227	2117	2449	1679	1997	2116	1783	2132	1878

*T: TWAS * M: Manure

Table D. 9. Soluble and particulate COD concentrations (mg/L) over time in hydrolysis/acidification of TWAS/Manure/SSO

Soluble COD concentrations (mg/L) ocer time

Time	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 5/2.5/2.5	T/M/SSO 2.5/5/2.5	T/M/SSO 2.5/2.5/5	T/M/SSO 4/4/2	T/M/SSO 2/4/4	T/M/SSO 4/2/4
0	1050	2803	9906	2322	4106	8975	4109	4886	7040	3945	5955	5536
2	1700	3169	9933	2936	4548	9100	4576	5299	7353	4404	6274	5917
4	1968	3375	9948	3198	4787	9172	4790	5507	7509	4622	6438	6094
6	2175	3945	9956	3443	5374	9263	5065	5906	7745	4972	6745	6319
8	2557	4243	9972	3814	5716	9350	5364	6200	7956	5280	6972	6564
10	2868	4677	9992	4137	6184	9448	5662	6553	8189	5618	7246	6808
12	3147	5163	9996	4437	6697	9534	5953	6923	8420	5962	7530	7044
24	3282	5320	10009	4574	6870	9576	6075	7059	8514	6095	7636	7146
48	3317	5757	10121	4665	7317	9725	6247	7361	8721	6332	7891	7310
72	3368	6837	11168	4944	8516	10751	6866	8322	9681	7074	8145	8044

Particulate COD concentrations (mg/L) over time

Time	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/SSO 1/8/1	T/SSO 1/1/8	T/SSO 5/2.5/2.5	T/SSO 2.5/5/2.5	T/SSO 2.5/2.5/5	T/SSO 4/4/2	T/SSO 2/4/4	T/SSO 4/2/4
0	23602	31182	23728	25372	29385	23980	26557	27793	25255	27501	22242	25756
2	22053	30915	23581	24958	28895	23746	26090	27380	24983	27042	21923	25576
4	21784	30659	23497	24496	28656	23573	25877	27072	24787	26824	21779	25198
6	21577	30240	23319	24350	28265	23482	25601	26472	24451	26574	21452	24974
8	21395	29942	23165	23990	27923	23395	25502	26279	24339	25966	21225	24329
10	21184	29788	22885	23837	27555	22998	25375	25726	23906	25828	20951	24084
12	20985	29586	22605	23755	27382	22770	25130	25455	23856	25584	20467	23948
24	20780	28875	21920	23120	25960	22349	24320	24919	23182	24550	19461	22750
48	20515	27730	21590	22776	25141	21880	23950	24108	22774	24124	19181	22110
72	20404	27511	21496	22665	24990	21794	23830	23978	22675	23972	19055	21955

*T: TWAS

* M: Manure

Soluble COD concentrations (mg/L) over time												
Time	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/M/SSO 1/8/1	T/M/SSO 1/1/8	T/M/SSO 5/2.5/2.5	T/M/SSO 2.5/5/2.5	T/M/SSO 2.5/2.5/5	T/M/SSO 4/4/2	T/M/SSO 2/4/4	T/M/SSO 4/2/4
0	1050	2803	9906	2322	4106	8975	4109	4886	7040	3945	5955	5536
2	1700	3169	9933	2936	4548	9100	4576	5299	7353	4404	6274	5917
4	1968	3375	9948	3198	4787	9172	4790	5507	7509	4622	6438	6094
6	2175	3945	9956	3443	5374	9263	5065	5906	7745	4972	6745	6319
8	2557	4243	9972	3814	5716	9350	5364	6200	7956	5280	6972	6564
10	2868	4677	9992	4137	6184	9448	5662	6553	8189	5618	7246	6808
12	3147	5163	9996	4437	6697	9534	5953	6923	8420	5962	7530	7044
24	3282	5320	10009	4574	6870	9576	6075	7059	8514	6095	7636	7146
48	3317	5757	10121	4665	7317	9725	6247	7361	8721	6332	7891	7310
72	3368	6837	11168	4944	8516	10751	6866	8322	9681	7074	8145	8044

Particulate COD concentrations (mg/L) over time												
Time	TWAS	Manure	SSO	T/M/SSO 8/1/1	T/SSO 1/8/1	T/SSO 1/1/8	T/SSO 5/2.5/2.5	T/SSO 2.5/5/2.5	T/SSO 2.5/2.5/5	T/SSO 4/4/2	T/SSO 2/4/4	T/SSO 4/2/4
0	23602	31182	23728	25372	29385	23980	26557	27793	25255	27501	22242	25756
2	22053	30915	23581	24958	28895	23746	26090	27380	24983	27042	21923	25576
4	21784	30659	23497	24496	28656	23573	25877	27072	24787	26824	21779	25198
6	21577	30240	23319	24350	28265	23482	25601	26472	24451	26574	21452	24974
8	21395	29942	23165	23990	27923	23395	25502	26279	24339	25966	21225	24329
10	21184	29788	22885	23837	27555	22998	25375	25726	23906	25828	20951	24084
12	20985	29586	22605	23755	27382	22770	25130	25455	23856	25584	20467	23948
24	20780	28875	21920	23120	25960	22349	24320	24919	23182	24550	19461	22750
48	20515	27730	21590	22776	25141	21880	23950	24108	22774	24124	19181	22110
72	20404	27511	21496	22665	24990	21794	23830	23978	22675	23972	19055	21955

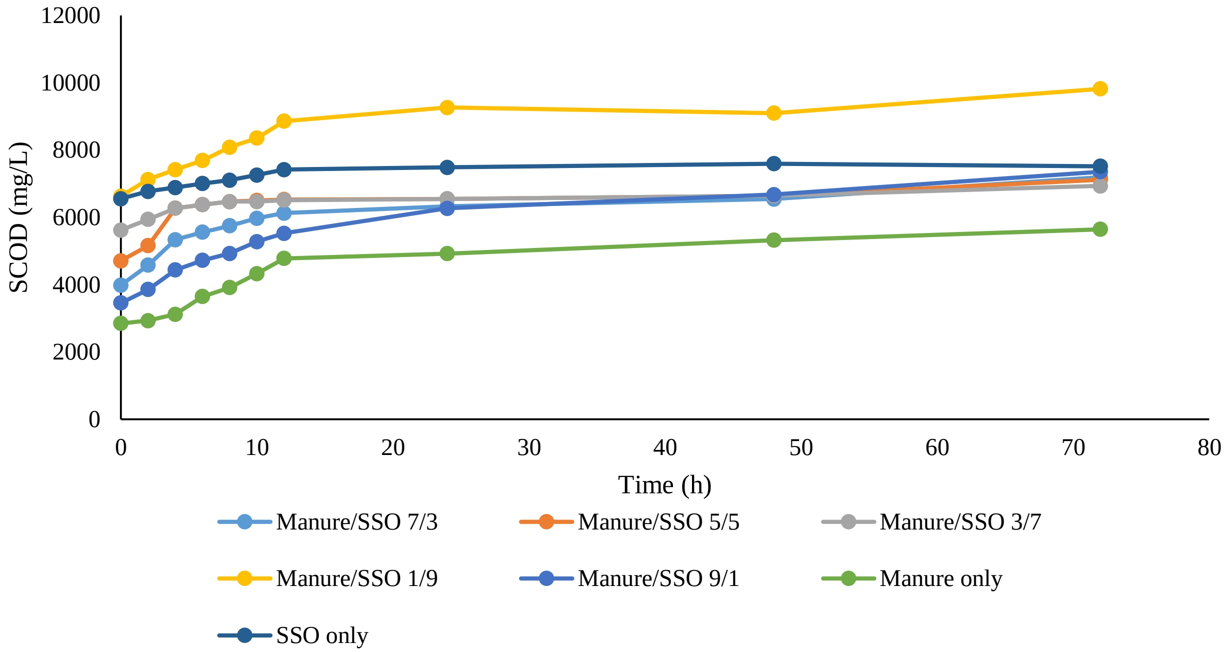


Figure D.2. Concentration of soluble COD over time at different mixing ratios

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There will come a time when you believe everything is finished.

That will be the beginning…

Luis L'Amour