OPTIMIZATION OF METHANE YIELD IN SOLID-STATE ANAEROBIC CO-DIGESTION OF DAIRY MANURE AND CORN STOVER

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Department:
Agricultural and Biosystems Engineering

March 2020

Fargo, North Dakota

North Dakota State University
Graduate School

Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Sole dependence on fossil fuel and the concomitant environmental concerns could be minimized through the optimization of green energy generation from the growing volume of on-farm organic wastes. In this mesophilic study, green energy, mainly methane, was optimized through the solid-state anerobic co-digestion (SSAD) of two on-farm organic wastes (dairy manure with corn stover). Factors considered to achieve the improved methane yield under a total solids of 16% were particle size of corn stover (0.18 – 0.42 and 0.42 – 0.84 mm), alkaline pretreatment type (thermo-chemical and wet state), alkaline-pretreatment reagent (NaOH, NH_4OH, and $Ca(OH)_2$) used for the corn stover, and the magnetite nanoparticles (20, 50, and 75 mg/L) thereafter added to the treatment with highest methane yield. Kinetic models were used to describe some of the high methane yield as well as the environmental impact investigated with life cycle assessment.

Results indicated that corn stover with particle size 0.42 - 0.84 mm blended with dairy manure under a C/N of 24 had the highest methane yield (106 L/ kgVS) under 60 days retention time. After pretreatment of the 0.42 - 0.84 mm corn stover with the three different alkaline reagents, methane yield improved under this wet state pretreatment relative to thermochemical. For instance, calcium pretreated corn stover blended with dairy manure (CaW) had the highest methane yield (176 L / kgVS) under a reduced retention time (79 days), overcame potential volatile fatty acids accumulation and digester upset relative to other pretreated treatments. Furthermore, addition of 20 mg of the nanoparticles to the CaW treatment further enhanced methane yield (191 L / kg VS), minimized digester upset, and reduced retention time to 52 days. Suitable process parameters for methanogenic activities were 0.1 - 0.5 for VFA/Ammonia and VFA/Alkalinity ratios. Free ammonia concentration between 258 – 347 mg/L does not affect methanogenic activities. Environmnetal impact aseessment indicated that pretreatment negatively influenced

human health factors and eutrophication potentials though reduced ozone depletion, global warming potential, and smog potentials.

The solid-state of dairy manure co-digested with corn stover has the potential to improve green energy generation that could complement fossil fuel and address waste management challenges.

ACKNOWLEDGEMENTS

My unwavering appreciation goes to my adviser, Dr. Shafiqur Rahman, who not only gave me the opportunity to have an international exposure, He equally trained me to become a dependent researcher. I am equally grateful to other members of my supervisory committee (Dr. Sumathy Krishnan, Dr. Larry Cihacek, and Dr. David Ripplinger) for their time and contribution to my success. Also, to appreciate are all the Professors who have taught me relevant skills and impacted knowledge gracefully, to mention a few are Dr. Katti Kaplana, Dr. Zhulu Lin, Dr. Ronald Deggies, Dr. Richard Horsley, and Dr. Ghasideh Pourhashem, I am indeed grateful to them. A couple of students were equally helpful during my PhD, to mention a few are Kelvin Betitame, Mustapha Yusuf, Luke Weldt, John Baldwin, Jide Ogungbe, Blessing Okafor, Kunle Owaniyi, Victor Oribamise, and the LCA group.

All my dreams would not have been achieved without the intervention of four class people that combined students parenting with their Professional duties, the Dean of Engineering (Dr. Michael Kessler), the Dean of College of Agriculture and Natural Resources (Dr. Greg Lardy), the Dean of Graduate School (Dr. Claudia Tomany), and the immediate past Agricultural & Biosystems Departmental Chair (Dr. Srikala Bajwa). I am indeed grateful to you all.

The support of my wife (Ibukunoluwa Ajayi-Banji) and daughter (Omobaara Ajayi-Banji) is very amazing, we took the enormous task together a day at a time, encouraging and improving ourselves. My best friend was also with me in all these, encouraging and challenging me, you are the best Oluwaseun Alajo. To my bosom friend. Ayodeji Iwayemi, who was always keeping in touch and praying for me. I am indeed grateful. Support from the Eromosele, and Rotimi Akinola during the tough times were also appreciated.

My siblings and parents were not left out, they prayerfully and financially supported me. I celebrate the families of Olomis, Ajayi, and Oyeleke, I am really grateful to them. I also appreciate CfaN for prayers during the challenging period and the international office NDSU for the input in my life.

Finally, I appreciate all whose actions drove my passion in pursuit of greatness, God bless you all. In addition, God bless North Dakota State University (NDSU) and America also for providing such an enabling environment that helped surmount my grandiose dream.

DEDICATION

This project and all my achievements at the North Dakota State University is dedicated to the Almighty God (Yahweh), for His mercy, kindness, protection, favor, promises, and provisions. He made this a reality. Thank you, God.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Interest on green energy has continued to grow due to environmental management and economic benefits. In green energy generation, biodegradable materials such as corn stover, wheat straw, animal manure, food waste, yard trimmings are utilized to produce hydrogen or methane gases which are renewable energy. Hence, one of the channels for green energy production is called anaerobic digestion (AD). This process employs non-oxygen loving microbes to harness methane gas from biodegradable materials. It also requires a synergetic balance among four groups microbes (fermentative bacteria, acidogens, acetogens, and methanogens) involved in the biodegradation. Furthermore, a number of factors (e.g. temperature, pH, feedstock composition) are highly important for optimal methane yield from the AD process. Hence, all these conditions make the AD process complex.

Recently, the focus has been on an anaerobic digestion process that requires low use of water and also combines more than one feedstock for green energy generation thus reduce waste generation and disposal. This process is termed solid-state anaerobic co-digestion (SSCoD). Benefits of co-digestion in SSCoD include nutrient balance with respect to suitable carbon to nitrogen ratio and possible toxicity reduction. Therefore, individual feedstock composition is very imperative. Other benefits of SSCoD include limited energy required for heating and mixing operations (Li et al., 2013) and easy handling of digestate.

With respect to feedstock, one of the major considerations for selection is cost and availability. On most of the feedstock considered for anaerobic digestion is often a waste or less competitive material in demand relative to agricultural produce. For instance, sewage sludge, animal manure, food waste, and crop residue are all feedstock with has low demand, nevertheless, they are available. Hence, in addition to the benefits of SSCoD earlier stated, the availability and affordability of these feedstocks equally drive interest for SSCoD, as more than one waste is utilized at the same time. Notably, waste from agricultural activities such as farm manure or food waste and crop residue are often blended together in SSCoD. For example, yard waste and food waste (Brown et al., 2013); corn stover and chicken manure (Li et al., 2013); hay and soybean processing waste (Zhu et al., 2014); distiller's grain and food waste (Wang et al., 2012); spent mushroom, yard trimming, and wheat straw (Lin et al., 2014); expired dog food and corn stover (Xu et al., 2012); and tomato residue, dairy manure, and corn stover (Li et al., 2018) are some of the feedstock recently co-digested under solid state anaerobic digestion. However, dairy manure and corn stover was considered as feedstock under this study due to their abundance in Fargo, North Dakota, USA. Furthermore, wastes from these productions are in large quantity in this region of the United States with low utilization rate (Prochnow et al., 2009; USDA–NASS, 2017). Hence in this project, a blend of dairy manure and corn stover were anaerobically co-digested under solid-state conditions with the intent to optimize methane yield.

1.1. Objectives

The general objective of this study was to optimize methane yield from the blend of dairy manure (DM) and corn stover (CS) under mesophilic (37 and 35 ºC) conditions.

The specific objectives of this study were to:

(1) determine the effect of CS particle size and carbon to nitrogen ratio on methane yield from the blend of DM and CS.

(2) determine the effect of NaOH, aqueous ammonia, and calcium hydroxide ($Ca(OH)_2$) pretreated CS (under either thermo-chemical or wet state pretreatment method) on methane yield from the co-digestion of DM and pretreated CS.

(3) determine the effect of nanoparticles (magnetite) on methane yield from the co-digestion of DM and the calcium-pretreated CS.

(4) investigate kinetic models to better understand methane production and the interaction among the process parameters such as pH, alkalinity, VFA, and free ammonia.

(5) perform life cycle assessment (LCA) of energy use analysis in SSAD of corn stover and dairy manure.

2. OVERVIEW AND BACKGROUND STUDY

2.1. Agricultural Waste Generation and Management

Animal production is a long-aged activity that has advanced with research and development. While a number of its products have found judicious applications in today's world, the enteric fermentation and manure management end-products have been subject of environmental concern. Manure comprises animal excreata mixed with bedding materials and it is an excellent source of organic fertilizer for agriculture. However, if it is not managed properly antropogenically, it produces odor (a nuisance) and pollutant gaseous emissions of ammonia, hydrogen sulfide, and greenhouse gases (GHGs) during the anaerobic storage. For example, in 2016, methane accounted for about 10% of total U.S. GHG emissions from human activities (Owen and Silver, 2014; USEPA 2016). Furthermore, methane emissions from enteric fermentation and manure management represent 26% and 10% of total CH_4 emissions from anthropogenic activities, respectively. Specifically, livestock production accounts for 20% of the non-CO_2 greenhouse gas (GHG) emissions and this is majorly from enteric fermentation (EPA, 2012). However, much attention has been focused on GHG emissions reduction from livestock manure, because only minimal modifications could only be carried out on the naturally occurring enteric fermentation.

In the US dairy industry, over 9,000,000 milk cows generate 19 million tons of manure per year, and about 43% GHG emissions is generated from dairy manure management (USDA 2011; Sakadevan and Nguyen, 2017; USDA–NASS, 2017). Another challenge with dairy manure is odor nuisance. Management practices often adopted to address these challenges include biochar production for remediation purposes, composting, and anaerobic digestion (Ahn et al., 2011; Wang et al., 2010; Xu et al., 2013). Nonetheless, the preference for anaerobic digestion (AD) has been induced by its rich microbial composition along with high buffering capacity.

Aside the dairy industry, corn is another major source of food for both humans and livestock. For instance, the United States produced 39% of the world's corn needs and a significant acreage of this corn (3.39 million acres) are from North Dakota, USA. According to the USDA, about 4.45 million bushels of corn produced from corn acreages are used for animal feed. This corn acreage also produces significant amounts of corn stover (non-grain portion of the corn plant), which is viewed as residue. Based on corn stover nutritional composition, it is generally rich in carbohydrates but low in nutrients. From the environmental standpoint, corn stover is better harvested and harnessed than being burnt in the open field (Li et al., 2009). However, when judiciously harvested, over 100 million tons of corn stover could be obtained annually (Prochnow et al., 2009). One interesting observation about corn stover and dairy manure is that both have a significant amount of lignin and in a few cases, the values are relatively close (Li e*t al.,* 2016: Yue et al., 2013). Generally, the presence of lignin makes accessibility of anaerobic microbes to cellulose and hemicellulose difficult. Due to its complex and tight structure or recalcitrant structure, the biodegradability of corn stover needs to be enhanced through some form of pretreatment in order to effectively convert it to biomethane.

2.1.1. Brief History and Background Information on Anaerobic Digestion

The discovery of flammable gases from organic matter by Jan Baptita Van Helmont in the 17th century was the start of anaerobic digestion, globally. Further studies by Count Alessandro Volta in 1776 concluded that there exists a direct nexus between methane production and organic matter degradation. These were the beginning of interest in AD. In the 18th century, Sir Humphry Davy discovered methane as one of the constituent gases produced from dairy manure digestion, and thus generated subsequent interests on animal waste as feedstock for AD. More recently, in 1859, development in AD led to the production of the first anaerobic digester plant in Bombay, India. This interest then gradually spread to other parts of the world like England, China, Denmark, and South America (Lusk, 1998).

Anaerobic digestion is the microbial degradation of complex organic compounds in an oxygen-depleted environment. The process relies on a syntrophic relationship between a number of bacteria and archaea (Lei et al., 2018). In the AD process, polymers of lipids, carbohydrates, and proteins are broken down biochemically to fatty acids, monomers, and oligomers and then, eventually, methane, carbon (iv) oxide, and other trace gases. Prominent advantages of the process are waste reduction and energy generation (Li et al., 2014). This long-known process occurs naturally or under anthropogenic influence and could be harnessed to obtain usable products such as biogas and digestate. Biogas, which is mainly composed of methane (55 - 75%) and carbon dioxide (25 -50%), and some other trace gases (Appels et al., 2008; Zheng et al., 2014), is often used as a heat source and for cooking. Furthermore, these alternative applications are economically viable when compared to being used for electricity generation. Aside from the biogas production, anaerobic digestion offers a number of advantages in sludge management. These include a reduction in disposable sludge volume, enhancement of sludge dewaterability, and sludge stability (Peng et al., 2018). Socio-economically, anaerobic digestion of feedstocks are low cost and the biogas produced has a low selling price compared with fossil-derived fuels (Mao et al., 2015). Furthermore, digestate obtained after the anaerobic process can be used as a nutrient source for crop production.

With regards to biochemical reactions in AD, the biological process is dependent on nutritional and mineral compositions of the substrate, digester temperature, ingestate pH, organic loading rate, and hydraulic retention time, amongst others (Khalid et al., 2011). All these play important roles in anaerobic digestion stage processes. Interestingly, they also make AD reaction non-linear and complex (Tan et al., 2018).

Generally, anaerobic digestion efficiency is evaluated through the volatile solids reduction, methane yield, and gas production while its process stability is examined with the total volatile fatty acid (TVFA), total ammonia (TA), and individual volatile fatty acid (VFA) (Li et al., 2014). Furthermore, there are three stages (start-up or lag stage, growth, and maturation) in an anaerobic digestion process and each stage plays an important role in the AD process. At the start-up or early stage, methane yield and gas production fluctuate, while the maturation stage is characterized with the steady state, low VFA production, and the efficiency parameters become stable. These efficiency parameters (VS, gas production, and methane yield) are often used to evaluate digester effectiveness. However, studies have shown that the parameters could produce divergent results. For example, in an AD study conducted on food waste, in which the volatile solids (VS) of the influent was reduced to within 72–96% range at the cessation of the experiment, such reactor was considered to have suitable solid waste reduction ability (Braguglia et al., 2018). However, in another similar study by Peng et al., (2018), methane yield and gas production were inconsistent though the reactor displayed good solid waste reduction ability. This decline in methane production and biogas yield was linked to acetate, propionate, and valerate accumulation Peng et al., (2018). Hence, conclusion on efficiency parameters should be critically viewed particularly in connection with VFAs.

Furthermore, continuous accumulation of these VFAs (acetate, propionate, and valerate) likely to lead the process inhibition in the digester as observed by Peng et al., (2018). Interestingly, at some point in the digester a pseudo-steady state could occur. According to Degueurce et al., (2015), under a pseudo-steady state, actions of acetogens and methanogens proceeded relatively well. Other conditions at which a pseudo-steady state that might occur during the previously stated stages or at some point during the AD process are quasi-steady state, inhibited steady state, and instability state.

2.2. Anaerobic Digestion Process

Anaerobic fermentation consists of hydrolysis, acidogenesis, acetogenesis, and methanogenesis processes. During the hydrolysis process, hydrolytic microorganisms (exoenzymes) convert complex compounds (biomass) such as carbohydrates, lipids, and proteins into simple organic compounds in the hydrolysis stage. They usually use up oxygen present.

$$\text{Biomass} + H_2O \rightarrow \text{Monomers/Oligomers} + H_2 \tag{2.1}$$

In the acidogenesis stage, metabolites produced by hydrolytic bacteria are converted into volatile fatty acids, ethanol, and other compounds by fermentative bacteria. These products control methanogenic population, pH variations, anaerobic digestion efficiency, and buffering capacity of the ingestate (Zhu et al., 2010). For instance, high VFA consequently results in low pH and hence affects methanogens' population. With respect to optimal environmental conditions, acidogens operate under a pH range of 4.0 – 6.5, which are optimal at pH of 5.5 (Yu and Fang, 2002). Also, of interest is what acidogens produce based on pH. When the pH is between 4.0 and 4.5, acidogens produce ethanol and propionate. While between 6.0 and 6.5 pH range, acidogens generate acetate and butyrate which are more important for methane production relative to propionate and ethanol (Yu and Fang, 2002).

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \tag{2.2}$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \tag{2.3}$$

In the acetogenesis stage, homoacetogenic or syntrophic bacteria make acetate and hydrogen. These bacteria are very active between 5.4 - 9.8 pH range and temperature range between 20 – 72 ^{o}C (Bengelsdorf et al., 2018). The same authors classified acetogens into different categories. For example, acetogens that produce acetic, butyric, and other organic acids are called acetogenic, and those that produce ethanol, butanol, and hexanol are called solventogenic. While those that use carbon monoxide as substance were classified as carboxydotrophic bacteria.

$$CH3CH2COO- + 3H2O \rightarrow CH3COO- + H+ + HCO3- + 3H2 \tag{2.4}$$

$$C6H12O6 + 2H2O \rightarrow 2CH3COOH + 2CO2 + 4H2 \tag{2.5}$$

$$CH3CH2OH + 2H2O \rightarrow CH3COO- + 2H2 + H \tag{2.6}$$

$$2HCO3- + 4H2 + H+ \rightarrow CH3COO- + 4H2O \tag{2.7}$$

The final stage of this biochemical process results in methane and carbon dioxide production by methanogenic archaea (Wei, 2016). Other potential gases from anaerobic digestion are hydrogen sulfide and ammonia as shown in the equations thereafter.

$$2CH3CH2OH + CO2 \rightarrow 2CH3COOH + CH4 \tag{2.8}$$

$$CH3COOH \rightarrow CH4 + CO2 \tag{2.9}$$

$$CH3OH \rightarrow CH4 + H2O \tag{2.10}$$

$$CO2 + 4H2 \rightarrow CH4 + 2H2O \tag{2.11}$$

$$CH3COO- + SO42- + H+ \rightarrow 2HCO3 + H2S \tag{2.12}$$

$$CH3COO- + NO- + H2O + H+ \rightarrow 2HCO3 + NH4 \tag{2.13}$$

Though each stage has a specialized group of microorganisms, methane production is influenced by the balance of the whole stages and would be affected by any rate-limiting steps such as hydrolysis and acidogenesis (Vanwonterghem et al., 2014). A quick view of these stages is shown in Figure 2.1. In terms of recovery response after some sort of injury to microorganisms, acid forming bacteria recover quickly when compared with methane forming bacteria (Millati et al., 2018). All the anaerobic digestion process equations previously stated arecredited to Clifford (2018).

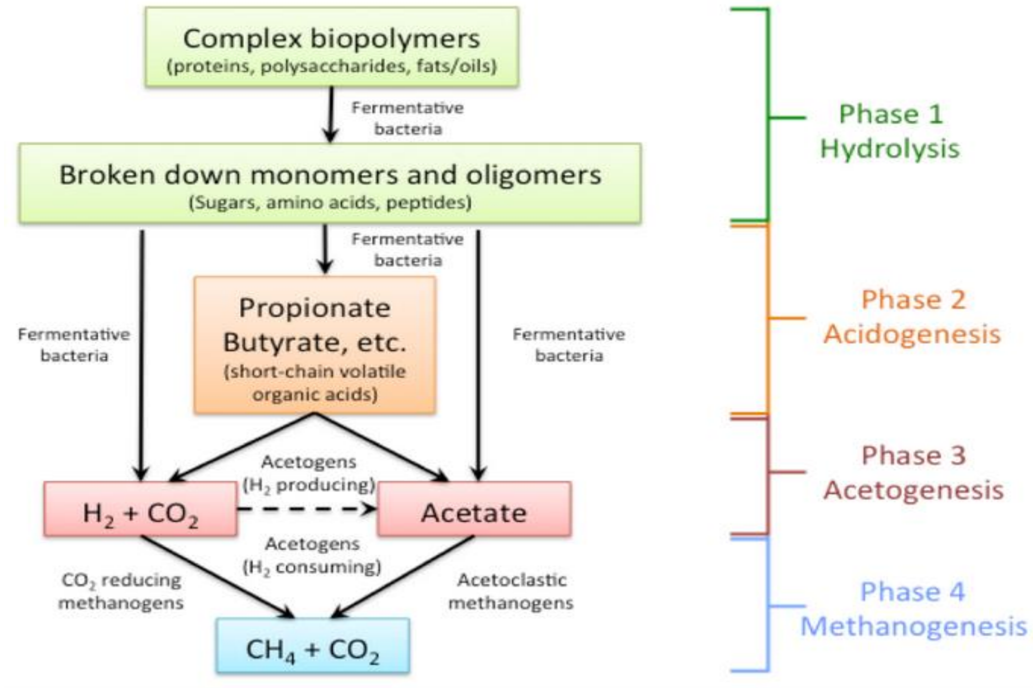

Figure 2.1: Anaerobic digestion process.
Source: Shi et al., (2012)

2.3. Anaerobic Digestion Reactors

The major type of reactors for AD process are covered storage, plug flow reactor, mixed plug flow reactor, complete mix, induced blanket reactor, up-flow anaerobic sludge blanket reactor, anaerobic sequencing batch reactor, anaerobic contact reactor, anaerobic filter reactor, continuously-stirred tank reactor, fixed film reactor and a host of others (Lee et al., 2014, Sakar et al., 2009). However, most common of these reactors are continuously stirred tank, anaerobic baffled reactor, and anaerobic sequencing batch reactor (Lee et al., 2014, Sakar et al., 2009). Factor that distinguishes various types is majorly energy input and dominant methanogen community (Lee et al., 2014). However, *Methanoculleus* was often dominant in the reactors except when the acetate concentration is low, about 0.1 g/L or the reactor is an upward anaerobic sludge blanket

(Lee et al., 2014). Also, in any reactor, at least one of these process stages described previously could occur; steady state, quasi - steady state, inhibited state, inhibited steady state, and instability state. Steady state

2.3.1. Method of Feedstock Introduction to Reactors

Batch and continuous flow methods are the two common ways to introduce feedstocks into reactors. Semi-continuous is another mode of feedstock introduction into biodigester. In the batched condition, new ingestate or feedstock mix, is introduced into the reactor only after the retention time is completed and digestate or anaerobically spent feedstock mix is completely evacuated while under the continuous system, new ingestate are introduced into the reactor based on the hydraulic loading rate. The batch reactor often operates under a smaller scale while the continuous flow reactor operates on a larger scale. However, the continuous flow reactors have to deal with an issue such as an inconsistent loading rate of feedstock and inconsistent feed composition. For instance, the chemical oxygen demand (COD) of swine slurry or wastewater can vary between 4786 and 20,180 mg/L (Deng et al., 2014; Wang et al., 2015). This significant variation in COD concentration may cause bacteria shock and will obviously affect the reactor performance in terms of effluent quality and biogas production. To ensure consistent biogas production despite this challenge, a predictive model was recommended by Tan et al., (2018) and can be used for predicting biogas yield of a reactor.

2.4. Types of Anaerobic Digestion

Total solid of ingestate is the differentiating factor between solid and liquid state anaerobic digestion. In AD, ingestate with total solids below 10% are considered liquid state anaerobic digestion (LSAD), those between 10-15% are considered hemi-solid-state anaerobic digestion

(HSS-AD) while those above 15% are considered solid-state anaerobic digestion (SSAD) (Li et al., 2018a).

Liquid state anaerobic digestion (LSAD) has been greatly explored with outstanding and innovative results, however, the method requires huge water volume and the digestate produced requires huge post treatment cost about $254–290 per ton output (Golkowska et al., 2014). Another prominent challenge is the high transport cost of liquid digestate (Lia, et al, 2018).

SSAD process is an innovative waste-recycling approach treating high-solid content bio-wastes. SSAD is presently gaining research interest because of the ease of transportation, storability, direct usability as biofertilizer, and conversion to heat and fuel (Fuchs and Drosg, 2013). Nevertheless, one of the major challenges with solid-state anaerobic digestion is that replicates might show different behaviors in terms of methane production, digester failure, etc. For instance, in an SSAD study conducted by Abbassi-Guendouz et al., (2012) in which four replicates of 30% TS ingestate were examined under 25 and 35% total solid conditions. Two of the 30% replicates behaved similarly to replicates with 25% TS while the other two had similar behavior of replicates with 35% TS in terms of methane production.

Another common challenge with SSAD is the high ammonia concentration in the reactor (Poirier et al., 2017; Tao et al., 2017). Li et al., (2015) also reported retarded microbial cell translocation as a major challenge in SSAD. However, the advantages of SSAD over LSAD are higher organics loading rates, lower energy requirements, a lesser degree of feedstock processing, and smaller digester volumes than conventional low-solids AD processes (Gao et al., 2015; Li et al., 2017). Interestingly, leachate recirculation has been reported to enhance some of the challenges in SSAD (Pezzolla et al., 2017). The result and process indicators of some SSAD processes are presented in Table 2.1.

Table 2.1: SSAD process and performance indicators (Sources: Li et al., (2016); Narra et al., (2016); Peng et al., (2018).

Ingestate	pH	C/N	TS (%)	Temp. (ºC)	FF	OLR	Methane yield=X (LCH_4g/VS)	HRT (days)	TVFA/Alk	TVA/Amm
Food waste	6.4	15	28	M-36	SC		0.2 -56/day	233		0.09 – 1.02
Tomatoes residue, corn stover and dairy manure	7.1 – 7.6	17- 28	12	M-35	B	NP	250 -425	45	0.0 – 2.1	0.003 – 0.340
Rice straw and cattle dung	NP	NP	25	M-32 & T -50	B	NP	120≤ X≤ 250	35-M & 21-T	NP	NP

C/N- Carbon to nitrogen ratio; TS – Total Solids; Temp. – Temperature; FF – Feeding form into the digester; OLR-Organic loading rate; HRT- Hydraulic retention time; TVFA/Alk – Total volatile fatty acids to Alkalinity ratio; TVFA/Amm – Total volatile fatty acids to ammonium-N ratio; M- Mesophilic temperature; T – Thermophilic temperature; SC- Semi continuous reactor; B-Batch reactor; NP- No data from the author

Aside from the LSAD, HSS, and SSAD, another classification of anaerobic digestion is based on the number of feedstocks introduced into the digester. In an experimental design when more than one feedstock is introduced into the digester, this AD set-up is called co-digestion. On the other hand, the introduction of a single type of feedstock in the digester is called mono-digestion. Co-digestion easily addresses nutrient imbalance which is one of the major limitations in mono-digestion. More detailed review on co-digestion and the benefits were discussed later in the study.

2.5. Factors Imperative in Anaerobic Digestion

Many factors play both direct and synergistic roles in biogas production and methane concentration (Figure 2.2). Some of the parameters or factors that impact AD process the most are discussed below:

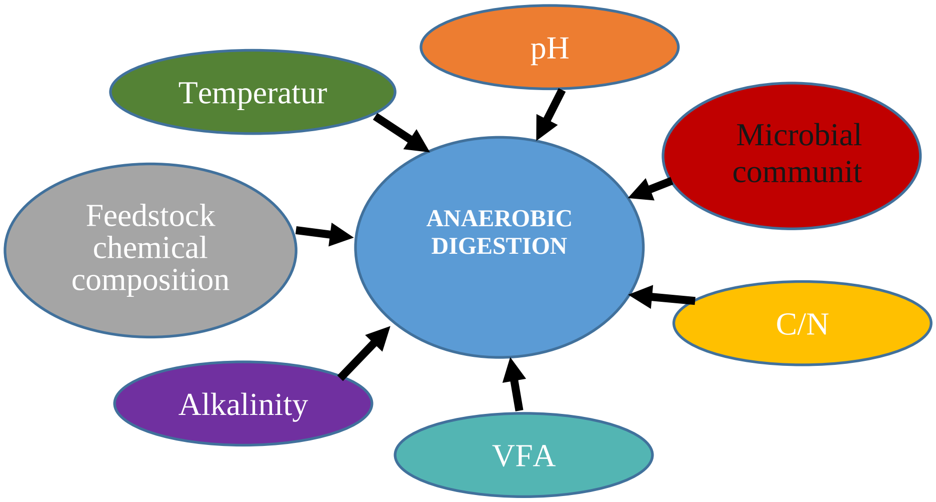

Figure 2.2: Factors that influence anaerobic digestion process.

2.5.1. Bioreactor Temperature

Bioreactor temperature for anaerobic digestion could be categorized as psychrophilic (20 ^{o}C), mesophilic (35 - 37 ^{o}C), thermophilic (55 - 70 ^{o}C), and temperature between 70 -80 ^{o}C for hyperthermophilic for (Alqaralleh et al., 2018; Jiang et al., 2018, Hu et al., 2018; Saidi et al., 2018). The mesophilic and thermophilic AD are the most prominent because others have low methane yield and are highly unstable, except for the reduced cost benefit because heat supply is not required for the psychrophilic (Mao et al., 2015). From these two, thermophilic has better reaction rates, load-bearing capacity and productivity over mesophilic AD process (Mao et al., 2015). On the other hand, acidification may be pronounced under thermophilic conditions, a situation that inhibits biogas production (Mao et al., 2015). Furthermore, decreased stability, low-quality effluent, enhanced toxicity and susceptibility to environmental conditions, high cost, high sensitivity to environmental changes, poor methanogenesis, and high energy input are some of the thermophilic AD drawbacks compared with mesophilic (Bowen et al., 2014). However, mesophilic systems are rich in bacteria and highly stable despite the low methane yield and biodegradability (Bowen et al., 2014). An innovative project on temperature phased mesophilic and thermophilic anaerobic digester was developed by Han et al., (1997). In the experiment, the digester integrated both the mesophilic and thermophilic advantages for upgraded process performance, which resulted in the complete destruction of both total and fecal coliform in the wastewater sludges below an acceptable limit. Furthermore, VS removal for this waste stream was doubled in the integrated digester relative to a conventional singled-staged system (Han et al., 1997). In addition to these, the integrated system ensured contact temperature in the digester relative to the conventional single-stage system and this was discussed in the next sub-section.

2.5.1.1. Constant Digester Temperature

Anaerobic microorganisms are highly sensitive to variation in temperature, hence maintaining a constant temperature is imperative throughout the digestion process. Consequently, temperature fluctuation adversely affects methane cum hydrogen production, and organic matter decomposition. Studies carried out by Bowen et al., (2014) and Mao et al., (2015) showed that a decrease in digester temperature ultimately reduces yield. It also decreases VFA production rate, microorganism metabolic rate, ammonia concentration and substrate utilization rate, while it elevates start-up time. Another study investigated the effect of an increase in digester temperature in an AD study; pH value, methane potential, and hydrolysis of particulate increased under this condition. For instance, the initial pH was 6.89 at 30 ^{o}C and 7.21 at 40 ^{o}C, while at the cessation of the experiment, the pH had increased to 7.12 under 30 ^{O}C and 7.44 under 40 ^{o}C (Wang et al., *2*014).

2.5.2. pH

Aside from temperature, pH is another very important parameter in anaerobic digestion. pH range depends on feedstock type and source, as listed in Table 2.2. Interestingly, feedstock pH outside the range of 5.5 - 8.0 will definitely affect methanogens. Instead of acetate production, ethanol was the major product under a pH < 4.5 and an ORP < -120 mV in a two-phase anaerobic co-digestion of food waste and rice straw study (Figures 2.3 - 2.5) Chen et al., (2015). In the same study, a pH > 5 with ORP lower than -120 mV led to butyric fermentation (Chen et al., 2015). All these might eventually lead to digester failure. Hence, pH should be maintained via alkalinity from the influent or by adding alkaline solution.

Table 2.2: Feedstock pH.

Feedstock	pH	Status	Author
Dairy manure	7.2	Fresh	Li et al., 2018a
Tomatoes residue	7.7	Not Fresh	Li et al., 2018a
Pig manure	8.4	Fresh	Zhang et al., 2014
Dewatered sewage sludge	7.5	Not Fresh	Zhang et al., 2014
Poultry manure	6.4	Fresh	Sánchez-García et al., 2015
Barley straw	5.8	Not Fresh	Sánchez-García et al., 2015

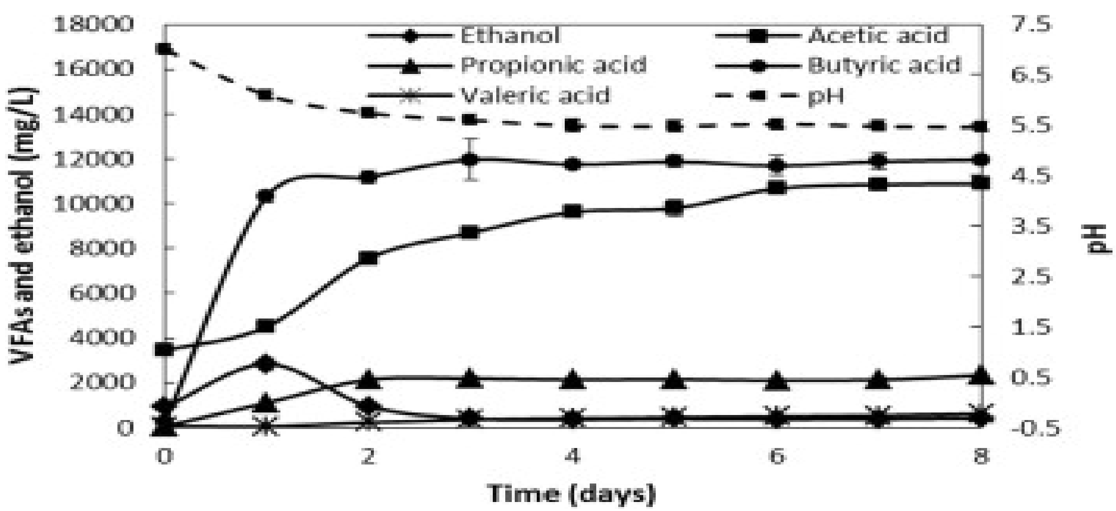

Figure 2.3: Relationship among VFA, ethanol, pH, and retention time from anaerobically co-digested food waste and rice straw.
Source: Chen et al., (2015).

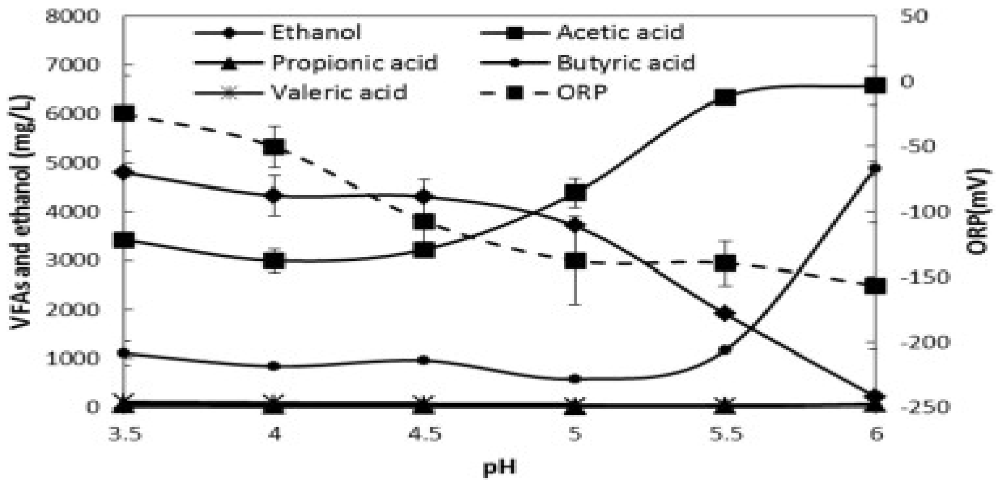

Figure 2.4: Relationship among VFA, ethanol, pH, and ORP in the co-digestion study.
Source: Chen et al., (2015).

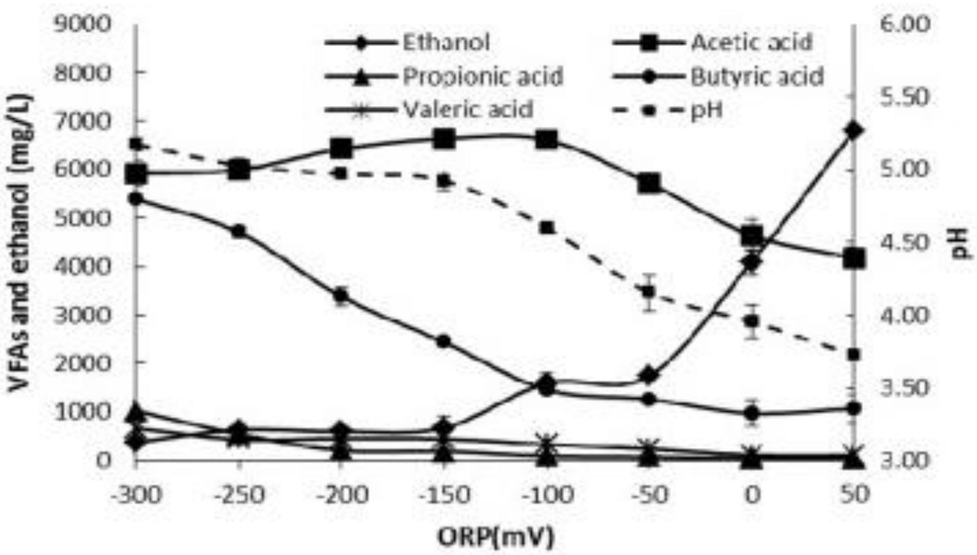

Figure 2.5: Relationship among VFA, ethanol, ORP, and pH in the co-digestion study.
Source: Chen et al., (2015).

Furthermore, Zhai et al., (2015) observed digester failure when the initial pH of cow manure co-digested with kitchen waste was 6.0. Nexus between pH and parameters such as alkalinity, temperature, and free ammonia nitrogen (FAN) have been reported. For instance, highly alkaline ingestate in AD process breaks down microbial granules and then lead to process failure

(Ward et al., 2008). Furthermore, a continuous flow anaerobic study of food waste carried out by Peng et al., (2018) showed that pH versus FAN/TAN ratio or pH versus the ratio of free ammonia nitrogen followed a similar trend. Hence, they can be used to monitor reactor condition. Succinctly, a low pH is an indication of VFA accumulation (Peng et al., 2018). However, under this circumstance, a chemical reaction takes place between ammonia and ammonium that causes a decrease in FAN when the pH is low. This reaction lessens the ammonia inhibition (Peng et al., 2018).

2.5.3. Feedstock Source and Composition

There are a variety of feedstocks used for AD depending on the geographical locations and availability. However, only a few of the feedstocks will be discussed in this section. Feedstocks serve as a food source and habitation for degrading bacterial in anaerobic digesters. However, the compositions of these feedstocks can either enhance or hamper the AD process. For example, the presence of antibiotic monensin in dairy cattle manure, a product used for milk production enhancement and coccidiosis treatment, impacts anaerobic digestion of dairy cattle manure negatively when in high concentration (Spirito et al., 2018).

Wastewater from the biofuel hydrothermal liquefaction process has also been considered as feedstock for the AD despite its inherent inhibitory compounds. In a study conducted by Fernandez et al., (2018), methane formation was adversely affected when the feedstock and wastewater stream from hydrothermal liquefaction concentration increased from 22 - 26.5 (v/v) to over 40 % (v/v). Furthermore, under this elevated concentration, low methanization indicators such as chloride, sulphate, phosphate, and nitrate levels had increased to over 800, 280, 140 and 60 ppm, respectively, compared to the initial condition of 610, 94, and 51 ppm. The increase is at least about 40, 77, and 202% for chloride, sulphate, and phosphate, respectively.

Sludge from wastewater treatment plants is another feedstock that has been prominently managed with anaerobic digestion. AD management of this waste is preferred to incineration because incineration accounts for about 60% of total operating costs for the wastewater treatment plant (Appels et al., 2008; Neyens and Baeyens, 2003).

Food waste has been specifically identified as suited for AD in terms of waste management compared with a number of wastes rich in organic content. Specifically, potential methane production from food waste is about 200 and 670 mL CH_4/g added feedstockVS (Ariunbaatar et al., 2015). Based on the prominent food waste nutritional composition, such as protein, carbohydrate, and lipid content, food waste could be classified as protein-rich food waste, starch-rich food waste or lipid-rich food waste in that order. However, one of the major challenges with proteinous food waste is the potential release of ammonia to undesirable concentrations. This is unlike food waste high in fiber or carbohydrate content (Gao et al., 2015; Tao et al., 2017).

Algae is another biomass feedstock with low lignin content an added advantage in anaerobic digestion. This compositional property also makes its degradation rate high (Wei et al., 2013). However, unprocessed algae are not suitable due to the high lipid content that will obviously lead to VFA accumulation. Hence, algae used as feedstock for the AD process already have the oil content extracted (Saratale et al., 2018). Another challenge with this feedstock is the high protein content which makes the carbon to nitrogen ratio typically below 11% and unsuitable for mono-digestion (Xia et al., 2015).

Municipal solid waste leachate is another feedstock that could be treated with AD though the resulting composition of the leachate such as high VFA, elevated ammonia, presence of calcium, and heavy metals makes the feedstock unattractive for anaerobic digestion. The use of non-biological and conducting materials such as carbon cloth and graphite rod had help solved some of the waste compositional challenges by provision of surface for bacteria biofilm formation, absorption of inhibitory substances, and also their extraction from the bioreactor environment (Lei et al., 2016; Zulkeflia et al., 2016; Gao et al., 2017). Furthermore, a recent study examined the management of a bioreactor with magnetite in order to overcome these harsh compositional challenges (Lei et al., 2018). However, the leachate is not recommended as feedstock for the AD process under a mono-substrate state or when being chemically pretreatment like lignocellulosic feedstock (Sežun et al., 2011). Interestingly, a study by Bougrier et al., (2018) has successfully provided a way to harness this huge available feedstock, about 38.6 $\times 10^6$ tons annually (Mussatto, 2013). In their study, they found COD removal rate to be about 60%, enhanced biodegradability was close to 65%, and yielded methane was approximately 285 CH_4 NL/kgVS. Furthemore, cobalt, magnesium, and phosphorus inclusion were in high concentrations in the study while iron and nickel were required in low concentrations.

2.5.4. Bioreactor Micro-organisms

Microorganisms in an anaerobic bioreactor are majorly categorized into polymer degraders or acidogens, acetogens and methanogens. Examples of polymer degraders, which convert complex organic matter into monomers and oligomers, are bacteroidetes and clostridia. All of these categorized microorganisms play an integral role in the bioreactor in accordance to their diversities. For example, predominant bacteria *phyla* observed in a continuous flow bioreactor fed with food waste were *firmicutes, bacteroidetes, actinobacteria, synergistetes, candidate_division_WS6, proteobacteria,* and *chloroflexi;* with *firmicutes* over half of the population of this list. In the same experiment, the predominant *archaeal phylum* was *euryarchaeota*, with about 98% population and its dominant class was *methanomicrobia* (Peng et al., 2018). This suggests that despite the presence of bacteria in an AD system, the most predominant species has a major impact on the AD process. In another study, the functionality of these microorganisms on methane fermentation was examined (Mustapha et al., 2018). The authors suspected that some of the waste sewage sludge microorganisms were suppressors while others accelerate methane production. Identified order of suppressors from some of the stated phyla were *Nitrosomonadaceae and Nitrospiraceae,* while the accelerators were *Clostridia, Cladilinea, Planctomycetes, and Alphaproteobacteria.* Notwithstanding, bioreactors with the same feedstock, process parameters, and environmental conditions are meant to produce similar microbial communities, diversity, and dynamics (Lucas et al., 2015). However, microbiomes diversity, especially as the anaerobic process deteriorates, is not germane in developing a successful microbial community (Li et al., 2016; Ros et al., 2017; Peng et al., 2018).

Environmental factors, particularly temperature and pH, have been noted to markedly affect AD microbial community (Shi et al., 2013; Wong et al., 2013). Recent investigation has shown that feeding sludge in continuous flow digesters could also affect microbial assemblage (Wang et al., 2018). Also noted in another study was how low pH (about 3.8 - 4.0) has been linked to low microbial degradation in anaerobic digestion (Xiao et al., 2018). Furthermore, seasonal temperature changes have also been reported to influence AD microbial communities. A study conducted by Wang et al., (2018) indicated that the numbers of archaea, bacteria, and fungi feeding on digested sludge were distinctly higher in the summer than in the winter. Furthermore, high nitrogen content seemingly encourages some specific methanogens. For instance, hydrogenotrophic dominates when the nitrogen concentration is high while acetropics overwhelms the system at low nitrogen content (Peng et al., 2018). Albeit this organic nitrogen is liberated from microbial degradation of total ammonia nitrogen especially when microbial mass is huge and highly active (Tampio et al., 2014).

Another aspect worth mentioning is the strong nexus between the microbial community and bioreactor performance. Though changes in the microbial community happen earlier in the digester compared with changes in process performance as observed by Peng et al., (2018). However, in this research, few microbial community analyses were carried out.

2.5.5. Nutrients

The most important nutrients in anaerobic digestion are carbon (C) and nitrogen (N) in the appropriate ratio. AD literature generally recommended a C/N ratio of 20-30:1 as the best range because microbes in the digester use up carbon up to 20-30 times than the quantity of nitrogen consumed. However, some researchers observed a bit different C/N ratio (14-25:1) for an effective anaerobic co-digestion (Li et al, 2016; Peng et al., 2018).

Furthermore, in an extensive study carried out by Li et al., (2018a) on the mesophilic co-digestion of dairy manure, corn stover and tomato residue under 9 mix categories and respective C/N ratios (between 19 and 29). Results from their study indicated that the success of any co-digestion process is not primarily dependent on C/N of the ingestate but also on the individual feedstock ratio/total feedstock ratio. For example, ingestates with tomatoes residues, which had 54% of the total ingestate volume (C/N of 19 and 21), failed. However, methane production for other mix categories, which had tomato residue fraction less than 41% of the total volume (C/N of 21, 22, 23, 24, and 28), produced peak methane concentration above 60%. Best and maximum methane yield of 415 L/kg VS was obtained from a ternary mix of 33% corn stover, 54% dairy manure, and 13% tomato residues at a C/N of 22. Furthermore, this best ternary mix had initial and final alkalinity, pH, total volatile fatty acid, and total ammonia nitrogen of 6.6 g/kg and 13.4 g/kg; 7.6 and 7.9; 0.67 g/kg and 0.07 g/kg; 2.29 g/kg, and 3.4 g/kg respectively.

2.5.6. Ammonia Concentration

The core process that generates ammonia nitrogen is the hydrolysis of nitrogenous feedstock. This process generates both ammonium ions and free ammonia (FAN). Excessive level of ammonia concentration, particularly FAN, in AD process will pose limitation to biogas production and invariably lead to digester failure, while low concentration of the gas will enhance the buffering capacity of substrate (Astals et al., 2013; Orhan and Demirel, 2013; Mahdy et al., 2017; Sun et al., 2016). In an AD process that ammonia nitrogen concentration was greater than 1700 mg/L, methanogens and their archaea diversity were inhibited (Demirel and Scherer, 2008; Rajagopal et al., 2013). Nevertheless, under a condition of high VFA production, which is relatively dependent on experimental design, high ammonia concentration in the bioreactor prevented significant pH change and also digester failure due to its buffering capacity (Pind et al., 2003). A lot of disparities have been noted in the relationship between ammonia concentration and methanogens activities. The limitation in these studies were that process failure were related to laboratory scale and not full-scale set-up (Franke-Whittle et al., 2014).

In a large scale set-up, elevated ammonia concentration has generally been a problem relative to pilot or lab scale in which the effect has been counteracted with dilution, air stripping, bioaugmentation, co-digestion, ammonia-binding ions, and struvite precipitation (Mahdy et al., 2017; Sun et al., 2016). Generally, total ammonia nitrogen at elevated concentration was attributed to substrate composition, volumetric ratio of feeding substrate to discharged digestate, gaseous emission, and microbial biomass fixation. Another process that leads to ammonia accumulation as stated by Gao et al., (2015) was reducing discharge volume as against consistently fed or input volume. However, in a continuous flow set-up study reported by Peng et al., (2018), this was only attributed to gaseous emission and microbial biomass fixation because all other stated factors were relatively stable and consistent.

2.5.7. Volatile Fatty Acids

Volatile acid acids (VFA) are an intricate and as well salient factor in anaerobic digestion (Wang et al., 2014). This is because carbon dioxide and methane, the composition of biogas, are mainly formed from the disintegration of VFA. Two important components of VFA are the composition and the respective concentration. Detail of how these affect AD processes are discussed in the subsequent subsection.

2.5.7.1. VFA Concentration

In anaerobic digestion, VFA concentration plays an important role in methanogens activities, pH trend in the bioreactor, and overall methane yield. Furthermore, Lee et al., (2014) reported that during organic waste fermentation, VFA concentration is influenced by pH, temperature, C/N, and hydraulic retention time.

In practice, high VFA concentration from acetogens and acidogens might leads to acid accumulation, if consumption rate by microbial metabolism or mesophilic acidogenic culture is not significant enough to prevent accumulation (Karadag and Puhakka, 2010). However, this accumulation might inhibit the anaerobic digestion process and eventually causes digester failure. In fact, it could occur owing to hydrogenogenensis and inhibited methanogenesis under a pH between 5.0 and 6.5 (Fang and Liu, 2002). Besides, from Yuan *et. al*., (2006) point of view, hydrolysis rate, acidification process, and methanogens reproduction are pivotal in order to modify the rate of VFA accumulation. For instance, oxidative-redox-potential under -350 mV is found to optimized methanogens microbe's reproduction (Wang et al., 2014). Hence, a higher ORP might limit methanogen procreation while enhancing VFA production. Additionally, related to acidogenesis, a process that leads to VFA formation, the bioprocess is inhibited when pH is less than 4 (Wang et al., 2014). They also observed a similar condition under a low ratio of soluble chemical oxygen demand (sCOD) to volatile fatty acid. Hence, this confirms that suitable pH range for effective methanogenic activity is between 6.5 - 8.0, as higher microbial activities were noted at pH of 6.0 compared with lower pH (Wang et al., 2014). Related to waste source, Wang et al., (2014) observed that substrate with large population of hydrolytic and acidogenic bacteria often generate more VFA than those with lesser hydrolytic and acidogenic bacteria population.

2.5.7.2. VFA Composition

Also, worth mentioning is the effect of pH on VFA composition. A large percentage of VFA produced during anaerobic digestion is acetic acid since VFA with more than three carbon atoms such as propionic, butyric, or valeric acids easily biodegrade to form acetate (Wang et al., 2014). Primarily, VFA concentration determines the pH (Zhang et al., 2014) and this subsequently affects VFA composition. For instance, acetic and butyric acids production is relatively high at pH between 6.0 - 6.5, while isovaleric acid is only produced from proteinous degradation relative to acetic, propionic and butyric acids which are produced from directly soluble proteins, carbohydrates and lipids fermentation (McInerney, 1988, Horiuchi et al., 2002). This is an indication that some specific anaerobic bacteria influence the overall conditions in the digester.

Impact of VFA in relation to activities of methanogens have been studied and found to be a more consistent indicator of process imbalance relative to other indicators like pH, alkalinity, and buffering capacity (Murto et al., 2004). In a separate study, Wang et al., (2009) reported that acetic and butyric acid at 2400 mg/L (40 mM) and 1800 mg/L (20.42 mM) concentration, respectively, do not pose any significant threat on methanogenic activities. However, propionic acid at 900 mg/L (12.15 mM) concentration significantly impacts methanogenic activities.

Nevertheless, this is not a rule of thumb for all conditions as other factors such as experimental design play a major role in predicting concentrations of specific VFA that is suitable for its process (Angelidaki et al., 1993). Preferably, the ratio of acetic to propionic acid, ammonia to total VFA, alkalinity to VFA, butyric to acetic acid might be better indicators of bioreactor performance based on operational conditions. On the contrary, Ehimen et al., (2011) observed that acetic to propionic acid of 1.4 was not a suitable predictor of digester performance.

Overloading the reactor has also been linked with VFA accumulation resulting in digester failure (Akuzawa et al., 2011). According to Franke-Whittle et al. (2014), an AD experiment with a working volume of 173 m^3 under an organic loading rate (OLR) of 2.8 kg VS m^{-3} d^{-1} was considered to be overloaded, as the propionic: acetic acids ratio was greater than 1.4. Ratio of the propionic: acetic acids greater than 1.4 is indicative of process instability (Hill et al., 1987). In another study, ratio of total volatile fatty acids and total alkalinity (TVFA/TA) greater than 0.3 - 0.4 was considered unsuitable for AD processes (Gao et al., 2015; Li et al., 2014).

2.5.8. Organic Loading Rate

Another operational and environmental parameter applicable to the continuous AD system is the organic loading rate. In a semi-continuously stirred digester study, Astals et al., (2013) attributed an increase in methane yield to increased organic loading in a study on thermophilic co-digestion of swine manure with glycerol among other reasons. In the experiment, the authors noted that doubling the organic loading rate (OLR), from 1.4 to 2.6 g VS L^{-1} d^{-1}, increased methane production by 180 %. This organic loading rate is still within the safe value of organic loading recommended by Li et al., (2014) and Shi et al., (2016) was 3 g VS L^{-1} d^{-1}. Interestingly in another liquid state anaerobic digestion study with food waste as influent, stable operation condition, high VS reduction (over 90 %), and significant methane yield (455 mL / g VS) was achieved at 9.2 g

VS L^{-1} d^{-1} (Luste and Luostarinen, 2010). Unfortunately, OLR is not often applied in solid state anerobic digestion due to difficulty in the continuous loading and unloading of feedstock with TS > 15% (Brown et al., 2012).

2.5.9. Hydraulic Retention Time

Relative to other stated factors or environmental conditions and operational parameters, this equally affects the kinetics of anaerobic degradation (Vandenbroucke and Largeau, 2007). Depending on the experimental design, and AD types, hydraulic retention time varies. For instance, HRT of 230 days was considered effective for a semi-continuous SSAD study on food waste (Peng et al., 2018). However, in a mesophilic batch ternary SSAD study of dairy manure, corn stover and tomatoes residue by Li et al., (2018a), hydraulic retention time was 45 days, this time was only extended when some of the failed digesters had to be recovered with $NaHCO_3$. In another co-digestion study, at a liquid state and at thermophilic temperature, the retention time for a blend of swine manure and maize stalk was 35 days (Zhang et al., 2015). Hence, hydraulic retention time seems dependent on the experimental design and influent source. For instance, in a liquid and solid-state digestion study on wheat straw and some other lignocellulose biomass, peak methane yield of 12 L / kg VS/day was reached on day 7 for SSAD and a peak methane yield of 13 L / kg VS/day) on day-10 for LSAD (Brown et al., 2012). However, after 30 days of retention time, the LSAD had a higher cumulative methane yield of 140 L / kg VS/day relative to 125 L / kg VS/day documented for the SSAD (Brown et al., 2012).

2.5.10. Agitation

Views on agitation has been divergent. Some researchers believe that continuous agitation of an anaerobic digester allows for even nutrient distribution, uniform microbial distribution and also solves mass transfer limitation, particularly in SSAD and hence improves biomethanization

(Li et al., 2016). On the contrary, some microbiologists have suggested intermittent agitation in order to allow for minimal disruption of bacterial granular structure that negatively impact methanogens (McMahon et al., 2001; Ong et al., 2002). In these studies, extracellular polymeric substances production was not affected, an indication that cell detachment did not occur during the digester agitation

2.6. Enhancement of Anaerobic Digestion

There are presently a number of methods employed to improve anaerobic digestion end-products such as methane concentration and digestate quality. These might include some process upgrade, ingestate modification or introduction of some nutrients. Examples of ways to enhance AD process and methane yield includes two-stage anaerobic digestion, pretreatment etc. A number of these examples are discussed in subsequent sections.

2.6.1. Two-stage Anaerobic Digestion

This design implies that the anaerobic process has two separate stages, often the hydrolysis stage in the presence of little or no oxygen and then the methane forming stage in a completely anaerobic environment. A more detailed procedure of the two-staged process is a thermophilic hydrolysis and acidification stage followed by mesophilic methanogenesis stage (Mao et al., 2015). A two-stage reactor was employed to address the negative impact from VFA formation and accumulation in AD process due to the presence of limonene from citrus waste, the process prevented reactor failure and enhanced optimum conditions (Millati et al., 2018).

2.6.2. Co-digestion

This method involves the blending of feedstocks together in order to achieve nutrient compositional balance, enhance buffering capacity of feedstock or in a bid to dilute some highly concentrated parameters that might inhibit methane forming bacteria. etc. Co-digestion has the potential to enhance system efficiency in anaerobic digestion (Kavacik and Topaloglu, 2010). Another merit of co-digestion is that digestion time is shortened, and biogas production is enhanced. In a study whereby corn stover was pretreated with sodium hydroxide prior to blending with swine manure, digestion time was shortened from 18 days to 13 days (You et al., 2014).

Yue et al., (2013) studied the effect of co-digestion of dairy manure with corn stover and they observed that co-digestion enhanced biogas productivity by 23, 20, and 21% for 30, 40, and 50 day's hydraulic retention times, respectively. In another study, tomato residues were co-digested with a mixture of dairy manure and corn stover in a solid-state anaerobic study with a 20% TS (Li et al., 2016). The peak daily methane yield was between 15 - 28 L/kg VS for all the categories investigated and peak methane content was between 55 - 80% for the 45-day hydraulic retention time experiment. In the same study, the liquid state anaerobic digestion of individual substrate (dairy manure, corn stover and tomatoes residue) considered failed and the methane concentration was rejuvenated by adding $NaHCO_3$ (Li et al., 2016).

Wei et al. (2015) studied co-digestion of corn stover prior to blending with cattle manure under various pretreatment at mesophilic condition and they found that, cumulative biomethane production for 2% sodium pretreated corn stover blended with dairy manure was 9769.46 mL/g TS, this was 23% greater than non-pretreated corn stover blended with the cattle manure. Hence, the following studies have shown that co-digestion enhances both methane yield and gas composition.

2.6.3. Pretreatment

The major challenge with the utilization of lignocellulosic biomass as a substrate in anaerobic digestion despite its potential availability is because of its high lignin content, strong cellulose crystallinity, and its thick vascular bundles and tissues (Song et al., *2012*). All these inhibit microbial degradation, lengthen retention time and invariably reduce biogas production. Hence, pretreatment has been adopted to improve the digestibility and degradability of lignocellulosic biomass in order to enhance anaerobic fermentation. One of the commonly adopted pretreatment methods for lignocellulosic biomass is alkaline pretreatment. Others are thermal pretreatment, microwave irradiation, and biological pretreatment methods. A few of them has been discussed in subsequent sections.

2.6.3.1. Alkaline Pretreatment of Lignocellulosic Biomass

Alkaline pretreatment has been recommended as the most effective method for lignocellulosic biomass pretreatment. The method solubilizes hemicellulose, modifies lignin and cellulose crystallinity, and equally dislocates lignocellulose tissues. Zheng et al., (2014) further reported that this pretreatment increases internal surface area, polymerization, porosity and causes structural swelling of the fiber content of the biomass. The advantages of this method over other pretreatment methods include low cost and easy usage. However, the process reduces the C/N ratio and hemicellulose quantity of the biomass (Hassan et al., 2016).

Furthermore, alkaline pretreatment method could be enhanced with thermal inclusion, especially to reduce pretreatment time. A study on thermo-chemical pretreatment of corn stover with hydrogen peroxide and sodium hydroxide under 1 hr pretreatment time and 80 ^{o}C heating temperature improved lignocellulose degradation by 45 and 42% in that order (Hassan et al., 2016).

2.6.3.1.1. Alkaline Pretreatment Reagent

A. Hydrogen peroxide as alkaline pretreatment agent for lignocellulosic biomass

Hydrogen peroxide is a de-lignifying agent. It attacks the inter-lignin bond in lignocellulosic biomass via its oxidative properties to yield digestible products. Another advantage is the reduction in retention time. In a thermo-chemical study conducted by Hassan et al., (2016), about 70% of the overall methane production was documented in the first 20 days of the anaerobic digesters set-up. Methane production was equally enhanced by 9% when hydrogen peroxide concentration was increased from 4.5 to 7.5%. These concentrations were suitable for anaerobic digestion, as they did not result in hydroxyl ion production and accumulation that might inhibit methanogens.

B. Calcium hydroxide as alkaline pretreatment agent for lignocellulosic biomass

Two different quantities (5 and 7 g/L) of $Ca(OH)_2$ were utilized as pretreatment reagent in order to improve corn stover digestibility and ultimately enhance methanogenesis (Hassan et al., 2016). Increase in reagent loading increased C/N reduction and lignin content. This trend was contrary to what was observed when both hydrogen peroxide and sodium hydroxide were utilized as reagents (Hassan et al., 2016). Methane production was higher with the lower $Ca(OH)_2$ quantity. This establishes Chen et al., (2008) observation that excess lime inclusion in anaerobic digester might result in system failure.

C. Sodium hydroxide as alkaline pretreatment agent for lignocellulosic biomass

The solubilization property of sodium hydroxide makes it very effective in lignocellulosic biomass delignification. A liquid state study that compared the delignification potential of 5 g/L of NaOH and $Ca(OH)_2$ on corn stover, NaOH had 46% delignification more than $Ca(OH)_2$ and about 23.1% less C/N reduction compared with calcium hydroxide (Hassan et al., 2016). They also compared the influence of $Ca(OH)_2$, H_2O_2 and NaOH pretreatment on methane production, there an inclination in shortened retention time, because 70% methane production occurred in the first 20 days. Nonetheless, NaOH had steady daily methane production, this was not the case with H_2O_2 and $Ca(OH)_2$. Further investigation relating to the effect of NaOH concentration on biogas production shows that using 10% NaOH concentration at 40 ◦C for a 1hr pretreatment enhanced methane production by 47% (Hassan et al., 2016).

Another important parameter that affects methane production is the volatile fatty acids content. From the previous study, NaOH and H_2O_2 pretreated corn stover had better overall maximum VFA stabilization compared with $Ca(OH)_2$. This was strongly linked to their high methane productivity. However, Zhang et al., (2015) reported that high concentration of NaOH could result in VFA reduction, when NaOH severity is greater than 40 mg, despite NaOH potential of reducing long chain fatty acids to shorter ones. The explanation for this reduction was that at elevated NaOH concentration, protective films were formed on the cell surface which inhibits lipid degradation.

2.6.3.1.2. Limitations in Alkaline Pretreatment

Some of the challenges with alkaline pretreatment includes a reduction in solid recovery with an increase in chemical loading. In a pretreatment study conducted by Zhao et al. (2014) observed that solid loss was lower when ammonia fiber expansion pretreatment was adopted for corn stover modification (2.3 - 3.8 %) compared with when hydrogen peroxide was used as the pretreatment agent (3.6 - 13.6%) irrespective of the chemical loading. However, delignification increased with chemical loading in the study.

2.6.3.2. Thermal Pretreatment

In thermal pretreatment or hydrothermal pretreatment, temperature and exposure time are the only required indices, hence it is majorly carried out in an incubator. The main advantage of the process is that chemical usage is not required, which makes the process simple and, hence a form of pretreatment cost minimization. While pretreatment temperature over100 ^{o}C results in toxic or inhibitory intermediate products (Wilson and Navak, 2009). Interestingly, thermal pretreatment is suitable for feedstock, such as sludge, in which a chemical method might not be appropriate. Furthermore, positive correlations have been established between pretreatment temperature and VFA degradation. In a thermal pretreatment study conducted by Zhang et al., (2015), there was strong correlation between thermal pretreatment temperature under 60 minutes and VFA degradation in the anaerobic study. This was because biodegradability increased with the severity of thermal pretreatment. For instance, feedstock thermally pretreated at 100 ^{o}C had higher VFA degradation (9 %) prior to digestion relative to the same feedstock thermally pretreated at 80 ^{o}C. An indication that temperature had significant effect on VFA degradation. However, when the pretreatment temperature was increased to 120 ^{o}C, there was no significant difference ($p > 0.05$) in VFA degradation prior to digestion compared with the 100 ^{o}C pretreatment temperature (Zhang et al., 2015). A similar effect on VFA degradation was observed when sludge was thermally pretreated at lower temperatures (70 - 90 ^{o}C) pretreated sludge on VFA degradation was observed by Appels et al., (2010). However, in the study, VFA degradation significantly increased with pretreatment temperature (70, 80, and 90 ^{o}C) considered (Appels et al., (2010).

2.6.3.3. Thermo-chemical Pretreatment

Most chemical pretreatment methods are combined with heat pretreatment to form thermochemical pretreatment. One of the advantages of the thermochemical pretreatment method is the reduction in pretreatment time as compared with chemical pretreatment. Relative to biological and mechanical pretreatment, thermo-chemical pretreatment feedstock has shorter processing time, high biogas yield, and lower energy requirements (Kumar et al., 2009). In a corn stover thermochemical pretreatment study conducted by Hassan et al., (2016), pretreatment time was reduced from day(s) under a chemical pretreatment method to one hour. Furthermore, methane yield from the pretreated corn stover was between 275 – 310 mL/g VS. Recently, freezing-thawing is another form of thermo-chemical pretreatment. However, this form of thermo-chemical pretreatment occurs under a temperature lower than ambient condition. In a study conducted by Yuan et al, (2018), corn straw soaked in ammonia solution was freeze-thawed under -20 ^{o}C prior to digestion. Results from the study shows that, cumulative biogas production of the influents or ingestates was about 250 ml/ g VS. This methane yield is indicative of the effectives of the freezing-thawing pretreatment (Yuan et al, (2018). However, there are high tendency for leachate (chemical and water) loss after this pretreatment process.

2.6.3.4. Wet State Pretreatment

Unlike the thermo-chemical pretreatment method, the advantages of wet state pretreatment method include minimal feedstock loss, no leachate production, no chemical waste, and less energy use since only ambient temperature is required during the biomass retention time (Zheng et al., 2009). Furthermore, wet state pretreatment method has gained huge attention as a prominent biomass pretreatment because it does not lead to intermediate toxic compound production like thermal pretreatment (Zheng et al., 2014). Additionally, using a wet state pretreatment method for biomass has enhanced lignocellulosic degradation and methane yield significantly. In a study conducted by Song et al., (2014) more than 100% increase in methane yield was observed when corn stover was chemically pretreated using the wet state pretreatment method relative to the untreated. Interestingly from the same study, wet state pretreatment was applied to the biomass with both acidic and alkaline reagents, though alkaline reagents such as NaOH and $Ca(OH)_2$ have outperformed most of the acidic reagents (Song et al., 2014).

2.6.3.5. Biological Pretreatment of Lignocellulosic Biomass

Biological Pretreatment is the use of microorganism or enzymes to enhance lignocellulose degradation. The method is environmental-friendly and inexpensive. Other advantages stated by

Sindhu et al., (2016) was that the process does not require chemical recycling, by-products of the process do not inhibit hydrolysis and there are no toxic chemical releases to the environment. Albeit, the process is time-consuming (Zheng et al., 2014) and on a large scale requires high operational cost accrued to sterile conditions needed (Caturvedi and Verma, 2013). One of the promising micro-organisms used to degrade lignin in lignocellulosic biomass is white rot fungi (Chen et al., 2010), this is because both lignin and hemicellulose restrain accessibility to cellulose. Recently, more studies have adopted the use of fungi and bacteria consortium. For example, Song et al., (2013) achieved 43 % lignin removal and seven-fold increase in hydrolysis when a fungal consortium was used for corn stover pretreatment.

2.6.4. Additives and Nanoparticles

2.6.4.1. Appropriate Quantity of Trace Elements.

A number of factors influence AD process but recent specificity on the effect of some elements at improving anaerobic digestion is worth mentioning. Cobalt, nickel, magnesium, iron, and potassium were introduced into a bioreactor loaded with brewery spent grains, which without these elements would have failed. The substrate not only became usable feedstock for anaerobic digestion, methanization and biodegradability was enhanced as the AD process was stable, while short chain and long chain fatty acid accumulation were avoided (Bougrier et al., 2018). It is interesting that some of these elements such as Mg and Ca were introduced via tap water, though in minute quantity.

2.6.4.2. Nanoparticles

Introduction of zero-valent iron (ZVI) into bioreactor is one of the recent suggestions to enhance anaerobic digestion. The uniqueness of the low-cost substance that originates from waste includes its ability to decrease oxidative–reductive potential (ORP) of the anaerobic digestion media and hence provide a more suitable condition for anaerobic digestion (Zhen et al., 2015). The presence of iron in ZVI, a cofactor in several enzymatic stages of the fermentation process was also an added advantage (Zhang et al., 2012). Additionally, ZVI has been found to enhance propionate fermentation. This propionate concentration regulation process improves methanogenic activities (Hao et al., 2017). However, corrosion of ZVI releases Fe^{2+} that affects physicochemical properties, lead to pyrite precipitation and phosphorus entrapment (Heiberg et al., 2012; An et al., 2014; Jia et al., 2017), as it is related to the concentration and quantity applied. An AD study on treatment plant waste sludge conducted with ZVI shows the introduction of 2.5 kg ZVIm^{-3} of ZVI enhanced methane production when compared with the control. However, when the concentration was increased to 10 kg ZVIm^{-3} biogas production reduced significantly (Puyol et al., 2018). However, introduction of 20 mg/L of magnetite (Fe_3O_4) with particle size of 7.0 nm into the bioreactor produced a higher methane yield than when ZVI (9.0 nm) in a mesophilic study. Precisely, while methane in Fe_3O_4 treated digester increased by 2.16-fold relative to control, only 1.67-fold increase was documented with ZVI treated digester (Abdelsalam et al., 2016).

2.7. Inhibition in Anaerobic Digestion

Inhibitors such as ammonia, sodium, long chain fatty acids, chlorophenols, and halogenated aliphatic retard anaerobic digestion and may even stop the process. Thus, they have to be monitored and their actions reduced (Feijoo et al., 1995, Yenigun et al, 2013; Chen, et al, 2014). The action of sulphur reducing bacteria in converting sulphate to an AD inhibitor called sulphide is notable. The bacteria equally compete with methanogens (Kao et al., 2008; Ruiz-Marin et al., 2010). VFA accumulation, particularly in terms of acetate, is another major inhibitor to the syntrophic acetogenic bacteria. This favors hydrolytic fermentative bacteria and jeopardizes the abundance of syntrophics, by resulting in more propionate, valerate, and some other long chain fatty acids in a food waste study (Peng et al., 2018). In the same study, an indirect positive relationship was established between VFA and free ammonia accumulation. This accumulation led to enormous foam formation that invariably led to the digester clogging. A study conducted on the relationship between total ammonia nitrogen inhibition and some VFAs, researchers observed that TAN inhibition causes acetate to initially rise and then fall while propionate continues to increase (Sun et al., 2016). Furthermore, to avoid system failure, TVFA/TA in an AD process should not exceed 0.3 – 0.4 (Gao et al., 2015; Li et al., 2014). Also, worth mentioning is the role trace elements play in preventing digester inhibition, this is discussed in a subsequent section. Chen et al., (2015) demonstrated stable process conditions in an LSAD study on the co-digestion of food waste and rice straw. In the study, pH (7.11 – 8.15), ammonia nitrogen (0.5 – 0.6 g/L), TVFA/Alkalinity (0.01 – 0.03) and ORP (- 328 to -367 mV) were maintained within these ideal conditions for methanogenesis. However, this ammonia nitrogen threshold was significantly lower than 7 g/L stated by Sun et al., (2016) in SSAD study on undiluted chicken manure and maize silage. Hence, the threshold for process parameter conditions might differ between SSAD and LSAD.

2.8. Models Adopted in AD

Predictive models have been used to ensure consistency in biogas production despite process variability in AD, especially in continuous flow bioreactors. The major benefit of this predictive model in thermophilic AD process as observed by Tan et al., (2018), is the significant reduction in repeated pilot testing. Some of the models that have been adopted for municipal and industrial wastes are anaerobic digestion model no. 1 (ADM1) and adaptive neuro-fuzzy inference system or adaptive network-based fuzzy inference system (ANFIS). With ADM1, correlations between parameters can be easily determined because all reactions and mechanisms were defined.

Similarly, the ANFIS model can equally adopt numerous data as well as historical data. Nonetheless, the model had to be modified to cater for feedstock variation due to seasonality and other factors. Recent studies are principally on a model modification for a more profound understanding of biogas production such as dynamics of AD processes, nutrient release, pH, and methanogenesis (Puyol et al., 2018).

2.9. Economics on AD

A number of factors affect production and maintenance or management costs in a typical AD process. For instance, most full-scale digester operates on an inhibited steady state that results in about 30% loss (Li et al., 2018a). This will adversely contribute to profitability which is a major concern in the biogas industry (Rajendran et al., 2014). In some studies, process design and energy estimation have been identified and considered as measures to address this economic challenge in this industry (Li et al., 2016; Li et al., 2018b). For example, the concept Li et al., (2018a) adopted was to enhance methane yield by introducing tomato residues as a ternary mixture in SSAD study. This inclusion improved methane yield, thereby compensating for extra energy input from tomato residue addition. Hence, to benefit more from the economic standpoint, feedstock with low VS and TS should be considered in SSAD.

3. IMPACT OF CORN STOVER PARTICLE SIZE AND DAIRY MANURE CO-DIGESTION ON METHANE AND BIOGAS YIELD IN SOLID-STATE ANAEROBIC CO-DIGESTION[1]

3.1. Abstract

Agricultural waste constitutes a significant fraction of global waste and hence a good management practice of this waste is imperative. In this study, the utilization of corn stover and dairy manure as substrates for solid-state anaerobic co-digestion was considered under mesophilic condition (37 ± 1.5 ^{o}C). Dairy manure and corn stover were blended to achieve three different C/N ratios (24, 28, and 32). The five blends produced (Experiment 1, Experiment 2, Experiment 3, Experiment 4, and Experiment 5) were analyzed before and after digestion for chemical composition, biogas yield and composition. Results from this study suggest that Experiments 1 and 2 had the highest methane yield (53 and 106 L/ kg VS), biogas yield (140 and 231 L/ kg VS) and peak methane concentration (55 and 60%) respectively. However, the particle size of corn stover in the blend of Experiments 1 and 2 had a significant impact ($p < 0.05$) on holocellulose degradation. Notably, failure in Experiments 3, 4 and 5 was attributed to low alkalinity in corn stover and high initial influent C/N. Conclusively, a binary mix of corn stover and dairy manure with C/N of and DM:CS of 1:1 based on VS, was considered most suitable for solid-state anaerobic co-digestion.

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[1] The material in this chapter was co-authored by Ademola. A. Ajayi-Banji, S. Rahman, S. Sunoj, C. Igathinathane. Ademola Ajayi-Banji had primary responsibility for collecting samples and analyzing laboratory data. Ademola Ajayi-Banji also drafted and revised all versions of this paper. Shafiqur Rahman, S. Sunoj and C. Igathinathane are contributing authors reviewed the manuscript and statistical analysis conducted by the primary author. Shafiqur Rahman is the corresponding author. It has been published in Journal of the Air & Waste Management Association Journal (https://www.tandfonline.com/doi/full/10.1080/10962247.2020.1729277)

3.2. Introduction

Agricultural waste is a significant fraction of biodegradable waste generated globally. Recently, the baseline estimated volume of the lignocellulosic fraction of this waste in the US is about 136 million dry tons (USDA, 2011). Biologically, the waste could either be treated anaerobically or in the presence of oxygen through microbial degradation. The benefits of the anaerobic waste treatment relative to the aerobic include odor mitigation and methane capture as green energy. However, a number of factors influence this green energy production in anaerobic digestion. This includes carbon to nitrogen ratio (C/N) of influent, digester temperature, feedstock particle size, ingestate pH, and alkalinity among others (De Vrieze et al., 2015; Izumi et al., 2010; Wang et al., 2014). For instance, C/N between 20 – 30 have been recommended for effective digester performance (Brown and Li, 2013). Notably, this range seems wider for a branch of anaerobic digestion called solid-state anaerobic digestion (SSAD). Furthermore, in a solid-state co-digestion (SSCOD) study on food waste and sewage sludge, effective ingestate C/N for optimal digester performance was between 6 - 15. In another SSAD study, the best C/N ratio was 19 -25 (Brown and Li, 2013). Interestingly, C/N modification could be achieved by co-digestion. In a study conducted by (Zhang et al., 2013), co-digestion of dairy manure with food waste adjusted the ingestate C/N to (15 - 18). Furthermore, this co-digestion process could improve biogas yield and also mitigate inhibition (Cuetos et al., 2013). In a ternary study conducted by (Wang et al., 2014), ingestates with C/N adjusted to 25 and 30 were found to outperform ingestates with C/N of either 15 or 20 in terms of biogas production and methane yield. Interestingly, another benefit of co-digestion is the diverse bacterial diversity in the reactor relative to mono-digestion (Wang et al., 2012).

With respect to particle size, studies have suggested that particle size affects methane yield. For instance, reduction of food waste particle size from 8.0 mm to 2.5 mm in a liquid-state semi-continuous co-digestion study of food waste and dairy manure enhanced methane yield by at least 9% (Agyeman and Tao, 2014). This improvement was attributed to the larger surface area of the ingestates that allows for more hydrolytic activities (Izumi et al., 2010). However, in a solid-state mono-digestion study of wheat straw with three average particle size grades (1.45, 0.67, and 0.11mm), the impact of C/N overrode the effect of particle size on methane yield (Motte et al., 2013). Ingestates with C/N more than 20 failed in the study irrespective of particle size grade, that significantly influenced soluble fraction concentration (Motte et al., 2013). However, in solid-state anaerobic co-digestion studies, the combined effect of particle size and C/N on methane yield has not been vastly studied.

In addition to particle size and C/N, feedstock to inoculum ratio (F/I) is another important factor that could significantly impact green energy production through anaerobic digestion. Hence, F/I between 0.9 – 1.1 (1 - 3 VS based) has been often suggested for suitable SSAD of lignocellulose under mesophilic condition (Liew et al., 2012; Zhu et al., 2014; Li et al., 2014). However, there was no methane inhibition when F/I ratio of 4 was considered in a SSAD study on tomato residue, corn stover and dairy manure (Li et al., 2018).

Hence, the objective of this SSCOD study was to investigate the effect of two corn stover particle size (0.18 – 0.42 mm and 0.42 – 0.84 mm) blended with dairy manure with C/N ratio (24, 28, and 32), on process parameters, biogas yield, biogas composition, and methane yield.

3.3. Material and Methods

3.3.1. Dairy Manure and Inocula Collection

Fresh dairy manure from North Dakota (ND) State University Dairy Farm, ND, USA was collected in a 20 liter plastic container. Inocula of about 7 liters each were obtained from mesophilic anaerobic digesters in either American Crystal Sugar Company, Moorhead, Minnesota, USA and Fargo Wastewater Treatment plant in ND, USA. All the collected substrates were used on the same day of collection. Characteristics of these substrates are presented in Table 3.1.

3.3.2. Corn Stover Collection and Pretreatment

Corn stover was obtained from Carrington Research and Extension Center, Carrington, North Dakota. The stover was crushed into < 3 mm particle size with a 3 mm-mesh-sieve-sized Schuttle Buffalo hammer mill (Model W6H, New York, USA). Crushed stover were sieved into 0.18 - 0.42 mm and 0.42 – 0.84 mm using a mechanical sieve shaker. The sieved stover was stored in 10 liters Ziploc bags under ambient condition (Figure 3.1). Characteristics of this substrate is presented in Table 3.1.

Table 3.1: Substrates characteristics.

Parameter	Corn stover	Dairy manure	Inoculum
pH	6.0±0.1	7.8±0.6	7.1±0.6
VS (%)	94.1±0.8	86.5±3.6	57±17.5
TS (%)	99.0±0.8	17.8±5.0	1.4±0.6
C (%)	41.0±0.1	38.9±3.3	24±8.8
N (%)	0.8±0.1	2.8±0.3	4.1±2.4
Alkalinity (g/L)	1.7±0.1[#]	8.3±1.8	1.6±0.3
C/N	51.3±0.1	14.4±2.2	7.5±0.7
Cellulose (%)	43.5±0.3	26.4±3.3	22.8±0.0
Hemicellulose (%)	34.2±0.1	20.9±0.5	14.0±0.0
Ash (%)	5.7±0.3	15.2±2.1	67.1±0.0
Lignin (%)	5.1±0.2	9.5±2.3	4.1±0.0
Amm-N (mM)	0.0±0.0	92.6±19	34.6±3

*Data represent the mean ± standard deviation, Amm-N represents ammonium nitrogen
[#]Alkalinity of corn stover used for Experiments 1& 2 was 5.5 g/L

A

B

Figure 3.1: (a) Unmilled and (b) milled corn stover.

3.3.3. Substrates Mix

These feedstocks (inoculum, dairy manure, and corn stover) were mixed based on carbon to nitrogen ratio (C/N) calculation as indicated in equation 3.1. Estimated quantities of the feedstocks based on the mix calculation are presented in Table 3.2. Furthermore, the resulting ingestates compositions are also presented in Table 3.3. Interestingly, this C/N estimation procedure was also adopted by Wang et al., (2014) for C/N adjustment. In this study, F/I ratio based on VS was between 1- 3 (Table not shown).

$$R = \frac{M1C1TS1 + M2C2TS2 + M3C3TS3}{M1N1TS1 + M2N2TS2 + M3N3TS3} \tag{3.1}$$

Where,

R represents the carbon to nitrogen ratio of the three feedstocks,
M1 denotes the moisture content (wet basis) of the dairy manure,
C1 represents the % carbon content (dry basis) of the dairy manure,
N1 denotes the % nitrogen content of the dairy manure,
TS1 represents the % total solids of the dairy manure,
M2 denotes the moisture content (wet basis) of the inoculum,
C2 represents the % carbon content (dry basis) of the inoculum,
N2 denotes the % nitrogen content (dry basis) of the inoculum,
TS2 represents the % total solids of the inoculum,
M3 denotes the moisture content (wet basis) of the corn stover,
C3 represents the % carbon content (dry basis) of the corn stover,
N3 denotes the % nitrogen content (dry basis) of the corn stover, and
TS3 represents the % total solids of the corn stover.

Table 3.2: Mix ratio for pretreated ingestate.

Experiment (Ingestate)	Quantity of dairy manure (%)	Quantity of corn stover (%)	Quantity of inoculum (%)
1 & 2	34.6	32.2	33.2
3	29.4	60.5	10.1
4	38.4	17.5	44.1
5^{R}	48.1	11.1	40.8

R indicates quantity of dewatered liquid from ingestate.

Table 3.3: Initial composition of ingestate.

Experiment	Experiment 1&2-Ingestate	Experiment 3-Ingestate	Experiment 4-Ingestate	Experiment 5-Ingestate
pH	$7.5 \pm 0.1^{*a}$	6.8 ± 0.0^{b}	7.5 ± 0.0^{a}	8.3 ± 0.0^{c}
Total VFA (g/L)	7.6 ± 0.1^{a}	2.4 ± 0.0^{b}	2.1 ± 0.0^{c}	5.5 ± 0.0^{d}
Ammonium- nitrogen (mg/L)	46.4 ± 2.1^{a}	8.9 ± 0.1^{b}	19 ± 0.0^{c}	51.8 ± 0.0^{d}
Alkalinity (g/L)	4.0 ± 0.4^{a}	4.1 ± 0.0^{a}	2.5 ± 0.0^{b}	5.7 ± 0.0^{c}
C/N	24 ± 2.5^{a}	34 ± 0.0^{b}	28 ± 0.5^{c}	24 ± 0.0^{a}

[*]Values are expressed in means ± standard deviation.
Means followed by the same letter in a row are not significant.

3.3.4. Experimental Set-Up

Based on the C/N calculation, required quantities of dairy manure, inoculum, and pretreated corn stover were thoroughly mixed to ensure ingestate was homogenous. After homogeneity is ensured through physical observation, 1000 g or 1500 g of ingestate containing a mixture of dairy manure, inoculum, and untreated corn stover was introduced into the digester. Detailed quantity and mix ratios of the ternary composition of ingestate for each digester was presented in Table 3.1. The 6 liter digesters used in this study were compartmentalized into two sections, the 3.5 liters working volume and the leachate collection volume. After loading, the sealed digesters were deoxygenated with nitrogen gas and then check for gas leak. Aside from the latter reason, the introduced nitrogen gas equally flushes out oxygen from the digester in order to create an enabling environment for the anaerobic microbes.

After the stated procedures were completed, the digesters used in this study were immersed vertically in 0.73 m × 0.49 m × 0.53 m plastic tub filled with water. The tub was coupled with a thermostatically controlled heater set at 37 ^{o}C (Figure 3.2). The water volume in the tub was daily maintained by manual water introduction. To quantify the biogas volume produced from the respective digesters, a rectangular glass chamber with a tipping bucket system was coupled with a sensor for real-time data collection. This chamber was filled with water to allow for easy bucket tip when about 12 – 13 mL of biogas is produced. Data obtained from this chamber were collected and stored every minute using a the CR-1000 data recorder.

A

B

C

D

Figure 3.2: Experimental set –up.
Figure 3.2A indicates the 6 litre digester with a air inflator to check for leak in digester prior to experimental set-up, Figure 3.2B shows the six gas collector chambers connected to a data logging system, Figure 3.2C and 3.2D show the complete and running anaerobic digestion set-up.

3.3.5. Gas Analyses

In order to analyze gas composition weekly, gas produced from the digester was collected from the head space of the digester by inserting a 20 mL syringe through a septum stopper attached to one of the gas outlets. The gas was analyzed for biogas (methane and carbon (iv) oxide) and hydrogen sulfide composition using a gas chromatograph (GC, 8610C, SRI California, USA) and hydrogen sulfide analyzer (Jerome 631X, Arizona Instrument LLC, Arizona, USA) respectively. The gas chromatograph used was equipped with a flame ionization detector (FID) for CH_4 and CO_2 analyses. It was equally equipped with an electron capture detector (ECD) detector for nitrous oxide (N_2O) gas analysis. The chromatograph was operated on a 20 PSI N_2 carrier for the ECD while hydrogen gas an air was supplied on a 20 PSI to the FID or methanizer. The calibration curves were generated from four different CH_4 (5, 10, 20, and 100 ppm) and CO_2 (100, 500, 1000, and 2500 ppm) concentrations were used to compute the values for the curve area. Also, worth mentioning was the 100 ^{o}C pre-set temperature of the gas chromatograph prior to any analysis. This ensured stability in results. On the hydrogen sulfide analyzer, the Jerome meter works through a thin gold film subjected to variation through electrical resistance that senses the gas concentration. Prior to both analyses, 5mL of the collected gas was introduced into a 0.5 L SKC Tedlar bag and then diluted with 500 mL of N_2 prior to manual mixing. This dilution factor was considered in the result presentation.

3.3.6. Ingestate, Leachate, and Digestate Analyses

In order to avoid crust formation due to seepage and filtration, the digesters were periodically agitated manually based on the experimental design. This process was a form of leachate return without air introduction. Subsequently, 4 – 7 mL of these leachates were collected periodically (weekly) through the digester outlet tap for ammonia, pH, volatile fatty acids (VFA), ammonia –nitrogen, oxidation redox potential (ORP), and electrical conductivity (EC) analyses using standard methods. Samples were also collected from the fresh dairy manure, inoculum, corn stover, ingestate and digestate for ammonia, nitrite, nitrate, iron, phosphorus, ash content, and alkalinity using standard methods. Carbon and nitrogen content of these samples were inspected with combustion analyzer- Elemental Vario Macro Cube –CNS analyzer following the protocol described by Pella (1990). In order to validate the accuracy of the combustion analyzer, total nitrogen was also inspected with Kjeldahl method (Isaac and Johnson, 1976). Ammonium–nitrogen was evaluated following the international organization of standardization procedure for water quality determination of ammonium nitrogen by flow analysis, ISO - 11732:1997 protocol. On the other hand, nitrate and nitrite nitrogen was analyzed with the EPA 353.2 revised method (1993). Total phosphorus content in these samples were also determined after the sample digestion was carried out with the method described by Watson et al., (2003). In this digestion procedure, nitric acid was digested with peroxide in a block digester and afterwards, the phosphorus content was measured with a Brinkmann PC 910 colorimeter. To quantify the iron concentration in all these samples, samples were first digested similarly to the procedure previously described for total phosphorus (Watson et al., 2003). Thereafter, iron concentration was measured with a Buck Scientific 210 VGP atomic absorption spectrophotometer.

Alkalinity was another important parameter that was measured for all the samples. This was carried out with the method described by APHA (1992) using the titration method with 0.01N sulfuric acid. Other parameters measured were dry matter, ash content, crude protein, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). Dry matter of these samples was measured with the procedure stated by Hoskins et al., (2003) and AOAC #934.01 with #930.15 (2010). Crude protein protocol followed AOAC official method #2001.11

(2010). ADL was also measured with AOAC official method #973.18 (2010). However, NDF and ADF followed a different procedure described by Goering and Van Soest, (1970).

Volatile fatty acids were measured with the procedure described by Baumgardt (1964), while ammonium nitrogen was determined with the colorimeter determination of urea nitrogen protocol described by Sigma Technical Bulletin. Brief description of the VFA protocol. Sample was introduced into a 25 mL centrifuge tube prior to vortex at 20000 X g for 10 minutes. 5 mL of the supernatant produced from this process was pipetted into another centrifuge tube and a 1 mL 25 % metaphosphoric acid solution added prior to another vortex for 30 minutes. Filtered liquid was collected and loaded into the Agilent 6890N Gas Chromatograph with an FID (flame ionization detector) and the 7683 Series auto injector and auto sampler. Column used was the Supelco brand, NUKOL Fused Silica Column (Column (Agilent Technologies, California, USA), 15 mm × 0.53 mm × 0.5 um GC. This GC analysis followed the protocol described by Goetsch and Galyean, (1983) and the acids analyzed with the GC were acetic, propionic, isobutyric, butyric, isovaleric, and valeric.

A similar initial procedure for VFA analysis was carried out for ammonium – nitrogen quantification prior to when the metaphosphoric acid solution was added and then vortex. The difference after these steps were a 10-fold fluid dilution, followed by the introduction of 100 µL of this diluted sample into three borosilicate tubes and then add 1.0 mL phenol nitroprusside solution, 1.0 mL alkaline hypochlorite solution, 5.0 mL deionized water prior to vortex of the three tubes. A detailed procedure is found in the protocol described by Sigma Technical Bulletin. pH, ORP, and EC of these samples were measured with HANNA HI 4522 dual channel benchtop meter (Hanna Instrument, USA).

3.4. Statistical Analysis

Statistically, the significant difference among treatments means for the completely randomized design used in this study was investigated with SAS 9.4. TS Level 1M4. X64_10PRO platform. Duncan's multiple range test was used to evaluate the significant treatments using the same SAS tool with a threshold p-value of 0.05.

3.5. Results and Discussion

Results presented in this section are dependent on the retention time at which each digester stop production, either due to low biogas production or digester failure.

3.5.1. Process Parameters

Figures 3.3 – 3.7 show that a combination of factors such as low alkalinity, minimal nitrogen content, and poor nitrogen mineralization to ammonia of influents were the major factors that contributed to VFA accumulation, which invariably led to low methane and biogas yield. In most SSAD studies, alkalinity required to maintain the SSAD process was provided by the inoculum. For example, inoculum with 12.9 – 15.6 g/kg $CaCO_3$ alkalinity content was considered suitable for a SSAD of switchgrass (Sheet et al, 2015). While inoculum with 19.8 g/kg $CaCO_3$ alkalinity was deemed suitable for a corn stover SSAD study (Li et al., 2011). The inocula used in this study had low alkalinity (1.6 g/kg $CaCO_3$), but with a high nitrogen content of about 4.1% (Table 3.1). Hence, we relied mainly on the alkalinity from dairy manure to serve as buffer. Alkalinity from this freshly collected dairy manure was 8.3 g/kg $CaCO_3$ (Table 3.1), which was close to the alkalinity value (10.3 g/kg $CaCO_3$) documented by Li et al., (2018a) for fresh dairy manure in an SSAD study of tomato residue, dairy manure, and corn stover blend. This slight variation could be attributed to the difference in the manure source. Another important observation from Li et al., (2018) co-digestion study was that, the acidic feedstock (tomato residue) has an alkalinity less than 2.4 g/kg $CaCO_3$, hence, this must be mixed with non-acidic ones with total alkalinity around (20 – 23 g/kg $CaCO_3$). Under this condition, non-acidic feedstocks would sufficiently prevent pH drop from the acidic feedstocks. Though, the alkalinity of the other feedstock (corn straw) used in Li et al., (2018) study was not quantified, the ingestates alkalinity were between 7.9 - 9.1 g/kg $CaCO_3$. Hence, it seems 7.9 - 9.1 g/kg $CaCO_3$ was the suitable alkalinity range for ingestates based on Li et al., (2018) SSAD studies. In this study, alkalinity for all the ingestates (Table 3.3 and Figure 3.3) were below 5700 mg/L (5.7 g/kg $CaCO_3$). This value was obviously lower than the alkalinity reported in another SSAD study by Li et al., (2018b) SSAD. The reasons for the low ingestates alkalinity content in this study was because, aside the low alkalinity from the inoculum as earlier stated, some of the corn stovers used in these experiments have alkalinity of about 1200 – 1700 mg/L. This value (1200 – 1700 mg/L) was about 3.0 – 3.5 folds lower than the values observed for the stover used for Experiments 1 and 2 and also significantly lower than the influents for Experiments 1 and 2 at $p < 0.05$ (Chart not presented).

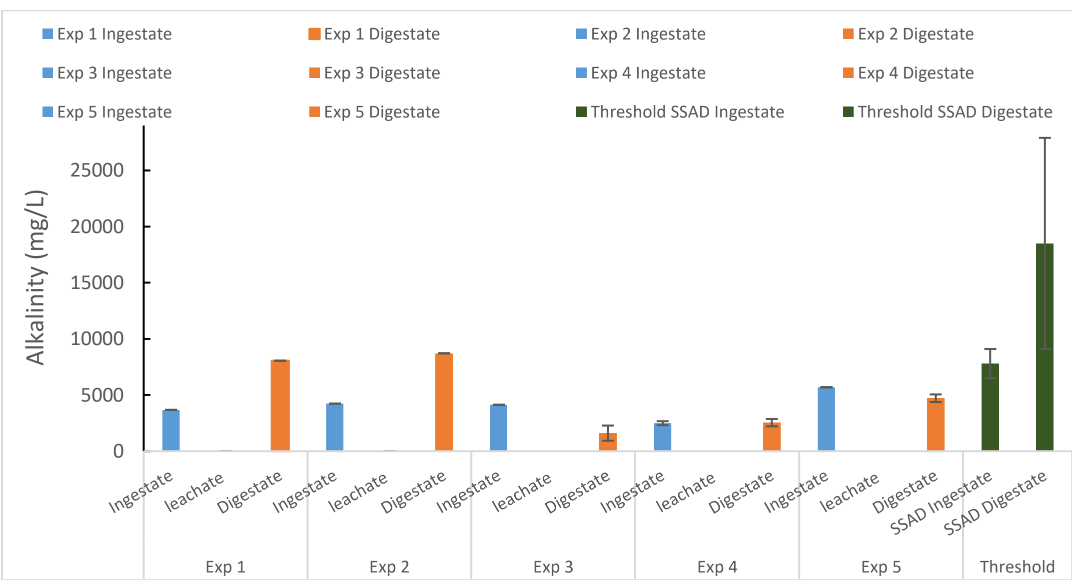


Figure 3.3: Ingestate and digestate alkalinity concentration for the 5 experimental treatments compared with threshold.

Interestingly from Figure 3.3, increase in dairy manure concentration in the ingestate elevated the alkalinity concentration by 25 – 56% (Experiment 5). This additional alkalinity could possibly be as a result of ammonia produced from nitrogen mineralization.

The outcome of the digestates, gave a good understanding of the influence of each feedstock composition on final alkalinity concentration. As previously stated, Experiments 1 and 2 had high corn stover alkalinity relative to other experiments in this study (about 3.0 - 3.5 folds). Hence, the digestate alkalinity for Experiments 1 and 2 had increased by 51 – 54 % after 60 days retention time. Nevertheless, between Experiments 1 and 2, alkalinity increment for Experiment 2 treatment was significantly higher than Experiment 1 ($P < 0.05$). On the other hand, at the end of the other treatments (Experiment 3, 4, and 5), the alkalinity for all these three influents had decreased by 1.8 – 54 %. At this point, the treatments (Experiment 3, 4, and 5) had either failed due to VFA accumulation or incurred significantly low methane concentration with respect to Experiments 1 and 2. The low alkalinity reported in this study was an indication that the SSAD studies might experience pH drop to at least less than 6.5, a threshold considered not suitable for methanogens.

Ammonia concentration is another important parameter in SSAD, which should not be neither too low to cushion VFA concentration nor be too high such that it inhibits methanogens. Precisely, ammonia concentration around 7000 mg N/L has been reported to negatively affect methanogenic activities, while at higher concentration (9000 mg N/L) methanogens are completely inhibited (Sun et al., 2016). In this study, ammonium-nitrogen concentration for the influents was only within the recommended value (> 1500 mg/L) for Experiments 1, 2, and 5 (Figure 3.4). Though we expected more ammonia to be produced through nitrogen mineralization during the digestion process in order to reach a desired concentration of 4600 mg/L stated by Li et al., (2018).

At this concentration, VFA production could sufficiently be mitigated and accumulation prevented. However, only Experiments 1 and 2 met this criterion after the digestates were analyzed, while almost all other digestates were way below 2000 mg/L (Figure 3.4). Experiments 1 and 2 met this criterion because ammonia is one of the wastes produced by methanogens so when methane yield was high (> 40%), there was parallel ammonia production from this methanogens Wang et al., (2012). This indicates that VFA accumulation was not sufficiently mitigated by ammonium-nitrogen concentration in Experiments 3, 4, and 5. This observation could be linked to the trend of both biogas production and peak methane concentration later discussed in this chapter.

With respect to the relationship between C/N and ammonium-nitrogen concentration, Experiment 3 with initial C/N of 33 had ammonium-nitrogen concentration less than 9 mg/L at the commencement of the experiment which increased by 4 folds at the end of the experiment (Figure 3.4). However, when influent C/N was reduced to 24 (Experiment 5 ingestate), the initial ammonium-nitrogen concentration relative to Experiment 3 had increased by almost 6-folds. This reason for this trend was because Experiment 3 had about 5.5-fold more corn stover than Experiment 5, though the dairy manure concentration was about 1.6-fold lower than Experiment 5 (Figure 3.4). Hence, Experiment 5 had enhanced ammonium nitrogen concentration. However, this ammonium-nitrogen concentration was not sufficient to prevent VFA accumulation as the dairy manure was also a source for VFA production (Figure for VFA accumulation is shown under VFA discussion). This latter conclusion was based on the holocellulose composition from these analyses stated later in this chapter (Table 3.1and Figure 3.5).

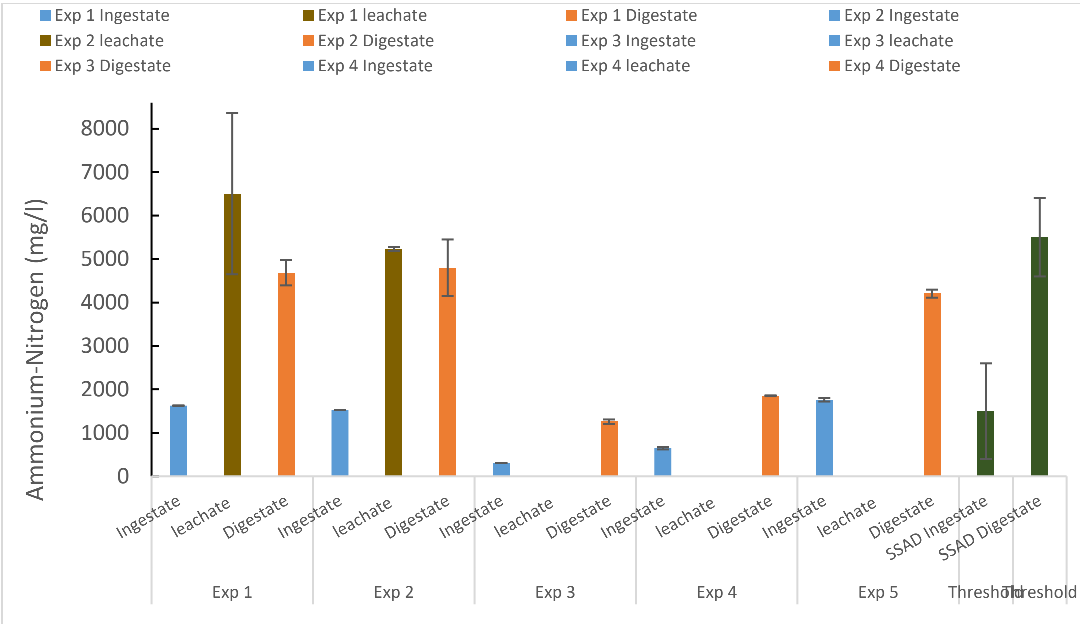


Figure 3.4: Ingestate and digestate Ammonium-Nitrogen concentration for the 5 experimental treatments compared with threshold.

Besides ammonium-N and alkalinity concentrations, VFA concentration and composition are good process indicators in SSAD studies. In all these experiments, initial TVFA concentration was higher than the threshold (400 - 800 mg/L) stated by Li et al., (2018) for solid state anaerobic co-digestion (Figure 3.5). This might be an indication of activities of acidogens in the ingestate or possibly high bioconversion of complex organic compound to simple ones. Synergetic processes that generate these simple compounds depend on three bacteria (hydrolytic, acidogenesis, and acetogenesis bacteria). In this study, VFA equally accumulated in the course of the digestion process for all the ingestates except for the Experiments 1 & 2 (Figure 3.5). Furthermore, the VFA concentrations for the digestates were greater than 13000 mg/L (Figure 3.5). These were above the stable 5800 – 12100 mg/L VFA concentration for digestates documented by Li et al, 2018. Also worthy of note was the decrease in acetic concentration in Experiments 1 and 2 compared with Experiments 3, 4, and 5, which had an increment of over 4 – 6 folds (Figure 3.8).

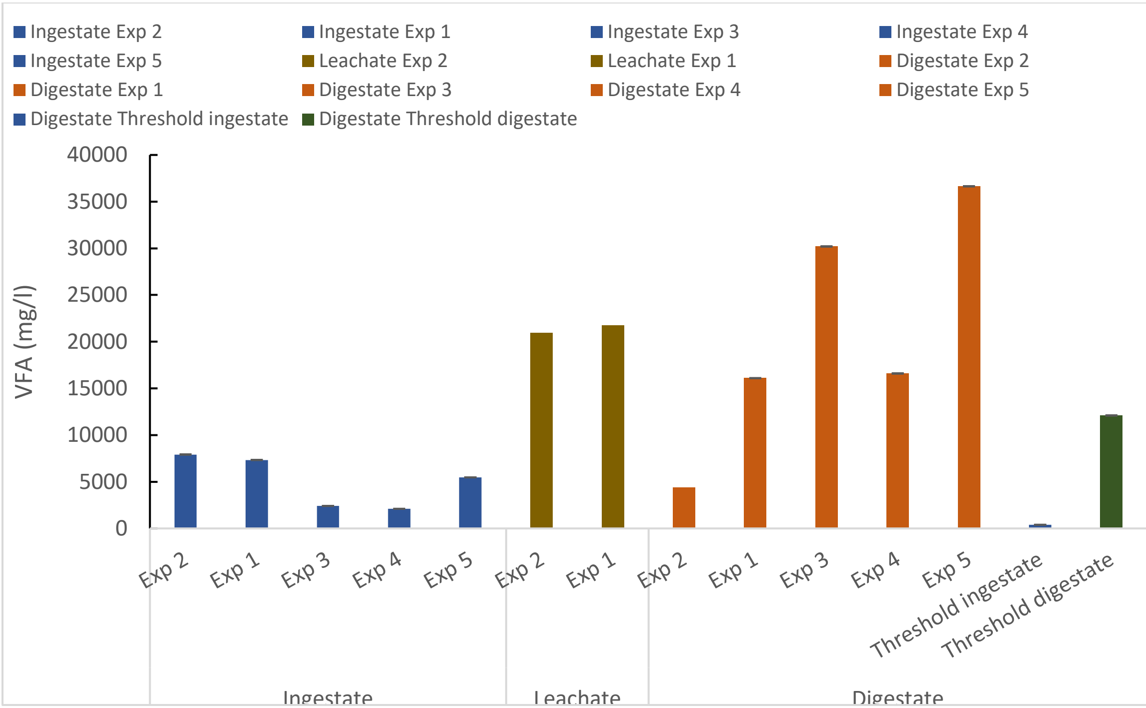


Figure 3.5: Ingestate and digestate VFA concentration for the 5 experimental treatments compared with threshold.

More importantly, is the TVFA/Alkalinity and TVFA/Ammonium-nitrogen relationship in predicting the suitability of ingestates for SSAD. According to Li et al., (2013), TVFA/Alkalinity of 0.4 was considered optimal for AD digestion. In another study (SSAD) conducted by Li et al., (2018), VFA/Alkalinity and VFA/Ammonium nitrogen ratio for the ingestates were within 0.04 – 0.12 and 0.2 – 0.4 respectively. While the digestates had VFA/Alkalinity and VFA/Ammonium-nitrogen ratio of 0.2 – 0.9 and 1.0 – 5.3, respectively. Relative to this study, VFA/Ammonium-nitrogen concentration in this experiment was between 3.1 – 7.9 for ingestates and between 0.91 - 24.0 for digestate (Figures 3.6 and 3.7). These ingestates results were influenced by feedstock composition, the concentration, and the C/N ratio of influent considered in these experiments. When the C/N were more than 26, VFA/Ammonium-nitrogen ratio was greater than 4.0 for ingestates and the respective digestates were greater than 7.0 (Figure 3.6). This might suggest that C/N greater than 26 is not at all suitable for SSAD under this experimental condition. Interestingly, ingestates and digestates for Experiments 4 and 5 behaved similarly under VFA/Ammonium-nitrogen ratio consideration despite the difference in C/N and dairy manure to corn stover ratio (Figure 3.6). This observation supports the claim that suggested ratios between process parameters are a better indicator relative to individual process parameters (Gao et al., 2015).

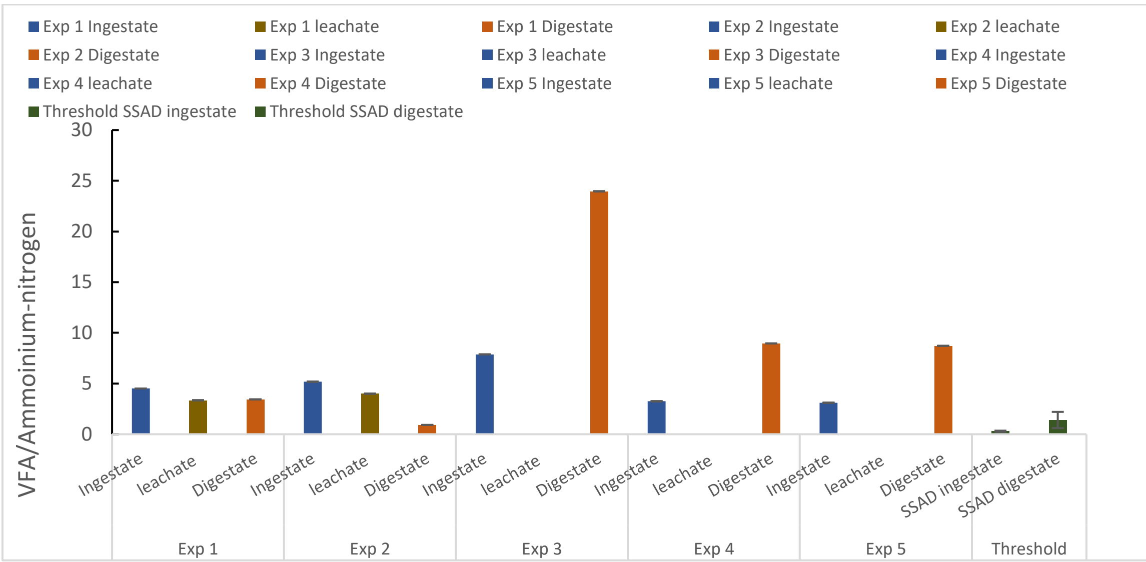


Figure 3.6: Ingestate and digestate VFA/Ammonium-nitrogen value for the 5 experimental treatments compared with threshold.

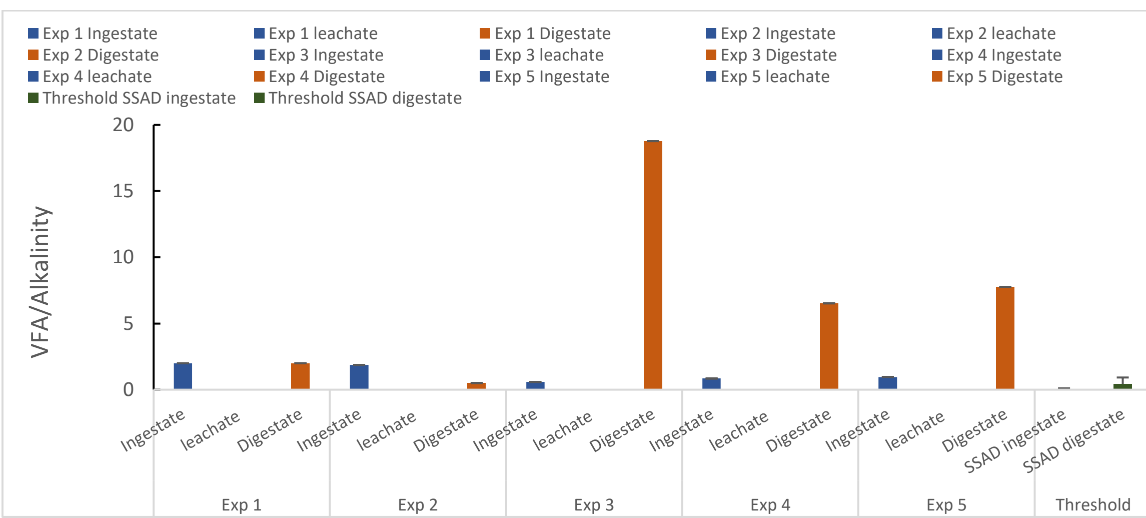


Figure 3.7: Ingestate and digestate VFA/Alkalinity value for the 5 experimental treatments compared with threshold.

3.5.2. VFA Composition

In anaerobic digestion, the most important VFA composition for methanogensis is acetate. Acetate is degraded by methanogens into carbon dioxide and methane. However, its high concentration can hamper methanogens. This could reduce both alkalinity and pH of influent, in addition, could make hydrolysis difficult if pH fall below 4.5 - 5.5 (Vavilin et al., 2008). Equally some other VFA compositions need to maintain certain concentrations, this is highly imperative in order to ensure steady process conditions and particularly prevent inhibition. For example, propionic acid concentration above 1.5 g/L is considered inhibitory to both anaerobic bacteria and methanogens (Barredo and Evison, 1991). However, Wang et al., (2006), suggested a pH above 5.5 as well as an ORP less than -300 mV to prevent propionic accumulation. Interestingly, Barredo and Evison (1991) noted that the presence of propionic does not affect methanogens at pH of 8. On oleic acid, oleic acid concentration more than 26 mg/L has also been considered inhibitory to methanogens (Al-Mallahi et al., 2015).

With respect to SSAD studies, propionic and valeric acid concentrations in this study were above standard recommended for stable SSAD process. Precisely at the end of the experiment, propionic and valeric acid concentrations in the study were greater than 900 mg/L and 1500 mg/L respectively (Figures 3.9 and 3.11). According to Wang et al., (2009), propionic acid concentration more than 900 mg/L negatively impacts methanogen.

Aside from the direct effect of these acids on methanogens, positive correlation has been established between temperature and acetic acid concentration. This infers that increase in temperature will increase acetic acid production, though decrease the pH (Li et al., 2015).

In this study, all the VFA composition (acetic, propionic, butyric, and valeric acids) increased significantly ($p < 0.05$) at the end of the experiment for most of the treatments except

Experiment 2. This was irrespective of the experimental conditions (Figures 3.8 – 3.11). An indication of VFA acids accumulation and potential digester failure. This might suggest that Experiment 2 with DM:CS of 1:1(VS) was the most suitable ingestate condition for solid-state anaerobic co-digestion of dairy manure and corn stover under this study.

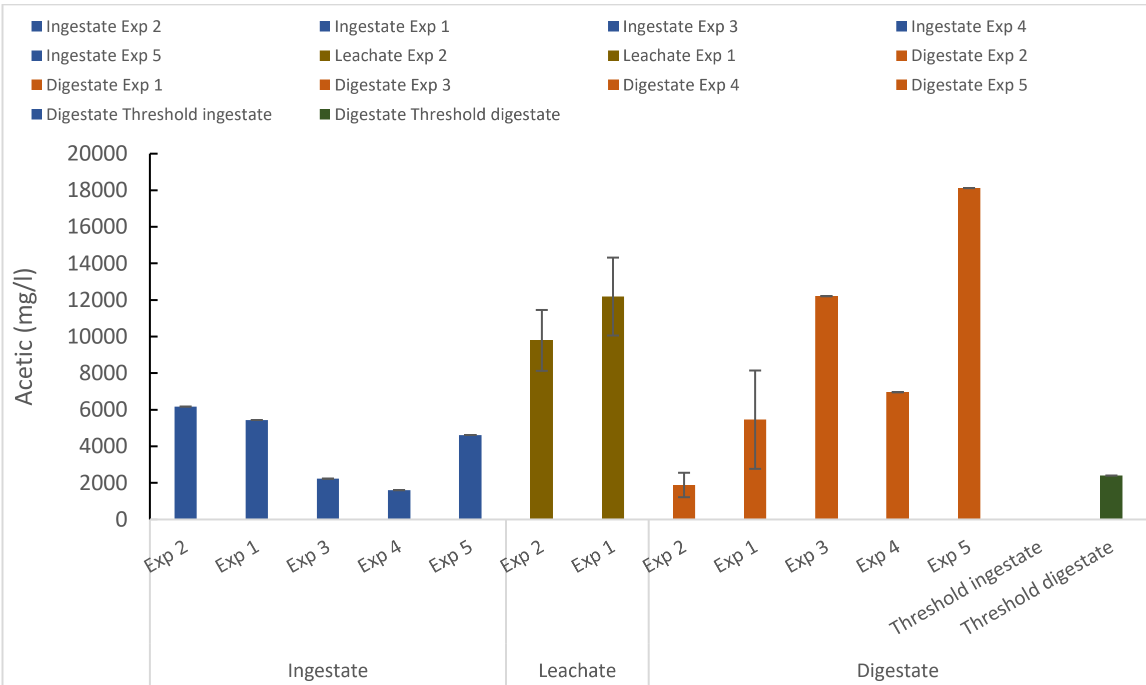


Figure 3.8: Ingestate and digestate acetic acid value for the 5 experimental treatments compared with threshold.

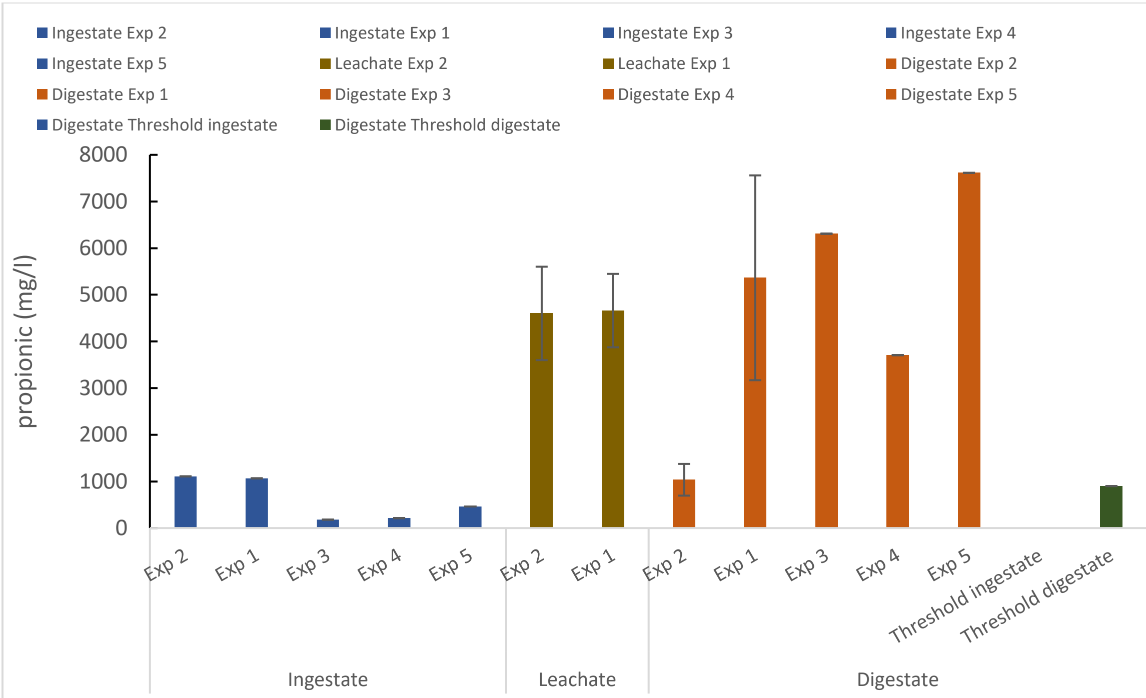


Figure 3.9: Ingestate and digestate propionic acid value for the 5 experimental treatments compared with threshold.

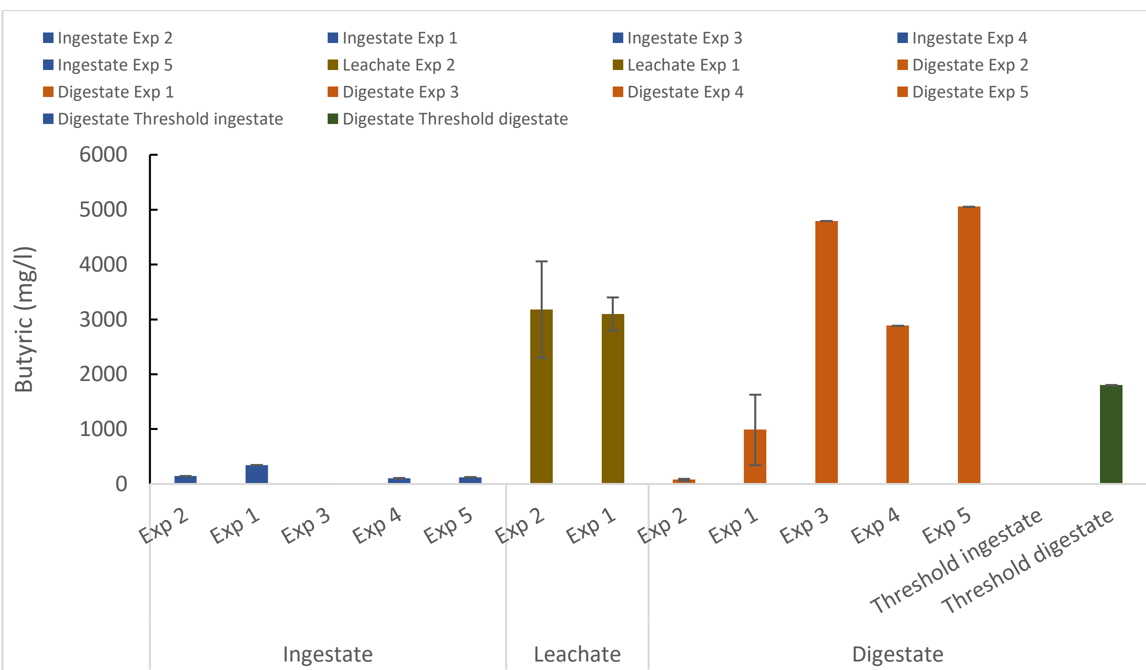


Figure 3.10: Ingestate and digestate butyric acid value for the 5 experimental treatments compared with threshold.

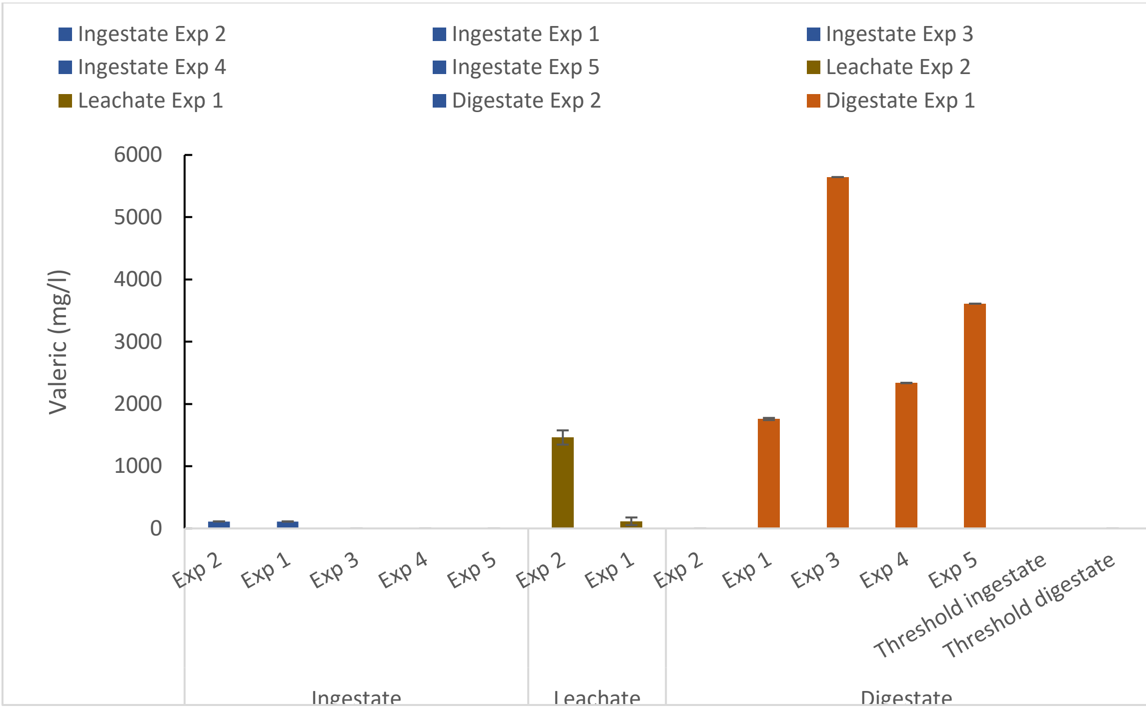


Figure 3.11: Ingestate and digestate valeric acid value for the 5 experimental treatments compared with threshold.

3.5.3. Holocellulose Degradation

Holocellulose degradation is the change in the sum of cellulose and hemicellulose after digestion referred to as polysaccharide degradation (Li et al., 2011). Since holocellulose degrades to produce VFA and acetate, it is invariably expected to reduce in quantity. However, degree of holocellulose conversion has a huge part in feedstock biogas yield. For biomass which are naturally recalcitrant, degradation of holocellulose is limited by its rigid structure. This rigid structure makes accessibility of both hydrolytic and enzymatic bacteria difficult in the AD process. In this study, polysaccharide, cellulose, and hemicellulose degradation were between 4.5 – 6.0, 0.7 – 6.9, and 8.8 – 53 %, respectively (Figure 3.12). The highest holocellulosic degradation occurred with Experiment 2 and then followed by Experiment 1. However, holocellulosic degradation was significantly different for these two treatments ($p < 0.05$). Holocellulose degradation in Exp 2 was at least 21% more than Exp 1. An indication that particle size influence holocellulose degradation.

As expected, cellulose degradation was lower than hemicellulose degradation. (Figure 3.12). We suspected that the hydrolytic and enzymatic bacteria could not access the cellulosic material because of the lignin structure. Also, worth mentioning was the low cellulosic degradation in Experiment 1 despite its low particle size (0.18 mm). This was an indication that increased surface area through size reduction does not necessarily increase hydrolytic and enzymatic bacteria accessibility to cellulose in this study (Figure 3.12). Also worthy of note was that much of the hemicellulose degradation seems to come from the corn stover and not the dairy manure. Hence, as the ratio of corn stover to dairy manure increases, hemicellulose degradation equally increased.

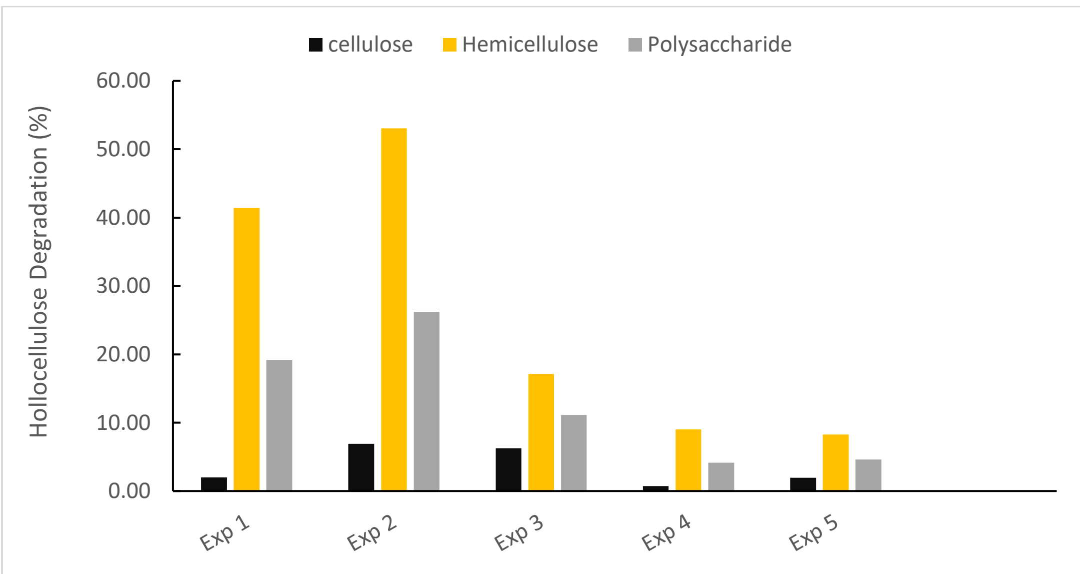


Figure 3.12: Holocellulose degradation for the 5 experimental treatments.

3.5.4. Total Solids and Volatile Solids

When anaerobic bacteria degrade complex organic matter and it is converted to gases such as methane, hydrogen, and carbon dioxide, then the volatile solids of the digestate is reduced. The extent of this reduction influences biogas yield. In this study, the VS reduction was unique. Infact Hill et al. (1987) noted that VS reduction of at least 50% after an experiment is an indication of healthy digester. For Experiments 1 and 2, biogas yield and methane yield were 100 and 50

L/kgVS, respectively, there was neither TS nor VS reduction after 60 days retention time, an indication of unhealthy digester (Figure 3.13). In fact, the TS increased by 50 - 65%, while the VS also increased by 4–5%; the reason for this trend was not fully understood. However, treatments Experiment 3 and Experiment 4 had slight TS reduction (16 -22 %), while treatments Experiment 4 and Experiment 5 had 4–7% VS reduction (Figure 3.13). Though in general, VS reduction in SSAD was relatively lower than either liquid or hemi-solid anaerobic digestion, about 75% VS destruction was achieved in an SSAD investigated by Li et al., 2018. The authors also observed that ingestate with TS between 8 – 15% does not have a significant effect on VS reduction. To further substantiate this, a study conducted by Forster-Carneiro et al., (2008) reported that high TS content could reduce substrate degradation since it affects the rheological behavior of ingestate. Hence, in this study, the high TS (15%) might probably affect VS degradation for those SSAD experiments that failed, particularly because lignocellulosic feedstock was not pretreated.

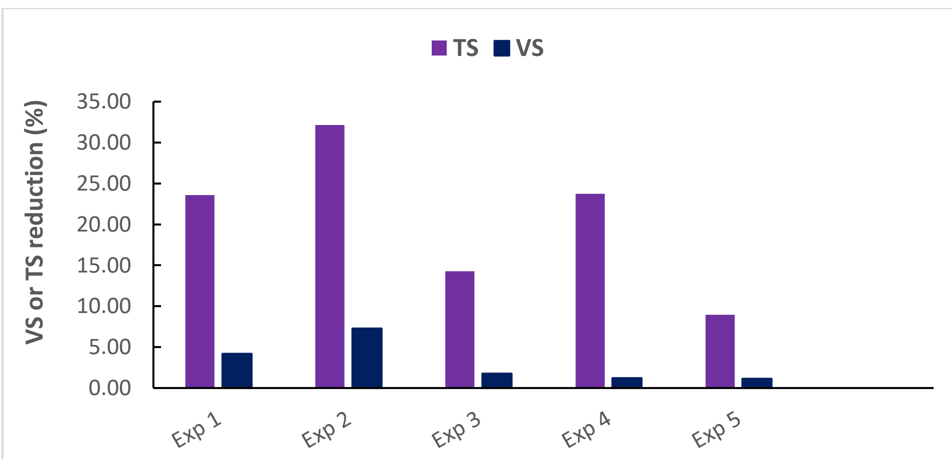


Figure 3.13: Volatile solids and total solids reduction for the 5 experimental treatments.

3.5.5. Biogas Composition, Yield, and Methane Yield

Methane concentration is very important in anaerobic digestion because it has a direct relationship with the potential energy that could be generated from the feedstock. High methane concentration indicates suitable environmental conditions for methanogens. Specific methane yield of 0.20 m^3 CH_4/kg VS to 50% VS reduction has been used as an indicator for a healthy digester (Ehimen et al., 2011). In this study, methane concentration had different trends for the experiments under consideration. For Experiments 1 and 2, methane concentration increased from 15 – 50% at the end of the experiments. While for other treatments (Experiments 3, 4, and 5), peak methane concentration was lower than 20% at the end of the experiment (Figure 3.14). Interestingly, the peak methane concentration for all the treatments was significantly different ($p < 0.05$).

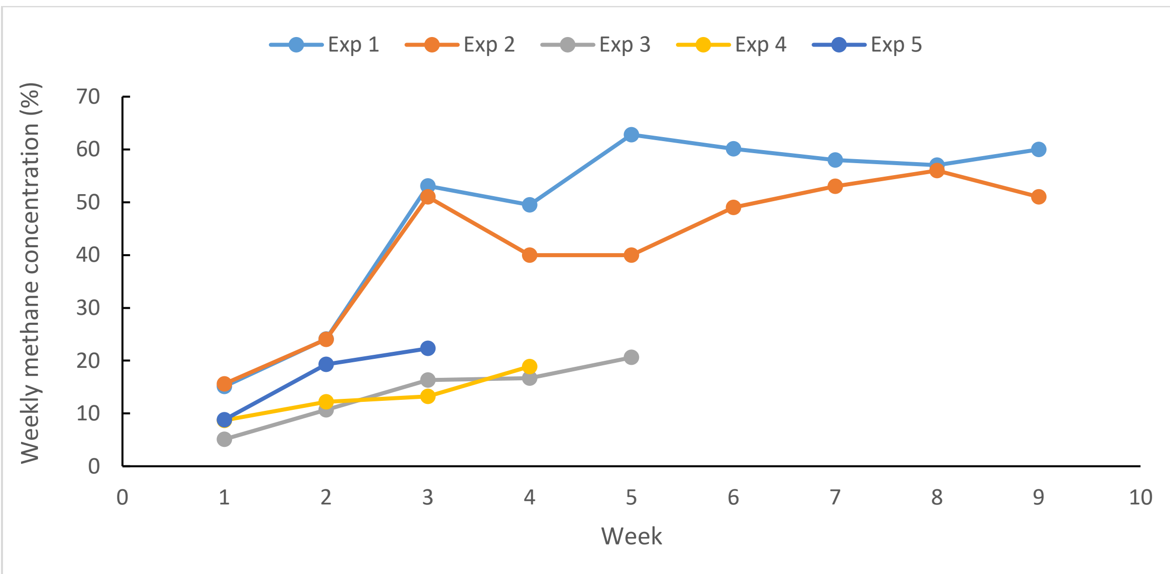


Figure 3.14: Methane production percentage for the 5 experimental treatments.

In relation to other gases produced from the anaerobic process, carbon dioxide oxide is likely to reduce with retention time under a stable anaerobic digestion. However, in this study, carbon dioxide content only significantly reduced to about 40 - 45 % for Experiments 1 and 2, while it remained between 75 – 80% for other treatments (Figure 3.15). An indication of either low methanogenic activities or VFA accumulation. Interestingly, high CO_2 production could probably contribute to VFA accumulation.

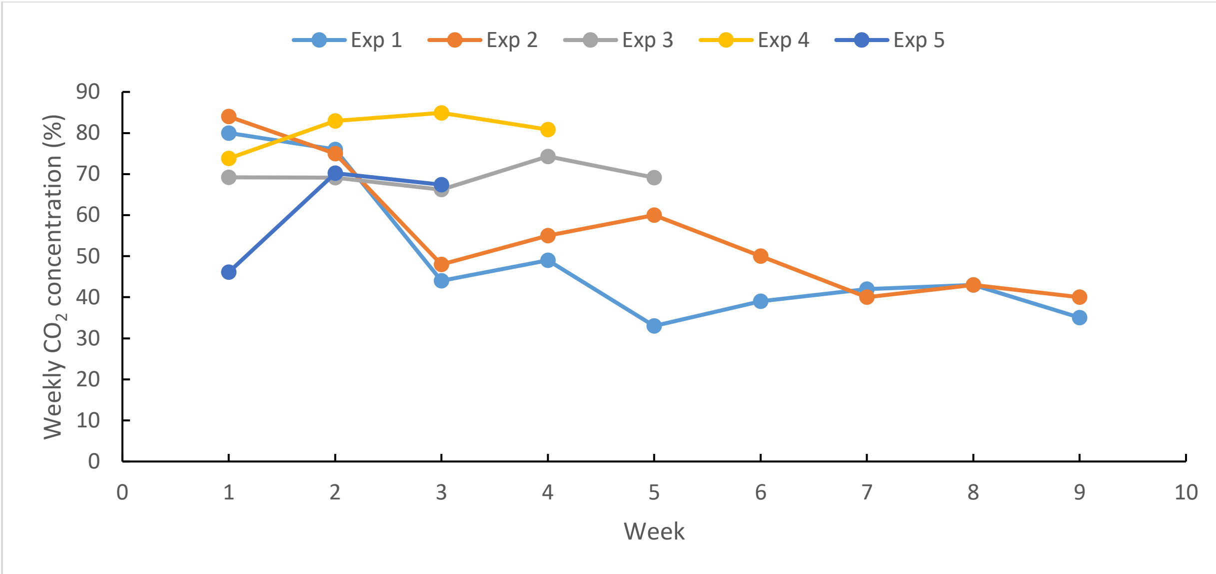


Figure 3.15: Carbon dioxide production percentage for the 5 experimental treatments.

Sulfur reducing bacteria seems to strive under the same environmental conditions with methanogens, hence they compete for nutrients with methanogens. In this study, all H_2S concentrations were less than 4000 ppm after the third week of retention time. However, Experiments 2 and 5 had over 4000 ppm concentration at the cessation of the second week (Figure 3.16). This indicates the activities of sulfur reducing bacteria was more predominant at this period (second week) relative to other periods for Experiments 2 and 5. However, this concentration was lower than the value (>5000 ppm) Isa et al., (1986) noted that could inhibit anaerobic processes.

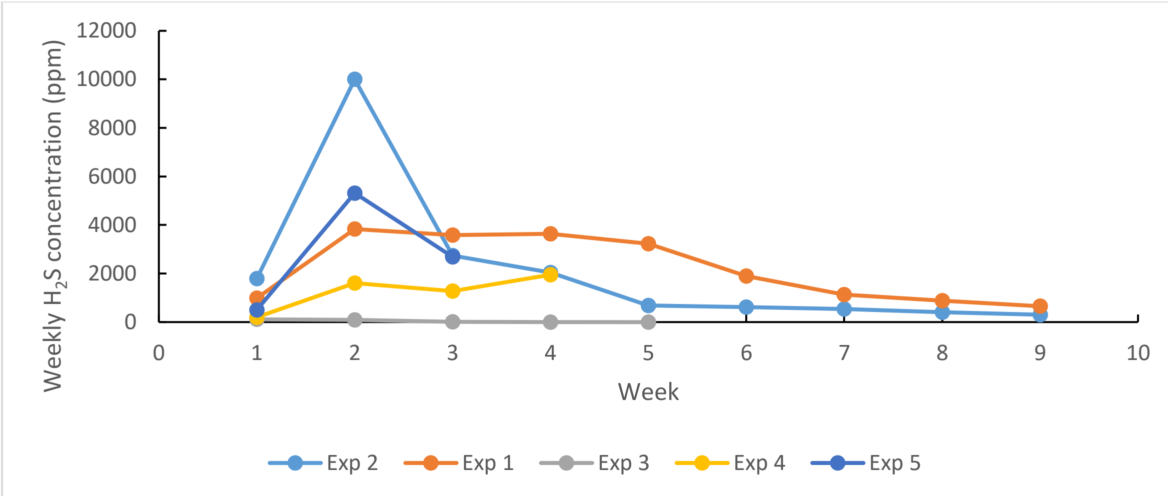


Figure 3.16: Hydrogen sulfide production percentage for the 5 experimental treatments.

As expected, cumulative methane and biogas yield was in tandem with the methane concentration for all the influents. Experiments with minimum peak methane concentration of at least 40% had relatively high biogas and methane yield. For instance, Experiments 1 and 2 had over 140 L/ kg VS biogas yield and at least 50 L/ kg VS methane yield at the cessation of the experiment (Figure 3.17). While for other experiments (Experiments 3, 4, and 5) with peak methane concentration less than 35%, biogas and methane yields were less than 60 L/kg VS and 10 L/kg VS, respectively (Figure 3.17). This latter result suggests an obvious poor anaerobic process as a result of VFA accumulation, low alkalinity, and low ammonia production rate. Nevertheless, Experiment 2 proved to be the best binary mixed influent with respect to methane yield under this study.

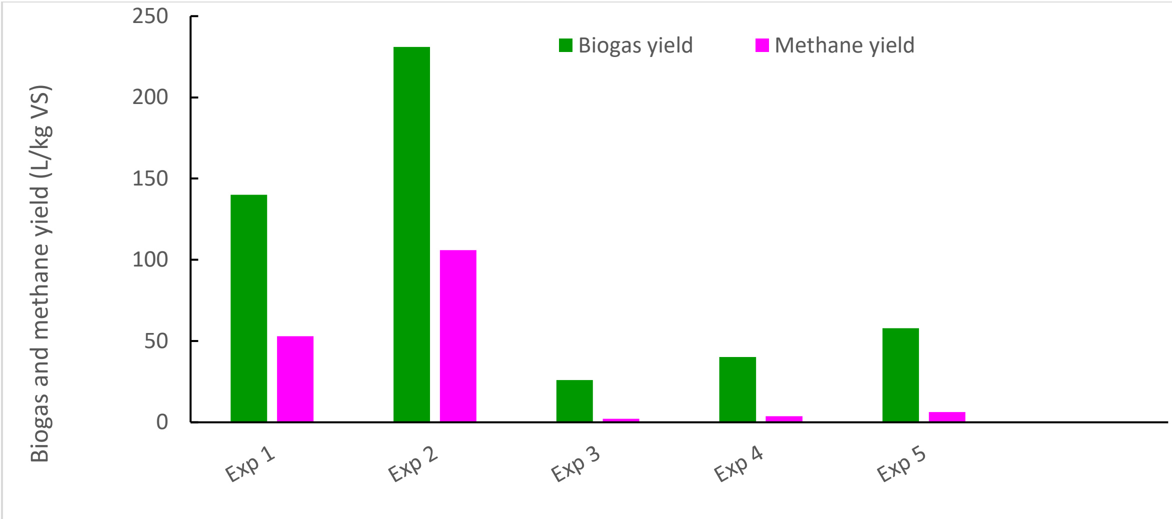


Figure 3.17: Biogas and methane yield for the 5 experimental treatments.

3.6. Conclusion

Binary mixture of dairy manure and corn stover mixed with inoculum was considered in this study for solid-state anaerobic co-digestion under a mesophilic temperature (37 ^{o}C). Five different experiments were conducted, which includes, Experiments 1 and 2 with the same mix but different corn stover particle size (0.42 and 0.18 mm). Experiments 3, 4, and 5 all had corn stover of the same particle size (0.42 mm), though mix ratio and C/N differ.

The results from this study show that Experiments 1 and 2 which had two different corn stover particle sizes followed the same pattern. However, VFA composition was more pronounced with Experiment 1. Furthermore, VFA composition of the digestate and peak methane concentration for this Experiment 1 increased significantly relative to Experiment 2 ($p < 0.05$). An indication that size reduction enhances acidogens action leading to more VFA production.

Results in this study further suggested that the negative and low volatile solids (< 25%) obtained for all the treatments at the end of the experiments was an indication of poor volatile solids destruction and also suggested that methanogens action on the influents was ineffective on the overall.

In general, a similar trend observed for Experiments 1, 3, 4, and 5 in terms of elevated propionic and valeric production at the end of the study suggests potential inhibition at some point in these treatments. Hence, Experiment 2 (treatment with 0.42 – 0.84 mm and DM:CS of 1:1 based on VS) was considered the best treatment under this study.

4. EFFECT OF WASHING AND CARBON TO NITROGEN RATIO ON BIOGAS YIELD IN A SOLID-STATE ANAEROBIC CO-DIGESTION OF DAIRY MANURE WITH NaOH-PRETREATED-CORN STOVER.

4.1. Abstract

Solid-state anaerobic co-digestion could be harnessed to manage the growing volume of agricultural wastes. In this study, two agricultural wastes (dairy manure and NaOH-pretreated-corn stover) were co-digested to enhance biogas yield under 37 °C mesophilic temperature and solid-state condition. Two factors that were used to describe the treatments involved in this study were washing (washed vs. unwashed following pretreatment) and C/N ratio (27 and 33), hence there were five treatments (WCN33, WCN27, WCN27B, UwCN33, and UwCN27). Results from these studies indicated that unwashed pretreated corn stover blended with manure (UwCN33 and UwCN27) outperformed the washed treatments (WCN33, WCN27, and WCN27B) in terms of biogas yield. The biogas yield for the treatments WCN33, WCN27, WCN27B, UwCN33, and UwCN27 were 40, 63, 77, 122, and 114 L/kg VS, respectively. Similarly, hemicellulose degradation in treatments UwCN33 and UwCN27 was at least 13 % significantly higher than all the WCN33, WCN27, and WCN27B treatments. Hence, co-digestion of animal manure and unwashed-NaOH-pretreated biomass in solid-state anaerobic digestion could be area to explore in order to enhance biogas yield.

4.2. Introduction

Agricultural waste management with anaerobic digestion has gained much attention in the last two decades. However, there are limitations in this treatment method based on waste composition and concentration. For instance, lignocellulosic biomass is sturdy and hence hermetic microbial degradation of such substrate is limited. This limits the potential biogas yield, prolong retention time, and result in poor digestate quality. Strategies that have been employed to address these potential challenges from utilizing agricultural wastes include pretreatment, co-digestion, use of nanoparticles, and carbon to nitrogen ratio (C/N) adjustment. Though, a lot of these strategies have been employed in liquid-state anaerobic digestion, only a few studies have combined these strategies in solid-state anaerobic digestion. For instance, Li et al., (2018), utilize co-digestion of tomato residue, corn stover, and dairy manure to enhance biogas yield. In the study, tomato residue volume more than 80 % of the influent composition resulted in poor reactor performance. In another related study, chicken manure was blended with corn stover to improve methane yield, this improved biogas yields by at least 3-fold relative to the control (Feng et al., 2018). Furthermore, in most of these studies, substrate to substrate ratio was used as the basis for comparison. However, in these co-digestion studies, the effect of C/N on biogas yield was not considered. Hence, this study investigated the effect of two C/N ratios on biogas yield. Also investigated was the impact of washing on pretreated corn stover blended with dairy manure on biogas yield.

4.3. Materials and Methods

4.3.1. Dairy Manure and Inocula Collection

Fresh dairy manure from North Dakota (ND) State University Dairy Farm, Fargo, ND, USA was collected in a 20-liter plastic container. Inocula of about 7 liters each were obtained from mesophilic anaerobic digesters in both American Crystal Sugar Company, Moorhead, MN, USA and Fargo Wastewater Treatment Plant, Fargo, ND, USA. All the collected substrates were used on the same day of collection, except the corn stover that had to be chemically pretreated. Characteristics of these substrates were presented in Table 4.1.

Table 4.1: Substrates characteristics for feedstocks and thermo-chemically pretreated corn stover.

				Thermo-chemical-Pretreated corn stover		
				Washed	Unwashed	
Parameter	Corn stover	Dairy manure	Inoculum	4%	4%	2%
pH	6.0±0.1	7.8±0.6	7.1±0.6	7.6±0.4		10.09±0.0
VS (%)	94.1±0.8	86.5±3.6	57±17.5	95.1±0.6	57.6±0.0	69.1±0.0
TS (%)	99.0±0.8	17.8±5.0	1.4±0.6	98.7±0.5	93.6±0.0	98.8±0.0
C (%)	41.0±0.1	38.9±3.3	24±8.8	39.1±0.7	34.88±0.0	39.2±0.0
N (%)	0.8±0.1	2.8±0.3	4.1±2.4	0.09±0.0	0.2±0.0	0.4±0.0
Alkalinity (g/L)	1.7±0.1	8.3±1.8	1.6±0.3	4.4±1.0	167.6±0.0	78.5±0.0
C/N	51.3±0.1	14.4±2.2	7.5±0.7	434.2±84	174±0.0	47.7±0.0
Cellulose (%)	43.5±0.3	26.4±3.3	22.8±0.0	77.4±1.4	41.6±0.5	48.79±0.0
Hemicellulose (%)	34.16±0.1	20.9±0.5	14.0±0.0	13.4±2.7	6.9±0.4	10.76±0.0
Ash (%)	5.7±0.3	15.2±2.1	67.1±0.0	5.5±0.0	NI	35.9±0.0
Lignin (%)	5.1±0.2	9.5±2.3	4.1±0.0	1.4±0.3	1.0±0.4	1.4±0.0
Amm-N (mM)	0.0	92.6±19	34.6±3	0.5±0.0	1.0±0.0	ND
VFA (g/L)	0.0	9.7±0.8	0.1±0.0	0.0±0.0	28.6±2.2	NI

*Data represent the mean ± standard deviation
* ND – Not Detected, NI – Not Inspected, Amm-N represents ammonium nitrogen

4.3.2. Corn Stover Collection and Pretreatment

Corn stover was obtained from the NDSU Carrington Research and Extension Centre, Carrington, ND. The stover was crushed into < 3 mm particle size with a 3 mm-mesh-sieve-sized Schuttle Buffalo hammer mill (Model W6H, New York, USA). Crushed stover was sieved into 0.42 – 0.84 mm using a mechanical sieve shaker. The sieved stover was stored in 10 liters Ziploc bags under ambient condition. Characteristics of this substrate were presented in Table 4.1.

4.3.3. NaOH-Pretreated-Corn stover

A thermo-chemical method was employed to pretreat the corn stover with some of the pretreated samples washed and other unwashed after this pretreatment, this was prior to oven drying. The detailed pretreatment process is presented in the subsequent section.

4.3.3.1. Thermo-Chemical Pretreatment

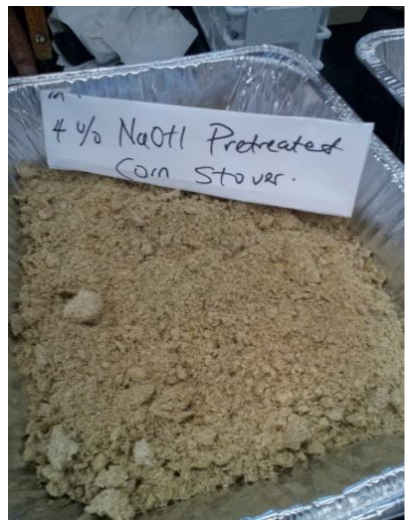
The thermo-chemical pretreatment was done by preparing a 2% and 4% concentration of NaOH solution. To achieve this, 40 g of pelletized NaOH (98% Assay) was dissolved in 1000 mL deionized water in order to produce 4% NaOH concentration. One liters of the prepared NaOH solution was mixed with 100 g (dry mass) of the 0.42 – 0.84 mm sieved corn stover inside a 2 L bottle (1:10 w/v solid loading of corn stover to NaOH solution). This mixture was thermally treated in an autoclave (Allen-Bradley Consolidated Sterilizer Systems, Boston, Massachusetts, USA) at 120 ^{o}C for 30 minutes. After this process, the pretreated samples were allowed to cool down before dewatering. To achieve dewatering, the pretreated sample was introduced into a funnel underlaid with doubled layer of cheese cloth in order to prevent loss of pretreated stover, the subsequent procedure was done under two different procedures. In one of the processes, deionized water was introduced to wash the pretreated stover until the pH was reduced by about 7.0 -7.5. The pH was examined using indicative litmus paper as cell indicator. While in the other process, deionized water was not introduced but excess leachate from the pretreated stover was allowed to flow through the funnel with the aid of suction. Dewatered samples were oven dried at 40 °C for 2 – 3 days and stored in 5 L Ziploc bag under ambient conditions. Pictorial view of these processes was presented in Figure 4.1. Characteristics of these substrates was presented in Table 4.1.

A

B

C

D



E

Figure 4.1: (a-e) Corn stover NaOH pretreatment processes.
Step A represents the sterilizatiion of the corn stover soaked in NaOH. Step B is the end product from the streilization process. Step C shows the process of washing the sterilized or NaOH-pretreated corn stover. In Step D, the washed pretreated stover was weighed prior to oven-drying and Step E shows the washed NaOH-pretreated corn stover after oven-drying.

4.3.4. Substrates Mix

All the feedstocks (inoculum, dairy manure, and the pretreated corn stover) used in this experiment were mixed based on carbon to nitrogen ratio (C/N) calculations indicated in equation 4.1. The carbon to nitrogen ratios used were 27 and 33.

$$R = \frac{M1C1TS1 + M2C2TS2 + M3C3TS3}{M1N1TS1 + M2N2TS2 + M3N3TS3}. \tag{4.1}$$

Where,

R - represents the carbon to nitrogen ratio of the three feedstocks,
M1 - denotes the moisture content (wet basis) of the dairy manure,
C1 - represents the % carbon content (dry basis) of the dairy manure,
N1 - denotes the % nitrogen content of the dairy manure,
TS1 - represents the % total solids of the dairy manure,
M2 - denotes the moisture content (wet basis) of the inoculum,
C2 - represents the % carbon content (dry basis) of the inoculum,
N2 - denotes the % nitrogen content (dry basis) of the inoculum,
TS2 - represents the % total solids of the inoculum,
M3 - denotes the moisture content (wet basis) of the corn stover,
C3 - represents the % carbon content (dry basis) of the corn stover,
N3 - denotes the % nitrogen content (dry basis) of the corn stover, and
TS3 - represents the % total solids of the corn stover.

4.3.5. Experimental Set-Up

The mix ratio of dairy manure and the pretreated corn stovers in this study was fixed at 1.2:1 (VS based). This ratio is close to the value (1.5:1) that has been considered in most co-digestion studies of animal manure and crop residue (Li et al., 2018, Seppala et al., 2013). Inoculum was then added to the mix (dairy manure and corn stover) to achieve a total solid content of 16%, C/N of 27 or 33 and substrate to inoculum ratio (S/I) less than 3.0 (Table 4.2). This S/I range was considered effective in solid state anaerobic co-digestion (Li et al., 2018). Hence, the following treatments WCN33, WCN27, WCN27B, UwCN33, and UwCN27 were used and the mix ratio for these treatments was presented in Table 4.2.

Table 4.2: Mix ratio for dairy manure, inoculum, and NaOH-pretreated corn stover for Ingestate.

Ingestate mix	Quantity of dairy manure (%)	Quantity of pretreated corn stover (%)	Quantity of inoculum (%)
WCN33	32.96	16.95	50.09
WCN27	38.35	11.68	49.97
WCN27B	45.65	6.99	47.35
UwCN33	35.52	13.85	50.62
UwCN27	40.82	10.20	48.98

Where: W represents washed NaOH-pretreated corn stover; Uw represents unwashed NaOH-pretreated corn stover; and CN27 & CN33 represents C/N ratio of 27 and 33 ingestate. WCN27 and WCN27B have the same C/N ratio but different ratio of dairy maure to corn stover.

Thereafter, 1000 g or 1500 g of ingestate containing a mixture of dairy manure, inoculum, and the pretreated corn stover was introduced into the digester. The disparity in weight of feedstock introduced into the digesters was adjusted with the methane and biogas yield calculation, hence results are comparable. The digesters with 3.5 liters working volume were deoxygenated with nitrogen gas after loading and thereafter checked for gas leak by submerging the digester in a water filled bath. The deoxygenation process made the digester environment anaerobic.

The digesters were immersed vertically in a 0.73 m × 0.49 m × 0.53 m plastic tub filled with water. The tub was coupled with a thermostatically controlled heater set at 37± 1.5 ^{o}C for all the ingestates. Volume of water in the tub was daily maintained by manual water introduction. To quantify the biogas volume produced from the respective digesters, a rectangular gas collector with a tipping bucket was programmed with CR-1000 in order to collect real-time data. This chamber was filled with water to allow for easy bucket tip. The gas collector was coupled to a digester with a 2 mm diameter Teflon tube. Data obtained from this chamber were collected and stored every minute and 24 hours using a CR-1000 data logger.

4.3.6. Gas Analyses

In order to analyze gas composition weekly, gas produced from the digester was collected from the headspace of the digester by inserting a 20 mL syringe through a septum stopper attached to one of the gas outlets. The gas was analyzed for biogas (methane and carbon (iv) oxide) and hydrogen sulfide composition using a gas chromatograph (GC, 8610C, SRI Instruments, California, USA) and hydrogen sulfide analyzer (Jerome 631X, Arizona Instrument LLC, Arizona, USA) respectively. The gas chromatograph used was equipped with a flame ionization detector (FID) for CH_4 and CO_2 analyses. It was equally equipped with an electron capture detector (ECD) detector for nitrous oxide (N_2O) gas analysis. The chromatograph was operated on a 20 PSI N_2 carrier for the ECD while hydrogen gas and air were supplied on a 20 PSI to the FID or methanizer. Calibration curves generated from four different CH_4 (5, 10, 20, and 100 ppm) and CO_2 (100, 500, 1000, and 2500 ppm) concentrations were used to compute the values for the curve area. Also, worth mentioning is the 100 ^{o}C pre-set temperature of the gas chromatograph prior to any analysis. This ensures stability in results. On the hydrogen sulfide analyzer, the Jerome meter works through a thin gold film subjected to variation through electrical resistance that senses the gas concentration. Prior to both analyses, 5mL of the collected gas was introduced into a 0.5 L SKC Tedlar bag and then diluted with 500 mL of N_2 prior to manual mixing. This dilution factor was considered in the result presented.

4.3.7. Ingestate, Leachate, and Digestate Analyses

The primary step taken during digestion so as to avoid crust formation due to seepage and filtration, was to periodically agitate the digesters manually based on the experimental design. This process was a form of leachate return without air introduction. Subsequently, 4 – 7 mL of these leachates were collected periodically (weekly) through the digester outlet tap for ammonia, pH, Volatile fatty acids (VFA), ammonium –nitrogen, oxidation redox potential (ORP), and electrical conductivity (EC) analyses using standard methods. Samples were also collected from the fresh dairy manure, inoculum, corn stover, ingestate, and digestate for ammonia, nitrite, nitrate, iron, phosphorus, ash content, and alkalinity analysis using standard methods. Carbon and nitrogen content of these samples were inspected with combustion analyzer- Elemental Vario Macro Cube –CNS analyzer following the protocol described by Pella (1990). In order to validate the accuracy of the combustion analyzer, total nitrogen was also evaluated with Kjeldahl method (Isaac and Johnson, 1976). Ammonium–nitrogen was evaluated following the international organization of standardization procedure for water quality determination of ammonium nitrogen by flow analysis, ISO - 11732:1997 protocol. On the other hand, nitrate and nitrite nitrogen were analyzed with the EPA 353.2 revised method (1993). Total phosphorus content in these samples were determined after sample digestion was carried out with the method described by Watson et al., (2003). In this digestion procedure, nitric acid was digested with peroxide in a block digester and afterward, the phosphorus content was measured with a Brinkmann PC 910 colorimeter. To quantify the iron concentration in all these samples, samples were first digested similarly to the procedure previously described for total phosphorus (Watson et al., 2003). Thereafter, iron concentration was measured with a Buck Scientific 210 VGP atomic absorption spectrophotometer.

Alkalinity was another important parameter that was measured for all the samples. This was carried out with the method described by APHA (1992) using the titration method with 0.01N sulfuric acid.

Other parameters measured were dry matter, ash content, crude protein, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). Dry matter of these samples was measured with the procedure stated by Hoskins et al., (2003) and AOAC #934.01

with #930.15 (2010). Crude protein protocol followed AOAC official method #2001.11 (2010). ADL was also measured with AOAC official method #973.18 (2010). However, NDF and ADF followed a different procedure described by Goering and Van Soest, (1970).

Volatile fatty acids were measured with the procedure described by Baumgardt (1964), while ammonium nitrogen was determined with the colorimeter determination of urea nitrogen protocol described by Sigma Technical Bulletin. Brief description of the VFA protocol. Sample was introduced into a 25 mL centrifuge tube prior to vortex at 20000 X g for 10 minutes. 5 mL of the supernatant produced from this process was pipetted into another centrifuge tube and a 1 mL 25 % metaphosphoric acid solution added prior to another vortex for 30 minutes. Filtered liquid was collected and loaded into the Agilent 6890N Gas Chromatograph with a FID (flame ionization detector) and the 7683 Series auto injector and auto sampler. Column used was the Supelco brand, NUKOL Fused Silica Column (Agilent Technologies, California, USA), 15 mm × 0.53 mm × 0.5 um GC. This GC analysis followed the protocol described by Goetsch and Galyean, (1983) and the acids analyzed with the GC were acetic, propionic, isobutyric, butyric, isovaleric, and valeric.

A similar procedure to the VFA analysis was carried out for ammonium – nitrogen quantification prior to when the metaphosphoric acid solution was added and then vortex. The difference after these steps were a 10-fold fluid dilution, followed by the introduction of 100 µL of this diluted sample into three borosilicate tubes and then the addition of 1.0 mL phenol nitroprusside solution, 1.0 mL alkaline hypochlorite solution, 5.0 mL deionized water in that order prior to vortex to one of the tubes. The detailed procedure could be found in the protocol described by Sigma Technical Bulletin. pH, ORP, and EC of these samples were measured with HANNA HI 4522 dual channel benchtop meter (USA).

4.3.8. Statistical Analysis

Statistical difference among treatments means for the completely randomized design used in this study was investigated with SAS 9.4. TS Level 1M4. X64_10PRO platform. Duncan's multiple range test was used to evaluate the significant treatments using the same tool with a threshold p-value of 0.05.

4.4. Results and Discussion

4.4.1. Process Parameters

The process parameters considered in this study were alkalinity, ammonium-nitrogen, and volatile fatty acids.

4.4.1.1. Alkalinity

The alkalinity for ingestates obtained in this study varied based on pretreatment conditions. All the treatments with pretreated-unwashed-corn stover as part of its constituents UwCN33 and UwCN27 had initial alkalinity over 9 g/L (Table 4.3). On the other hand, the initial alkalinity for treatments with pretreated-and-washed-corn stover WCN33, WCN27, and WCN27B were below 5.3 g/L (Table 4.3). This was despite the significant difference among all the treatments ($p < 0.05$). This is an indication that washing pretreated corn stover reduces the alkalinity for most of the ingestates by at least 0.9 fold (Table 4.3). Also observed was the effect of C/N on initial alkalinity concentration, C/N ratio increases with alkalinity. Interestingly, the increase in the dairy manure to the pretreated corn stover ratio under the same C/N (27) ratio also enhanced the initial alkalinity concentration of the treatment. Precisely, treatment WCN27B, which has a C/N of 27, was about 38 % more than treatment WCN27 with respect to initial alkalinity concentration (Table 4.3). An indication that both concentration of corn stover and dairy manure enhanced initial alkalinity of influent.

Relative to a number of successful SSAD studies, alkalinity increases with retention time. For instance, in Li et al., (2018) study, alkalinity increased by 2 – 3 folds at the end of the experiment (35 days), which was about 67 – 200 % of the initial values. However, the trend in this study was contrary to all the treatments, as there was a decline in alkalinity concentration at the cessation of the experiment (Table 4.3). A trend that may suggest either VFA accumulation or unfavorable methanogenic environment. Precisely, all the treatments with C/N ratio of 33 (WCN33 and UwCN33) had the final alkalinity concentration decreased by at most 32% (Table 4.3). While treatments with C/N of 27 (WCN27, WCN27B, and UwCN27) show more significant reduction ($p < 0.05$) in final alkalinity concentration (about 43%). A trend that suggests C/N ratio had more impact on alkalinity concentration relative to dairy manure proportion.

Table 4.3: Alkalinity of the treatments.

Experiment	Initial Alkalinity (g/L)	Final Alkalinity (g/l)
WCN33	5.2 ± 0.0^{a}	3.8.± .8^{a}
WCN27	2.9 ± 0.2^{b}	1.7 ± 0.0^{b}
WCN27B	4.7 ± 0.0^{c}	3.2 ± 0.1^{c}
UwCN33	24.1 ± 0.0^{d}	13.5± 0.0^{d}
UwCN27	9.9 ± 0.0^{e}	5.3± 0.3^{e}

Values are expressed in means ± standard deviation.
Means followed by the same letter in a column are not significant.
Where: W represents washed NaOH-pretreated corn stover; Uw represents unwashed NaOH-pretreated corn stover; and CN27 & CN33 represents C/N ratio of 27 and 33 ingestate. WCN27 and WCN27B have the same C/N ratio but different ratio of dairy maure to corn stover.

4.4.1.2. Ammonium-Nitrogen

Ammonia in this study was mainly derived from the dairy manure. As the ratio of dairy manure to pretreated corn stover in the ingestate increases, the ammonium-nitrogen concentration also increases irrespective of whether the corn stover was washed or not after pretreatment (Table

4.4). Hence, treatments WCN27B and UwCN27 had high initial ammonium-nitrogen concentration of at least 1.6 g/L while WCN27, WCN33, and UwCN33 ingestates had concentration lower than 1.6 g/L (Table 4.4). This was simply because dairy manure to pretreated corn stover ratio was significantly higher ($p < 0.05$) in all of the WCN27B and UwCN27 treatments than the WCN27, WCN33, and UwCN33 treatments. However, methanogens produce ammonia as by-products and also nitrogen mineralization occurs during the anaerobic digestion process, hence ammonia was expected to be produced and hence, the concentration was deemed to rise. In this study, as expected the ammonium-nitrogen concentration after the retention time had increased by at least 24% for the treatments with dairy manure blended with pretreated -and-washed-corn stover (Figure Table 4.4). Furthermore, at the cessation of the experiments, all the treatments had ammonium-nitrogen concentrations less than 2.9 g/L (Table 4.4). The peak ammonium-nitrogen concentration value obtained after the experiment value was still within the range (1.5 – 2.6 g/L) Li et al., (2018) observed in a solid-state study. It is also less than the value of 7 g/L considered being tolerable by methanogens (Sun et al., 2016). However, Chen et al., (2008) reported that this ionic ammonia inhibition is strongly linked with temperature and pH, though in general hydrogenotrophic is more tolerable to this inhibition compared with acetoclastic methanogens. The authors equally emphasized the stronger inhibitory effect free ammonia has on anaerobic process compared with ionic form of ammonia. In this study, we suggest that a relatively high ammonia concentration could mitigate VFA accumulation. Hence a more robust nitrogen mineralization or ionic ammonia generation by methanogens would have possibly enhanced a stable anaerobic digestion process via VFA neutralization.

Table 4.4: pH, ammonium-N and initial C/N of the treatments.

Experiment	Initial pH	Final pH	Initial Ammonium-nitrogen (g/L)	Final Ammonium-nitrogen (g/L)	Initial C/N
WCN33	7.2 ± 0.0[a]	5.2 ± 0.2[a]	0.7±0.0[a]	1.1 ± 0.0[a]	33± 0.0[a]
WCN27	8.0 ± 0.1[b]	4.8 ± 0.1[b]	1.4±0.0[d]	1.9 ± 0.2[b]	27 ± 0.5[b]
WCN27B	8.1 ± 0.0[b]	5.1 ± 0.2[a]	1.7±0.0[b]	2.8 ± 0.0[c]	27 ± 0.0[b]
UwCN33	8.6 ± 0.0[c]	6.2 ± 0.4[c]	1.0 ± 0.0[c]	2.3 ± 0.0[d]	33± 0.0[a]
UwCN27	8.6 ± 0.0[c]	5.4 ± 0.3[d]	1.6± 0.0[b]	2.6 ± 0.3[e]	27± 0.0[b]

DM represents dairy manure and IN represents inoculum.
All the values are expressed in means ± standard deviation.
Means followed by the same letter in a column are not significantly different.
Where: W represents washed NaOH-pretreated corn stover; Uw represents unwashed NaOH-pretreated corn stover; and CN27 & CN33 represents C/N ratio of 27 and 33 ingestate. WCN27 and WCN27B have the same C/N ratio but different ratio of dairy maure to corn stover. C/N represents carbon to nitrogen ratio.

Also, notably was the pH of all the treatments at the cessation of the experiments, which were all below (< 6.0) the threshold required for suitable methanogenic activities (Table 4.4). An indication of VFA accumulation and hence an unsuitable environmental condition for methanogens.

4.4.1.3. VFA

Volatile fatty acids are very intricate in anaerobic process, they are converted to methane and carbon dioxide by both acetoclastic and hydrogenotrophic methanogens. In this study, VFA for all the ingestates were less than 13.5 g/L (Table 4.5) with more than 87% of this value being acetic acid (Data not shown) for the ingestates with pretreated-unwashed corn stover. This percentage was lower (66 – 80 %) with the ingestates that had pretreated-washed corn stover (Table 4.5). From related studies (Li et al, 2018; Li et al., 2011), VFA concentration of ingestates was usually between 1 – 2.2 g/L. A previous study stated that VFA above 6 g/L could inhibit the digestion process (Li et al., 2013b). Hence, the high initial VFA values, which McCarty and McKinney (1961) considered as an upset reactor, might be the sole cause of digester failure in this experiment. It was equally observed in this study that initial VFA concentration in the treatments increased with C/N (Table 4.5). However, it is unclear which of the substrates or anaerobic factors contributed largely to VFA production in this study.

Table 4.5: Initial and final VFA concentration and initial C/N ratio of the 5 treatments.

Experiment	Initial Total VFA (g/L)	Final Total VFA (g/L)	Initial C/N
WCN33	13.4±0.0[a]	21.0±0.0[a]	33.± 0.0[a]
WCN27	7.3 ±0.0[b]	21.6±0.0[b]	27 ± 0.5[b]
WCN27B	6.0±0.1[c]	36.3±0.1[c]	27 ± 0.0[b]
UwCN33	5.5 ± 0.0[d]	76.9± 0.0[d]	33± 0.0[a]
UwCN27	4.7± 0.0[e]	19.0± 0.0[e]	27± 0.0[b]

Means followed by the same letter in a column are not significant. Treatments WCN27 and WCN27B have the same C/N ratio but different ratio of dairy maure to corn stover. VFA represents volatile fatty acids and C/N represents carbon to nitrogen ratio.

On VFA composition, lower initial propionic acids content of the ingestates with pretreated-unwashed corn stover (0.2 – 0.7 g/L) relative to the pretreated-washed corn stover (0.7 – 1.8 g/L). An observation that suggests washing pretreated corn stover might enhance propionic production. Generally, propionic acid accumulation is not desirable in anaerobic digesters. According to Barredo and Evison, (1991) propionic concentration between 20 – 80 mM (1.5 – 6.0 g/L) affects methanogens population. The only observed exception was when the pH was 8.0. Dogan et al., (2005) also noted that 50% methane inhibition commences when propionic and acetic concentrations were about 3.5 g/L and 13 g/L, respectively. At the end of this experiment, propionic concentration in all the treatments, except treatment WCN27B, were more than 3.5 g/L, while the acetic acid concentration only exceeded 13 g/L for treatments WCN27B (Data not shown). Hence, poor reactor performance in all these digesters could be attributed to either high acetic or high propionic concentration in these earlier stated treatments.

On VFA composition ratio as process indicator, when propionic acid to acetic acids ratio in treatment is greater than 1.4, Hill et al., (1987) noted that the failure of such digester is imminent.

Gao et al., (2015) also stated that a safe anaerobic process for acid should have a propionic acid to acetic acids ratio 0.08. In this study, propionic-to acetic-ratio was only less than 0.08 for treatments with unwashed-pretreated-corn stover (Data not shown). However, after the digestion process, all the aforementioned treatments had propionic-to-acetic acid ratio that were above 0.08 but less than 1.4 (Data not shown). Hence, we concluded that propionic acid to acetic acids ratio of 0.08 was a better indicator of acid accumulation than when at 1.4. The upsurge in propionic acid to acetic acid value in this study could explain why the peak methane concentration and cumulative biogas yield in this study was below 40 % and 140 L/kgVS respectively for all the treatments. Furthermore, in most of the treatments, there was obvious sign that VFA had accumulated, because almost all the pH values of the digestates were less than 6.4 (Table 4.4). According to Ahring et al., (1995), this increment in VFA was as a result of an imbalance among hydrolytic bacteria, acidogens, acetogens, and methanogens. This was because the production rate of acidogens outmatched the bioconversion rate of both acetogens and methanogens. Interestingly, unlike the final propionic concentration, most of the acetic acid concentration in this study was within the tolerable range stated earlier. Hence, propionic acid concentration is a better reactor performance indicator than acetic acid concentration.

4.4.1.4. VFA/Ammonium-N and VFA/ Alkalinity

Other prominent process indicators are VFA/Ammonium-N and VFA/Alkalinity. This is because they allow for easy comparison among treatments even if the composition are different or experimental conditions are divergent. Generally, in successful solid-state anaerobic digestion and co-digestion experiments, VFA/Ammonium-Nitrogen ratio has been between 0.14 – 0.45 for ingestates and 0.96 – 2.26 for digestates (Li et al., 2018a, Li et al., 2018b). With respect to VFA/Alkalinity ratio, treatments that performed well had ingestates values between 0.04 – 0.12

and digestates values between 0.21 – 0.93 (Li et al., 2018a, Li et al., 2018b). Unfortunately, in most of the treatments, the VFA/Ammonium-Nitrogen and VFA/Alkalinity ratio were significantly higher than these stability ranges (Data not shown). This established the reason for the low methane concentration and biogas yield experienced in this study (As discussed later in this chapter).

4.4.2. Holocellulose Degradation

Extend of microbial decomposition of organic waste could be monitored with holocellulose degradation. In this study, treatments with pretreated and unwashed corn stover had consistent holocellulosic degradation (Figure 4.2). In these treatments, cellulose degradation was between 30 - 51%, while hemicellulose degradation was between 52 - 73% (Figure 4.2). The lower cellulosic degradation relative to the hemicellulose was because of the high recalcitrance nature of cellulose compared with hemicellulose. Hence microbial degradation of cellulosic was limited. Furthermore, washing had effect polysaccharide degradation, treatments with unwashed-pretreated corn stover (UwCN33 and UwCN27) had significantly ($p <0.05$) higher polysaccharide degradation relative to the WCN33, WCN27, and WCN27B treatments (Figure 4.2).

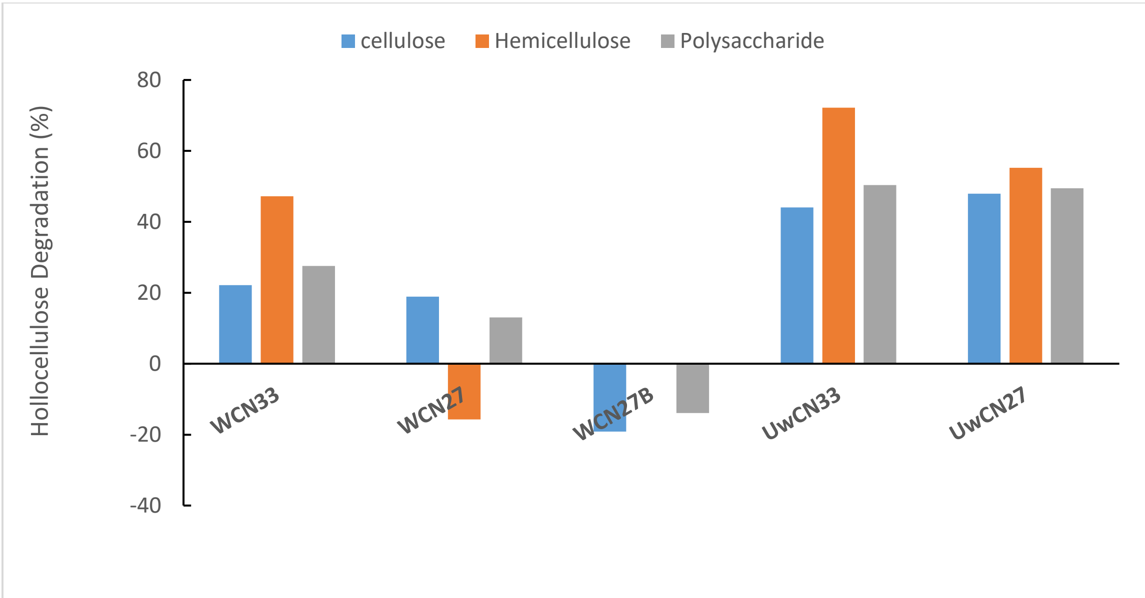


Figure 4.2: Holocellulose degradation in treatments.
W represents washed NaOH-pretreated corn stover; Uw represents unwashed NaOH-pretreated corn stover; and CN27 & CN33 represents C/N ratio of 27 and 33 ingestate. WCN27 and WCN27B have the same C/N ratio but different ratio of dairy maure to corn stover. C/N represents carbon to nitrogen ratio.

4.4.3. Total Solids and Volatile Solids

Percentage of volatile solids (VS) destroyed is one of the metrics to quantify the effectiveness of microbial degradation in SSAD. According to Hill et al., (1987), anaerobic digester with VS reduction of at least 50% was considered healthy. In this study VS destruction was less than 25% for all the treatments (Figure 4.3). Furthermore, this low VS reduction indicates poor microbial degradation. The suspected reason for this trend of VS was likely due to poor microbial balance in the digesters. Poor microbial balance hampers methanogens, leading to VFA accumulation. Furthermore, when VFA accumulates, it does not only inhibit methanogens, it equally kills the acetogens.

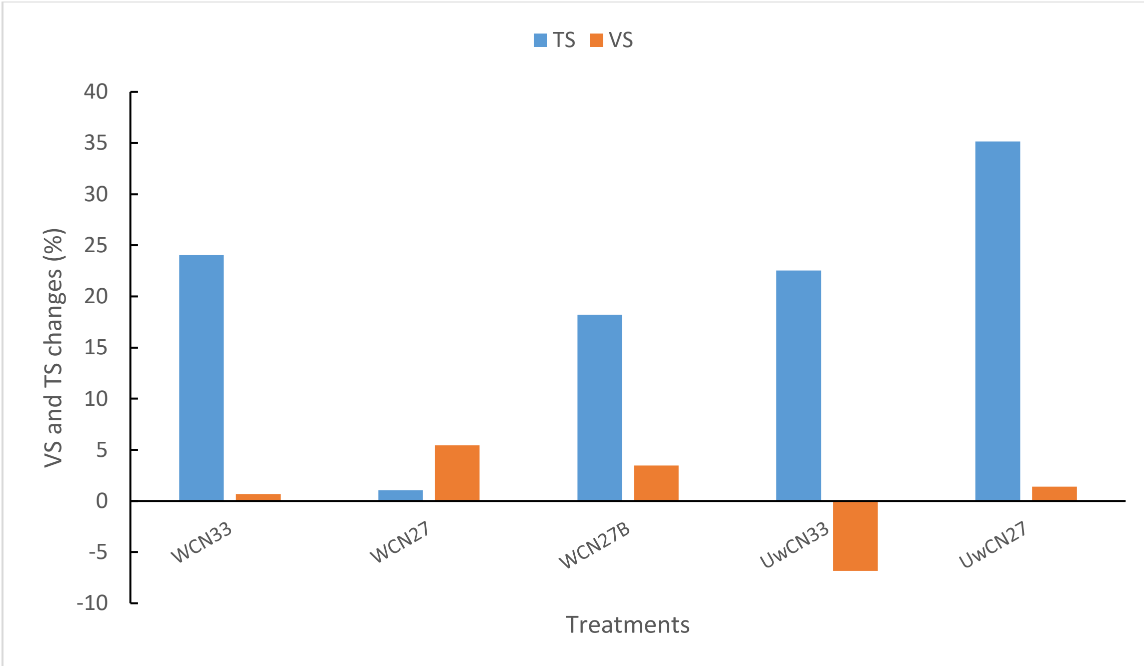


Figure 4.3: Volatile solids and total solids changes in treatments.
W represents washed NaOH-pretreated corn stover; Uw represents unwashed NaOH-pretreated corn stover; and CN27 & CN33 represents C/N ratio of 27 and 33 ingestate. WCN27 and WCN27B have the same C/N ratio but different ratio of dairy maure to corn stover. C/N represents carbon to nitrogen ratio.

4.4.4. Biogas and Methane Yield

Figure 4.4 shows cumulative methane yield at the end for most of the experiments. Cumulative methane yield was less than 20 L/kg VS for all the treatments. Basically, the low methane and biogas yield in the other treatments could possibly be attributed to inadequate buffering capacity from the influent, which does not provide enough alkalinity within the influent to suppress VFA accumulation. Hence, the digesters were stressed and then failed. In fact, the addition of $NaHCO_3$ could not rejuvenate the system. On another thought, low methanization experienced for the failed digesters (with methane yield less than (20 L/kg VS) could be linked to the high initial VFA concentrations of the treatments (> 5 g/L) which were higher than the value recommended in anaerobic digestion studies. For instance, Ehimen et al., (2011) suggested an initial VFA concentration of less than 5 g/L for a healthy anaerobic process.

Despite these, methane yield increased slightly under one instance. Methane yield and biogas yield increased by at least 66% and 8%, respectively, with treatments which has pretreated-unwashed-corn stover (UwCN33 and UWCN27) as one of the feedstocks blends rather than with the pretreated-and-washed-corn stover (Figure 4.4), this was irrespective of the C/N. The latter case could probably be attributed to the loss of nitrogen and some portion of the pretreatment reagent (NaOH) to washing. In this experiment, though pretreatment reduced nitrogen concentration by 30%, washing after pretreatment further reduced nitrogen concentration by 83.5% (Data not shown). Overall, treatments with unwashed-pretreated-corn stover (UwCN33 and UWCN27) shown a better outcome than the ones with washed-pretreated-corn stover (WCN33, WCN27, and WCN27B).

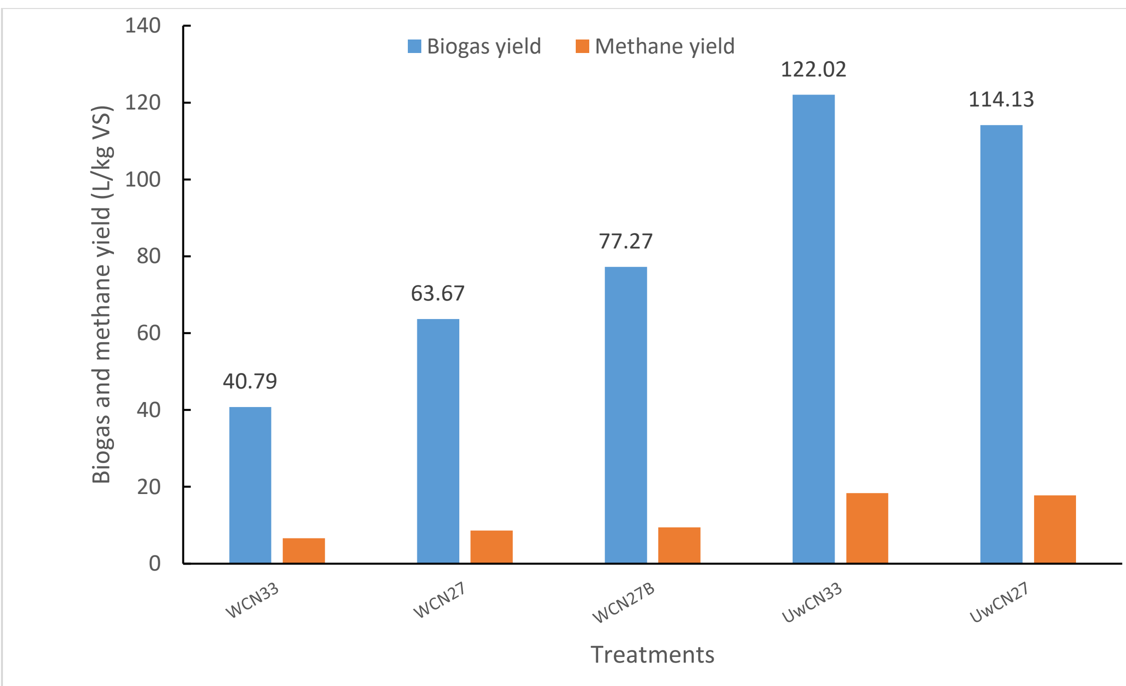


Figure 4.4: Biogas and methane yield in treatments.
W represents washed NaOH-pretreated corn stover; Uw represents unwashed NaOH-pretreated corn stover; and CN27 & CN33 represents C/N ratio of 27 and 33 ingestate. WCN27 and WCN27B have the same C/N ratio but different ratio of dairy maure to corn stover. C/N represents carbon to nitrogen ratio.

On biogas composition, the concentration of gases produced (methane, carbon dioxide, and hydrogen sulfide) from the SSAD study on the blend of dairy manure and pretreated-corn stover were influenced by the quantity of dairy manure as well as the concentration of the alkaline reagent. As shown in Figure 4.5, ingestates with unwashed-NaOH-pretreated corn stover blended with dairy manure had higher methane peak concentration (30 – 65 %) relative to the washed blended with dairy manure (12 – 22 %). However, the ingestates with this unwashed-alkaline-pretreated corn stover had very low methane concentration during the start-up stage (0.2 - 1.8%), before a significant increase between the second and third week of the experiments (Figure 4.5). This was suspected to be as a result of the high concentration and the harsh nature of the pretreatment reagent (NaOH) in the unwashed-alkaline-pretreated corn stover blended with dairy manure. NaOH might have a harsh effect on methanogens due to its corrosive nature. However, a simple general conclusion could be the high initial VFA concentration of all the ingestates despite difference in C/N, pretreatment type and pretreatment condition. Furthermore, on NaOH, at over 5% concentration, NaOH inhibits methanogenic activities due to faster production of VFA during the hydrolysis and acidogenesis stages (Zhu et al., 2010). For instance, in Zhu et al., (2010) study, VFA concentration for 7.5% pretreated corn stover was about 10 folds compared with 1.0 – 5.0% pretreated corn stovers. In another solid-state co-digestion study, the author reported that the low methane yield could possibly be due to the combined effect of NaOH-pretreatment and solid-state co-digestion (Feng et al., 2018).

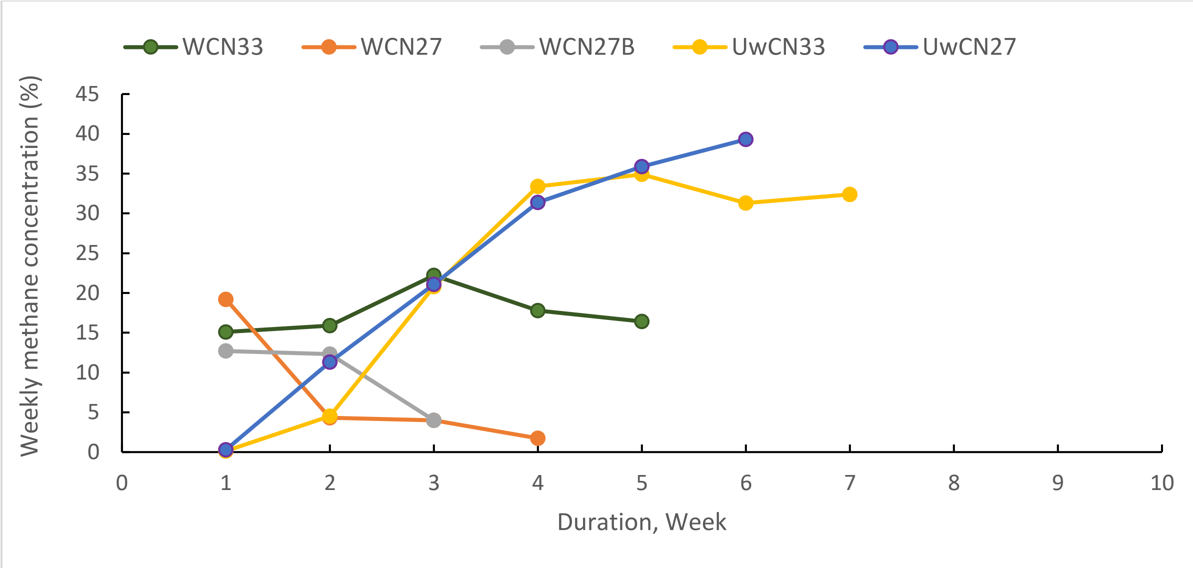


Figure 4.5: Methane concentration in treatments.
W represents washed NaOH-pretreated corn stover; Uw represents unwashed NaOH-pretreated corn stover; and CN27 & CN33 represents C/N ratio of 27 and 33 ingestate. WCN27 and WCN27B have the same C/N ratio but different ratio of dairy maure to corn stover. C/N represents carbon to nitrogen ratio.

Also obvious was the correlation between hydrogen sulfide concentration and the volume of dairy manure included in ingestate constituent. From Figure 4.6, the highest peak of H_2S (6000 ppm) was observed with ingestate which has the highest dairy manure concentration to pretreated corn stover ratio (7.0 – 7.5 fold). This indicates that most of the H_2S produced in the study were primarily from the dairy manure. It has been noted that H_2S produced by sulfur reducing bacteria at a concentration between 5,000 – 10,0000 ppm hampers anaerobic process (Isa et al., 1986). This is because it makes non-alkali metals, which are an important nutrient for biogas microbial growth, to precipitate and become unavailable for microbial consumption (Isa et al., 1986). Specifically, acetoclastic methanogens metabolic process are inhibited when sulfide concentration is about 50% at 50 mg/L and 100% at 200 mg/L (Kroiss and Wabnegg, 1983). These sulfur reducing bacteria also compete with methanogens for acetate but not analyzed for in this study.

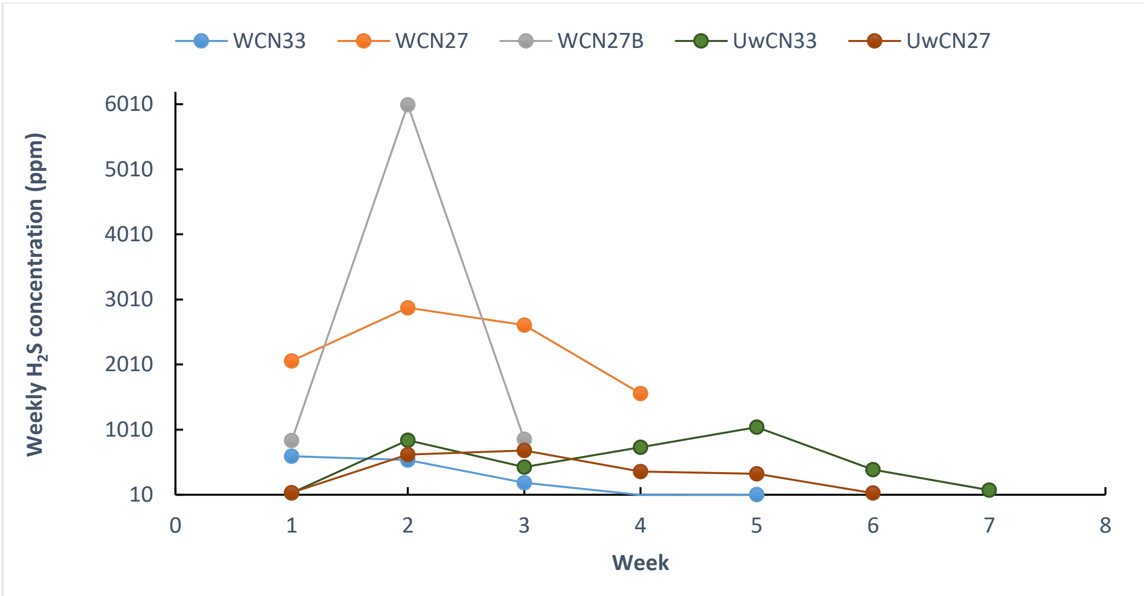


Figure 4.6: Hydrogen sulfide concentration in treatments during the digestion process. W represents washed NaOH-pretreated corn stover; Uw represents unwashed NaOH-pretreated corn stover; and CN27 & CN33 represents C/N ratio of 27 and 33 ingestate. WCN27 and WCN27B have the same C/N ratio but different ratio of dairy maure to corn stover. C/N represents carbon to nitrogen ratio.

In this study, it is also worth mentioning that the high concentration of NaOH in the ingestates with unwashed corn stover seems to impact H_2S concentration. After two days from set-up, treatments with unwashed-pretreated-corn stover (UwCN33 and UWCN27) had H_2S concentration lower than the treatments with washed-pretreated-corn stover (WCN33, WCN27 and WCN27B). This was despite the ratio of the dairy manure volume to the pretreated corn stover ratio being about 2 – 4 (Figure 4.6).

As expected in a low methane producing anaerobic system, all the failed treatments considered in this study had high carbon dioxide concentration between 64 - 83% after the fifth week of the experiment (Data not shown). An indication of either low methanogens growth or unsuitable environment for these microbes.

4.5. Conclusion

The potential of NaOH-pretreated-corn stover blended with dairy manure as co-substrate was examined in this mesophilic solid-state anaerobic study. Of the five treatments, WCN33, WCN27, WCN27B, UwCN33, and UWCN27 considered, washing pretreated corn stover prior to co-digestion with dairy manure influenced both holocellulose degradation and biogas yield. Precisely, hemicellulose degradation for the treatment with washed and pretreated corn stover was between 13 – 33 % significantly ($p < 0.05$) greater than the treatments with washed and pretreated corn stover (WCN33, WCN27, and WCN27B). While biogas yield for the unwashed treatments (UwCN33 and UWCN27) was 66 % more than the washed treatments (WCN33, WCN27, and WCN27B). On the two C/N ratios (27 and 33), influent alkalinity was affected by C/N ratio. As the influent C/N ratio increased from 27 to 33, the initial influent alkalinity concentration increased by at least 9 and 59% for treatments with washed and unwashed pretreated corn stover, respectively. However, this does not have a significant effect on biogas yield. Hence, this study has shown that unwashed-NaOH-pretreated-corn stover blended with dairy manure (UwCN33 and UWCN27) has a better potential to improve biogas yield relative to the treatments with washed-NaOH-pretreated-corn stover blended with dairy manure.

5. EFFECT OF ALKALINE-PRETREATED-CORN STOVER AND DAIRY MANURE CO-DIGESTION ON METHANE AND BIOGAS YIELD IN SOLID-STATE ANAEROBIC CO-DIGESTION[2]

5.1. Abstract

Renewable energy generation from agricultural waste is a potential complementary energy source to address the growing energy need. In this study, pretreated corn stover blended with dairy manure was harnessed to improve biogas yield under 35 ^{o}C mesophilic temperatures. Hence, corn stover was subjected to three different alkaline wet-state pretreatments (NaOH, NH_4OH, and $Ca(OH)_2$) prior to co-digestion with dairy manure under solid-state condition. Hence, the treatments were NaW, 2NHW, 4NHW, and CaW. Results from this study show that the combination of wet state pretreatment method enhanced biogas yield and methane yield. Precisely, methane yield from treatments CaW, 2NHW, 4NHW, and NaW were 176, 173, 117, and 97 L/kg VS, respectively. Retention time was lower in CaW treatment (79 days) relative to other treatments (100 days). Interestingly, unwashed calcium hydroxide pretreated corn stover mixed with dairy manure in this solid-state mesophilic study is a suitable feedstock for renewable energy generation, particularly with a potentially upset feedstock.

[2] The material in this chapter was co-authored by Ademola. A. Ajayi-Banji, S. Rahman, L. Cihacek and N. Nahar. Ademola Ajayi-Banji had primary responsibility for collecting samples and analyzing laboratory data. Ademola Ajayi-Banji also drafted and revised all versions of this paper. Shafiqur Rahman, L. Cihacek and N. Nahar are contributing authors reviewed the manuscript conducted by the primary author. Shafiqur Rahman is the corresponding author. It is currently under review in the Journal of the Waste and Biomass Valorization

5.2. Introduction

Interest on renewable energy has continued to grow globally in order to reduce carbon footprint from activities related to fossil utilization and also sole dependence on fossil fuel (Kwietniewska and Tys, 2014). For instance, renewable energy options such as anaerobic digestion technologies have shown enormous reception to a number of input materials (Kwietniewska and Tys, 2014) in the last 2 decades. This does not only complement fossil fuel, it is also a means of waste management. However, with at least 20% yearly global growth in organic waste generation (Appels et al., 2011), present anaerobic digestion technology needs to be upgraded to accommodate this growth. Some possibilities recently considered include reduced retention time of the digester through pretreatment of lignocellulosic biomass (Zhu et al., 2010), increasing digester temperature (Bowen et al., 2014; Mao et al., 2015), and co-digestion of various waste (Li et al., 2018). Other feasible option that has barely been studied is the solid-state co-digestion of pretreated lignocellulosic biomass and animal manure. This option will not only reduce the retention time, it has the possibility to utilize more organic material. It equally improves volumetric methane productivity. One of the few reported studies on this includes a failed reactor as a result of VFA accumulation when NaOH-pretreated corn stover and chicken manure was co-digestion under a solid-state study (Feng et al, 2018). Hence, we considered pretreatment of lignocellulose waste prior to co-digestion with dairy manure in order to enhance biogas yield and reduce retention time. Precisely, NaOH-pretreated-corn stover or $Ca(OH)_2$-pretreated-corn stover or NH_4OH-pretreated-corn stovers were blended with dairy manure under a mesophilic condition (35 ± 1.5 ^{o}C).

5.3. Materials and Methods

5.3.1. Dairy Manure and Inocula Collection

Fresh dairy manure was collected in a 20 liters plastic container from North Dakota State University (NDSU) Dairy Farm, ND, USA. About 7 liters inoculum was obtained from a mesophilic anaerobic digester located at Fargo Wastewater Treatment Facility, Fargo, ND, USA. All the collected substrates were used on the same day of collection, except the corn stover that had to be chemically pretreated.

5.3.2. Corn Stover Collection and Pretreatment

Corn stover were obtained from Carrington Research and Extension Centre, Carrington, North Dakota. The stover was crushed into < 3 mm particle size with a 3 mm-mesh-sieve-sized Schuttle Buffalo hammer mill (Model W6H, New York, USA). The crushed stover was sieved into 0.42 – 0.84 mm using a mechanical sieve shaker. The sieved stover was stored in 10 liters Ziploc bags under ambient condition. Characteristics of this substrate were presented in Table 5.1.

Table 5.1: Substrates characteristics.

Parameter	Corn stover	Dairy manure	Inoculum
pH	6.0±0.1	7.89±0.0	7.4±0.0
VS (%)	94.1±0.8	86.6±0.0	68.1±.0
TS (%)	99.0±0.8	15.6±0.0	1.5±0.0
C (%)	41.0±0.1	39.6±0.2	35.7±0.1
N (%)	0.8±0.1	2.7±0.0	1.53±0.0
Alkalinity (g/L)	1.7±0.1	5.3±1.0	1.7±0.0
C/N	51.3±0.1	14.7±0.0	23.3±0.0
Cellulose (%)	43.5±0.3	25.2±0.0	-
Hemicellulose (%)	34.16±0.1	22.6±0.0	-
Ash (%)	5.7±0.3	-	-
Lignin (%)	5.1±0.2	8.0±0.0	-
Amm-N(mM)	0.0	92.6±19	34.6±1.3
VFA (g/L)	0.0±0.0	9.7±0.8	0.1±0.0

*Data represent the mean ± standard deviation, Amm-N denotes ammonium-Nitrogen, C represents carbon, N represents nitrogen, C/N represents carbon to nitrogen ratio, VS represents volatile solids, TS represents total solids, and VFA represents volatile fatty acids.

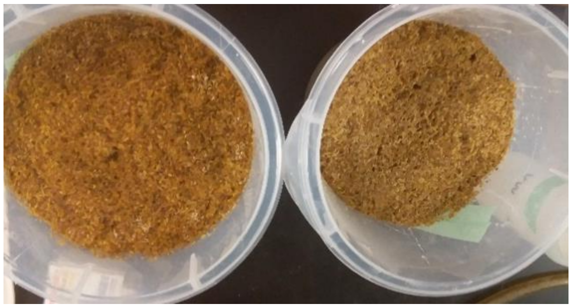
5.3.3. Wet State Pretreatment of Corn Stover

In a previous thermo-chemical pretreated corn stover blended with dairy manure SSAD resulted in low methane yield (Chapter 4 of this dissertation). Literature suggested that wet-state pretreatment of corn stover may likely overcome some of the issues encountered in the thermo-chemical pretreated SSAD. The benefits of the wet state pretreatment method are optimal water use, no leachate generation, low energy use, low chemical waste and high effectiveness in terms of result Zheng et al., (2009). However, it takes at least 5 days to complete the process. The procedure used to carry out this pretreatment followed the protocol described by Zheng et al., (2009).

Briefly, calculated volumes of alkaline solutions (8% $Ca(OH)_2$, 4% NaOH, 2%NH_4OH, and 4%NH_4OH were used based on the literature) the required to mix with 100 g of dried corn stover (TS based) were prepared. However, an initial experiment was carried out to know the appropriate volume of alkaline solution to corn stover that will generate a minimum volume of leachate possible using the formula stated by Zheng et al., (2009) in equation 5.1.

$$Moisture\ Content(\%) = 1 - (\frac{Dry\ matter\ weight\ of\ corn\ stover}{Weight\ of\ cornstover + Water\ added}) \quad (5.1)$$

Hence, 79% moisture contents were considered appropriate for the NaOH, 2%NH_4OH, and 4%NH_4OH wet state pretreatment method. While 83% was considered appropriate for the $Ca(OH)_2$ wet state pretreatment method. The means 372.9 mL of one of NaOH or 2%NH_4OH or 4%NH_4OH solutions was mixed with 100 g of corn stover (TS based), while 508.65 mL of $Ca(OH)_2$ solution was mixed with 100 g of corn stover (TS based). After mixing the required volume of alkaline solution with the corn stover, the mix was stirred, cover with aluminum foil and then stored under the ambient condition for 5 days (Figures 5.1a - c). However, for corn stover pretreated with $Ca(OH)_2$ and NaOH, 7 days storage time was considered. During this period, the mix was stirred daily and the pH was equally monitored. After the respective retention time, the samples were oven dried at 40 ^{c}C for 2 – 3 days and stored in 5 L Ziploc bag under ambient condition. Hence, the $Ca(OH)_{2,}$ NaOH, 2%NH_4OH and 4%NH_4OH wet state pretreated corn stovers were denoted as 8CaCs, 4NaCs, 2NHCs, and 4NHCs respectively. Characteristics of the pretreated substrates were presented in Table 5.2.



A B

C

Figure 5.1 : a-c NaOH and $Ca(OH)_2$ wet state pretreatment process for the crushed corn stover.

Figure 5.1a shows the solutions of NaOH and Ca(OH)2 after mixing with corn stover, Figure 5.1b shows the pretreated mix of alkaline solutions and corn stover in Fig 5.1a prepared for pH test, and Figure 5.1c shows the pretreated mix of corn stover and respective alkaline solution (NaOH and Ca(OH)2) ready for storage.

5.3.4. Scanning Electron Micrograph

Modification of morphological structure of corn stover was investigated prior to and after pretreatment with scanning electron microscope (Model: JEOL JSM-6490LV, Massachusetts, USA).

5.3.5. Substrates Mix

All the feedstocks (inoculum, dairy manure, and the unwashed pretreated corn stover) used in this experiment were mixed based on carbon to nitrogen ratio (C/N) calculations indicated in equation 5.2. Furthermore, the quantity for all the different mix ratios is shown in Table 5.2.

$$R = \frac{M1C1TS1 + M2C2TS2 + M3C3TS3}{M1N1TS1 + M2N2TS2 + M3N3TS3} \tag{5.2}$$

Where,

R - represents the carbon to nitrogen ratio of the three feedstocks,
M1 - denotes the moisture content (wet basis) of the dairy manure,
C1 - represents the % carbon content (dry basis) of the dairy manure,
N1 - denotes the % nitrogen content of the dairy manure,
TS1 - represents the % total solids of the dairy manure,
M2 - denotes the moisture content (wet basis) of the inoculum,
C2 - represents the % carbon content (dry basis) of the inoculum,
N2 - denotes the % nitrogen content (dry basis) of the inoculum,
TS2 - represents the % total solids of the inoculum,
M3 - denotes the moisture content (wet basis) of the corn stover,
C3 - represents the % carbon content (dry basis) of the corn stover,
N3 - denotes the % nitrogen content (dry basis) of the corn stover, and
TS3 - represents the % total solids of the corn stover.

The F/I was 2.5 for all treatments, except the controls (Table 5.2). This value was within the range that Li et al., (2018) and Li et al., (2103) considered suitable for SSAD experiments.

Table 5.2: The mix ratio for pretreated ingestate containing dairy manure, pretreated corn stover and inoculum.

Ingestate mix	Quantity of dairy manure (%)	Quantity of pretreated corn stover (%)	Quantity of inoculum (%)
8CaW	59.33	7.71	32.96
2NHW	40.0	8.30	33.80
4NHW	46.9	7.90	33.80
4NaW	58.92	7.70	33.39
Control (DM+IN)	63.80	-	36.20
Control (IN)	-	-	100.00

Where:
Control (DM+IN) and Control (IN) were both liquid state, TS for Control (DM+IN) was 11.5% while the TS for Control (IN) was 1.47 %.
8CaW: Influent with F/I of 2.5 and contained a mixture of dairy manure, inoculum and 8% $Ca(OH)_2$-wet state pretreated corn stover, and
4NaW: Influent with F/I of 2.5 and contained a mixture of dairy manure, inoculum and 4%-NaOH-wet state pretreated corn stover.
2NHW: Influent with F/I of 2.5 and contained a mixture of dairy manure, inoculum and 2%-NH_4OH-wet state pretreated corn stover.
4NHW: Influent with F/I of 2.5 and contained a mixture of dairy manure, inoculum and 4%-NH_4OH-wet state pretreated corn stover.

5.3.6. Experimental Set-Up

One thousand grammes of ingestate containing a mixture of dairy manure, inoculum, and the unwashed pretreated corn stover was introduced into the digester. Initial chemical composition of the ingestate in each digester is presented in Table 5.3. The 6 liters digesters with 3.5 liters working volume were deoxygenated with nitrogen gas after loading and thereafter checked for gas leak. This oxygen flush made the digester environment anaerobic. A similar step was taken for the two controls. Equally, the volume of biogas produced from the inoculum was deducted from all the treatments.

The digesters were immersed vertically in a 0.73 m × 0.49 m × 0.53 m plastic tub filled with water. The tub was coupled with a thermostatically controlled heater set at 35± 1.5 ^{o}C for all the treatments. Volume of water in the tub was daily maintained by manual water introduction. To quantify the biogas volume produced from the respective digesters, a rectangular gas collector with a tipping bucket was programmed with a CR-1000 data logger in order to collect real-time data. This chamber was filled with water to allow for easy bucket tip. The gas collector was coupled to a digester with a 2 mm diameter Teflon tube. Data obtained from this chamber were collected and stored every minute and 24 hours through the CR-1000.

5.3.7. Gas Analyses

In order to analyze gas composition weekly, gas produced from the digester were collected from the headspace of the digester by inserting a 20 mL syringe through a septum stopper attached to one of the gas outlets. The gas was analyzed for biogas (methane and carbon (iv) oxide) and hydrogen sulfide composition using a gas chromatograph (GC, 8610C, SRI Instruments, California, USA) and hydrogen sulfide analyzer (Jerome 631X, Arizona Instrument LLC, Arizona, USA) respectively. The gas chromatograph used was equipped with a flame ionization detector (FID) for CH_4 and CO_2 analyses. It was equally equipped with an electron capture detector (ECD) detector for nitrous oxide (N_2O) gas analysis. The chromatograph was operated on a 20 PSI N_2 carrier for the ECD while hydrogen gas and air were supplied on a 20 PSI to the FID or methanizer. Calibration curves generated from four different CH_4 (5, 10, 20, and 100 ppm) and CO_2 (100, 500, 1000, and 2500 ppm) concentrations were used to compute the values for the curve area. Also, worth mentioning is the 100 ^oC pre-set temperature of the gas chromatograph prior to any analysis. This ensures stability in results. On the hydrogen sulfide analyzer, the Jerome meter works through a thin gold film subjected to variation through electrical resistance that senses the gas concentration. Prior to both analyses, 5mL of the collected gas was introduced into a 0.5 L SKC Tedlar bag and then diluted with 500 mL of N_2 prior to manual mixing. This dilution factor was considered in the result presentation.

5.3.8. Ingestate, Leachate, and Digestate Analyses

The primary step taken during digestion so as to avoid crust formation due to seepage and filtration, was to periodically agitate the digesters manually based on the experimental design. This process was a form of leachate return without air introduction. Subsequently, 4 – 7 mL of these leachates were collected periodically (weekly) through the digester outlet tap for ammonium-Nitrogen, pH, oxidation redox potential (ORP), and electrical conductivity (EC) analyses using standard methods. Samples were also collected from the fresh dairy manure, inoculum, corn stover, ingestate, and digestate for ammonium-Nitrogen, nitrite, nitrate, iron, phosphorus, ash content, and alkalinity analysis using standard methods. Carbon and nitrogen content of these samples were inspected with combustion analyzer- Elemental Vario Macro Cube –CNS analyzer following the protocol described by Pella (1990). In order to validate the accuracy of the combustion analyzer, total nitrogen was also inspected with the Kjeldahl method (Isaac and Johnson, 1976). Ammonium–nitrogen was evaluated following the international organization of standardization procedure for water quality determination of ammonium nitrogen by flow analysis, ISO - 11732:1997 protocol.

Alkalinity was another important parameter that was measured for all the samples. This was carried out with the method described by APHA (1992) using the titration method with 0.01N sulfuric acid.

Other parameters measured were dry matter, ash content, crude protein, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). Dry matter of these samples was measured with the chemical procedure stated by Hoskins et al., (2003) and AOAC #934.01 with #930.15 (2010). Crude protein protocol followed AOAC official method #2001.11

(2010). ADL was also measured with AOAC official method #973.18 (2010). However, NDF and ADF followed a different procedure described by Goering and Van Soest, (1970).

Volatile fatty acids were measured with the procedure described by Baumgardt (1964), while ammonium nitrogen was determined with the colorimeter determination of urea nitrogen protocol described by Sigma Technical Bulletin. Brief description of the VFA protocol. Sample was introduced into a 25 mL centrifuge tube prior to vortex at 20000 ×g for 10 minutes. 5 mL of the supernatant produced from this process was pipetted into another centrifuge tube and a 1 mL 25 % metaphosphoric acid solution added prior to another vortex for 30 minutes. Filtered liquid was collected and loaded into the Agilent 6890N Gas Chromatograph with a FID (flame ionization detector) and the 7683 Series auto injector and auto sampler. Column used was the Supelco brand, NUKOL Fused Silica Column (Agilent Technologies, California, USA), 15 mm × 0.53 mm × 0.5 um GC. This GC analysis followed the protocol described by Goetsch and Galyean, (1983) and the acids analyzed with the GC were acetic, propionic, isobutyric, butyric, isovaleric, and valeric.

A similar procedure to the VFA analysis was carried out for ammonium– nitrogen quantification prior to when the metaphosphoric acid solution was added and then vortex. The difference after these steps were a 10-fold fluid dilution, followed by the introduction of 100 µL of this diluted sample into three borosilicate tubes and then the addition of 1.0 mL phenol nitroprusside solution, 1.0 mL alkaline hypochlorite solution, 5.0 mL deionized water prior to vortex to the tubes. The detailed procedure could be found in the protocol described by Sigma Technical Bulletin. pH, ORP, and EC of these samples were measured with HANNA HI 4522 dual channel benchtop meter (USA). Total and volatile solids were equally analyzed using standard methods (Motte et al., 2013).

5.3.9. Statistical Analysis

Statistical difference among treatments means for the completely randomized design used in this study was investigated with SAS 9.4. TS Level 1M4. X64_10PRO platform. Duncan's multiple range test was used to evaluate the significant treatments using the same tool with a threshold p-value of 0.05.

5.4. Results and Discussion

5.4.1. Characteristics of Alkaline-pretreated-cornstover

Suitable process parameters are important indicators of feedstocks potential in anaerobic digestion, of which pH is an example. In this study, pH of corn stover increased after pretreatment with any of the three alkaline reagents (2% or 4% aqueous ammonia, 4% sodium hydroxide, and 8% calcium hydroxide; Tables 5.1 & 5.3) considered. For instance, pH value for the wet-state-2% and 4% ammonia pretreated corn stover, denoted with 2NHCs and 4NHCs respectively, were within the suitable range (6.5 – 8.0) indicated for methanogens in AD (Table 5.3). On the contrary, corn stover pretreated with 8% calcium hydroxide and 4% sodium hydroxide respectively, denoted with 8CaCs and 4NaCs, had pH values outside this range. Notwithstanding, it is expected that co-digestion of the 8CaCs and 4NaCs with dairy manure will neutralize excessive alkalinity as indicated in Table 5.4 for all the treatments.

On fiber content degradation or exposure, ammonia pretreatment simply exposed the cellulose content in the corn stover by at least 10% (Tables 5.1 & 5.3), irrespective of the concentration. In addition to this, minimal disintegration of the hemicellulose content (< 24 %) occurred in the ammonia pretreated corn stovers (2NHCs and 4NHCs) relative to 8CaCs and 4NaCs (> 65%). Hence, ammonia reagent had a minimal harsh impact on the corn stover (Tables 5.1 & 5.3). This trend could be attributed to the manner in which ammonia pretreatment disrupts pretreated corn stover structure by swelling. Unlike the ammonia pretreated corn stovers, significant cellulose content disintegration occurred under both calcium and sodium hydroxide pretreatment. Precisely, the percentage of cellulose degradation into smaller oligomers and monomers was 28% and 23% under both calcium and sodium hydroxide pretreatment, respectively (Tables 5.1 & 5.3). The reason for the generally low cellulose degradation in 8CaCs and 4NaCs relative to hemicellulose in this study after pretreatment was due to the recalcitrant nature of cellulose.

As expected, alkaline pretreatment increased alkalinity significantly, alkalinity for all the pretreated corn stover increased by at least 2-folds after pretreatment (Tables 5.1 & 5.3). However, volatile nature of ammonia reagent seems to impact alkalinity of the pretreated corn stover. We suspected that due to the high volatility of aqueous ammonia relative to calcium and sodium hydroxides, alkalinity concentration in ammonia pretreated corn stover were less than 5.0 g/L (Table 5.3).

Table 5.3: Wet state method-pretreated corn stover characteristics treated with 8CaCs, 4NaCs, 2NHCs, and 4NHCs.

	Wet State Method Pretreated corn stover			
Parameter	8CaCs	4NaCs	2NHCs	4NHCs
pH	11.3±1.2	13.4±0.0	7.4±0.1	7.4±0.0
VS (%)	62.5±0.0	63.6±0.0	95.0±0.8	94.7±0.2
TS (%)	98.1±0.0	97.3±0.3	37.3±0.1	13.0±0.8
C (%)	34.1±0.0	39.8±0.0	44.9±0.3	45.2±0.0
N (%)	0.55±0.0	0.64±0.0	1.3±0.1	0.98±0.0
Alkalinity (g/L)	39.42±1.0	10.2±4.5	4.0±0.1	4.9±0.2
C/N	79.5±0.0	68.6±0.0	34.5±0.3	44.3±0.0
Cellulose (%)	30.85±0.0	33.36±0.0	48.0±0.5	49.1±0.5
Hemicellulose (%)	10.46±0.0	11.93±0.0	26.6±1.0	30.0±0.1
Lignin (%)	2.4±0.0	2.3±0.0	5.3±0.4	5.6±0.5
Amm-N (mM)	46.9±1.2	27.0±0.9	34.6±3.0	289.5±4.9

*Data represent the mean ± standard deviation. 8CaCs represents 8% $Ca(OH)_2$-wet state pretreated corn stover, 4NaCs represents 4%-NaOH-wet state pretreated corn stover, 2NHW represents 2%-NH_4OH-wet state pretreated corn stover, and 4NHW represents 4%-NH_4OH-wet state pretreated corn stover. Amm-N represent ammonium-Nitrogen

5.4.2. SEM

Aside from the chemical and physical characterization of the pretreated corn stover, the morphologically changes observed were also very informative. Surface erosion in pretreated corn stover was predominant with sodium hydroxide pretreated corn stover (Figure 5.2). This trend is similar to the impact sonification pretreatment had on wheat straw in a study conducted by Prajapati et al., (2018). On the contrary, undissolved particles of the calcium hydroxide, pretreatment reagent, clustered on the surface of the biomass (Figure 5.2). For the ammonia pretreated stovers, there seems to be slight erosion of the surface structure and some observed pores (Figure 5.2). In summary, all the aforesaid trends are an indication that the impact of alkaline pretreatment on corn stover differs with reagent solubility, harshness, and also the reagent concentration.

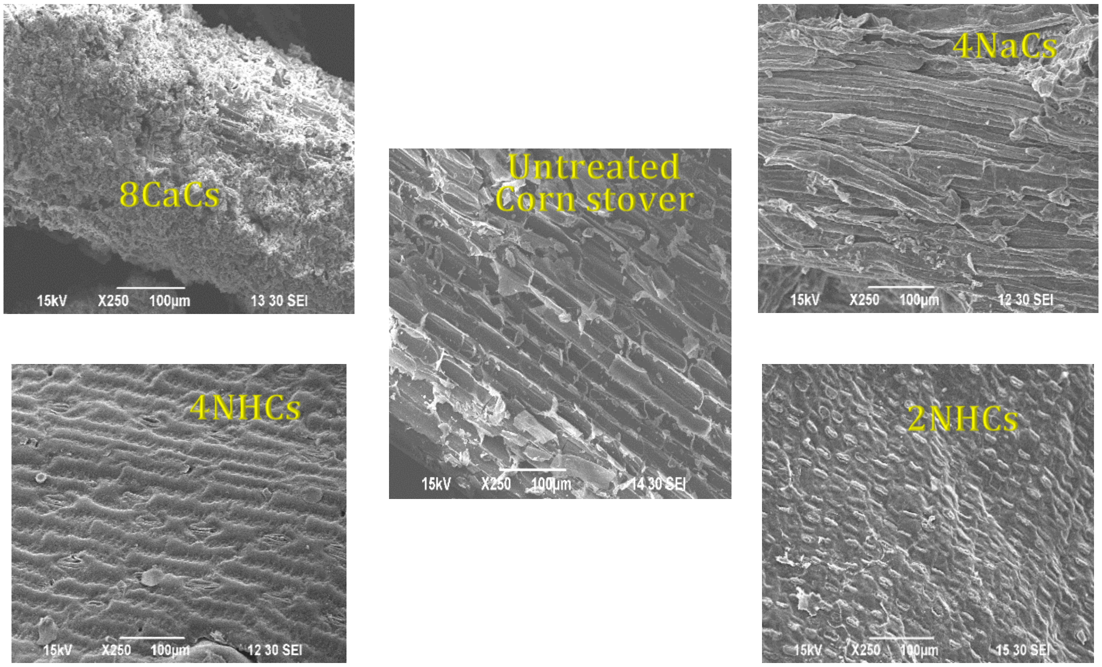


Figure 5.2: Scanning electron micrograph for the untreated and alkaline-wet-state pretreated corn stovers.

8CaCs represents 8% Ca(OH)2-wet state pretreated corn stover, 4NaCs represents 4%-NaOH-wet state pretreated corn stover, 2NHCs represents 2%-NH4OH-wet state pretreated corn stover, and 4NHCs represents 4%-NH4OH-wet state pretreated corn stover.

5.4.3. Chemical Properties of the Treatment

One of the benefits of co-digestion is to ensure treatments have nutrient balance, reduced environmental toxicity to micro-organisms, and ensure process parameters are within a suitable range. In most successful SSAD studies, pH, VFA/Amm, and VFA/Alk of ingestates are within the range of 7.1 – 8.0, 0.20 – 0.85, and 0.22 – 0.73 respectively (Li et al. 2018a, Li et al. 2018b, Li et al. 2019, Wang et al., 2018, Rouches et al., 2019). In this study, all the ingestates had VFA/Amm and VFA/Alk outside this range, except the VFA/Alk for 4NaW treatment (Table 5.4). This is an indication of a potentially upset or stressed digester. However, at the end of the experiments, almost all the treatments had stabilized, as these process parameters were within the suitable range for methanogens (Table 5.2). The only exception was found with 4NaW, an indication that the system was unstable during the AD process as a result of VFA accumulation. Obviously from the digestate propionic acid concentration, only 4NaW had an inhibitory propionic acid concertation (> 1.5 g/L, Barredo and Evison, 1991, Table 5.2). Hence, co-digestion of sodium pretreatment corn stover with diary manure might not be a suitable option due to the unsafe propionic acids concentration. This latter result is in line with Feng et al., (2018) observation, the author attributed the failed state of similar digester to rapid hydrolysis leading to VFA accumulation. Similarly, in this study, VFA accumulated in 4NaW treatment (7.1 g/L), a trend contrary to other treatments (Table 5.4).

Other notable observation was the mass of ingestates consumes by the anaerobic microbes. For all the treatments, ash content decreased by at least 35% at the cessation of the experiments (Table 5.4). In addition, ammonia pretreated treatments had the highest ash content reduction (50 - 54 %) at the cessation of the experiments (Table 5.4). Low ash content reduction in treatment 8CaW (38%) seems to be linked with low pretreatment agent solubility and low retention time of the treatment relative to other treatments (Table 5.4). In the case of 4NaW, the low ash content reduction (35%) could be attributed to the inhibition in the treatment during digestion, as previously noted in this section.

Table 5.4: Characteristics of the chemically treated pre-ingestate and post-ingestate.

	4NaW		2NHW		4NHW		8CaW	
	ING	DIG	ING	DIG	ING	DIG	ING	DIG
Alk (g/L)	12.2 ± 0.4	13.2 ± 1.0	3.4 ± 0.5	5.1 ± 1.0	3.5 ± 0.0	5.6 ± 0.3	5.9 ± 0.4	6.1 ± 0.3
VFA (g/L)	6.3 ± 0.0	7.1 ± 0.0	3.3 ± 0.0	1.0 ± 0.0	3.9 ± 0.0	1.1 ± 0.0	7.5 ± 0.0	1.5 ± 0.0
Amm (g/L)	0.9 ± 0.0	2.7 ± 0.0	0.7 ± 0.0	2.1 ± 0.0	0.9 ± 0.0	2.7 ± 0.0	1.6 ± 0.0	2.9 ± 0.0
ORP (mV)	-406 ± 0.0	-307 ± 3.0	-329 ± 11	-249 ± 6.5	-378 ± 5.7	-262 ± 10	-270 ± 0.5	-205 ± 17
CP (%)	11.0 ± 0.3	14.7 ± 0.6	10.1 ± 0.1	13.9 ± 0.3	10.8 ± 0.3	9.5 ± 0.1	10.1 ± 0.0	13.9 ± 0.4
Ash (%)	26.2 ± 1.2	35.4 ± 2.5	10.8 ± 0.3	23.9 ± 0.1	11.8 ± 0.0	23.7 ± 3.3	24.1 ± 0.3	39.2 ± 0.1
pH	8.6 ± 0.0	8.2 ± 0.0	7.2 ± 0.0	7.5 ± 0.2	7.4 ± 0.1	7.8 ± 0.2	9.4 ± 0.0	7.8 ± 0.0
Acetic (g/L)	5.6 ± 0.4	1.9 ± 0.2	2.7 ± 0.0	1.0 ± 0.0	2.9 ± 0.1	1.0 ± 0.0	6.6 ± 0.2	1.4 ± 0.1
Butyric (g/L)	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.0
Propionic (g/L)	0.3 ± 0.0	2.1 ± 0.3	0.3 ± 0.0	0.0 ± 0.0	0.5 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	0.0 ± 0.0
Free NH_3 (mg/L)	390± 0.7	640 ± 0.3	22± 0.4	1220 ± 1.	42 ± 0.4	295 ± 0.9	1274 ± 0.	318 ± 0.3
VFA/Amm	6.8	2.6	4.5	0.5	4.4	0.4	4.7	0.5
VFA/Alk	0.5	0.5	1.0	0.2	1.1	0.2	1.3	0.2

Note: ING represents ingestate, DIG represents the digestate, CP represents crude protein, Alk denotes alkalinity, Amm represents ammonium-nitrogen concentration, VFA/Amm denotes ratio of volatile fatty acid to ammonium nitrogen ratio, VFA/Alk represents ratio of volatile fatty acid to alkalinity. 4NaW, 8CaW, and NHWs are sodium hydroxide, calcium hydroxide, and ammonia hydroxide pretreated corn stover blended with dairy manure respectively.

5.4.4. Degradation

Aside from process parameters, how much of the ingestate fibre content that degrades is another reactor performance indicator**.** Hemicellulose degradation for all the treatments seems to have a direct nexus with VS degradation (Figure 5.3). However, both VS degradation and cellulose degradation was low (less than 30%). This latter trend possibly suggests that high cellulosic degradation in this study would have resulted in high VS degradation. As indicated in Figure 5.3, a large fraction of the biogas produced from this study was from the easy-to degrade (hemicellulose) fraction of the treatment. Precisely, hemicellulose degradation was at least 40% for all the treatments with alkaline pretreated corn stover. Interestingly, unlike solid-state mono-digested of dairy manure that failed (Data not shown), liquid state mono-digested dairy had about

65% hemicellulose degradation (Figure 5.3). This latter trend suggests that total solids of ingestates play a significant role in reactor performance.

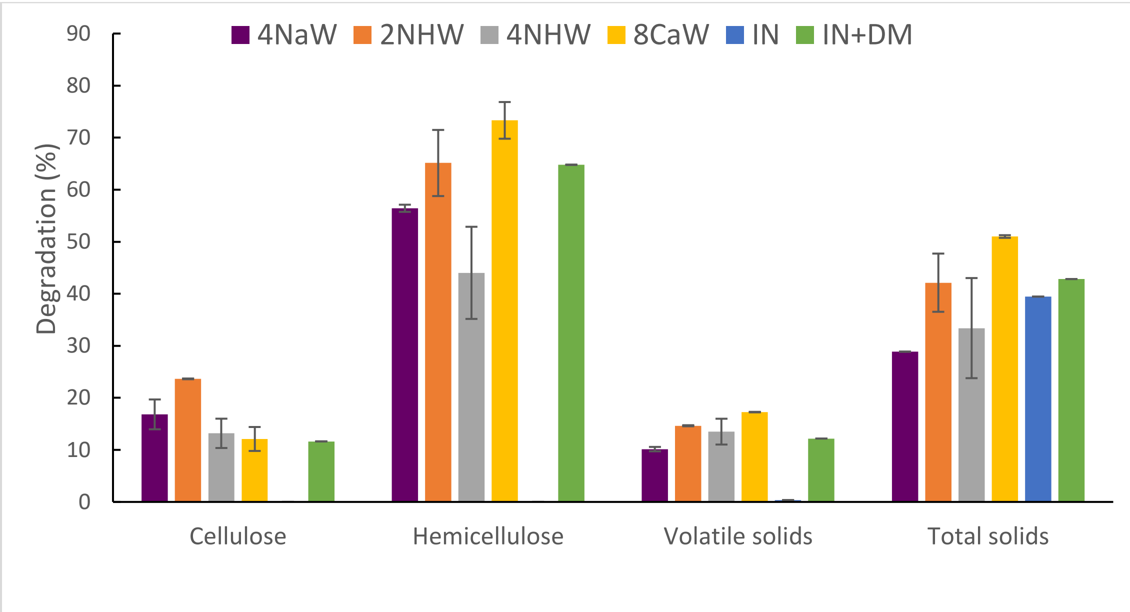


Figure 5.3: Substrate degradation in treatments as affected by the chemical pretreatment. 4NaW, 8CaW, and NHWs are sodium hydroxide, calcium hydroxide, and ammonia hydroxide pretreated corn stover blended with dairy manure respectively. IN represents the inoculum only and IN+DM represents the blend of inoculum and dairy manure.

5.4.5. Retention Time

For all the treatments, digesters were dismantled when the daily biogas production was considerably and consistently low (<140 mL or 1.1 mL/ g VS/day). For instance, treatment 8CaW was dismantled after 79 days hydraulic retention time, while all other treatments were dismantled after 100 days. Hence, a shorter retention time was achieved with 8CaW treatment relative to other treatments. Though, this retention period was longer than most studies on solid-state anaerobic co-digestion (Feng et al., 2018), the high initial VFA observed in this study was suspected to be the reason for the delay in microbes acclimatization, growth, and low initial methane yield, that led to a longer retention time. Precisely, in most solid-state studies with lower retention time than the one observed in this study, initial VFA concentration was often less than 5 g/kg (Shi et al., 2013;

Wang et al., 2012; Li et al., 2018). Early digester stress or failure could be another possible reason for the short retention time during anaerobic digestion as observed with the treatments with NaOH-thermochemically-pretreated-corn stover (chapter 4 of this dissertation).

5.4.6. Biogas Composition

Hydrogen sulfide, carbon dioxide, and methane concentration were inspected in this study with respect to retention time. These compounds are the most predominant in biogas composition.

5.4.6.1. Biogas and Methane Yield

Cumulative methane yield at the cessation of the experiment was greater than 90 L/kg VS for all the treatments except the inoculum (IN) as shown in Figure 5.4. This suggests that inoculum might not have significantly affected methane yield. Hence was not further considered in subsequent discussion in this section. However, the average highest cumulative methane yield (176 L/kg VS) was obtained with 8CaW treatment which was 39, 33, and 49% significantly ($p < 0.05$) greater than DM+IN, 4NHW, and 4NaW treatments, respectively (Figure 5.4). A similar trend was observed for the cumulative biogas yield (Figure 5.4). Interestingly, cumulative methane yield for 8CaW was about half of the 340 L/kg VS cumulative biogas yield (Figure 5.5), this trend suggests a suitable reactor performance for this treatment, unlike the treatments with thermochemically pretreated corn stover that had cumulative methane yield less than 20 L/kg VS (chapter four of this dissertation). Furthermore, with respect to both cumulative biogas and methane yield, 8CaW and 2NHW treatments which were solid-state condition outperformed the liquid state mono-digested dairy manure (DM+IN). Remarkably, cumulative methane yield in this study was greater than the values documented by Feng et al.., (2018) in a solid state co-digested study of NaOH-pretreated corn stover blended with chicken manure. Furthermore, it was close to the cumulative methane yield obtained when NaOH-pretreated-corn stover and dairy manure were co-digested in a liquid-state study conducted by Li et al., (2009).

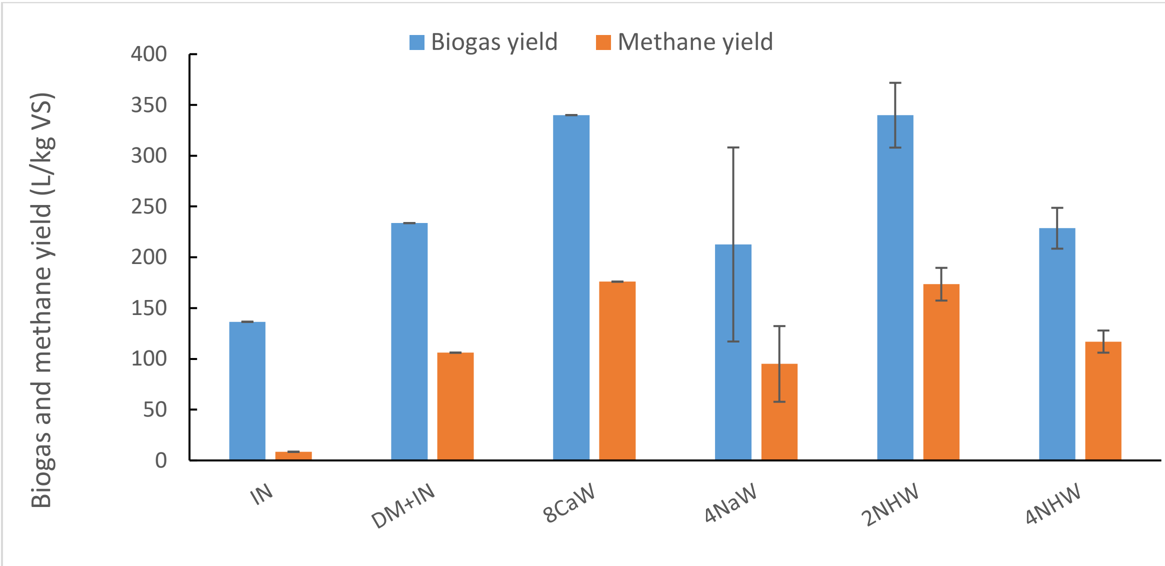


Figure 5.4: Biogas and methane yield in treatments.
4NaW, 8CaW, and NHWs are sodium hydroxide, calcium hydroxide, and ammonia hydroxide pretreated corn stover blended with dairy manure respectively. IN represents the inoculum only and IN+DM represents the blend of inoculum and dairy manure.

5.4.6.2. Biogas Composition

On biogas composition, the concentration of gases produced (methane, carbon dioxide, and hydrogen sulfide) from the SSAD study on the blend of dairy manure and pretreated-corn stover were influenced by the alkaline reagent used for pretreatment. As shown in Figure 5.4, 4NaW and 4NHW treatments had low methane concertation (< 27 %), until the 6th week of the experiment. This suggests some inhibition in both 4NaW and 4NHW digesters. Although all other treatments started with low methane concentration, after the 3rd week, methane concentration continued to increase significantly for treatments DM+IN, 2NHW, and 8CaW (Figure 5.5). Furthermore, 8CaW and 2NHW digesters reached methane peak concentration (65%) between the 5th and 6th week before a slight decline. While both DM+IN (65%) and 4NaW (54%) reached peak methane concentration at the 10th week (Figure 5.5). Hence, treatments 8CaW and 2NHW were suspected to have better methanogenic activities than other treatments in this study. Furthermore, treatments 8CaW, 2NHW, and 4NHW peak methane concentration in this study were significantly higher ($p < 0.05$) than the values (< 36%) obtained from all the treatments with thermochemically pretreated corn stover (chapter 4 of this dissertation).

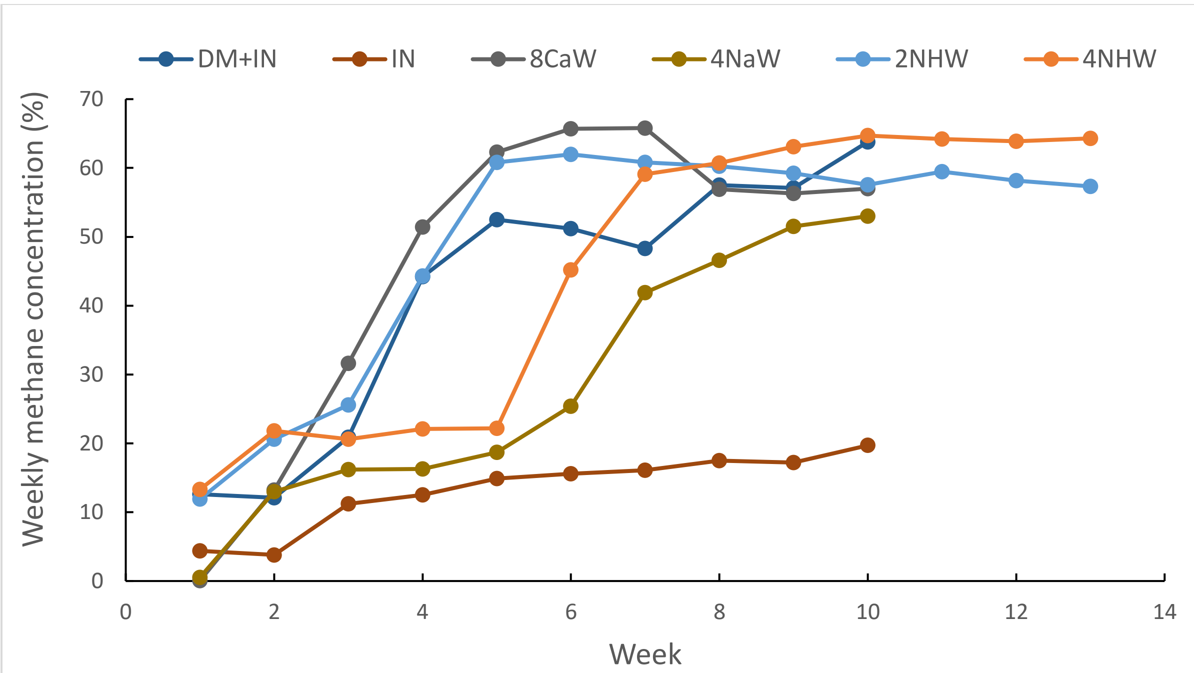


Figure 5.5: Methane concentration in treatments.
4NaW, 8CaW, and NHWs are sodium hydroxide, calcium hydroxide, and ammonia hydroxide pretreated corn stover blended with dairy manure respectively. IN represents the inoculum only and IN+DM represents the blend of inoculum and dairy manure.

Also obvious was the nexus between hydrogen sulfide concentration and the methane concentration for all the treatments except the inoculum. For instance, with treatment NaW, when the methane concentation was below 27%, (Figure 5.5), hydrogen sulfide concentration (Figure 5.6) was significantly low (between 23 – 150 ppm) relative to other treatments (40 – 900 ppm). A trend that suggests Na ions might have impacted methanogenic activities. Though the H_2S concentration was less than the range 5,000 – 10,0000 ppm stated to impact methanogens for all the treatments (Isa et al., 1986), it seems there was also a reaction between Na concentration and the organic compounds in the 4NaW digester that inhibits H_2S concentration, as this was equally noticed in our previous studies (Chapter 4). However, as hydrogen sulfide concentration increased from 92 to 300 ppm for 4NaW treatment, the methane concentration started picking up above 30% after the 6th week (Figures 5.5 and 5.6). On the contrary, treatments 8CaW, 2NHW, 4NHW, and DM+IN all had high H_2S at the beginning of the digestion process (700 – 2500 ppm), which gradually declined with retention time. The initial H_2S concentration in these treatments (8CaW, 2NHW, 4NHW, and DM+IN) might suggest a favorable environment for methanogens. This is because sulfur reducing bacteria and methanogens could survive under similar environmental condition. Interestingly, at the first week of the experiment, ammonia pretreated treatments (2NHW and 4NHW) had significantly high initial H_2S concentration ($p < 0.05$) relative to 8CaWand DM+IN (Figure 5.6). The reason for this trend is not clear. However, there might be a nexus between ammonia pretreated treatments and sulfur reducing bacteria.

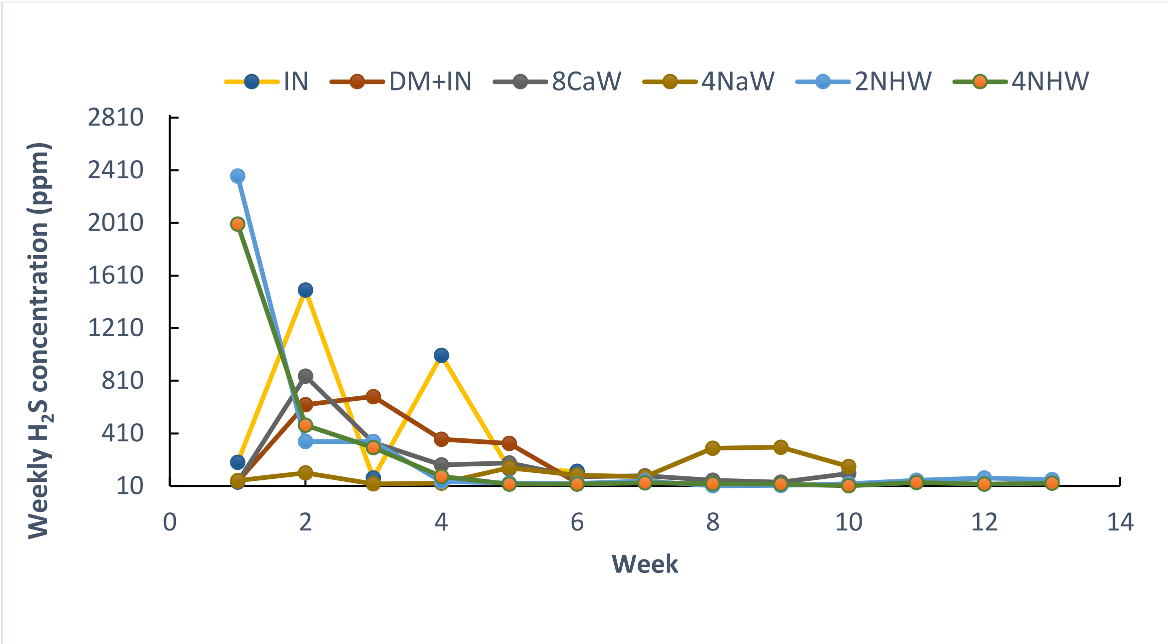


Figure 5.6: Hydrogen sulfide concentration related to the treatments during the digestion process.

4NaW, 8CaW, and NHWs are sodium hydroxide, calcium hydroxide, and ammonia hydroxide pretreated corn stover blended with dairy manure respectively. IN represents the inoculum only and IN+DM represents the blend of inoculum and dairy manure.

Hence, the feasibility of calcium hydroxide-pretreated-corn stover blended with dairy manure as ingestate was established in this solids-state anaerobic co-digestion study for renewable energy generation under mesophilic temperature within a 79 days retention time. The methane and biogas yield for this treatment (8CaW) was 176 L/kg VS and 340 L/kg VS respectively, which was encouraging relative to most SSAD studies (Li et al., 2018). Furthermore, this treatment (8CaW) show a quicker recovery relative to the other treatments.

5.5. Conclusion

The potential of unwashed pretreated corn stover blended with dairy manure was examined in this study for optimal methane yield. Of the five treatments (DM+IN, 8CaW, 2NHW, 4NHW, and 4NaW) considered, CaW outperformed mono-digested liquid state dairy manure and other treatments in terms of biogas and methane yields under shortest retention time. Specifically, cumulative methane yield and biogas yield for 8CaW, 2NHW, 4NHW, 4NaW and DM+IN were 176, 173, 117, 88, and 106 L/kg VS and 340, 339, 228, 202, 233 L/kg VS respectively, an indication of effective digestion process relative to Chapter 4. Furthermore, cumulative methane to biogas yield ratio was highest for CaW(0.52), followed by the NHW treatments (0.51),and relatively close for both DM+IN and 4NaW(0.46). This further suggests more active methanogenic activities with treatment 8CaW. This study has suggested a novel way to utilize potentially upset waste under a solid-state anaerobic co-digestion process of dairy manure and unwashed-alkaline-pretreated corn stover.

6. IMPACT OF MAGNETITE NANOPARTICLES ON BIOGAS AND METHANE YIELD IN SOLID-STATE ANAEROBIC CO-DIGESTION OF CALCIUM-PRETREATED-CORN STOVER AND DAIRY MANURE[3]

6.1. Abstract

Introduction of nanoparticles could improve methane yield and reactor performance in solid-state anaerobic co-digestion. Three concentrations of magnetite (20, 50, and 75 mg/L) were included in the blend of calcium-pretreated-corn stover and dairy manure to produced three treatments. These treatments are blend of wet-state-calcium-pretreated-corn stover and dairy manure with 20 mg of magnetite (CaW20), blend of wet-state-calcium-pretreated-corn stover and dairy manure with 50 mg of magnetite (CaW50), and blend of wet-state-calcium-pretreated-corn stover and dairy manure with 75 mg of magnetite CaW75) under mesophilic temperature. Relative to the control (CaW), the presence of nanoparticles reduced the hydraulic retention time by 27 days. Early sulfur reducing bacteria predominance was noticed with the treatments relative to the control, though this did not pose a significant threat to methanogens. At the end of the experiment, treatment CaW20 had the highest methane yield (191 L/kg VS), cellulose (30%) and hemicellulose degradation (93%). Magnetite presence influenced microbial diversity. This result suggests that the inclusion of nanoparticles at micro-scale could significantly improve methanogens activities, fiber content degradation, and ultimately the reactor performance in solid-state AD.

[3] The material in this chapter will be co-authored by Ademola. A. Ajayi-Banji and S. Rahman. Ademola Ajayi-Banji had primary responsibility for collecting samples and analyzing laboratory data. Ademola Ajayi-Banji will draft and revise all versions of this intended paper. Shafiqur Rahman will review the manuscript conducted by the primary author and will also act as the corresponding author. It is being processed.

6.2. Introduction

Clean and green energy from the solid-state anerobic digestion (SSAD) of on-farm organic wastes has attracted much attention. Aside the capability of this energy source to complement the rising energy demand, it is an environmental beneficial approach to manage the growing on-farm organic wastes such as livestock manure and crop residue. On-farm organic solid wastes volume and energy demand in the United States are both on the high side in the last 5 years (FAO, 2019, IEA, 2019). Nevertheless, harnessing biogas from a large fraction of the on-farm waste through SSAD or high-solid AD is a major concern. Though few food related AD researches have documented over 89 – 94% of biomethane potential obtained as actual methane yield (Holliger et al., 2017), only a few on-farm wastes have attained this high threshold. In addition to this limitation, high methane concentration is also desired in biogas composition to improve the calorific value of gas from SSAD. Hence, the need to optimize biogas and methane yield from this form of anaerobic digestion. Interestingly, some methods have been adopted to address these AD challenges including co-digestion, pretreatment, use of additives, conductive material, modification of process parameters (Li et al., 2018, Li et al., 2019, Lin et al., 2017, Vasco-Correa et al., 2015). For instance, the co-digestion of cucumber residues, corn stover, and pig manure improved methane yield by 8 - 30% relative to the any of the substrate's mono-digestion (Wang et al., 2018). Other benefits of co-digestion are substrates toxicity reduction and nutrient balance (Brown et al., 2012, Sun et al., 2016). In addition to co-digestion, on-farm organic waste pretreatment, especially lignocellulosic biomass, has equally improved methane yield in a number of SSAD studies (Mustafa et al., 2017, Vasco-Correa et al., 2015). Pretreatment simply disrupts substrate structure to enhance microbial access to the cellulose and hemicellulose fractions. Aside co-digestion and pretreatment, additives such as nanoparticles, particles less than 100 nm, are recently introduced into anaerobic reactors to improve reactor performance. These nanoparticles include zero valent iron, nickel, iron, cobalt and magnetite (Abdelsalam et al., 2016, Abdelsalam et al., 2017, Yang et al., 2013). Benefits of these nanoparticles in AD bioreactors include H_2S reduction, excessive prevention of acids formation, reduction in lag phase, and hydrogen accumulation (Abdelsalam et al., 2016, Al Mamum and Torii, 2015, Yang et al., 2013). To the best of our knowledge no SSAD studies has combined on-farm wastes, co-digestion, alkaline pretreatment, and inclusion of nanoparticles. Hence the objective of this study is to investigate the effect of Fe_3O_4 on methane yield and overall reactor performance in SSAD of calcium-pretreated-corn stover blended with dairy manure.

6.3. Materials and Methods

The methodology in this section is as described for calcium pretreated ingestate in the Chapter five of this thesis. Nevertheless, substrates properties are presented in Table 6.1. The only additional information to this methodology is the inclusion of nanoparticles which will be discussed in the next subsection.

Table 6.1: Substrates characteristics for corn stover, calcium pretreated corn stover, dairy manure and inoculum.

Parameter	Corn stover	Calcium pretreated corn stover	Dairy manure	Inoculum
pH	6.0±0.1	11.3±1.2	7.78±0.0	7.4±0.0
VS (%)	94.1±0.8	62.5±0.0	84.7±2.4	60.0±.5.7
TS (%)	99.0±0.8	98.1±0.0	14.9±0.7	1.7±0.1
C (%)	41.0±0.1	34.1±0.0	43.3±0.5	29.6±0.1
N (%)	0.8±0.1	0.55±0.0	2.6±0.3	3.7±0.0
Alkalinity (g/L)	1.7±0.1	39.42±1.0	8.1±0.4	1.7±0.3
C/N	51.3±0.1	79.5±0.0	16.7±0.0	8.0±0.0
Cellulose (%)	43.5±0.3	30.9±0.0	29.0±1.0	24.4±0.0
Hemicellulose (%)	34.2±0.1	10.5±0.0	20.0±1.0	13.7±0.0
Ash (%)	5.7±0.3	-	14.6±1.8	-
Lignin (%)	5.1±0.2	2.4±0.0	9.0±0.2	12.4±0.0
Amm-N (mM)	0.0	46.9±1.2	68.6±5.0	38.0±1.2
VFA (g/L)	0.0±0.0	-	9.0±1.2	-

*Data represent the mean ± standard deviation, Amm-N represents Ammonium-Nitrogen, VS represents volatile solids, TS represents total solids, C represents carbon content, N represents nitrogen content, C/N represents carbon to nitrogen ratio.

6.3.1. Nanoparticles

Iron oxide or magnetite (Fe_3O_4) nanopowder or nanoparticle (Figure 6.1) was considered in this SSAD study because it outperformed zero valent iron and cobalt nanoparticles in terms of methane yield (Abdelsalam et al., 2016 Abdelsalam et al., 2017). Relative to other nanoparticles such as nickel and cobalt, Fe_3O_4 has non-carcinogenic potential. In addition, the application of digestate with macro quantity of nickel and cobalt could lead to contamination of soil with metals. Hence Fe_3O_4 nanoparticles was considered in this study. The Fe_3O_4 nanoparticle was procured from the US Research Nanomaterials, Inc. The nanoparticle has a 15 – 20 nm particle size and 99.5+% purity (Figure 1).

Figure 6.1: Fe_3O_4 nanoparticles used in this study.

6.3.2. Treatment Preparation and Analyses

Three masses of Fe_3O_4 (20, 50, and 75 mg) were introduced into 1 kg of the blend of calcium-pretreated-corn stover and dairy manure. Hence, the treatments were CaW20, CaW50, and CaW75. The choice of the aforementioned concentrations was based on recent literature (Abdelsalam et al., 2017, Ali et al., 2017). All the treatments were duplicated and subjected to some chemical and physical analyses using standard methods stated in Chapter 5. These analyses include total solids (TS), volatile solids (VS), ammonium-Nitrogen, alkalinity, cellulose, hemicellulose, lignin, pH, ash content, crude protein, carbon, nitrogen, and volatile fatty acids concentrations. Biogas volume and composition were equally inspected. Treatment without nanoparticles was considered as the control in the study. Also investigated was the microbial diversity.

6.3.3. Extraction, PCR amplification and 16S rDNA sequencing

Microbial community diversity and poplution was also investigated in this study. After the extraction of the samples considered in this section following standard protocol. The V3-V4 region of the prokaryotic (including bacterial and archaeal) small-subunit (16S) rRNA gene was amplified with 338F and 806R. PCR amplification was performed using standard methods. In addition, ultrapure water was used as the negative control to exclude false positives. PCR products were purified by AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified by Qubit (Invitrogen, USA). The size and quantity of the amplicon library were assessed with Agilent 2100 Bioanalyzer (Agilent, USA) and Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA), respectively. PhiX control library was combined with the amplicon library and then sequenced on Illumina MiSeq) using the standard Illumina sequencing primers.

6.3.4. Statistical Analysis

Data obtained in this study were also statistically analyzed with SAS software (Version 9.4, SAS Institute Inc., Cary, NC, USA). Pairwise comparisons of the treatment means were conducted with Duncan multiple range tests (DMRT) at a 5% p-value threshold.

6.4. Results and Discussion

6.4.1. Volatile and Total Solids Ratio of Individual Substrate

Biodegradability of individual substrate has been described with the ratio of VS/TS. For instance, in the event that a substrate has higher VS/TS than another substrate. The substrate with the higher VS/TS ratio is considered to have high biodegradability relative to the other. In this study, VS/TS for corn stover, calcium-pretreated corn stover, dairy manure, and inoculum was 95, 63, 568, and 3529%, respectively. This suggests that inoculum had the highest biodegradability. Nevertheless, this does not suggest high biogas yield, which is primarily linked with the bioconversion of the cellulose and hemicellulose contents. Relative to literature, dairy manure in this study had 7-folds more VS/TS than the dairy manure considered in a study by Degueurce, et al., (2016), which ia an indication that VS/TS is influenced by substrate source.

6.4.2. Process Parameters of Treatment before and after Digestion

Prior to the digestion of the treatments, initial alkalinity concentration increased significantly as the concentration of Fe_3O_4 in the treatment (CaW20, CaW50, and CaW75) increased. On the contrary, VFA concentration decreased significantly relative to the control for most of the treatments ($p < 0.05$, Table 6.2). This trend might suggest the presence of nanoparticles in treatment CaW20, CaW50, and CaW75 had some effect on the chemical properties of the treatments prior to digestion. In addition to this, the presence of nanoparticles could also influence microbial community during AD. Relative to the control, nanoparticles had impact on the initial VFA/Ammonium nitrogen and VFA/Alkalinity concentrations for all the treatments CaW20, CaW50, and CaW75 ($p < 0.05$, Table 6.2).

On oxidation redox potential (ORP), the ORP values for all the treatments were within a suitable range (- 204 to -367, Table 6.2, Chen et al.,2015, Wang et al., 2006) for methanogens at the on-set and cessation of the experiment. This suggests a stable digestion process for all the treatments. In addition to this, VFA/Alk or VFA/Amm, and pH of the treatments at the end of the experiments were within suitable range < 0.8 and 6.5 – 8.5 (Table 6.2) often documented in successful SSAD studies (Li et al. 2019, Wang et al., 2018).

Of the three VFA compositions considered in this study (Table 6.2), only the acetic acid concentration of the control differs significantly ($p < 0.05$) from the other treatments (CaW20, CaW50, and CaW75). An indication that suggests the presence of Fe_3O_4 nanoparticles might influenced the conversion of acetic to acetate, CO_2, H_2, and CH_4.

Table 6.2: Treatment at both pre-digested and post-digested stages at 4 levels of Fe_3O_4 nanoparticles.

	CaW (Control)		CaW20		CaW50		CaW75	
	ING	DIG	ING	DIG	ING	DIG	ING	DIG
Alk (g/L)	5.9 ± 0.4	6.1 ± 0.3	6.5 ± 1.4	7.9 ± 0.3	7.0 ± 0.6	7.4 ± 0.0	8.6 ± 1.7	8.2 ± 0.4
VFA (g/L)	7.5 ± 0.0	1.5 ± 0.0	6.7 ± 0.5	0.9± 0.1	5.6 ± 0.4	1.9 ± 0.0	5.5 ± 0.0	1.1 ± 0.0
Amm (g/L)	1.6 ± 0.0	2.9 ± 0.0	1.9 ± 0.0	3.9 ± 0.0	1.8 ± 0.0	3.6 ± 0.0	1.8 ± 0.0	3.7 ± 0.0
ORP (mV)	-270 ± 0.5	-205 ± 17	-266 ± 27	-205 ± 6.2	-326 ± 8.4	-229 ± 9.0	-367 ± 45	-243 ± 9.0
CP (%)	10.1 ± 0.0	13.9 ± 0.4	10.1 ± 1.0	15.1 ± 0.2	10.4 ± 0.1	14.8 ± 0.3	10.5 ± 0.2	15.1 ± 2.4
Ash (%)	24.1 ± 0.3	39.2 ± 0.1	25.2 ± 2.0	38.9 ± 2.0	23.8 ± 2.1	38.4 ± 0.7	24.8 ± 0.1	40.1 ± 0.4
pH	9.4 ± 0.0	7.8 ± 0.0	9.2 ± 0.0	7.7 ± 0.1	9.0 ± 0.0	7.6 ± 0.2	9.2 ± 0.0	7.6 ± 0.0
Acetic (g/L)	6.6 ± 0.2	1.4 ± 0.1	4.7 ± 0.6	0.7 ± 0.0	4.7 ± 0.0	1.8 ± 0.1	4.5 ± 0.0	1.0 ± 0.1
Butyric (g/L)	0.2 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.0
Propionic (g/L)	0.4 ± 0.1	0.0 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	0.1 ± 0.0
Free NH_3 (mg/L)	1274 ±0.3	318 ± 0.3	1434± 25	346 ±0.7	1188 ±9.0	258 ± 1.9	1358 ±3.8	265 ± 3.7
VFA/Amm	4.7	0.5	3.5	0.2	3.1	0.5	3.1	0.3
VFA/Alk	1.3	0.2	1.0	0.1	0.8	0.3	0.6	0.1

Note: ING represents ingestate, DIG represents the digestate, CP represents crude protein, Alk denotes alkalinity, Amm represents ammonium nitrogen, concentration, VFA/Amm denotes ratio of volatile fatty acid to ammonium nitrogen ratio, VFA/Alk represents ratio of volatile fatty acid to alkalinity. 8CaW, 8CaW20, 8CaW50 and 8CaW75 are all calcium hydroxide pretreated corn stover blended with dairy manure with the addition of 0, 20, 50, and 75 mg of Fe_3O_4 respectively.

6.4.3. Treatment Degradation

Obviously from this study, there were few clear distinctions among CaW20, CaW50, and CaW75 treatment in terms of process parameters (Table 6.2). Interestingly, considering degradation, cellulose and hemicellulose degradation for treatment CaW20 was significantly higher ($p < 0.05$) than for the treatments CaW50 and CaW75 (Figure 6.2). An indication that suggests that more fiber content of the CaW20 treatment was converted for methane production relative to the treatments CaW50 and CaW75. In general, it was also observed that the presence of nanoparticles in treatments CaW20, CaW50, and CaW75 significantly improved cellulose and hemicellulose degradation by at least 2.5-fold and 17%, respectively, relative to the control (Figure

6.2). This trend could be related to the availability of micro-nutrient to anaerobic bacteria which possibly improved the feedstock bioconversion process. However, the generally low VS degradation (< 21%) documented in the study could be attribute to the mild impact of calcium pretreatment on the cellulosic fraction of the biomass and manure (Figure 6.2). In relation to other SSAD studies, hemicellulose degradation was higher than the value obtained in the mono-digestion of corn stover under solid-state (Brown et al., 2012), while the cellulose degradation was close to the value obtained in the previously stated study. As earlier mentioned, feedstock source, co-digestion, and pretreatment conditions might account for the noticed variations.

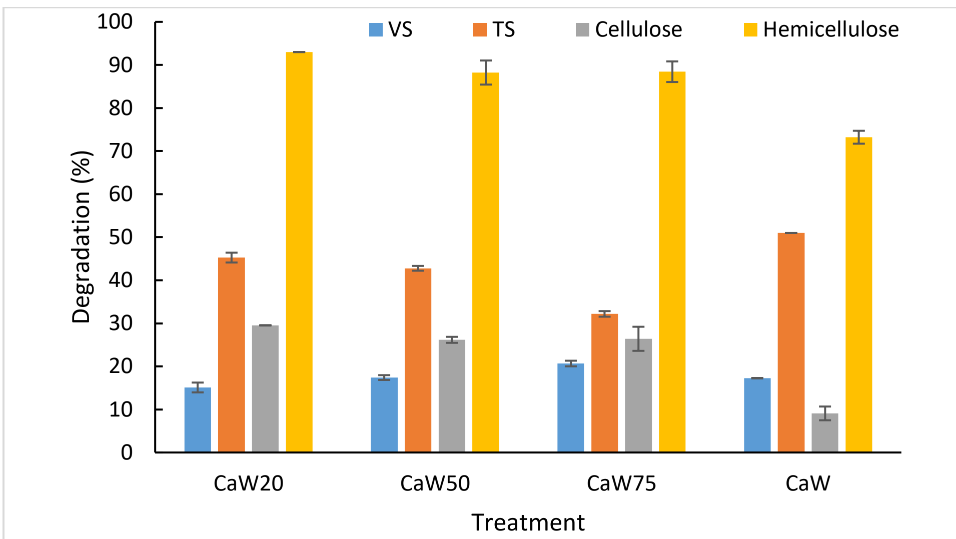


Figure 6.2: Degradation of VS, TS, cellulose and hemicellulose as affected by Fe_3O_4 nanoparticles.

8CaW, 8CaW20, 8CaW50 and 8CaW75 are all calcium hydroxide pretreated corn stover blended with dairy manure with the addition of 0, 20, 50, and 75 mg of Fe_3O_4 respectively.

6.4.4. Gas Composition and Yield

Methane concentration for all the treatments followed a similar trend until the 5th week, when the methane concentration for the control declined. This trend was suspected to be as a result of depletion of micro-nutrient, iron, which was possibly still available for the treatments with nanoparticles. Also noticed was the early adaptation of methanogens to the feedstock for the treatments CaW20, CaW50, and CaW75 relative to the control on the 7th day (Point not shown in Figure 6.3).

On the contrary, H_2S concentration for all the treatments with nanoparticles (CaW20, CaW50, and CaW75), increased significantly ($p < 0.05$) in the first week relative to the control (Figure 6.4). This might suggest an increase in sulfur reducing bacteria activities. In addition, this trend is contrary to trends reported in the literature (Al Mamun and Torii, 2015, Chaung et al., 2014), in which the presence of zero valent iron nanoparticles reduced H_2S concentration irrespective of the retention time. However, Chaung et al., (2014) observation on sulfide removal was a function of pH. Hence, the result obtained in this study could be attributed to nanoparticle type or pH of the treatment at each stage of the anaerobic digestion (Chaung et al., 2014). However, H_2S concentration reported in this study did not pose any threat to the digester performance, as it was lower than the inhibitory range (< 5000 ppm) noted in the literature (Isa et al., 1986).

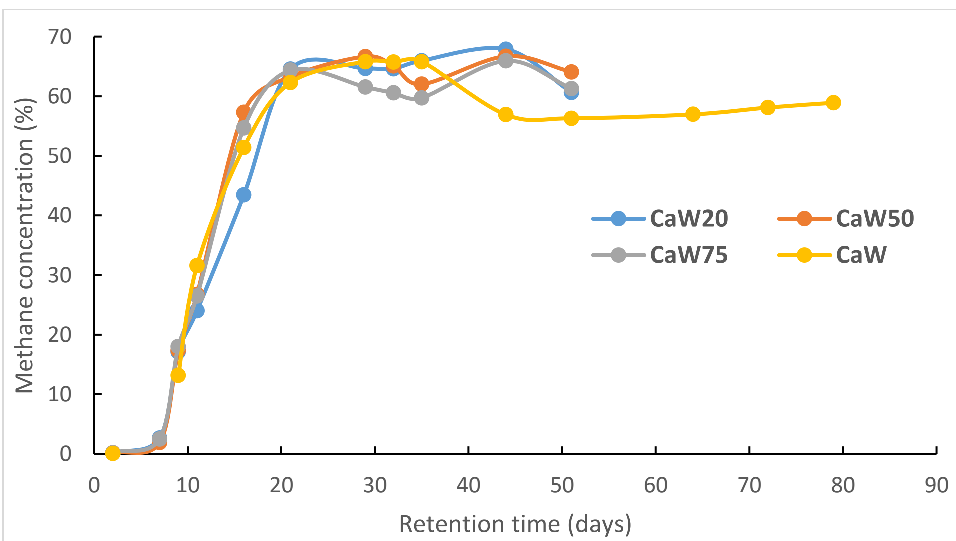


Figure 6.3: Periodic methane concentration.
8CaW, 8CaW20, 8CaW50 and 8CaW75 are all calcium hydroxide pretreated corn stover blended with dairy manure with the addition of 0, 20, 50, and 75 mg of Fe3O4 respectively. Treatments CaW was terminated at 79 days while treatments CaW20, CaW50, and CaW75 were terminated after 52 days detention time due to low methane production.

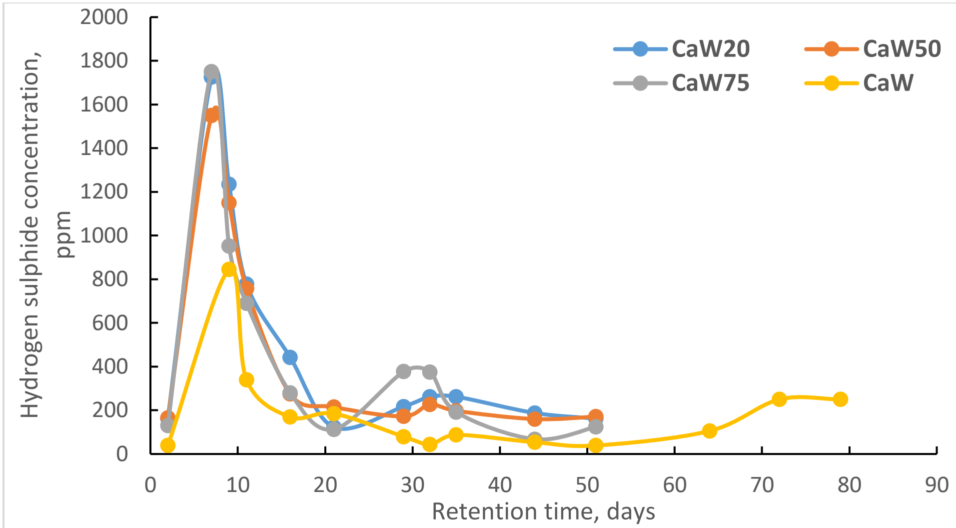


Figure 6.4: Hydrogen sulphide concentration as influenced by Fe_3O_4 nanopartilces treatment.

8CaW, 8CaW20, 8CaW50 and 8CaW75 are all calcium hydroxide pretreated corn stover blended with dairy manure with the addition of 0, 20, 50, and 75 mg of Fe_3O_4 nanoparticles respectively.

On yield, treatment CaW20 outperformed other treatments in terms of biogas and methane yield. Interestingly, there was not significant different ($p > 0.05$) among treatment CaW50, CaW75, and the control. However, a lower hydraulic retention time (52 days) was noted with all the treatments with nanoparticles relative to the control (79 days, Figures 6.3 & 6.4). This trend is an indication that anaerobic activities were improved with the inclusion of magnetite. In addition, ratio of biogas to methane yield for the control and these treatments (CaW20, CaW50, and CaW75) were 0.51, 0.53, 0.52, and 0.53. This result further established that CaW20 and CaW75 outperformed other treatment and control in terms of optimal methane yield.

Based on the methane yield and fiber content degradation, the best trace mass of magnetite required to optimized anaerobic microbes' activities in order to improve methane yield, reactor performance, and decrease retention time in this study was 20 mg.

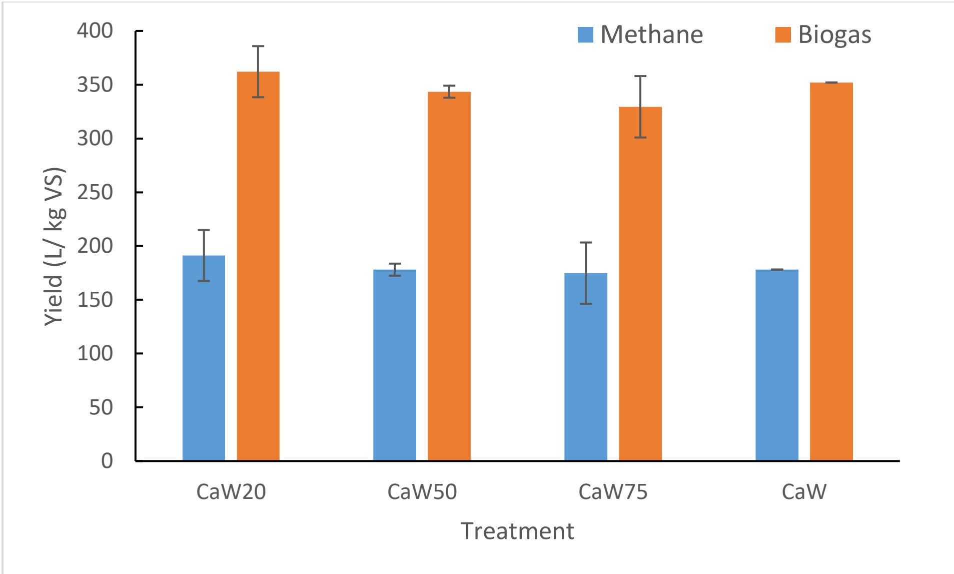


Figure 6.5: Biogas and methane yield as influenced by Fe_3O_4 nanopartilces treatment.
N.B. Hydraulic retention time for Treatment CaW was 79 days while other treatments (CaW20, CaW50, and CaW75) had 52 days retention time.
8CaW, 8CaW20, 8CaW50 and 8CaW75 are all calcium hydroxide pretreated corn stover blended with dairy manure with the addition of 0, 20, 50, and 75 mg of Fe3O4 respectively.

6.4.5. Microbial Distribution

A good understanding of the microbial community in an anaerobic digester could provide insightful information on the impact of the nanoparticles on microbial diversity. Results from this study suggest that bacteria community at genus level varied significantly at the cessation of the experiments and the nanoparticles presence influenced both bacteria diversity and abundance. However, only CaW20 and the control were considered for microbial taxonomy. Pre-digested treatment and control (CaW20 and CaW) contained similar genus (*Bifidobacterium, Rikenellacea, Atoposites, Eubacterium, and Proteinphilum),* however the abundance differs (Figure 6.6). Precisely, the significantly high ($p < 0.05$) presence of *Rikenellacea* and *Atoposites* and low population of *Bifidobacterium* in treatment with nanoparticles (CaW20) relative to the control suggests that the presence of magnetite influence the abundance of some of the bacteria (Figure 6.6). On the contrary, the presence of nanoparticles in the ingestates did not affect the abundance of *Eubacterium, and Proteiniphilum* (Figure 6.6). At the end of the experiment, the bacteria community has not only changed, the abundance was influenced by the presence of magnetite in treatment CaW20. Bacteria genus found after treatment digestion irrespective of magnetite addition now contained *Hydrogenispora, Fermentimonas, Ruminofilibacter*, *PeH15, Candidatus, and Caldicoprobacter*. Nothwithstanding, *PeH15 and Caldicoprobacter* bacteria genus were significantly higher ($p < 0.05$, Figure 6.6) at the end of the experiment in treatment with magnetite (CaW20 or Digestate_2) relative to the control (Digestate_1 or CaW). This trend established that the presence of the nanoparticles influenced the microbial diversity.

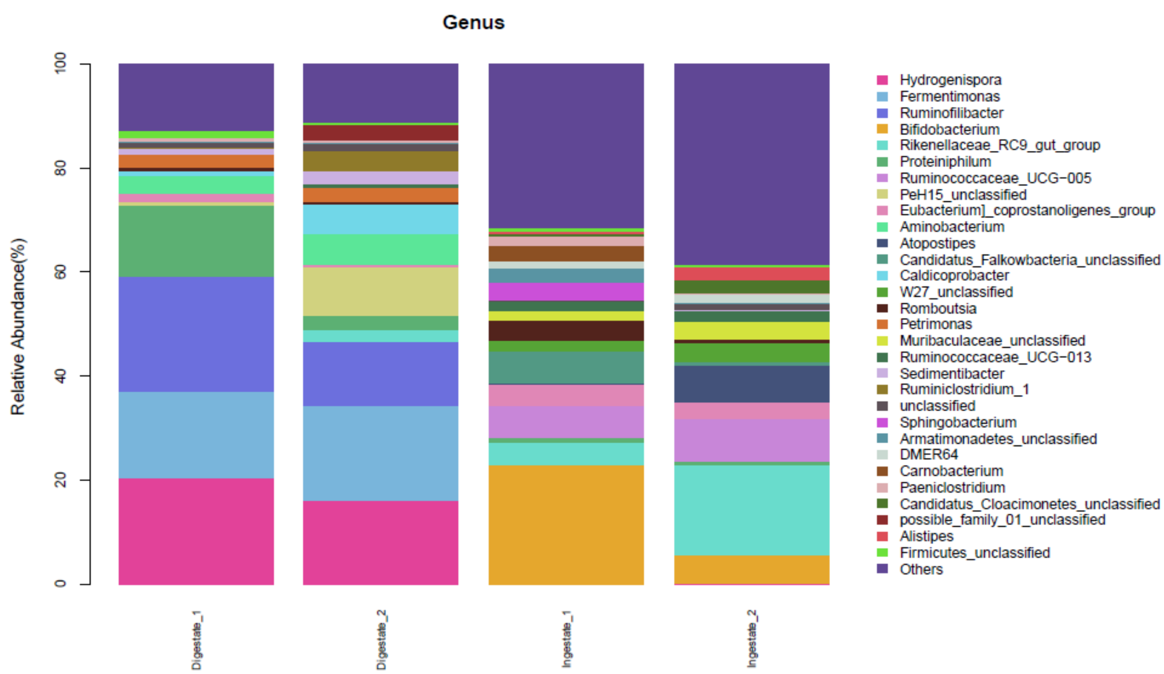


Figure 6.6: Taxonomy of bacteria genus for the control and CaW20.
Ingestate_1 represents calcium pretreated corn stover blended with dairy manure (CaW) at pre-digestion stage, digestate_1 represents calcium pretreated corn stover blended with dairy manure (CaW) at post-digestion stage, Ingestate_2 represents calcium pretreated corn stover blended with dairy manure and 20 mg of Fe_3O_4 nanopartilces (CaW20) at pre-digestion stage, digestate_2 represents calcium pretreated corn stover blended with dairy manure and 20 mg of Fe_3O_4 nanopartilces (CaW20) at post-digestion stage.

6.5. Conclusion

Nanoparticles could improve microbial activities and hence reactor performance in solid state anaerobic digestion. In this SSAD study, three different masses of magnetite (20, 50, and 75 mg) was introduced into bioreactors containing a blend of calcium-pretreated corn stover mixed and dairy manure. The inclusion of magnetite did not impact free ammonia concentration. Furthermore, the presence of magnetite minimized the upset nature of the ingestate by lowering the VFA/Alk and VFA/Amm concentration by at least 23 and 25% respectively. In addition, H_2S concentrations were within suitable range (< 5000 ppm) throughout the study. Of the three treatments, treatment CaW20 had the highest methane yield (191 L/ kg VS) with suitable values for all the process parameters. Hence, this SSAD study shows that the optimal and economical micro mass of magnetite to improve reactor performance was 20 mg/L.

7. SOLID-STATE ANAEROBIC CO-DIGESTION OF ALKALINE-PRETREATED-CORN STOVER AND DAIRY MANURE: KINETIC ANALYSIS[4]

7.1. Abstract

Four existing kinetic models were employed to describe anaerobic digestion process in the solid-state co-digestion of alkaline-pretreated-corn stover blended with dairy manure. The models were first order (FO), modified-first order (MFO), modified Gompertz (MGO), and cone models (CM). The treatments considered under these four kinetic models are as mentioned in the chapter 5 of this dissertation. These include 2% wet-state-sodium hydroxide pretreated-corn stover blended with dairy manure (2NaW), 2% wet-state-aqueous ammonia-pretreated-corn stover blended with dairy manure (2NHW), 4% wet-state-aqueous ammonia-pretreated-corn stover blended with dairy manure (4NHW), and 8% wet-state-calcium hydroxide-pretreated-corn stover blended with dairy manure (8CaW). Results from this study suggest that the linear FO and MFO models are not suitable to describe methane yield for all these treatments, an indication that acidification and not hydrolysis was the rate limiting step under pretreatment of lignocellulosic biomass blended with dairy manure. For the non-linear (CM and MGO) models, MGO had the better fit and lower error function values, which indicate the suitability for methane yield description and prediction. Of the four treatments, 8CaW had the shortest lag phase time (20 days), highest potential methane yield (180 L/kg VS), and highest maximum methane production rate (5.7 L/ kg VS/day). Hence, treatment 8CaW was validated has the best treatment in the study and also relative to results obtained from the Chapters 3, 4, and 5 of this Thesis. This also substantiated why only this treatment was considered for further studies in the Chapter 6 of this thesis.

[4] The material in this chapter was co-authored by Ademola A. Ajayi-Banji, , S. Sunoj, C. Igathinathane, S. Rahman. Ademola Ajayi-Banji collected the samples, analyzed data, and drafted the manuscript.S. Sunoj, C. Igathinathanen developed the model. S. Rahman reviewed the draft and is the corresponding author.

7.2. Introduction

Energy is key for any nation building and the present global growing energy demand has necessitated the need to source for other potential alternatives. One of the ways researchers have strived to advance this course is through solid-state anaerobic digestion (SSAD) of on-farm organic solid waste. The choice is due to the availability of the waste, low moisture content, and the environmental benefits from the stated waste management technology. In SSAD, moisture content of feedstock introduced into the digester must be less than 85%. This high solid content procedure allows for the utilization of large volume of on-farm waste and lowers water usage during AD. Other benefits of SSAD includes higher volumetric methane productivity, organic loading rate, and small digester volume (Yang and Li, 2014) relative to AD with feedstock moisture more than 85% (liquid state AD). Kinetic studies in AD has been very helpful in decision making and in the design of digester volume (Syaichurrozi, 2018). Other applications of kinetic studies include quantitative analysis of methane production, (Luo et al., 2015), and detection of optimal process variables (Gadhamshetty et al., 2010). However, kinetic studies on SSAD that combines pretreatment with co-digestion are limited. For instance, a modified first order model was considered suitable to describe methane production in a solid-state co-digestion of corn stover and chicken manure (Li et al., 2018). However, when the corn stover was chemically pretreated prior to co-digestion with dairy manure, the Cone and modified Gompertz models were the models suitable for methane yield description (Feng at al., 2018). Nevertheless, this kinetically described experiments failed due to system acidification (Feng et al., 2018). Hence, firm conclusion on the suitability of these models for methane yield description cannot be ascertained for chemically pretreated corn stover co-digested with dairy manure. Interestingly, the physicochemical properties of any feedstock also impact the kinetic characteristic (Li et al., 2015).

On kinetic models, the first order model (FO) could be harnessed to determine organic matter degradation rate. However, this model is limited in its simulation because it does not consider the non-degradable fraction (Vavilin et al., 2008). This is unlike the modified first order model (MFO). In addition, the first order model and modified first order models are linear models generally considered when the hydrolysis process in AD is a rate-limiting step (Mao et al., 2019). Hence, the first order model does not predict either reactor failure or suitable conditions in the system for optimal biological activities (Kafle and Chen, 2016).

Besides, MFO and FO models, modified Gompertz equation (MGO) are often appropriate to compute the cumulative methane yield when the rate limiting steps also include either acidogenesis or methanogenesis (Li et al., 2015, Ma et al., 2013). These rate limiting steps are attributed to either the digester temperature or the high lipid and protein content of the digested substrate (Li et al., 2015, Miron 2000). MGO model suggests that some kind of inhibition exist within the reactor that makes methane production dependent on the microbial growth (Bolado-Rodríguez et al., 2016). Hence, the model is non-linear unlike FO and MFO. Similar to MGO, the Cone model is another non-linear kinetic model which can be used for the description and prediction of AD process. The model has been harnessed for digester volume design and to predict volatile solids rate (Syaichurrozi, 2018). Based on the interest in these four stated models, the objective of this study is to kinetically describe methane production from the co-digestion of wet-state-alkaline-pretreated-corn stover blended with dairy manure.

7.3. Materials and Methods

Ingestates considered for this kinetic modelling are corn stover pretreated with 3 alkaline solutions under wet state condition prior to blending with dairy manure as previously described in the Chapter 5. Hence, the treatments are mixture of dairy manure, inoculum and 8%-Ca(OH)2-wet state pretreated corn stover (8CaW), mixture of dairy manure, inoculum and 4%-NaOH-wet state pretreated corn stove (4NaW), mixture of dairy manure, inoculum and 2%-NH4OH-wet state pretreated corn stover (2NHW), and mixture of dairy manure, inoculum and 4%-NH4OH-wet state pretreated corn stover (4NHW).

Methane yield and respective retention time obtained for these treatment in the Chapter 5 of this dissertation were the data processed with POLYMATH 6.10 software using mrgmin() approach to describe methane yield. The following kinetic models listed in Table 1 were considered for this analysis. After this analysis, the models were validated with coefficient of determination (R^2), root mean square error (RMSE), normalized root mean square error (NRMSE), and Akaike's information criterion (AIC) (Lima et al., 2018; El-Mashad et al., 2011; Akaike, 1998; Motulsky and Christopoulos, 2004, equations 7.5 – 7.8).

Table 7.1: Kinetic models evaluated for this study.

Kinetic Model	Mathematical expression	Source
First order	$Y(t) = Ymx \times (1 - e^{-kt})$	Li et al., (2014)
Modified First order	$Y(t) = Ymx[(1 - Y) - (1 - Y) \times e^{-kt})]$	Li et al., (2013)
Cone	$Y(t) = Ymx/(1 + (pt)^{-n})$	Syaichurrozi, (2018)
Modified Gompertz	$Y(t) = Ymx\, exp\{-\exp [\frac{\mu j}{Ymx}(\lambda - t_h) + 1]\}$	Syaichurrozi, (2018)

Note: *Y(t)* represents the cumulative methane yield at the cessation of the experiment (mL/gVS); *Ymx* is the methane potential of the ingestate (mL/gVS); *k* is the methane production rate constant or first order rate constant or hydrolysis constant (1/day); *t* is the digestion time in days; *p* is the hydrolysis rate constant for the cone model (1/day); *Y* is the non-biodegradable fraction of the treatment; *n* is the shape factor (dimensionless); μ is the maximum methane production rate (mL/gVS/day), λ is the lag phase time in hours which represents the minimum time required for either bacteria to adapt to the environment or for the gas production; *j* represents a mathematical constant (2.71821828); and t_h represents digestion time in hours

$$R^2 = 1 - \frac{\sum_i (Yo_i - Yp_i)^2}{\sum_i (Yo_i - Y_{\mathrm{mn}})^2} \tag{7.5}$$

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n} (Yo_i - Yp_i)^2}{n_m}} \tag{7.6}$$

$$\mathrm{NRMSE} = \left[\frac{\mathrm{RMSE}}{(Y_{\max} - Y_{min})}\right] \times 100 \tag{7.7}$$

$$\mathrm{AIC} = n_m \ln\left(\frac{\mathrm{RSS}}{n_m}\right) + 2v \tag{7.8}$$

where Yo_i represents the measured data point for the methane yield (mL/gVS), Yp_i is the represents data point for the methane yield (mL/gVS), Y_{mn} represents the mean of the measured data points for the methane yield (mL/gVS), n_m is the number of the experimented data point for the methane yield, $Y_{\max}$ and $Y_{\min}$ are the maximum and minimum experimental value for the methane yield, respectively, RSS represents the residual sum of squares, and v is the number of model parameters.

7.4. Results and Discussion

7.4.1. Relationship between Measured and Predicted Methane Yield

Coefficient of determination (R^2) values obtained between the predicted and measured values for methane yield under the four models were all greater than 0.85 (Figure 7.1). These values are close to the correlation coefficient value considered high (0.90) by Brown et al., (2012). In addition, the R^2 values for the non-linear models (CM and MGO) were all closer to unity than for the linear models (FO and MFO, Figure 7.1). This trend suggests that the non-linear models had a better fit than the linear models, and as such might be preferred in describing methane yield. A trend suggesting the rate-limiting step was not hydrolysis. Overall, MGO had the highest r^2 values suggesting that it was the best model predictor for methane yield in this study (Figure 7.1). Interestingly, pretreatment reagent type and concentration also influenced the model fitness.

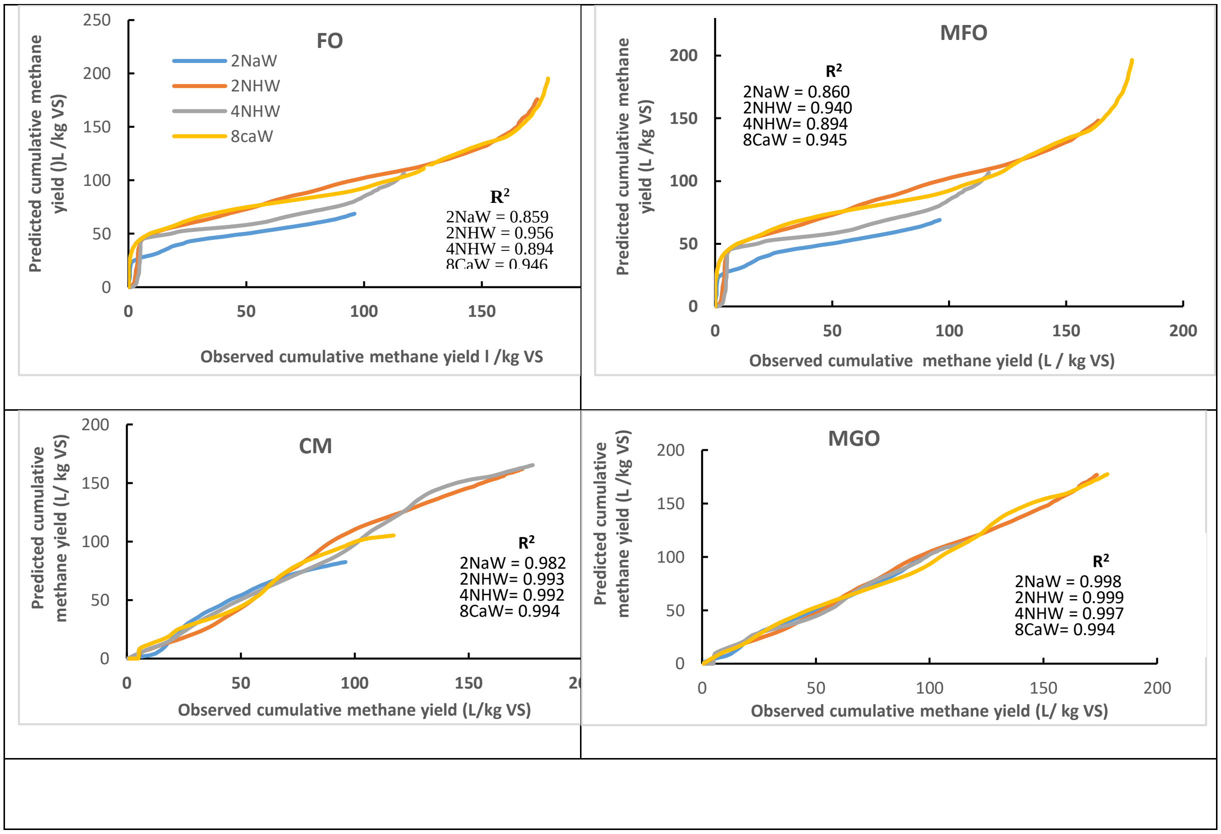


Figure 7.1: Predicted and observed methane yield for the four treatments evaluated with four kinetic models.

Note: CM represents Cone model, MGO represents the modified Gompertz model, MFO represents the modified first-order model, and FO represents the first-order model. Treatment 8CaW represents corn stover pretreated with 8% concentration of Ca(OH)2 solution and then blended with dairy manure, 4NaW represents corn stover pretreated with 4% concentration of NaOH solution and then blended with dairy manure, 2NHW represents corn stover pretreated with 2% concentration of NH4OH solution and then blended with dairy manure, and 4NHW represents corn stover pretreated with 4% concentration of NH4OH solution and then blended with dairy manure.

7.4.2. Kinetic Model Validation and Description

Aside correlation coefficient, r^2, the combination of more than one error function has been often advocated for model validation (El-Mashad et al., 2013, Lima et al., 2018). Nevertheless, decision making on model prediction in this study was not only centered on the combination of high r^2 value (> 91%) coupled with low RMSE, NRMSE, and AIC values. It was as well based on the practicality of the methane potential. Clearly, first order and modified first order kinetic had methane potential (Ymx) values that were not feasible relative to literature (> 600 L / kg VS, Li et al., 2013b, Wang et al., 2012). Hence, the two models were not further considered in this study to describe methane production. On the contrary, Cone and modified Gompertz models well fitted the cumulative methane yield with feasible potential methane yield (Table 7.2). Hence, were considered suitable to describe methane production in addition to meeting the criteria of low values of RMSE (< 2.3), NRMSE (< 1.2), and AIC (< 360) and high values of R^2(> 0.90, Table 7.2) relative to the linear models. Nevertheless, modified Gompertz model had better fit than Cone, a trend contrary with most literature without dairy manure as substrate (Syaichurrozi, 2018, Zhen et al., 2015.Li et al., 2015). Cone better fitness to modified Gompertz in most literature was attributed to the presence of the shape factor (n) that gives it flexibility in modelling various patterns (Syaichurrozi, 2018). However, on the account that dairy manure was part of the substrates, the predictability of methane production with Cone model was constraint. Hence, the presence of dairy manure in this study could be the sole reason why cone model outperformed modified Gompertz model in term of methane yield prediction. Furthermore, the shape factor for the cone model was between 5.0 – 8.1 (Table 7.2), which was very close to the values Li et al., (2015) reported for dairy manure. On the contrary, this value is significantly higher ($p < 0.05$) than the values (0.9 – 1.9) reported by Li et al., (2014) in a mesophilic study on pretreated and untreated corn stover. This trend confirms the constraint attributed to cone model in methane yield prediction in this study. The cone model hydrolysis rate constant, *p*, obtained in this study was between 0.013 – 0.029. High hydrolysis rate constant infers high substrates degradability. The values obtained in this study were outside the range (0.006 - 0.017) Feng et al., (2018) reported, an indication that feedstock source, and type could impact substrates degradation in AD. Interestingly, Syaichurrozi, 2018 observed that *p* values in Cone model are inversely proportional to lag phase time (λ), the time for microbial adaptation to substrate**,** in modified Gompertz model. This nexus was also applicable in this study (Table 2.2). The lag phase time required for anaerobic microbes' adaptation in treatment 8CaW was the least (20 days), an indication of early stability in the reactor relative to the other treatments. This further suggests that calcium pretreated corn stover blended with dairy manure is the most suitable combination under this study. Relative to literature, the lag phase time in this study was at least 15-fold higher than literature (Li et al., 2014). The reason could be the presence of high proteinous substrate, dairy manure, in the feedstock which is well known to require prolonged time for microbial degradation (Syaichurrozi, 2016, Kafle et al., 2012). Interesting the methane production rate, μ, in this study was in line with most mesophilic co-digestion studies (Feng et al., 2018). From this study, high values of μ suggest more actual methane production and not additional potential methane yield as presented by Syaichurrozi, (2018). Hence, treatment 8CaW and 4NaW with methane production rate of 5.7 and 2.0 respectively had the highest and lowest measured methane yield, respectively (Table 7.2). Potential methane yield was another interesting aspect of the result. Contrary to expectation, treatment 2NHW treatment had the highest methane potential (189 mL/ kg VS, Table 7.2). An indication that some form of inhibition might have affected the measured methane yield during this experiment, which were not applicable with treatment 8CaW.

Table 7.2: Model parameters and validation of four kinetic models.

Treatments							
	FIRST ORDER MODEL						
	Ymx		K	R^2	RMSE	NRMSE	AIC
2NaW	3209		0.0002	0.734	1.7	1.7	110
2NHW	5095		0.0004	0.900	2.0	1.2	143
4NHW	5275		0.0002	0.810	1.9	1.7	132
8CaW	2868		0.0009	0.910	2.3	1.3	136
	MODIFIED FIRST ORDER MODEL						
	Ymx	Y	K	R^2	RMSE	NRMSE	AIC
2NaW	2827	-2.41	0.000	0.740	1.7	1.7	112
2NHW	8752	-6.68	0.000	0.900	2.0	1.2	145
4NHW	752	-8.33	0.000	0.810	1.9	1.7	134
8CaW	1903	-2.68	0.000	0.910	2.3	1.3	138
	CONE MODEL						
	Ymx	P	N	R^2	RMSE	NRMSE	AIC
2NaW	86.8	0.014	7.6	0.906	1.0	1.0	3.0
2NHW	167.0	0.020	5.1	0.927	1.8	1.0	119
4NHW	106.3	0.018	8.0	0.934	1.1	0.9	32.0
8CaW	167.3	0.029	5.4	0.922	2.2	1.2	164
	MODIFIED GOMPERTZ MODEL						
	Ymx	µ	Λ	R^2	RMSE	NRMSE	AIC
2NaW	156.5	2.0	47.5	0.997	0.2	0.2	357
2NHW	189.0	3.6	28.1	0.998	0.3	0.2	265
4NHW	117.8	3.3	40.9	0.995	0.3	0.3	222
8CaW	180.6	5.7	20.3	0.998	0.3	0.2	226

Note: *Ymx* is the methane potential of the ingestate (mL/gVS); *k* is the methane production rate constant or first order rate constant or hydrolysis constant (1/day); *p* is the hydrolysis rate constant for the cone model (1/day); *Y* is the non-biodegradable fraction of the treatment; *n* is the shape factor (dimensionless); *µ* is the maximum methane production rate (mL/gVS/day), and *λ* is the lag phase time in days;. Treatment 8CaW represents cornstover pretreated with 8% concentration of $Ca(OH)_2$ solution and then blended with dairy manure, 4NaW represents cornstover pretreated with 4% concentration of NaOH solution and then blended with dairy manure, 2NHW represents cornstover pretreated with 2% concentration of NH_4OH solution and then blended with dairy manure, and 4NHW represents cornstover pretreated with 4% concentration of NH_4OH solution and then blended with dairy manure.

This kinetic study has shown that methane yield from the co-digestion of alkaline pretreated corn stover blended with dairy manure could best be described using the modified Gompertz model. Cone model could also be applicable for this purpose.

7.5. Conclusion

The kinetic studies of alkaline-pretreated corn stover blended with dairy manure was examined in this study. The four models considered were first order, modified first order, Cone model, and modified Gompertz model. Results from this study suggest that the first and modified-first order models were not suitable to describe cumulative methane yield. High lag phase time (> 19 days) in the study irrespective of treatment generally suggests retarded start-up attributed to some form of inhibition and the inclusion of proteinous substrates in the ingestate mix. Nevertheless, modified Gompertz model had the best methane yield description due to lowest value of error functions and the highest coefficient of determination. From the MGO model, maximum methane production rate (5.7 L/kg VS/ day) and lowest lag phase time (20 days) was observed with treatment 8CaW. On the contrary, treatment 2NHW had the highest potential methane yield (186 L/ kg VS). These results suggest that modified Gompertz model is the most suitable model to predict methane yield in solid-state co-digestion of dairy manure and alkaline pretreated corn stover.

8. ENVIRONMENTAL IMPACT ASSESSMENT OF ON-FARM WASTE MANAGEMENT THROUGH SOLID-STATE ANAEROBIC CO-DIGESTION[5]

8.1. Abstract

On-farm waste, if not effectively managed, can cause environmental pollution. This paper investigated the environmental impact from the on-farm wastes (dairy manure and corn stover) management practices through three solid-state anaerobic digestion scenarios. The investigated scenarios in our study include corn stover blended with dairy manure (SYM1), pretreated corn stover blended with dairy manure (SYM2), and pretreated corn stover blend with dairy manure and nanoparticles (SYM3). The environmental impacts of global warming potential, acidification potential, eutrophication potential, fossil fuel depletion, smog, ozone depletion, carcinogenic, non-carcinogenic, respiratory effects, and ecotoxicity potential were assessed. The global warming potential result indicated environmental gain from pretreatment, over 99% reduction was observed with SYM2 and SYM3 relative to SYMS 1. However, pretreatment contributed substantially to some environmental concern such as human health factors (carcinogenic, respiratory effects, and ecotoxicity potential) were influenced by at least 45%. The inclusion of nanoparticles, however, cushioned these impacts due to improved methane yield.

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[5] The material in this chapter was co-authored by Ademola. A. Ajayi-Banji, G. Pourhashem S. Rahman. Ademola Ajayi-Banji had primary responsibility for collecting samples and analyzing laboratory data. Ademola Ajayi-Banji also drafted and revised all versions of this paper. G. Pourhashem and Shafiqur Rahman are contributing authors reviewed the manuscript conducted by the primary author. Shafiqur Rahman and Pourhashem are the corresponding authors. It is being processed.

8.2. Introduction

Agricultural practices such as livestock housing and ration have received substantial boost in the last 20 decades with emerging technologies owing to ongoing research and development. Those practices, however, have led to more on-farm organic wastes generation. For instance, the amount of animal manure, an on-farm organic waste, generated in the US in the last decade was approximately between 6.64 – 7.03 Tg, with 884 - 907 Gg of the manure solely from dairy farms (FAO, 2019). Important challenges associated with this considerable manure volume are their further management cost for farmers, in addition to the environmental concerns from directly spreading the manure on the field (Kumar et al., 2013). For instance, dairy manure volume in the US could account for at least 22.7 million kg CO_2e (Liebrand and Ling, 2009). Another potential challenge with this on-farm waste disposal is their tendency for harmful pathogen spread to the environment (Kumar et al., 2013). To mitigate these problems, anaerobic digestion (AD) of dairy manure is suggested. AD can destroy manure pathogens, improve manure quality, and reduce its odor while offering the generation of heat and energy at a low cost (Orzi et al., 2015). These benefits make AD a preferred waste management technology option compared to other alternatives such as pyrolysis and composting (Aguirre-Villegas et al., 2017).

AD is the degradation of organic matter by hermetic microbes (bacteria and archaea) that mainly produces biogas and digestate. Despite the described AD benefits, low methane yield attributed to low carbon to nitrogen ratio (C/N) is a key limitation when only dairy manure is digested (mono-digestion of the manure) (Li et al., 2018). The low methane yield makes dairy manure AD uneconomical. Hence, the co-digestion of dairy manure with lignocellulosic residues has been suggested to improve C/N ratio and subsequently methane yield (Zhang et al., 2013). However, to improve accessibility of anaerobic microbes to the fiber content of harvested lignocellulosic residue, pretreatment or modification of the fiber structure prior to anaerobic digestion is beneficial (Carrere et al., 2016, Zheng et al., 2014).

In addition to co-digestion and pretreatment, studies have shown that the application of nanoparticles, micro-element or compounds smaller than 100 nm, can further improve the methane yield in AD (Abdelsalam et al., 2016). Other recent development in AD includes more efficient utilization of the available dairy manure volume and higher volumetric methane production through solid-state anaerobic digestion (Brown et al., 2012, Li et al., 2018). In solid-state anaerobic digestion (SSAD), moisture content (MC) of the feedstock introduced into the digester is less than 85%, which is an indication of low water usage relative to most AD studies with MC of about 92%.

From an environmental perspective, AD can reduce potential GHG emissions from dairy manure storage in an open space by at least 23% and possible marine eutrophication by 8.1% (Battini et al., 2014). However, storage of digestate in enclosed container should be discouraged after anaerobic digestion is completed to reduce NH_3 emissions (Aguirre-Villegas et al., 2017). Instead, further environmental benefits could be derived from composting the digestate (Di Maria and Micale 2015). Unlike liquid state AD, there is no report on the environmental impacts of solid-state anaerobic co-digestion (SSAD) of on-farm organic wastes under optimized condition such as pretreatment and the inclusion of nanoparticles. For instance, Li et al.(2018) reported that co-digestion of corn stover, dairy manure, and tomatoes residue in SSAD reduced acidification, eutrophication, and ecotoxicity potentials by at least 40% relative to the AD of these substrates. Additionally, anaerobic co-digestion of corn stover with these substrates, rather than the incineration approach contributes to the GWP environmental credits (Li et al., 2018). However, their study did not consider pretreatment despite using a lignocellulosic waste. As such, informed decision cannot be made in relation to the environmental benefits or constrains attributed to pretreatment or inclusion of nanoparticles in SSAD. In a previous study, we demonstrated that the addition of nanoparticles to pretreated lignocellulosic feedstock prior to co-digestion with animal manure can substantially reduce detention time for digestion and overall improve reactor performance (Ajayi-Banji et al., (2020), PhD Thesis). In this study, we aim to evaluate the environmental impacts of corn stover co-digested with dairy manure under 3 scenarios. The objectives of this study are to: (1) quantitatively evaluate the holistic environmental impact of producing methane from the blend of either corn stover or calcium corn stover and dairy manure through solid-state co-digestion, and (2) assess the impact of nanoparticles (magnetite or Fe3O4) on some environmental indicators with the highest methane yield treatment in objective 1. The methane production through the investigated system will then be compared to conventional methane production.

8.3. Methodology

We use life cycle assessment (LCA) method following ISO 14040 standards (ISO, 2006-second edition) to investigate the environmental performance of 3 scenarios of anaerobic solid-state co-digestion of manure with corn stover. Data used in this study were mainly 1) primary data obtained from the pilot-scale solid-state anaerobic co-digestion experiments conducted at the Animal Nutrition and Physiology Centre of NDSU, North Dakota agricultural weather network (NDAWN), and 2) secondary data obtained the Ecoinvent database. Other relevant data were sourced from literature. To assess the environmental impact, Tool for the reduction and assessment of chemical and other environmental impact (TRACI 2.1, Version 1.05) method and SimaPro software (Version 9.0.0.35) were used. Impact assessment with TRACI was limited to 10 environmental indicators such as global warming potential (GWP), acidification potential (AcP), eutrophication potential (EuP), fossil fuel depletion (FFD), smog, ozone depletion (OzD), carcinogenic (Cc), non-carcinogenic (NCc) respiratory effects (RpE), and ecotoxicity potential (EcP).

8.3.1. Goal and Scope Definition

The goal of this LCA study was to quantify and compare environmental impacts of energy production within solid-state anaerobic co-digestion of corn stover or calcium-pretreated corn stover and dairy manure with or without the inclusion of nanoparticles. Biogas generated from this study was considered for both heat generation and cooking while the other product (digestate) from AD could be harnessed as manure for either soil amendment or organic fertilizer. The functional unit for this study was then considered 1 MJ of methane produced during the SSAD study. This functional unit is used to compare the three scenarios considered in this study.

8.3.2. System Description

This study investigates three scenarios of anaerobic co-digestion of manure and corn stover. In all scenarios, produced methane is considered the main product, while digestate will be a coproduct of the system. The study was intended for an integrated farming system with the digester located about 4km from the farmland (Li et al., 2018). The environmental footprint from the feedstock (dairy manure and corn stover) from the point of collection to processing the feedstock such as milling, sieving, pretreatment, and drying of the corn stover, were also included within the system boundary of the analysis (Figure 8.1). Furthermore, influent considered as inoculum in this study was at first collected from a mesophilic liquid-state anaerobic digester operated by Fargo wastewater in Fargo, USA. For subsequent digester run, the liquid fraction of the digestate from this study was considered as inoculum. Electricity source for heating the digester was assumed USA average energy grid. Furthermore, weather data used for energy required to maintain the digester was extracted from NDAWN.

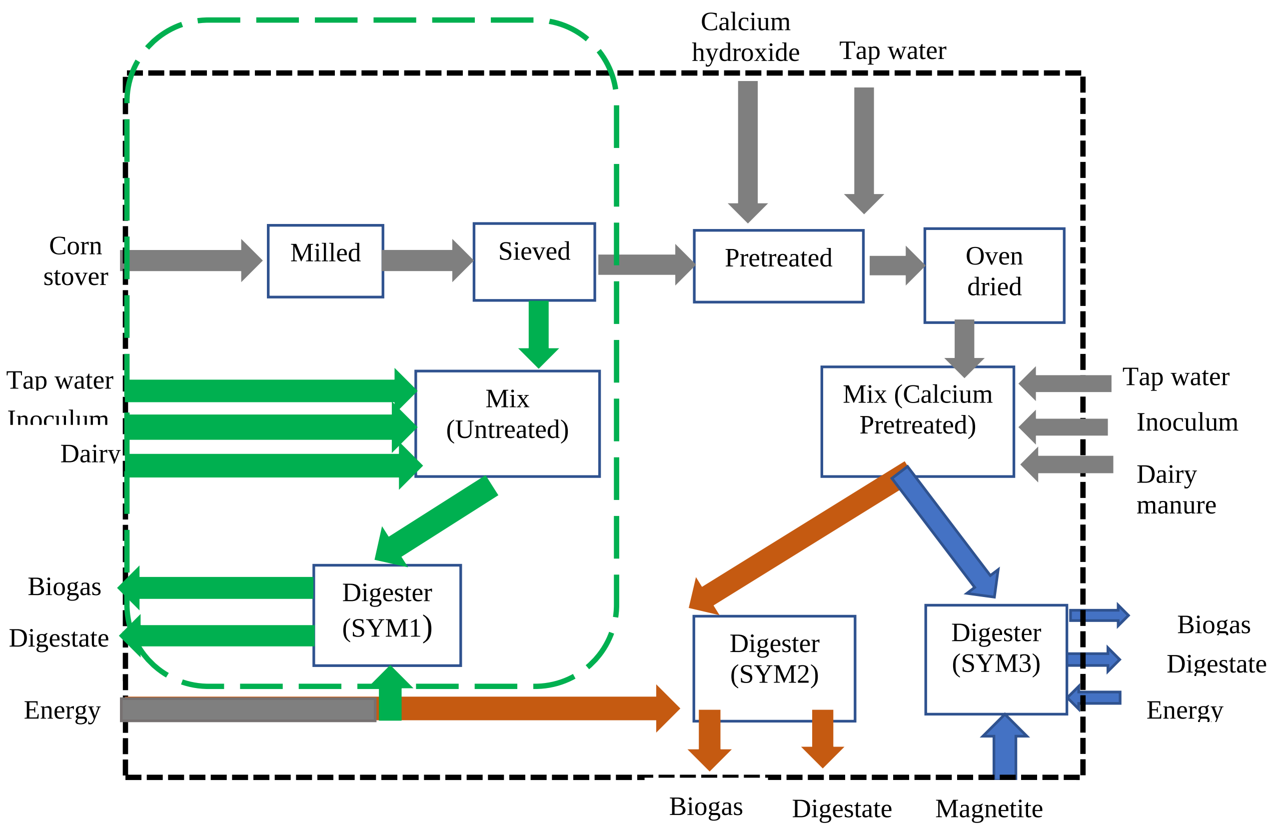

Figure 8.1: System boundary.

8.3.3. System Scenarios

Three systems with different solid-state anaerobic co-digestion mix under a total solid (TS) of 16% were compared in this study. The detailed mix ratios are presented in Table 8.1. Variation in the mix ratio as shown in Table 8.1 was basically due to secondary effect of the calcium hydroxide (pretreatment reagents) on the carbon to nitrogen ratio (C/N ratio) of the pretreated corn stover as well as the differences in the moisture content of the stover. These were catered for in the targeted 20 - 24: 1 carbon to nitrogen ratio computation. Furthermore, disparity in the hydraulic detention time was considered in the data computation of this study (Table 8.1).

8.3.3.1. System 1 (SYM1)

Corn stover was blended with dairy manure in a semi-continuous stirred solid state-anaerobic reactor. The detention time for the substrate mix in the reactor was 60 days under 35-37 ^{o}C mesophilic temperature. As previously stated, co-digestion addresses the challenges of nutrient imbalance (Sun et al., 2016), as well as help achieve a TS > 15% required for solid-state digestion (Li et al., 2018b). Furthermore, high TS in AD minimizes energy use and results in high volumetric methane production (Li et al., 2013).

8.3.3.2. System 2 (SYM2)

In the second scenario, corn stover pretreated with 8% $Ca(OH)_2$ concentration was co-digested with dairy manure under a solid-state condition. This pre-digestate had a hydraulic retention time (HRT) of 76 days under 35-37 ^{o}C mesophilic temperature. As previously stated, pretreatment was carried out due to the lignocellulosic nature of the stover, which makes its degradation difficult. Hence, pretreatment was used to enhance the anaerobic microbes' accessibility to the cellulose and hemicellulose fraction of the stover.

8.3.3.3. System 3 (SYM3)

Based on the high methane yield and lower detention time with SYM2, pretreated corn stover with 8% $Ca(OH)_2$ concentration was co-digested with dairy manure together with the addition of 20 mg of Fe_3O_4 nanoparticles (Fe_3O_4NPs). This pre-digestate was retained in the digester for 52 days under 35-37 ^{o}C mesophilic temperature. The availability of some micro-nutrients such as Fe from Fe_3O_4 could boost methanogens activities (Abdelsalam et al., 2016). Hence, Fe_3O_4NPs was introduced into the similar treatments as SYM2 for this purpose.

Notably for all the scenarios, a semi-continuous state was also assumed in which the initial and subsequent mass of the influent (41.8 Mg) was subjected to a continuous 300 days digestion period. These basically influenced energy required to maintain the temperature of the digester between 35 – 37 °C. Outside this startup time, other months represent the digester maintenance period. The digester considered in this study was a “garage-type” batch digester which as previously stated runs for approximately 10 months annually.

Table 8.1: Feedstock mix for the scenarios considered in this solid-state study based on TS.

Treatment	Dairy manure (%)	Inoculum (%)	Corn stover/ Pretreated corn stover (%)	Fe_3O_4 NPs (% of 1kg ingestate)	C/N[a] ratio	Hydraulic retention time (days)
SYM1[b]	34.6	32.2	33.2	NI[c]	24	60
SYM2[d]	55.7	33.8	7.2	NI	20	79
SYM3[e]	55.7	33.8	7.2	0.002	20	52

Note: Feedstock to inoculum ratio for all the treatment was between 1 – 2 TS basis to ensure suitable anaerobic digestion process, 1.5 kg of ingestate for SYM1 and 1.0 kg of ingestate for SYM 2 & 3 were used.
[a]C/N ratio denotes carbon to nitrogen ratios, [b]Ajayi-Banji et al., (2020), [c]NI represents Not Included, [d]Ajayi-Banji et al. (Under Review), [e]Ajayi-Banji & Rahman (ASABE 2020,(Accepted)).

8.3.4. Feedstock Constituents

Pretreatment of corn stover with 8% calcium hydroxide solution had significant impact on volatile solids, it reduced volatile solids by 34% ($p < 0.05$, Table 8.2). This suggests that polymeric degradation had occurred during pretreatment and the fiber structure of the stover was modified. For other parameters investigated (Table 8.2), the differences between the pretreated and untreated corn stover were marginal.

Table 8.2: Feedstock composition.

	Dairy manure	Corn stover	Pretreated corn stover	Inoculum
Carbon (%)	41.0	38.3	39.2	28.7
Nitrogen (%)	2.8	0.8	0.5	2.7
Total solids (%)	19.3	97.1	98.0	1.2
Volatile solids (%)	87.3	94.3	62.6	56.7

Ajayi-Banji et al., (2020), Ajayi-Banji & Rahman (ASABE 2020, (Accepted))

8.3.5. Life Cycle Inventory

8.3.5.1. Feedstock Transportation

As earlier indicated, this model considered dairy farm and corn field as an integrated mixed farming system with a distance of 4 km from the anaerobic digester and corn stover processing units. Water volume indicated in Table 8.3 was also provided from the farm. Inoculum used in this model was to be initially sourced from a liquid-state household waste management digester operating under mesophilic temperature, as previously stated in this study.

8.3.5.2. Corn Stover Processing

Corn stover used in this study was harvested from the field and crushed with a 3 mm mesh size Schuttle Buffalo hammer mill (Model W6H, New York, USA) prior to grading of the crushed stover with a RO-TAP Testing Sieve Shaker (Ohio, USA). Corn stover particle size of 0.42 - 0.83 mm was considered for AD and chemical pretreatment, based on our previous study (Ajayi-Banji et. al., 2020). Amount of energy required to carry out both the milling and grading processes as presented in Table 8.3 was calculated using standard procedure (Miao et al., 2011).

For scenarios SYM 2 & 3 that required pretreated corn stover, procedure for wet-state alkaline pretreatment method was followed (Song et al., 2014). Data on the water and pretreatment reagent (8% calcium hydroxide) quantity introduced during the pretreatment process are presented in Table 8.3. In addition, energy utilized to oven-dry the calcium-pretreated-corn stover under 40 ^{o}C for 24 hours was estimated with standard procedure for hot-air convection drying (Motevali et al., 2011).

Table 8.3: Annual input from technosphere.

Input from technosphere	Units	SYM1	SYM2	SYM3
Feedstocks				
Dairy manure	ton	24.0	93.1	142.1
Corn stover	ton	27.0	12.2	18.6
Corn stover production inputs				
Urea[a]	kg	216.3	96.4	146.9
Phosphate fertilizer[a]	kg	59.4	26.8	40.9
Potassium sulphate[a]	kg	118.8	53.7	81.8
Digester inputs				
Calcium hydroxide	ton	-	5.0	7.7
Inoculum	ton	13.6	13.6	13.6
Tap water used for pretreatment	ton	-	62.7	95.7
Tap water used for digestion at TS of 16%	ton	112	1.9	2.9
Magnetite	kg	-	-	4.9
Energy				
Electricity				
Hammer mill	kwh	1300	600	900
Testing sieve shaker	kwh	64.7	28.8	44.0
Oven dryer	kwh	-	4500	7400
Agitator	kwh	648.0	518.4	668.2
Fuel				
Diesel[a, b]	GJ	24.5	10.1	16.6
Propane [c]	GJ	2.9	2.9	2.9

Note: a: Pourhashem et al (2013), b: For corn harvest and traction, c: Used to start heating the digester.

8.3.5.3. Anaerobic Digestion

In this mesophilic semi-continuous SSAD study, required volumes of the inoculum, dairy manure, water, and corn stover or calcium pretreated corn stover mix, depending on the scenario, were fed into the 250 m^3 garage-type digester (Li et al., 2018). The working volume adopted for this digester was 80% of the digester volume with approximately 41.8 Mg of ingestate per run. For scenario 3, additional 4.9 kg of magnetite was added to the digester to improve reactor performance (Table 8.3). Gas leak was not suspected in the digester thus fugitive emissions due to gas leak was not accounted for in this model. Amount of energy used to heat-up the digester and maintain the digester temperature was investigated with standard procedure (Table 8.3, Sheets et al., 2015). However, heat generated by microbes was not considered in this estimation. Energy required to agitate the digester considering a long shaft agitator (Table 8.3) was equally estimated for 300 days based on the procedure described by Naegele et al., (2012). On the digestate volume, it was assumed that 4 - 7% of the volume of the digestate were used up for gas production. Hence the remaining fraction (approximately 40 Mg) was considered as digestate or effluent. Before the experiment began, propane was used as fuel to heat up the digester to the desired temperature (35-37 ^{o}C), and during the experiment, methane energy produced from the systems was used as fuel to maintain the digester temperature.

Table 8.4: Annual outputs of the studied systems to technosphere.

Output to technosphere	Units	SYM1	SYM2	SYM3
Products and co-products				
Total methane	Mwh	147.1	222.0	452.9
Surplus methane	Mwh	90	164.9	395.9
Digestate	tons	159.2	124.8	177.6
Emission from Digester				
Carbon dioxide	Mtons	3.5	2.7	2.2
Hydrogen sulphide	Ktons	15.5	1.0	3.5

Note: # , TS represents total solids

8.4. Results and Discussion

8.4.1. Relating Environmental Impact from SSAD

The following section describe the investigated environmental impacts including global warming potential, acidification potential, eutrophication potential, fossil fuel depletion, smog, ozone depletion, carcinogenic, non-carcinogenic, respiratory effects, and ecotoxicity potential., and smog considered for the three scenarios in this study (Figures 8.2 – 8.10).

8.4.1.1. Ozone Depletion

Biogas, in particular methane, contribute to ozone depletion due to their chemical bond with hydrogen, hence the environmental impact study is imperative. Ozone depletion was less than 3.7×10^{-9} kg CFC-11 eq / MJ Methane irrespective of scenarios considered in this study (Figure 8.2). The reason for the low values of ozone depletion in AD was generally because transportation requirement, which is regularly linked to ozone depletion, is often low (< 5 km) in AD (De Meester et al., 2012, Li et al., 2018). However, in this study, SYM3 had the least ozone depletion potential (1.89×10^{-9} kg CFC-11 eq / MJ Methane), while SYM1 had the highest (3.61×10^{-9} kg CFC-11 eq / MJ Methane, Figure 8.2). This trend indicates the combination of corn stover pretreatment and addition of nanoparticles in SSAD could reduce ozone layer depletion by 91%. While wet-state pretreatment of the corn stover with $Ca(OH)_2$ solution prior to co-digestion with dairy manure in SSAD will only achieve 26% reduction. Hence, the inclusion of magnetite nanoparticles in SSAD principally mitigated potential ozone layer depletion.

Magnetite nanoparticles mainly contain iron, a required nutrient by methanogens in trace quantity and also by AD bacteria for propionic acid fermentation, however, it is sparsely found in livestock manure.

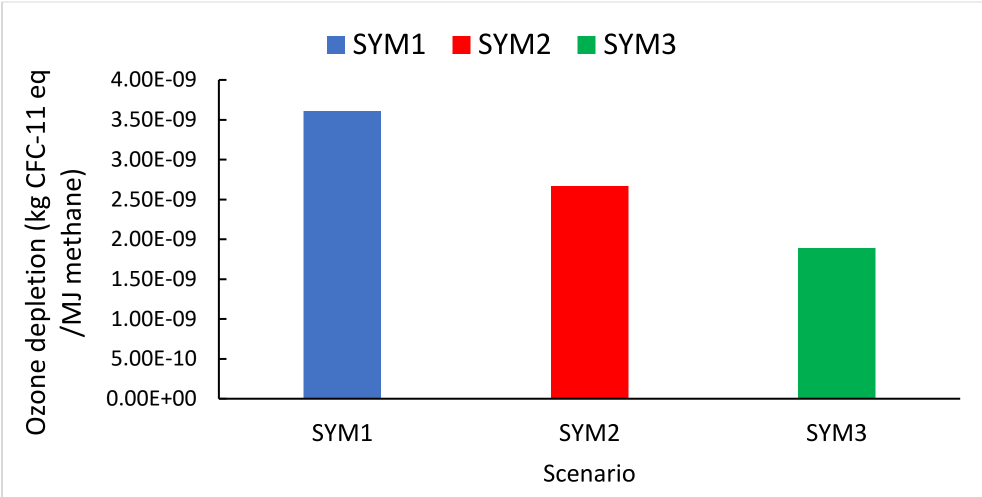

Figure 8.2: Ozone depletion for the three scenarios.

8.4.1.2. Global Warming Potential

Of the known waste management practices for biomass and livestock manure, AD generates lower GWP relative to incineration or composting (Moller et al., 2009, Evangelisti et al., 2014). In this SSAD study, SYM1 has the highest GWP (1.08×10^4 kg CO_2 eq / MJ Methane) compared with other scenarios (< 0.4 kg CO_2 eq / MJ Methane, Figure 8.3). However, this value was lower than the values (2.0×10^4 kg – 14×10^4 kg CO_2 eq) reported for either dairy manure AD only or dairy manure co-digested with plant waste for year-round average over a time horizon of one month (Zhang et al., 2013). This substantially high GWP in SYM1 could possibly be attributed to the environmental impact from producing over 27 tons of corn stover, which is at least 1.5-fold higher than the stover quantity required for other scenarios (SYMs 2 and 3, Table 8.3). Hence, to minimize GWP in SSAD ascribed to cornstover production, pretreatment of corn stover prior to co-digestion with manure could be adopted as seen in SYMS 2 and 3 (Figure 8.3). Similarly, increase in GWP (about 100%) was observed when corn stover was co-digested with dairy manure relative to dairy manure mono-digestion in a SSAD study (Li et al., 2018).

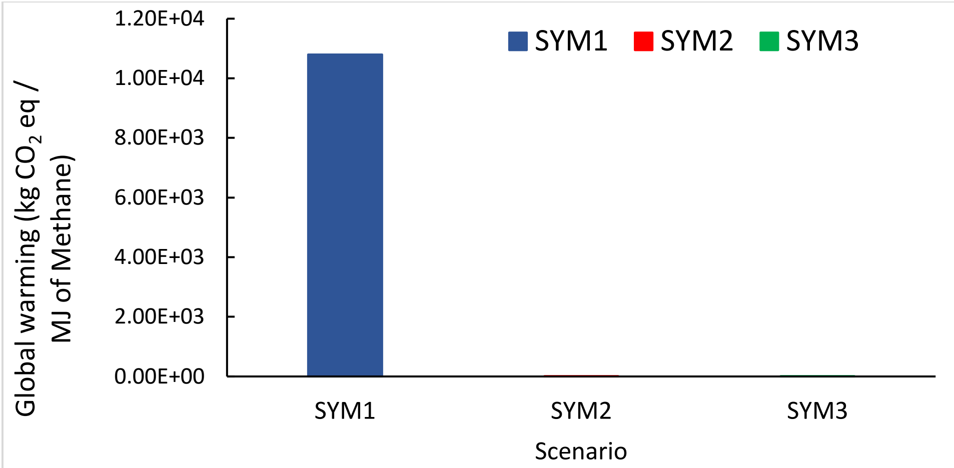

Figure 8.3: Global warming potential for the three scenarios.

8.4.1.3. Smog or Photochemical Oxidation

Smog is an environmental impact that is majorly linked with transportation and combined heat and power (CHP) engine (Slorach et al., 2019), hence we do not expect a substantial impact from smog in this study, due to the low contributions of these parameters. Nevertheless, smog potential in this study ranges between 0.0015 – 0.0030 kg O_3 eq / MJ Methane, with SYM3 having 107 % reduction relative to SYM1 (Figure 8.4). The substantial smog potential from SYM1 could be linked with corn stover production as previous noted in the global warming potential section (Section 3.1.2, Table 8.3).

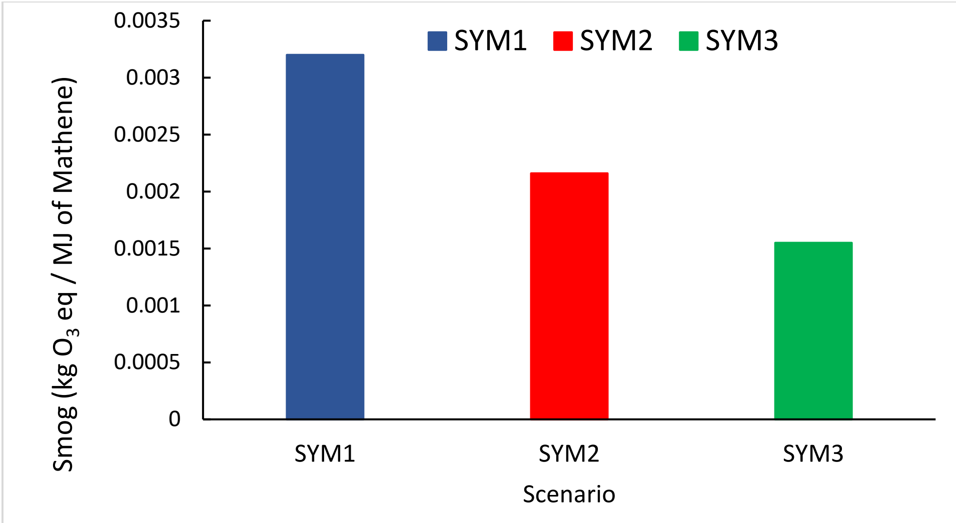

Figure 8.4: Smog for the three scenarios.

8.4.1.4. Acidification and Eutrophication potential

Acidification and eutrophication potentials have been strongly linked to ammonia emission. For instance, ammonia emission account for at least 94% of both acidification and eutrophication potentials from AD of agricultural and food wastes (Whiting & Azapagic 2014). In this co-digestion solid state study, we suspected similar trend. SYM2 had the highest acidification (0.218 g SO_2 eq / MJ Methane) and eutrophication (0.0055 g N eq / MJ Methane) potentials respectively (Figures 8.5 & 8.6). Interestingly, aside the high volume of dairy manure for SYM 2 and 3 (Table 8.3), pretreatment contributed substantially to eutrophication potential. SYM1 without pretreatment had at least 3-fold reduction in eutrophication potential (Figure 8.6). Relative to literature, AcP value reported for liquid state digestion of dairy manure and the co-digestion with plant waste (800 – 2300 kg $\times 10^4$ kg SO_2 eq, Zhang et al., 2013), for year-round average over a time horizon of one month, were higher than the ones reported in this study. This disparity indicates that total solid might substantially affect AcP and EuP. In another study, AcP of dairy manure was close to 100 g SO_2 eq, this further suggests that co-digestion of dairy manure with corn stover under solid state could minimize AcP (Li et al., 2018).

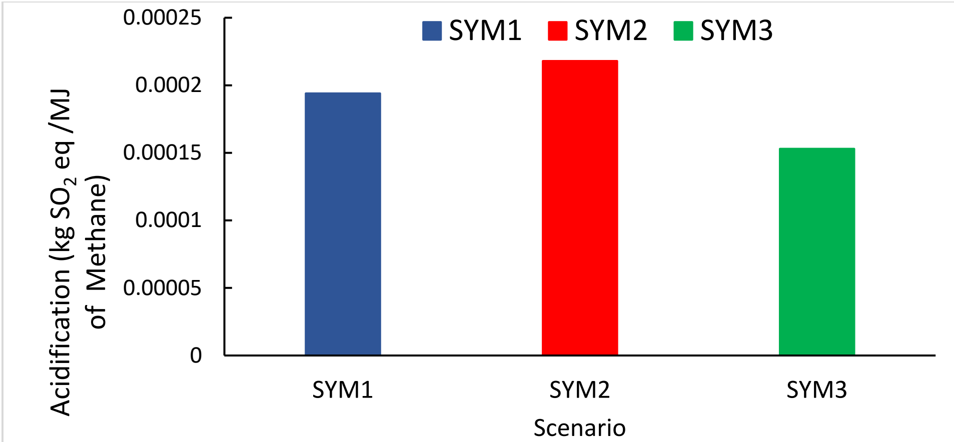

Figure 8.5: Acidification potential for the three scenarios.

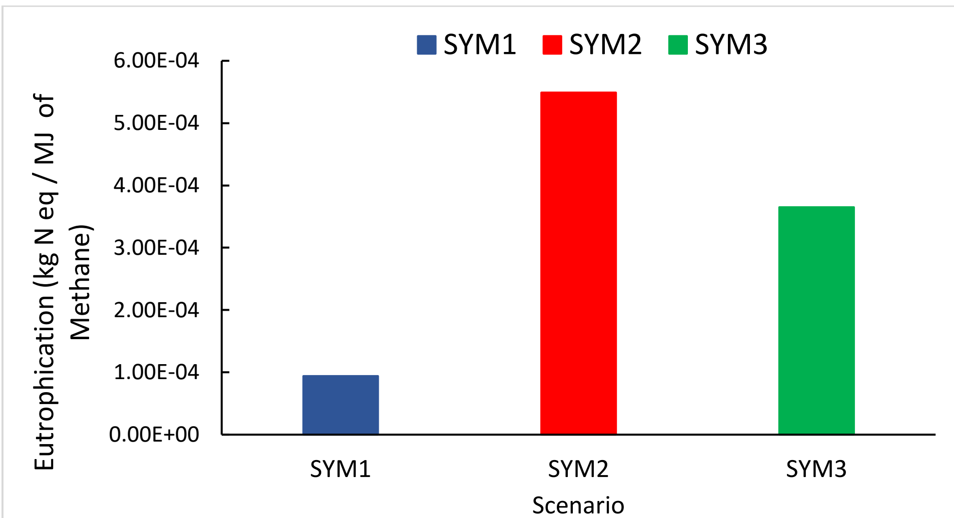

Figure 8.6: Eutrophication for the three scenarios.

8.4.1.5. Carcinogenic, Non-carcinogenic, Respiratory, and Ecotoxicity Potential

Aside from eutrophication, pretreatment also had negative impact on human health factors such as carcinogenicity, respiratory, and ecotoxicity potentials. In an event that pretreatment was not considered in a scenario (SYM1), the negative influence of any of these human health factors was low or mild relative to the pretreated (SYM2 and SYM3; Figures 8.6 – 8.9). The trend was due to the low carcinogenic substances in the ingestate with untreated corn stover blended with dairy manure (SYM1, Figure 8.7). Additionally, production of toxic substances during SSAD, which could be carcinogenic, have been linked to chemical pretreatment (Feng et al., 2018).

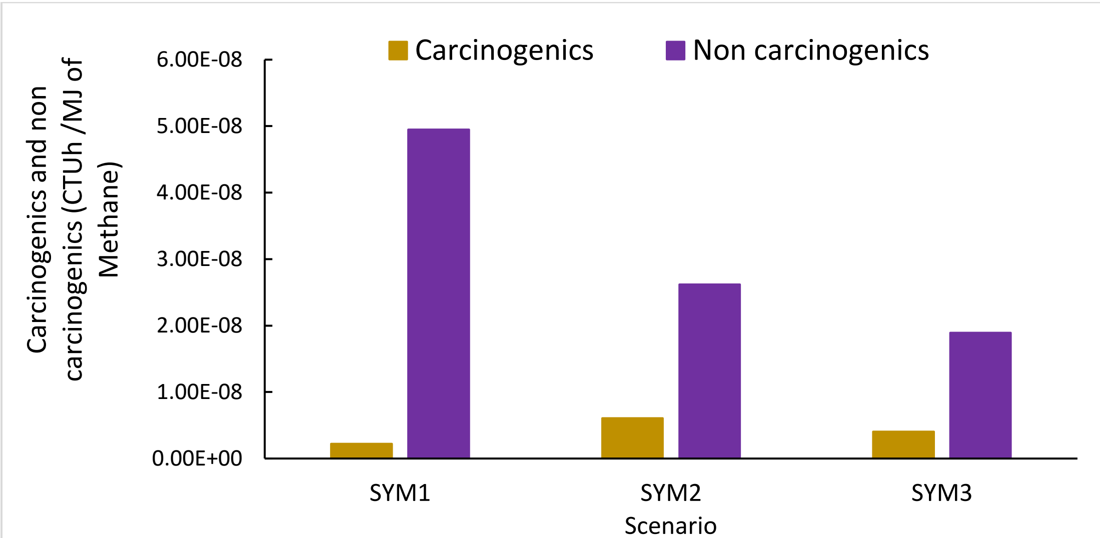

Figure 8.7: Carcinogenic and non-carcinogenic for the three scenarios.

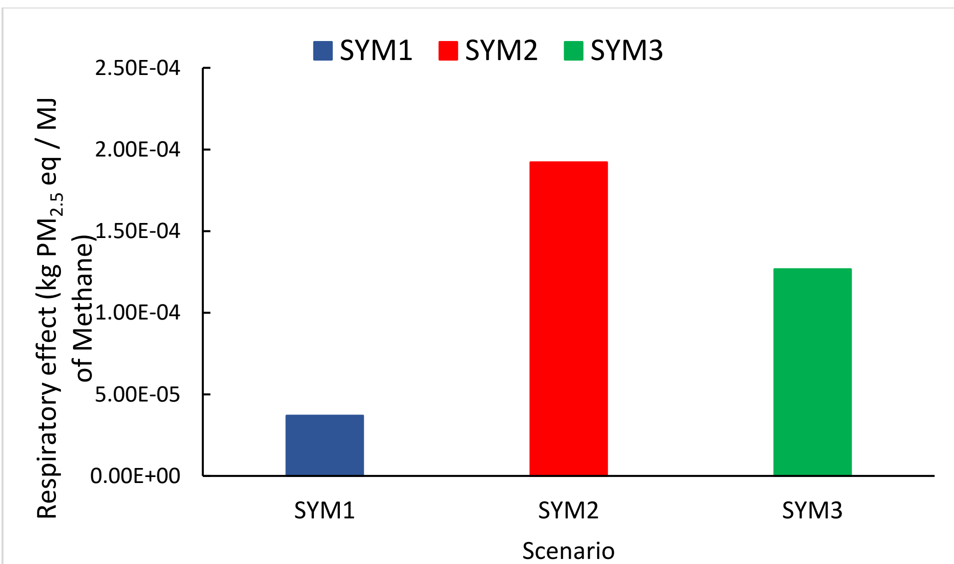

Figure 8.8: Respiratory effect for the three scenarios.

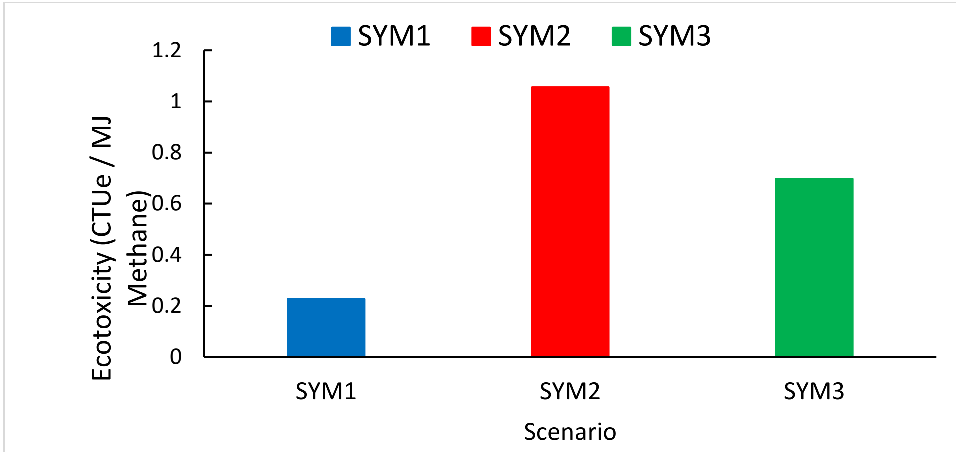

Figure 8.9: Ecotoxicity for the three scenarios.

8.4.1.6. Fossil Fuel Depletion

On fossil fuel depletion, it is not surprising that the utilization of high ratio of corn stover to dairy manure volume was directly related to fossil fuel depletion (Table 8.3). The impact on fossil fuel depletion in SYM1 is at least twice as high as the values obtained for SYM 2 & 3 (Figure 8.10). Transportation processes involved in corn stover production considerably impacted fossil fuel depletion in this study: when more corn stover was utilized for any scenario, the fossil fuel depletion increased. This trend is also in line with Li et al. (2018) observation.

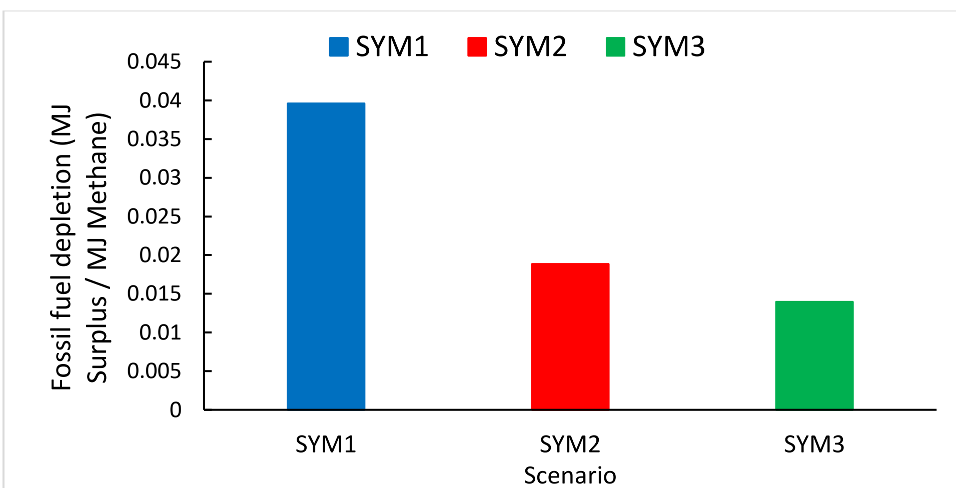

Figure 8.10: Fossil fuel depletion for the three scenarios.

In summary, this life cycle analysis indicates that the presence of magnetite in anaerobically digested corn stover blended with dairy manure could mitigate GWP substantially and AcP, OzD, and smog by at least 26%, 28% and 41% respectively. Pretreatment of corn stover negatively influenced human health factors and eutrophication potential by at least 45%.

8.5. Conclusion

Solid-state anaerobic co-digestion of dairy manure and pretreated corn residue could substantially reduce smog, fossil fuel depletion, ozone depletion, and global warming potential. The addition of magnetite nanoparticles to these combined feedstocks could further lead to more environmental gain due to higher methane yield. However, pretreatment of the corn residue prior to blending with other feedstocks principally contributed to eutrophication, and all the human health factors considered in this study. Future study should consider resource allocation to the digestate and also consider it as a functional unit. In addition, comparison of the environmental impact of this pretreated SSAD with pretreated liquid state AD should be investigated. Impact of post-digestion activities such as composting on the presented scenarios could also be investigated.

9. RECOMMENDATIONS

9.1. Recommendation from This Study

The following recommendatons are suggested from this study:

1. For more understanding of the impact of pretreatment and nanoparticles on reactor performance in this studies:
2. Weekly analyses of the chemical concentrations and microbial taxonomy should also be examined.
3. Other macro and micro-nutrients (cobalt, nickel, magnesium, and phosphorus) should be introduced into the digester and the utilization rate monitored.
4. Dairy manure with low initial VFA concentration (<1 g/L) should be considered for similar experiments in this study for a better understanding of the impact of VFA concentration on solid-state anaerobic digestion.
5. Similar quantities of nanoparticles should be added to treatments with wet state aqueous ammonia and sodium hydroxide pretreated corn stover blended with dairy manure. This will give more understanding on the effect of the magnetite nanoparticles on methane yield for these treatments.
6. Semi-continuous and continuous SSAD digestion of working treatments considered in this study.
7. Future study should consider resource allocation to the digestate and also consider it as a functional unit. In addition, comparison of the environmental impact of this pretreated SSAD with pretreated liquid state AD should be investigated.
8. Impact of post-digestion activities such as composting on the presented scenarios could also be investigated.

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