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By

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ABSTRACT

Anaerobic digestion is a widely used, convenient and long-standing method of sludge treatment before disposal. The process includes hydrolysis, acidogenesis/acetogenesis and methanogenesis. A single or multiple reactor (tank) can be used. However, fluidized bed reactors (AnFBRs) are increasingly becoming popular to promote anaerobic digestion when digesting sludge before final disposal. AnFBRs are characterized by a uniform temperature gradient in the reactor. However, this study has been carried out to establish which media are most optimal for the AnFBRs. Likewise, research has been done to establish the efficiency of the process when either mesophilic or the thermophilic organisms are dominant, keeping in mind the optimum temperature range where these organisms are most efficient. The third part of the study was devoted to investigate how how vomitoxin (deoxynivalenol [DON]), associated with plant pathogens, affected biological methane production kinetics during mesophilic anaerobic digestion.

The role of different media on mesophilic anaerobic digestion of simulated thin stillage using fluidized bed reactors was investigated in the first phase. Zeolite, a mineral based medium and plastic, a synthetic media was employed in two lab-scale AnFBRs. The reactor with zeolite showed an increase in the amount of attached biomass (32.4 mg VSS/g of media) when compared with plastic media (19.7 mg VSS/g of media). The maximum thickness of biomass in zeolite was 370 μm compared with 200 μm in the plastic media. From an operational perspective, zeolite was more suitable for the AnFBRs with less floating issue. The zeolite media was better in resisting floatation compared to the plastic media, which floated to the top even with 50% less superficial velocity that was sustained by the zeolite media.

The second part compares the operational parameters and efficiency between mesophilic and thermophilic anaerobic digestion of simulated thin stillage conducted in AnFBRs. Two 7-liter working volume AnFBRs were operated mesophilically (37±1˚C) and thermophilically (55 ±1˚C) over different hydraulic retention times (HRT). The plastic media with a diameter of (d_m) in the range of 600-2000 μm was employed as carrier media. Each experimental run continued over a six-month period. At 16, 6 and 4-days HRT, while maintaining the constant chemical oxygen demand (COD) concentration in the feed, Methane composition in biogas from the thermophilic reactor was between 10-15 % more than the amount of methane produced by the mesophilic reactor. Results also suggest that the thermophilic reactor could be operated at lower HRT without the loss of performance. Also, during this operation, it was observed that there was no foaming issue in the reactor.

The third part of the study investigated the effects of vomitoxin on biogas and methane production. The anaerobic digestion was conducted in batch reactors. Results indicate that the presence of vomitoxin, even at a high level of 20 ppm, did not harm the anaerobic digestion process of the liquefied corn. The cumulative methane production from reactors with vomitoxin and without vomitoxin remained almost the same. Liquefied corn with 0 g/L vomitoxin (control) produced around 458 mL of methane, while the samples with 1, 5, 10, and 20 ppm of vomitoxin produced around 443, 456, 453, and 459 mL, of methane, respectively. Methane fractions in the biogas for all the bottles were between 51 and 56%. Biogas production and the methane content both remain unaffected even under the presences of 20 ppm of vomitoxin.

DEDICATION

To

My wife, Mina who supported me through this journey

My dear dad, Ahmad Karimi and

my dear mom Shahnaz Ahmadi, and my sisters who inspired me

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

A	cross sectional area
BMP	Biomethane Potential
COD	chemical oxygen demand (ML^{-3})
COV	coefficient of variation
CSTRs	continuously stirred-tank reactors
dm	average particles diameter sizes
DON	deoxynivalenol
AnFBR	anaerobic Fluidized bed reactor
FBR	fluidized bed reactor
FA	free ammonia
g	gravitational constant (LS^{-2})
H	height of expanded bed in a fluidized bed (L)
HRT	hydraulic retention time (T)
kd_r	detachment rate coefficient in the downer (T^{-1})
M, Mm	total weight of bare particle weight (M)
OLR	organic loading rate ($ML^{-2}T^{-1}$)
Q	flow rate (L^3T^{-1})
SEM	scanning electron microscope
So/X	Initial substrate to microorganisms' ratios
SRT	sludge retention time (T)
SWW	synthetic wastewater
T	time (T)
TAN	total ammonium nitrogen

TSS	total suspended solids (ML^{-3})
VFA	volatile fatty acid
VSS	volatile suspended solids (ML^{-3})
WWTP	wastewater treatment plant
ρ	liquid density
ψ_T	total porosity
ρ_{mt}	bulk particle density

CHAPTER 1

INTRODUCTION

1. Introduction

More than two-thirds of bioenergy comes from the first-generation of biofuels, land-based feed stocks, leading to growing environmental concerns related to land-use changes and over competition for land and water required for food and fiber production (Gasparatos et al., 2013; International Energy Agency [IEA], 2010). Therefore, the use of residues and wastes for bioenergy production has attracted increasing interest, as they are often readily and locally available in most countries. The potential of lignocellulosic biomass varies and depends on the efficiency of the available processing technologies, the pattern of energy demand, and the type, abundance, and cost of biomass feed stocks (Ho et al., 2014). The ethanol production process produces ethanol and other byproducts. The type and quantity of byproducts strongly depends on the bio-ethanol plant input and production chain. The economic viability of the bio-ethanol industry depends largely on the ability of the industry to derive value from the bio-ethanol it produces as well as the byproducts that are generated during the process. About two thirds of each tonne of grain (i.e., the starch) is converted to ethanol (Popp et al., 2016). The remaining grain ends up as a byproduct of the process, which is known as whole stillage.

Moreover, weather damaged, contaminated, and immature grains, which are less suitable for human and livestock use, are excellent for ethanol production. However, livestock and ethanol producers need to blend corn that contains vomitoxin with corn that does not, to make it suitable for feed when toxin levels are high (Reuters Environment, 2018).

Anaerobic digestion is the preferred treatment process for organic wastes due to its low nutrient requirements, low biomass yield, and biogas (methane) production. However, current conventional anaerobic digestion processes require a hydraulic retention time (HRT) of up to 40 days to achieve the necessary quality for stillage recirculation (Lee et al., 2011).

1.1 Thin stillage

Corn whole stillage is the organic residue that is left over after ethanol is distillated from the fermented corn mixture in bio-ethanol production plants. In a traditional process, the whole stillage is centrifuged to separate the liquid fraction, which is called thin stillage, from the solid fraction, or the wet distillers' grains (Lee et al., 2011).

Thin stillage in corn ethanol plants contains soluble solids, suspended solids due to imperfect solid/liquid separation, and fermentation byproducts, such as lactic and acetic acids. Although the soluble/suspended solids of thin stillage are potentially a food source for animals, the high cost of the evaporation processes makes this use uneconomical. Whole stillage includes the fiber, oil, and protein components of the grain, as well as the non-fermented starch. Although it is possible to feed whole stillage to animals, it is usually processed further before being sold as feed. First, the "thin stillage" is separated from the insoluble solid fraction using centrifuges or presses/extruders. This coproduct of ethanol manufacture is a valuable feed ingredient for livestock, poultry, and fish (Bothast et al., 2005). The stillage leaving the fermentation column is centrifuged with a decanter, and this comprises between 15% and 30% of the liquid fraction (thin stillage). The remainder is concentrated further by evaporation, and the thick, viscose substance is known as condensed distiller's soluble (CDS). CDS is dried in the next step and dried distiller's grain with soluble is produced (DDGS) Eskicioglu et al., (2010). Thin stillage management is critical to the economics of an ethanol plant, where tradeoffs between operating, water consumption, capital costs, and environmental aspect must be made. The incorporation of thin stillage anaerobic digestion can reduce the energy required for the plant by over 30% through the elimination of the thin stillage evaporation (Wang et al., 2013) and decreased drying, as well as decreased natural gas usage by utilizing produced biogas. The AD of thin stillage may also increase the value of the feed products by producing higher protein DDG as opposed to DDGS and is expected to reduce the requirement of a supplemental nitrogen source for yeast fermentation.

1.2 Anaerobic Digestion and Fluidized Bed Reactors

Anaerobic Digestion (AD) is a biological process that happens naturally when bacteria break down organic matter in the absence of oxygen. Anaerobic digestion (AD) is a microbial decomposition of organic matter into methane, carbon dioxide, inorganic nutrients and compost in an oxygen-depleted environment and in the presence of hydrogen gas.

The conventional aerobic processes that are widely used for the treatment of domestic wastewater have at least three distinct disadvantages: their relatively high electrical requirement, the high operation cost and the high excess sludge production which requires treatment and disposal that further increases the operational cost (Lee et al., 2011). The fluidized bed technology presented a series of advantages compared to other kinds of anaerobic processes (Sowmeyan, 2008), like high organic loading rates and short hydraulic retention times. As a result of expanded knowledge, anaerobic digestion systems, especially fluidized bed reactors, have grown in maturity, occupying an outstanding position in industries as a result of their capabilities to handle a high range of loading rates and performed uniform temperature gradient in around reactors. Therefore, a number of design modifications have been tested or adopted in order to improve the performance of the system. In the classic case of fluidized systems, the solid particles have a higher density than the fluid. In the fluidized bed systems using carrier media, biofilms have a critical role for optimal performance. In biofilm reactors, the development of the biofilm is determined by the difference between biofilm growth and detachment processes. Biofilm growth mainly relies on the carrier characteristics such as particle size, sphericity porosity, density, and specific surface area (SSA) (Heijnen., 1988).

1.3 Deoxynivalenol (DON) contaminated grain crops

All grain crops are susceptible to fungal infection when specific weather patterns occur during the growing season. The fungi are capable of producing toxins known as mycotoxins. Deoxynivalenol (12,13-epoxy-3,4,15-trihyroxytrichothec-9-en-8-one or DON; chemical formula: $C_{15}H_{20}O_6$) is the best known and most commonly detected trichothecene mycotoxin (Pestka & Smolinski, 2005; Pestka, 2007). DON is also known as "vomitoxin" because of its association with human and animal toxicoses. Exposure to this toxin can cause vomiting, feed refusal, growth retardation, and affect the immune system in pigs (Pestka & Smolinski, 2005). DON causes nausea, diarrhea, and vomiting as primary symptoms among humans (Pestka, 2007), and it is an unavoidable contaminant in crops. Therefore, it occurs in commodities entering the marketing chain, including grains used in ethanol production. The AD may increase the economics of the plant by allowing it to process corn that contains concentrations of mycotoxins that may have previously been rejected by IGPC, which benefits both the plant as well as local farmers.

1.4 Research Objectives

Although high-rate anaerobic digesters, such as UASB, AnMBR, EGSB, and FBR are not suitable for high solids or thickened wastes, the high-rate systems can be used as a part of multi-stage system for treating high solids wastes (Angelidaki et al., 2003). This research was conducted to study the possibility, stability, and efficiency of anaerobic digestion for treatment of simulated thin stillage under thermophilic condition and compare that with mesophilic conditions that employ fluidized bed reactors, with two kinds of different support media. Thus, the current research will perform three key investigative tasks:

1. An evaluation of the treatment of simulated thin stillage with natural and synthetic carriers, employing AnFBRs under mesophilic temperature conditions.
2. Treatment of simulated thin stillage using AnFBRs under thermophilic temperature conditions.
3. An investigation of the effect of varied concentration of liquified corn with deoxynivalenol (DON) on biogas production in anaerobic digestion using batch reactors.

1.5 Scope of Work

To achieve its goals, the study will involve four critical tasks:

1. Operate AnFBRs under mesophilic and thermophilic temperature conditions at varying organic loading rates.
2. Investigate biogas production and content in mesophilic and thermophilic process.
3. Compare carriers in terms of operation parameters like fluidization, biomass attach, detachment and energy consumption, and
4. Design batch reactors to evaluate the effect of different concentrations of DON-contaminated liquified corn on biogas production in anaerobic digestion.

CHAPTER 2

LITERATURE REVIEW

2. Literature review

2.1 Thin stillage by product of Ethanol industries

Ethanol, which is a renewable biofuel from corn and is easy to transport and store, has been considered as an alternative fuel to petroleum oil. The steps in ethanol production include hydrolysis, saccharification, fermentation, and distillation and dehydration (Wilkie et al., 2000), and it can be fermented from sugar-based or starch-based feedstock. Corn and wheat are the primary feedstock in the Canadian ethanol industry: facilities in Ontario, Quebec, and Manitoba take in corn, while plants in Saskatchewan and Alberta rely primarily on wheat. In 2016, it was estimated that 77% of domestic ethanol production would be derived from corn, and 23% would be derived from wheat [(2016 August 24). Ethanol Producer Magazine.http://ethanolproducer.com/articles].

North America, however, corn is an abundant agricultural product; thus, it is more readily available than all other feedstocks combined (Roukas et al., 1996). Corn whole stillage is the organic residue after ethanol is distilled from the fermented corn mixture. In a traditional process, the whole stillage is centrifuged to separate the liquid fraction, or thin stillage, from the solid fraction, or the wet distillers' grains (WDG). In the dry grind process, the clean corn is ground and mixed with water to form a mash. The mash is cooked, and enzymes are added to convert starch to sugar. Afterwards, yeast is added to ferment the sugars, producing a mixture containing ethanol and solids. This mixture is then distilled and dehydrated to create bioethanol. The solids remaining after distillation are dried to produce distillers' dried grains with protein and are sold as an animal feed supplement (Bothast et al., 2005). The conventional corn ethanol plant uses a significant amount of energy for treatment of thin stillage and processing of co-products. The high chemical oxygen demand (COD) of thin stillage prevents it from being able to be discharged directly to the environment. Some alternatives have been introduced as a means to decrease the energy use and increase revenues of corn ethanol plants. Some of these technologies include the extraction of high value chemicals from thin stillage including glycerol, phytic acid and beta-carotene (Reis et al., 2017). This sought to increase the costs for processing ethanol and instead focus on co-products with a higher commodity price.

Normally, with the evaporation of thin stillage into syrup, nutrients and chemicals in the stillage can be concentrated to up to three times their initial concentration, which can cause issues in the animal feed (K. Liu, 2011). Anaerobic digestion can avoid this concentration step while treating the organic matter within the stillage. In a typical ethanol plant, up to 20 L of thin stillage can be generated per liter of ethanol (Rosentrater, 2006) and studies indicate that its balanced composition of COD, BOD, volatile solids and carbohydrates make it a strong candidate for AD substrate (Nasr et al., 2012). Usually, due to the solids build up and toxicity to yeast caused by lactic acid, acetic acid, glycerol, and sodium, only 50% or less of thin stillage is recycled as backset for fermentation (Andalib et al., 2012). This limits the ability to facilitate water reuse and nutrient recycling in the conventional plant. The anaerobic digestion of treated thin stillage can be expected to improve the water and energy efficiencies of dry grind corn ethanol plants. The analysis showed that the digestate would be of suitable quality for process water and the incorporation of AD improved energy efficiency by eliminating evaporator and producing biogas that can be used for drying (Alkan-Ozkaynak & Karthikeyan, 2011). The volatile solids reduction of thin stillage lends to improved water recycling, and natural gas displacement of between 43-57% for a dry grind facility (Reis et al., 2017). A schematic of the processes is illustrated in Figure. 2.1.

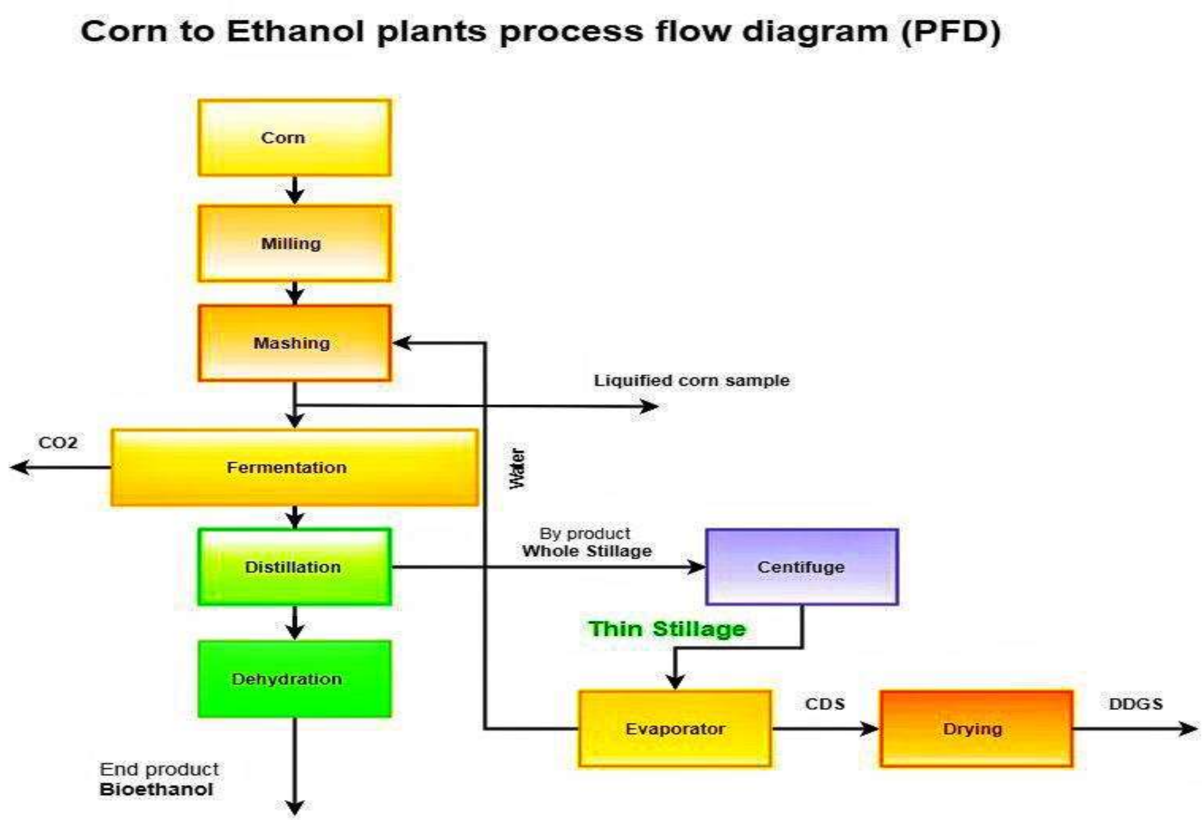


Figure 2.1 Corn to Ethanol plants process flow diagram (PFD)

Table 2.1 presents Canada ethanol plants' report. It shows that during the last seven years around 3.2 billion tones corn per year were used. The report indicates that Canada's ethanol plants used 3.250 billion tonnes corn in 2017, with 1.1 billion tonnes of DDGs as co-product for the same year. Canada currently has 14 ethanol refineries.

Table 2.1 Canadian annual biofuels report from ethanol industry (Voegele, 2016)

Ethanol Used as Fuel and Other Industrial Chemicals (Million Liters)								
Calendar Year	2010	2011	2012	2013	2014	2015	2016 (e)	2017(f)
Beginning Stocks	108	128	127	130	131	131	131	131
Fuel Begin Stocks	108	128	127	130	131	131	131	131
Production	1,530	1,790	1,780	1,815	1,820	1,820	1,800	1,820
Fuel Production	1,445	1,700	1,695	1,730	1,730	1,725	1,750	1,750
Imports	449	1,008	1,087	1,119	1,157	1,234	1,000	1,000
Fuel Imports	11	450	803	1,079	1,143	1,094	1,000	1,000
Exports	94	77	53	57.00	63	68	65	65
Fuel Exports	0	0	0	0	0	0	0	0
Consumption	1,865	2,722	2,811	2,876	2,914	2,986	2,735	2,755
Fuel Consumption	1,436	2,151	2,495	2,808	2,873	2,819	2,750	2,750
Ending Stocks	128	127	130	131	131	131	131	131
Fuel Ending Stocks	128	127	130	131	131	131	131	131
Production Capacity								
Number of Refineries	15	15	14	15	15	15	14	14
Nameplate Capacity	1,429	1,818	1,815	1,760	1,800	1,800	1,775	1,774
Capacity Use (%)	101%	94%	93%	98%	96%	96%	99%	99%
Co-product Production (1,000 MT)								
DDGs	980	1,220	1,075	1,100	1,100	1,100	1,100	**1,100**
WDG	575	550	635	650	650	650	650	650
Corn Oil	2	2	3	6	6	6	6	6
Feedstock Use for Fuel (1,000 MT)								
Corn	2,800	3,201	3,285	3,200	3,250	3,375	3,250	3,250
Wheat	770	970	850	1,000	1,000	1,000	950	1,000
Market Penetration (Million Liters)								
Fuel Ethanol	1,436	2,151	2,495	2,808	2,873	2,819	2,750	2,750
Gasoline	44,186	44,555	43,065	44,009	45,501	44,698	46,000	45,540
Blend Rate (%)	3.2%	4.8%	5.8%	6.4%	6.3%	6.3%	6.0%	6.0%

Source: Canadian government and industry sources with FAS/Ottawa analysis

The “thin stillage” is separated from the insoluble solid fraction using centrifuges or presses/extruders. The stillage leaving the fermentation column is centrifuged with a decanter, and this comprises between 15% and 30% of the liquid fraction which is called thin stillage.

2.2 The Anaerobic Digestion Process fundamental

Anaerobic digestion of the thin stillage is an alternative approach to recovering more energy from the corn (Wilkie et al., 2000), in addition to treating the stillage.

Anaerobic digestion is often believed to be a multifaceted process, and the digestion itself is based on a reduction process that contains several biochemical reactions that occur under anoxic conditions (Aslanzade, 2014). Methane formation in anaerobic digestion includes four different steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

- Hydrolysis

Hydrolysis is the first stage in the anaerobic digestion process and consists of the enzyme-mediated changes in insoluble organic materials including lipids, polysaccharides, proteins, fats, and nucleic acid—into soluble organic materials, such as compounds appropriate for use as sources of energy and cell carbon. These include monosaccharides, amino acids, and other modest organic compounds. This step is fulfilled due to strict anaerobes, such as bacterizes, clostridia, and facultative bacteria such as streptococci (Christy, 2014). This first step is vital because large organic molecules are basically too large to be directly absorbed and applied by microorganisms as a substrate/food source.

- Acidogenesis

The second stage is acidogenesis, during which the monomers shaped in the hydrolytic step are taken up by a wide variety of facultative and obligatory anaerobic bacteria and are degraded into short-chain organic acids, including butyric acids, propanoic acids, acetic acids, alcohols, hydrogen, and carbon dioxide. The concentration of hydrogen shapes as an intermediate result in this stage affects the type of final product formed during the fermentation process.

- Acetogenesis

The products formed in the acidogenic stage are used as substrates for the other microorganisms that are active in the third phase. In this, also referred to as the acidogenic phase, anaerobic oxidation is performed (Aslanzade, 2014). Products that cannot be directly changed into methane by methanogenic bacteria are formed into methanogenic substrates, while volatile fatty acids and alcohols (VFAs) are oxidized into methanogenic substrates, such as acetate, hydrogen, and carbon dioxide. VFAs with carbon chains longer than one unit are oxidized into acetate and hydrogen (Elseadi, 2008).

- Methanogenesis

In the methanogenic stage, methanogenic bacteria produce methane and carbon dioxide from intermediate products in strict anaerobic conditions (Aslanzade, 2014). Methanogenesis is an important step in the whole anaerobic digestion process as it is the slowest biochemical reaction of the process (Elseadi, 2008). All steps are presented in Figure 2.2.

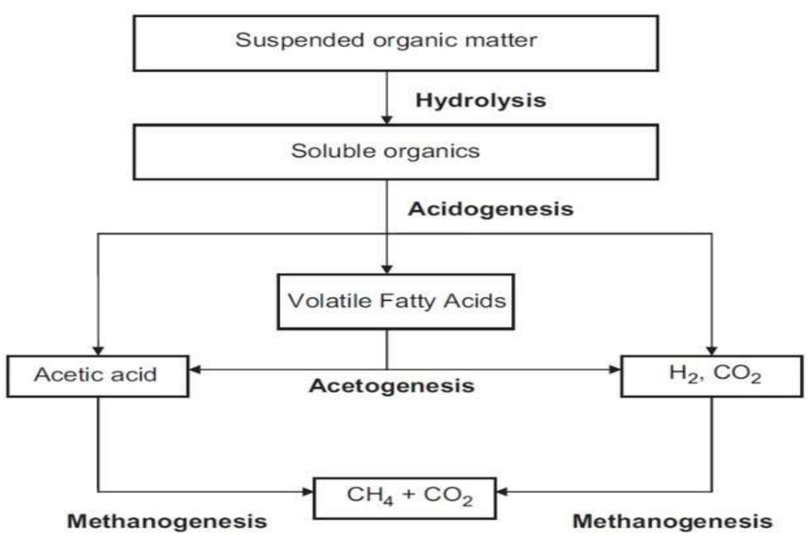


Figure 2.2 Steps of anaerobic digestion process

2.3 Effect of process variables on Anaerobic Digestion

Degradation of a waste contaminant in the anaerobic treatment depends on a number of parameters. The main parameters are related to reactor operating conditions (pH, temperature, organic loading rate (OLR), gas production and composition, VFAs and alkalinity ratio, COD removal and CH_4 production, and C/N Ratio. Some of the operational parameters and their effects are discussed in the following paragraphs.

2.3.1 pH

The optimum pH for the AD process is between pH 6.7 and 7.6, which is favorable for methane-producing archaea (Parkin & Owen, 1986; Speece, 2008). Methanogens grow very slowly at pH lower than 6.6 (Angelidaki et al., 2003). Low pH can be caused by an imbalance of conditions in the digester due to the domination of the acid-producing to the acid-consuming bacteria (Speece, 2008). Therefore, in an AD system, pH is usually maintained between methanogenic limits to inhibit the predominance of acid-forming bacteria and avoid VFA accumulation (Rajeshwari et al., 2000; Khalid et al., 2011). Veeken et al., (2000) found that pH influenced the hydrolysis rate in AD of organic solid waste, and an accumulation of VFAs may result in a decrease of pH and/or vice versa, but this depends on the composition of the waste or substrates. Methane-producing microorganisms experience optimum growth in the pH range of 6.6 and 7.4, although stability may be achieved in the formation of methane in a wider pH range between 6.0 and 8.0; pH values below 6 and above 8.3 should be avoided, as they can inhibit the methane–forming microorganisms (Lettinga el al.,1996).

The operation of anaerobic reactors with the pH constantly below 6.5 or above 8.0 can cause a significant decrease in the methane production rate. In addition, sudden changes in pH can adversely affect the anaerobic digestion process, and recovery would depend on a series of factors related to the type of damage caused to the microorganism (either permanent or temporary). According to Lettinga et al., 1996, recovery will be quicker if changes in pH (drop or rise) are not significant and also not for a long period of time. The bio methanation process takes place in a relatively narrow pH range, from 6.5 to 8.5 (Weiland P. 2003). The level of pH is necessary to be in a desired range because it directly affects the growth of microbes. The optimal pH of methanogenesis is around pH 7.0 (Liu et al., 2008). The pH value increases due to ammonia accumulation during the degradation of protein, while the accumulation of VFA (volatile fatty acid), resulting from degradation of organic matter (1 g of volatile acids produces per gram of volatile solids) (Sansone FJ 1982) decreases the pH value. A pH value below 6.6 is toxic to methanogenesis, it is important to maintain pH in the desired range for efficient gas production (Ward AJ et al., 2008). The level of pH, if necessary, can be maintained by adding calcium hydroxide (Weiland 2003). The anaerobic fluidized bed reactor (AFBR) was operated as a mesophilic methanogenic reactor with temperature of 37.1 ± 0.8 ˚C. The pH of the feed was 3.46, while the pH inside the AFBR remained constant at 7.2 throughout the experiment due to the high rate of VFA degradation in the column as well as ammonia production (Andalib et al., 2014).

Gavala et al., 2003 conducted a study on mesophilic and thermophilic anaerobic digestion conditions. They conducted experiments in two digesters. One was mesophilic (A) and the other was thermophilic (B). They found that for successful operation, digester A and digester B were operated at 7.6± 0.3 and 7.8± 0.2 respectively. Platsi (2009) also found that for a thermophilic digester, pH was slightly higher that mesophilic with 8.25 and 7.9, respectively. A similar trend was also observed by Eskicioglu et al., 2010, where pH in reactors for mesophilic conditions was 7.2-7.5 and 7.9 for thermophilic condition. Another study by Achu et al., 2010 also found a slight difference between two mesophilic and thermophilic reactors with a pH of 7.5-7.7 and 7.8-8, respectively. It appears from the literature that the pH value for the mesophilic digester was always slightly lower than for the thermophilic digester. This could be contributed to N-NH_3 and alkalinity concentration for the mesophilic and thermophilic operations.

2.3.2 Temperature

Methanogens are particularly affected by sharp and/or frequent fluctuations in temperature, therefore, sustaining a stable temperature in an AD system is critical (Appels et al., 2008; Ward et al., 2008). Angelidaki et al. (2003) stated that not only temperature in the AD process influences microbial growth, but also physical parameters such as viscosity, surface tension, and mass transfer properties. Other studies have also observed that temperature has a significant effect on the AD process, including accumulation of VFAs, biogas and methane production, and methanogenic activity (Lyberatos &Skiadas, 1999; Sánchez et al., 2001; Sanders et al., 2003; Appels et al., 2008). Some other studies have reported that for certain types of substrate, however, such as manure and food waste, operation at mesophilic temperature is favorable. They reported that mesophilic temperature leads to more stable digestion with very little inhibition from VFAs (Angelidaki and Ahring, 1994; Hansen et al., 1998; Banks et al., 2008). Increasing the rate of processing through a general increase in biochemical reaction rates was also reported by Mackie and Bryant, 1995; de la Rubia et al., 2002, 2006; and Appels et al., 2008.

2.3.3 Gas production and composition

In anaerobic systems, most of the biodegradable organic matter present in the waste (about 70 to 90%) is converted into biogas, which is removed from the liquid phase and leave the reactor in a gaseous form (Metcalf & Eddy, 2003). Only a small portion of the organic material is converted into microbial biomass (about 5 to 15%), which then constitutes the excess sludge of the system; besides the small amount produced, the excess sludge is usually more concentrated, with better dewatering characteristics (Metcalf & Eddy, 2003). In the absence of hydrogen, cleavage of acetic acid leads to the formation of methane and carbon dioxide.

$$CH_3COOH \rightarrow CH_4+CO_2$$

When hydrogen is available, most of the remaining methane is formed from the reduction of carbon dioxide.

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

It is important to monitor pH, alkalinity, and concentration of volatile acids in the effluent and compare these values with the influent. More interestingly, a sudden fluctuation in the biogas composition, specifically an increase in the CO_2 percentage, can be a sign of operational instability. The overall composition of the biogas produced during anaerobic digestion varies according to the environmental conditions prevailing in the reactor. For reactors operating in a stable manner the composition of the biogas produced is reasonably uniform, however, the carbon dioxide/methane ratio can vary substantially, depending on the characteristics of the organic compound to be degraded. In the anaerobic treatment of domestic sewage, typical fractions of CH_4 and CO_2 present in the biogas are 70-80 % and 20-30 %, respectively (Metcalf & Eddy, 2003). Zábranská et al., 2000 conducted a study on both mesophilic and thermophilic conditions. They found that methane yield were 0.23 ± 0.02 L and 0.26 ± 0.04 L CH_4/g TCOD (Total Chemical Oxygen Demand) respectively. Some other researchers calculated the methane production per VS added. Achu et.al.,2010 found the methane content in biogas fluctuated between 55% and 65% and the methane yield ranged from 0.314 to 0.348 m^3 CH_4 kg of VS added per day for both thermophilic and mesophilic digestion.

Andalib et al., 2014 performed a study using thin stillage and primary sludge. For thin stillage the maximum methane production yields was of up to 0.31 L of CH_4/g COD. For primary sludge, the maximum methane production was 0.25 L CH_4 /g COD. They also calculated biogas production per reactor volume and observed a biogas production rate per reactor volume of 15.8 L gas/L /d and 1.22 L CH_4 /L/ d. They found methane production rates of up to 160 L/d at steady state equivalent to 40 L CH_4/ (L $_{\text{thin stillage}}$/d). Biogas production rate per reactor volume of 15.8 L gas/$L_{reactor}$/d has been achieved by Andalib et al., 2012.

Researchers have compared the biogas yield between AnFBR as well as CSTR digesters. The biogas yield of the AnFBR in a different study was 345 mL CH_4/g COD removed compared to the anaerobic CSTR digester biogas yield of 200–350 ml CH_4/g COD removed (Nasr, N., Gupta, M., Elbeshbishy, E., Hafez, H., El Naggar, M.H., Nakhla, G. (2014)). It appears in this research that AnFBR produced a higher level of methane per grams of COD removed consistently than CSTR.

2.3.4 VFAs/Alkalinity ratio

The interaction between alkalinity and volatile acids during anaerobic digestion is based on whether the alkalinity of the system is able to neutralize the excess acids formed in the process and provide the buffering capacity. Another indicator for determining the stability of anaerobic digestion is the VFAs/alkalinity ratio (Andalib et al., 2012). There are three critical levels for this:

1. <0.4 stable.

2. 0.4–0.8, some instability will occur.

3. >0.8, significant instability (Callaghan et al., 2002).

Andalib et al., 2012 studied steady-state VFAs (as acetate)-to-alkalinity ratio. They reported the ratio in 5 phases, with phases I-IV for synthetic thin stillage and phase V thin stillage. They stated that the ratio was consistently below 0.2 in phases I–IV, although during the transition from one phase to another it increased due to a sudden doubling of OLRs. However, in phases IV and V, an increase in the value to 0.5 was observed due to the accumulation of the VFAs (Andalib et al., 2012). Another study by Nizar (2014) also found the variation of a VFAs/alkalinity ratio between 0.29 and 0.41, which clearly suggests that digestion stability at OLR of 28–39 kg/m^3/d was compromised.

2.3.5 COD removal and CH_4 production

Research has shown that maximum methane production yields of up to 0.31 L CH_4/g COD and 0.25 L CH_4/g COD were achieved for thin stillage and primary sludge, respectively (Mao et al., 2015). Lee et al., 2011 studied the treatment of thin stillage using a CSTR digester. They reported a COD removal efficiency of 85% at HRT of 24–40 d and OLR of

1.8–3.9 kg COD/m^3/d. Bolzonella et al.,2012 conducted a study for waste activated sludge and source sorted biowaste in mesophilic and thermophilic conditions. They reported the COD removal increased from 35% in mesophilic conditions, to 45% in thermophilic conditions.

In another study, thin stillages were efficiently digested anaerobically with high COD removal under mesophilic and thermophilic conditions and with an organic loading rate of 15–21 g COD/L / d (Osterkamp et al., 2016). Osterkamp et al., 2016 also reported the methane production rate was 0.2 L/g COD removed. They also reported methane percentage of 60 and 64, and 92 and 94 % soluble COD removed, respectively, by the mesophilic and thermophilic reactors. In another study an AnFBR has been demonstrated for the digestion of primary sludges. The AnFBR successfully treated the primary sludge at OLR of 19 kg/ m^3/ d, achieving COD removal efficiency of 68% (Mustafa, N., Elbeshbishy, E., Nakhla, G., & Zhu, J. (2014)).

2.3.6 C/N Ratio

The C/N ratio reflects the nutrient levels of a digestion substrate, and thus, digestion systems are sensitive to the C/N ratio. A high C/N ratio induces protein solubilization rate and leads to low total ammonium nitrogen (TAN) and free ammonia (FA) concentrations within a system. Thus, ammonia inhibition may be avoided by optimizing the C/N ratio in the AD process (Angelidaki et al., 2003). However, an excessively high C/N ratio provides insufficient nitrogen to maintain cell biomass and leads to fast nitrogen degradation by microbes, resulting in lower biogas production and vice versa (Giovanna et al., 2016). Substrates with an excessively low C/N ratio increase the risk of ammonia inhibition. The optimal C/N ratio for anaerobic digestion has been shown to be between 20 and 30 or between 20 and 35, with a ratio of 25 being the most common (Zhang et al., 2009; Punal A et al., 2000; YenH-W et al., 2007). Wang et al., 2014 studied primary and thickened waste activated sludges. They found C/N ratios of 25:1 and 30:1 provided the highest cumulative biogas production levels, approximately threefold compared with a C/N ratio of 15:1.

2.4 High-rate Anaerobic Digestion and Fluidized bed reactors

In recent years, many studies have been performed to evaluate different methods for the treatment of high organic load wastewaters (Hidalgo et al., 2005). The anaerobic treatment of high-strength wastewaters with high biodegradable content presents some advantages. For example, a high degree of purification with a high load of organic material can be achieved, requires few nutrients, and usually produces small amounts of excess sludge (Shida et al., 2009). A conventional anaerobic digester (for wastewater) was conducted in anaerobic ponds complemented with facultative and aerobic ponds to ensure sufficient removal of organic materials and pathogens (Hsu, 1996). However, this method requires a long retention time and large treatment area. As such, high-rate anaerobic reactors were introduced to provide high sludge retention and sufficient contact between bacteria and substrate (wastewater), which minimizes the duration of this treatment and the space requirement (Hsu, 1996).

Fluidization technology has been used for the pastcentury. Studies have long shown that fluidization provides many advantages to the processes, such as significantly enhanced mass and heat transfer rates, improved inter-phase contact efficiency, ease in handling a large number of particles, and uniform temperature distribution (Wang, 2016). These characteristics have led to increased productivity and the wide application of fluidized bed reactors. (JX et al., 2000).

Fluidization in liquid-solid systems is controlled by the circulation flow rate. With an increasing circulation flow rate, the liquid-solid system passes through some flow regimes; when the liquid flow rate is lower than the minimum fluidization velocity, the bed regime is fixed, the regular particulate fluidization regime where a clear borderline between the fixed bed region and the top freeboard region exists. There is also a transition region from regular fluidization to circulating fluidization regimes where the boundary between the two phases becomes blurred while the depth of the dense phase increases more and some particles are moved from the bed. It is necessary to continuously feed particles into the bottom to maintain the bed (Andalib et al., 2011). The fluidized bed reactor is a digester configuration which has been demonstrated in various studies to be feasible for the treatment of both low- and high-strength industrial wastewaters (Fernández et al., 2001; García-Encina & Hidalgo, 2005; Shida et al., 2009).The microorganisms in a fluidized bed reactor are attached to an inert support material, which is fluidized by a high liquid up flow velocity achieved by a high recycle rate . The substrate diffuses from the bulk liquid to the biofilm on the carrier surface and metabolic products diffuse back. In such a fluidized bed reactor, it is possible to have a higher surface area per unit reactor volume to support microorganisms, which increases the reactor microorganism concentration (Jeris, 1983). The larger specific surface area allows for shorter hydraulic retention times for the same degree of treatment in a given volume, or a higher removal capacity (Heijnen et al., 1988), compared with other high-rate anaerobic reactors.

In the field of anaerobic treatment processes, anaerobic fluidized bed reactors have emerged as a good alternative for the treatment of wastewater. This anaerobic fluidized bed reactor utilizes small, fluidized media to induce extensive cell immobilization, thereby achieving a high reactor biomass hold-up and a long mean cell residence time (Shieh and Hsu, 1996). Fluidized bed reactors have been used in various biotechnological applications utilizing low suspended solids streams e.g., treating food-processing, digesting paper industry wastewater, and purifying fermentation wastewater (Heijnen et al., 1988).

The mesophilic anaerobic fluidized bed reactor (AnFBR) with zeolite as carrier media (425–610 μm) developed by Nakhla and colleagues, achieved up to 88% TCOD and 78% TSS removal at an organic loading rate (OLR) of 29 g COD / L/day during the treatment of thin stillage with a TCOD of 130 g/L and TSS of 47 g/L (Andalib., 2012). Additionally, the AnFBR has recently been demonstrated for the digestion of primary sludges (Andalib., 2014) with a TSS destruction efficiency of 82% at an OLR of 9.5 g COD/L/day. Important operational parameters such as an OLR, superficial velocity and hydraulic retention time (HRT) are discussed in the following subsections.

2.4.1 Organic Loading Rate (OLR)

An organic load can be defined as the amount of volatile organic dry matter entering the anaerobic digester over time - measured in mass (kg or pound) per m^3 digester volume per day (http://www.renewable-energy-concepts.com). The organic loading provides information on nutrient supply levels of the microorganisms involved, overload or undersupply of the system, as well as resulting technical and process control measures to be taken. Furthermore, it gives an indication of the biological degradation of the substrates, which can be related to the efficiency of an AD. Overloading may cause accumulation of fatty acids, which can act as an inhibitor and result in low biogas yield. This may cause proliferation of acidogenesis, a decrease in pH, and mass death of methanogenic bacteria (Ray et al., 2013). At an organic loading of 24.32 g COD/L/day and HRT of 0.74 days, a high COD removal efficiency of up to 84% was achieved in all tested reactors (Balaguer et al., 1997). Eldyasti et al, 2012 conducted a study for four different media, namely, maxi-blast plastic (MX), lava rock (LR), multi-blast plastic (MB) natural zeolite (NZ). They achieved COD removal efficiencies for MX and LR in the range of 78%, while the MB and NZ media achieved a COD removal of 88% at an organic loading rate (OLR) of 5.9 ± 0.5 g COD/L/day.

The highest COD removal and VSS destruction efficiencies for primary sludge of 85% and 88%, respectively, were achieved at an HRT of 8.9 days and OLR of 4.2 g COD/L/day (Nizar et al., 2014). Another AnFBR has been demonstrated for the digestion of primary sludge (PS) (Wang et al., 2016). They reported PS feeding to the AnFBR started at an OLR of 9 g COD/L/day and increased to 18 g COD/L/day after 90 days.

Under mesophilic AD, VS destruction decreased with an increase in OLR, with average values of 85% and 77% for OLR 4 and 5 g VS /L/d, respectively. In thermophilic AD, however, the average VS destruction at both loadings was 88% (Tian et al., 2015). Santos et al., 2014 conducted a study on treating stillage using fluidized thermophilic bed reactors. They observed that when continuous flow operation was used for whole corn stillage at full strength (254 g TCOD/L), the thermophilic digester was unable to cope with an organic volumetric loading rate of 4.25 g TCOD/L at SRT of 60 days.

2.4.2 Superficial Velocity

The superficial velocity is calculated based on flow rate and the cross section of the reactor, as follows:

V= Q/A

where:

V= superficial velocity (cm/sec)

Q=Flow (cm^3/sec)

A= Section area of the reactor (cm^2)

The velocity is related to the type of sludge, the type of media, and how much loading is applied.

Zhu et al., 2000 studied superficial liquid velocity and reported that normally, there is an interface between a dense phase and a dilute phase. With a further increase of superficial liquid velocity, the axial bed voidage distribution becomes uniform throughout the bed and a definite particle circulation rate is established. Under this condition, the bed enters the circulating fluidization regime. In the circulating fluidization regime, researchers found that the bed voidage is always uniformly distributed in the axial direction regardless of the superficial liquid velocity and the particle circulation rate (Zhu et al., 2000).

Liang et al., (1997) showed the variation of the axial distribution of the bed voidage with an increase of superficial liquid velocity for glass beads before and after the transition of the fluidization regime. When the superficial liquid velocity is low, e.g., at U = 0.009 m/s and U = 0.018 m/s, the bed is in the conventional particulate fluidization regime and there exists a clear distinction between the bottom dense region and the freeboard region. Zheng et al. (1999) also reported that the flow phenomena at the transition are quite different for particles of different densities. With increasing solids density, the transition becomes more gradual; that is, the range of the liquid velocity for the transition is widened. On the other hand, the transition of the steel shot (very heavy particles) is much more gradual and the liquid velocity range over the transition is much wider (Zheng et al., 2000). Although a detailed comprehensive economic analysis between the AnFBR and conventional digestion is beyond the scope of this study, the superficial liquid up-flow velocity required for fluidization, which constitutes 94% of the operational cost of FBR, is 0.35 cm/s (Eldyasti et al., 2012). Zhu et al. (2000) reported that with a lack of significant particle clustering in the liquid-solid fluidization system, the fluid drag begins to overcome the particle gravity when the liquid velocity reaches the particle terminal velocity, resulting in obvious particle entrainment. The superficial liquid velocity maintained in the system was reduced from 1.4 cm/s, at low attached biomass of less than 15 mg VSS/g media, to 0.35 cm/s at high attached biomass of up to 38 mg VSS/g media (Andalib et al., 2014). The recirculation was maintained at a rate of 133 L/h (expansion 30%), and a superficial velocity of 1.30 times the minimum fluidization velocity was maintained (Santos et.al., 2014). The liquid at the top of the reactor was recycled and pumped back to the bottom of the fluidized bed to maintain an up flow velocity at 0.8 cm/s as an energy saving concern (Wang, 2015).

2.4.3 Hydraulic Retention Time (HRT)

Two significant types of retention time are herein discussed: SRT (Sludge Retention Time), which is defined as the average time the bacteria (biosolids) spend in a digester, and HRT, which is defined by the volume of a reactor with the flow rate. Anaerobic digestion is the preferred treatment process for organic wastes due to its low nutrient requirements, low biomass yield, and biogas (methane) production. However, current conventional anaerobic digestion processes require a long hydraulic retention time (HRT) of up to 40 days to achieve the necessary efficiency for stillage treatment (Lee et al., 2011). An average retention time of 15–30 days is required to treat wastes under mesophilic conditions (Metcalf & Eddy, 2003). Obtaining an effective HRT can depend on the substrate composition and OLR; typically, it requires two weeks (Lee et al., 2011). Decreasing the HRT usually leads to VFA accumulation, whereas a longer than optimal HRT results in insufficient utilization of digester components. For algal type biomass, an HRT below 10 days can result in low methane productivity (Kwietniewska et al., 2014).

The use of small, porous, fluidized media enables the reactor to retain high biomass concentrations and thereby to operate at significantly reduced HRTs (Montalvo et al., 2012). Based on the digestion kinetics, in order to achieve the typical 50% VSS destruction efficiency, the AnFBR should be sized for an HRT of only 2 days when treating primary sludge and 1.5 days for thin stillage with an estimated SRT of 30 days. (Andalib et al., 2014). Suhartini et al. (2014) conducted a study on digestion on sugar beet pulp. They suggested operating digesters at least 3 days HRT in thermophilic operation. Based on the digestion kinetics, in order to achieve the typical 50% VSS destruction efficiencies, the AnFBR should be sized for an HRT of only 2 days when treating primary sludge and 5.2 days when treating thickened waste activated sludge (TWAS) (Mustafa et al., 2014). Wang et al., 2016 studied the digestion of PS and reported that COD and VSS removal for PS was 62% and 63%, respectively, at an organic loading rate (OLR) of 18 kg COD $/m^3/$ d and a hydraulic retention times (HRTs) of 2.2 days.

2.5 Mesophilic and Thermophilic Anaerobic Digestion

Waste organic solids are widely produced by domestic and industrial wastewater treatment plants. An AD is a common stabilization method used to treat these solids, which is environmentally beneficial due to production of renewable energy (Ge et al., 2009). The performance of microbiological processes is closely related to the temperature of the system since the metabolic activity of microorganisms is possible only in a certain temperature range and because a maximum activity is obtained within this interval for pure species. However, AD is developed by a complex mixed population, and as a result, several temperature ranges may be possible for the development of the process. The two main ranges of temperature for AD are mesophilic (M) and thermophilic (T), whose optimum temperatures are 37 ˚C and 55 ˚C, respectively (Romero García et al., 1991; Fdez-Rguez et al., 2010; Fdez-Güelfo et al., 2010; Vindiset al., 2009). These processes have been widely studied and applied to different wastes. In general, the mesophilic anaerobic digestion of sewage sludge is more widely used compared to thermophilic digestion, mainly because of the lower energy requirements and higher stability of the process (Gavala et al., 2003). However, the thermophilic anaerobic digestion process is usually characterized by accelerated biochemical reactions, higher growth rate of microorganisms, and accelerated interspecies hydrogen transfer resulting in an increased methanogenic potential at lower hydraulic retention times (Zábranská et al., 2000). Moreover, the enhanced hygienization effect of the thermophilic process complies with the European Union (EU) policy for elimination of pathogens originating mainly from humans and animals (Oropeze et al., 2001). It has been reported that thermophilic anaerobic digestion of sewage sludge can lead to EPA (Environmental Protection Agency) class A biosolids, which are suitable for subsequent land application (Watanabe et al., 1997). Thermophilic digestion between 50-

55˚C offers attractive advantages, such as higher VS and pathogen destruction efficiency, higher biogas generation, less foaming, and better dewaterability over mesophilic plants (Peddie et al., 1996; Rimkus et al., 1982). In addition to affecting the reaction rate and required SRT to achieve a certain process efficiency (i.e., solids removal and methane production), process temperature also plays a key role in the stability of the process. Methanogenic archaea are especially sensitive to temperature fluctuations, even to changes as low as 1˚C/d (Metcalf & Eddy, 2003). This can be particularly critical for the thermophilic processes since they are reported to be less stable than mesophilic ones (Buhr & Andrews, 1977).

The normal functioning and stability of an anaerobic digestion system depends on the presence of viable bacterial groups, and SRT is a significant factor with regard to ensuring the growth and maintenance of various populations of microorganisms in the reactor (Zhang et al., 1994; Li et al., 1999; Solera et al., 2001).

In general, the increase in process temperature means a higher microbiological activity and, hence, the substrate consumption and the methane generation rates are higher. However, it obviously carries an increased expenditure of energy. In short, the thermophilic range shows some advantages, such as high biogas production, removal of pathogens, and the specific growth rate of microorganisms, and it thus increases the speed of the process. The mesophilic range has a higher process stability and lower operating costs (Fernández-Rodríguez et al., 2013).

It has been observed that higher temperatures in the thermophilic range reduce the required retention time, which implies that lower retention times are required in digesters operated in the thermophilic range. Moreover, the thermophilic bacteria are more sensitive to environmental conditions than those mesophilic bacteria (Fernández-Rodríguez et al., 2013). From an economical point of view, it would be most effective to operate at a minimum SRT to allow for optimizing methane production and solids removal whilst assuring process stability (Ferrer et al., 2010).

In anaerobic digesters, biogas production depends on the amount of organic matter biodegraded by anaerobic microorganisms (Ferrer et al., 2010). Thus, it depends on the composition of the substrate and the presence of an equilibrium between anaerobic consortia in the reactor (Ferrer et al., 2010). Design and operation parameters of the process include sludge retention time (SRT), organic loading rate (OLR), and temperature and reactor flow (Ferrer et al., 2010).

Garcia et al. (1998) found that the input organic loading rate could be increased from 6.11 to 35.09 g COD/L/day within less than 75 days, even when there was a delay of 15 days due to a nutrient deficiency problem. The COD removal was around 84% and the hydraulic retention time was as low as 0.19 days. A high-rate anaerobic fluidized bed bioreactor (AFBR) with zeolite as carrier media (425–610 μm) was tested for the treatment of thin stillage with TCOD of 120 g/L and TSS of 60 g/L (Andalib et al., 2012).The AnFBR successfully treated the primary sludge at OLR of 19 g/L/day, achieving COD removal efficiency of 68% and VSS destruction efficiency of 70%, with VSS destruction efficiency dropping to 42% and 31% at OLR of 28 and 39 kg /m^3/d, respectively (Mustafa et al., 2014).

Despite the very high strength of the thin stillage with chemical oxygen demand of TCOD of 130,000 mg /L and TSS of 47,000 mg /L, the AnFBRs achieved up to 88% TCOD and 78% TSS removal at very high organic and solids loading rates (OLR and SLR) of 29 g

COD/L/day and 10.5 kg TSS/m^3/d, respectively; hydraulic retention time (HRT) was 3.5 days (Andalib et al., 2014).

2.6 Thin stillage treatment with mesophilic and thermophilic anaerobic digestion conditions in AnFBRs

Thin stillage is characterized by high total chemical oxygen demand (TCOD) of up to 122 g/L, biological oxygen demand (BOD) of up to 70 g/L, volatile solids (VS) of 60 g/L (Nasr et al., 2011) and total carbohydrates of 65% (based on dry mass) (Mustafa et al., 2000). Therefore, it is a strong candidate for anaerobic digestion. Despite the very high strength of thin stillage, with chemical oxygen demand of 130,000 mg TCOD/L and suspended solids of 47,000mg TSS/L, the AFBR showed up to 88% TCOD and 78% TSS removal at very high organic and solids loading rates (OLR and SLR) of 29 g COD/L/day and 10.5 g TSS/L/day, respectively, at hydraulic retention time (HRT) of 3.5 days (Andalib et al., 2012).

The use of small, porous, fluidized media enables the reactor to retain high biomass concentrations and thereby to operate at significantly reduced HRTs. Fluidization also overcomes operating problems, such as bed clogging and high-pressure drops which could be encountered if such high surface area media were used in a packed bed reactor (Haroun & Idris, 2009). A further advantage of using media to retain the biomass within the reactor is the possible elimination of the secondary clarifier.

Among the most commonly used bioreactor configurations for increasing the microbial population density are fluidized bed reactors, where bacteria colonize particles of a support medium, thereby increasing the surface available for bacterial growth (Borja et al., 1994; Fernández et al., 2007 a, b; Kuba et al., 1990; Montalvo et al., 2008). The fluidized bed reactor is a digester configuration which has been demonstrated in various studies to be feasible for the treatment of both low and high strength industrial wastewaters (Fernández et al., 2001; García-Encina & Hidalgo, 2005; Shida et al., 2009).

The carrier material was found to be a very important parameter because biomass accumulation has brought about changes in particle volume and density, affecting the whole system (Sowmeyan et al., 2008). Supporting carrier particles' characteristics (i.e. size, shape, density, porosity, roughness, and surface area) play a significant role in the adhesion/detachment rate, all of which significantly impact biological nutrient removal (BNR) process performance (Eldyasti et al., 2012). Different carrier particles have already been tested in anoxic/anaerobic fluidized bed bioreactors such as: sand, sepiolite, pumice stone, zeolite, lava rock, quartzite, alumina, resin, arlita, and kaolinite bead (Hao-Ran et al., 1983; Jeris, 1983; Rockey and Forster, 1983; Balaguer et al., 1997; Chowdhury et al., 2009). Perlite was an interesting carrier when compared to others like cork, polyethylene or polypropylene (Garcia-Calderon et al., 1998) and has been found to be a good carrier for the anaerobic digestion of distillery wastewater in the inverse fluidized bed (Sowmeyan et al., 2008).

AD is normally carried out in one of two temperature ranges: mesophilic (30-45 ˚C) and thermophilic (50-60˚C) (Angelidaki et al., 2003; Speece, 2008; Weiland, 2010). Operating AD at the higher temperature range (thermophilic digestion) may increase the rate of processing through a general increase in biochemical reaction rates (Mackie and Bryant, 1995; de la Rubia et al., 2002, 2006; Appels et al., 2008). For certain types of substrate, however, such as manure and food waste, operation at mesophilic temperature is favourable because it gives more stable digestion with very little inhibition from VFAs, as indicated by an increase in biogas yield (Angelidaki and Ahring, 1994; Hansen et al., 1998; Banks et al., 2008).

2.7 Deoxynivalenol (DON) contaminated corn treatment

During the corn-to-ethanol production process, approximately two-thirds of the grain, mainly starch, is fermented by yeast to produce ethanol and carbon dioxide, neither of which would contain mycotoxins if contaminated corn was used (Ingledew, 2006). However, the remaining coproduct, dried distiller's grain with soluble (DDGS), could potentially contain a higher concentration of any mycotoxin that was present in the grain prior to fermentation. The increased level of a given mycotoxin in DDGS was reported to be approximately three times as high as the level in the grain (Bennett, G. A et al., 1996).

Little is known about the effects of anaerobic digestion on the survival of plant pathogens like fungi and their secondary metabolites. Several studies have provided information about microbial degradation or transformation of mycotoxins. For example, microbiota and pure cultures of microbial isolates from chicken intestine have shown the capacity for degrading DON (Young et al., 2007). Guan et al. (2009) showed that the microbial community from catfish gut completely transformed DON at 15 °C in an artificial medium after 96 h incubation. In another study, Goux et al. (2010) examined contaminated wheat grain flour samples under mesophilic anaerobic digestion in two-liter batch digesters. They experimented on seven naturally contaminated flour samples (DON = 0; 1,976; 4,586 and 10,470 μg/kg) or artificially spiked commercial flour (DON = 0; 8,000 and 80,000 μg/ kg). The volume and composition of the biogas produced, as well as the progress of the DON concentration were monitored (Goux et al., 2010). They reported that DON contamination does not harm the anaerobic digestion process of wheat flour, and that the process may present a good alternative to incineration to treat such substrates since it allows for the recovery of energy and nutrients. However, little is known about the fate of DON from contaminated corn during mesophilic anaerobic digestion. By degrading DON, this could reduce issues surrounding mycotoxins in the corn, and allow the IGPC facility to accept corn that would previously have been rejected by the plant. Drosg et al., (2008) reiterates the advantage of AD in the ability to use grains contaminated with mycotoxins, as these toxins have no negative effect on ethanol production and their residues can be converted to biogas with an AD.

CHAPTER 3

MATERIALS AND METHODS

3. Materials and methods

3.1 Systems description

The lab-scale AnFBRs shown in Figure 3.1 were fabricated using a 10 and 5 cm internal diameter (ID) and a 1.2 and 1.1m high plexiglass, respectively. Reactors had either a 78.5 or 19.6 cm^2 cross section area. The heating tape on the steel component provided sufficient temperature, and the feed solution was pumped into the bottom of the anaerobic column by a peristaltic pump (Masterflex I/P, Masterflex AG, Germany).

To ensure fluidization in the anaerobic column, the large AnFBRs had an average recirculation flow of 5440–6500 L/d, while the smaller AnFBRs saw 855–1650 L/d. These rates corresponded to a superficial liquid velocity of 0.8–0.95 and 0.5–1 cm/sec, respectively.

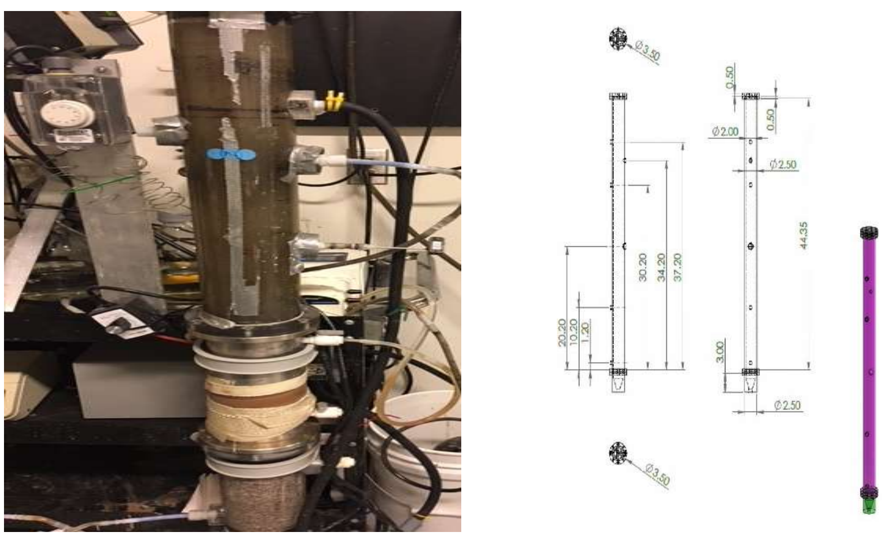


Figure 3.1 Schematic of anaerobic fluidized bed reactor.

3.2 Seed characteristics for different media

Anerobic digested sludge from the secondary digester was collected from a wastewater treatment plant (London, Ontario, Canada) and used as the seed sludge for the AnFBRs. The total suspended solids (TSS) and volatile suspended solids (VSS) concentrations of the ADS were 37.9 g/L and 19.7 g/L, respectively.

Table 3.1 Seed sludge characteristics for plastic and zeolite media experiment

Parameter (g/L)	ADS (AV± SD)
TS	39.7 ± 1.5
VS	20.6 ± 0.9
TSS	37.9 ± 1.4
VSS	19.7 ± 0.8
TCOD	27.6 ± 0.5
SCOD (mg/L)	710 ± 15
VFAs (mg/L)	105 ± 1
Total Carbohydrates (mg/L)	6,300 ± 55
Soluble Carbohydrates (mg/L)	85 ± 2
pH	7.31
Alkalinity (mg/L) as $CaCO_3$	3690 ± 200

3.3 Seed sludge characteristics for mesophilic and thermophilic

Attempts to secure thermophilic sludge were not successful. As a result, the anaerobically digested sludge (ADS) collected from the secondary digester at Stratford's wastewater treatment plant (London, Ontario, Canada) that operated under mesophilic conditions and was used for both AnFBRs. This sludge was used as seed sludge for the start-up of AnFBRs, operated under both mesophilic and thermophilic conditions. The total suspended solids (TSS) and volatile suspended solids (VSS) concentrations of the ADS were 28.9 and 16.8 g/L, respectively.

Table 3.2 shows various characteristics of the seed sludge, measured three times to ensure accuracy.

Table 3.2 Seed sludge characteristics for mesophilic and thermophilic experiment

Parameter (g/L)	ADS (AV± SD)
TS	40 ± 0.5
VS	24 ± 0.5
TSS	28.9 ± 1.2
VSS	16.8 ± 0.6
TCOD	28 ± 0.5
SCOD (mg/L)	560 ± 5
VFAs (mg/L)	40 ± 1
Total Carbohydrates (mg/L)	5,500 ± 55
Soluble Carbohydrates (mg/L)	68 ± 2
pH	8.3
Alkalinity (mg/L) as $CaCO_3$	6,050 ± 300

3.4 Sludge characterization for vomitoxin experiment

The ADS was collected from the secondary anaerobic digester at the Chatham wastewater treatment plant (Ontario, Canada). Table 3.3 lists the various characteristics of the secondary sludge, measured three times.

Table 3.3 Seed sludge characteristics for vomitoxin experiment

Parameter (g/L)	ADS (AV± SD)
TS	24.9 ± 850
VS	13.2 ± 140
TSS	24.3 ± 635
VSS	13.1 ± 495
TCOD	20.4 ± 395
SCOD (mg/L)	265 ± 5
VFAs (mg/L)	45 ± 2
Total Carbohydrates (mg/L)	5,500 ± 5
Soluble Carbohydrates (mg/L)	75 ± 5
pH	7.12
Alkalinity (mg/L) as $CaCO_3$	3,825±155

3.5 Wastewater feed and trace element solution

Simulated thin stillage was used as wastewater in the experiment. The characteristics of the simulated thin stillage and trace elements are shown in Table 3.4.

Table 3.4 Composition of synthetic wastewater

[a] Feed Comp.	CH_3COOH (mL/ L_F)	[*]UREA (g / L_F)	K_2HPO_4 (g/L_F)	$MgSO_4.7H_2O$ (g/L_F)	$CaCl_2.2H_2O$ (g/L_F)	$NaHCO_3$ (g/L_F)	Trace element (mL/L_F)
Concentration	9.5	0.27	0.1	0.03	0.03	6.2	1

Trace element Comp.(mg/L)	$FeCl.4H_2O$	$MnCl_2.4H_2O$	H_3BO_3	$ZnCl_2$	$CuCl_2$	$AlCl_3$	$CoCl_2.6H_2O$	$NiCl_2$
Conc.	2000	500	50	50	50	50	50	50

[a] All the above reagents were ACS Grade, 99.5 % min.

Note: The present study used UREA instead of NH4Cl for nitrogen sources.

3.6 Liquified corn (substrate) characterization

Liquified corn waste was collected from Greenfield Global (Chatham, Ontario, Canada) and used as the substrate to evaluate the methane production rates. Table 3.5 lists the various characteristics of the liquified corn waste from the analysis, done in triplicate.

Table 3.5 Liquified corn waste characteristics

Parameter (mg/L)	Av ± SD
TCOD	389,435 ± 3,100
SCOD	273,465 ± 2,385
TSS	374,300 ± 4,670
VSS	365,750 ± 4,600
TS	378,850 ± 21,000
VS	369,500 ± 20,505
PO4	1,850 ± 75
NH3-N	165 ± 7
pH	4.45 ± 0.03
Alkalinity ($CaCO_3$)	NM

3.7 Batch experiment

Vomitoxin was purchased from Toronto Research Chemicals (Ontario, Canada) (25 mg) in order to analyze the degradability of artificially contaminated liquified corn with Vomitoxin (DON). The vomitoxin was prepared at 392 ppm DON in de-ionized water (19.6 mg Vomitoxin dissolved in 50 mL de-ionized water). Eight bottles were spiked with 1, 5, 10, and 20 ppm of vomitoxin solution (duplicated) (see Figure 3.2).

Figure 3.2 Batch reactors preparation.

Batch anaerobic studies were conducted in bottles with a liquid volume of 100 mL and a head space volume of 50 mL. Experiments were done in duplicate for F/M of 1 $TCOD_{Sustrate} / VSS_{seed}$.

$$\frac{F}{M} = \frac{TCOD_{feed} * V_{feed}}{VSS_{seed} * V_{seed}} \qquad \text{Equation (3.1.7)}$$

$\frac{F}{M}$	Food to microorganism's ratio
V_{feed}	Volume of Liquified corn
V_{seed}	Volume of sludge
$TCOD_{feed}$	Liquified corn TCOD
VSS_{seed}	Sludge VSS

V_{feed} = 5 ml

V_{seed} = 145 ml

14 batch reactors were prepared:

- Blank

- Control
- Liquified corn
- Liquified corn + 1 PPM DON
- Liquified corn + 5 PPM DON
- Liquified corn + 10 PPM DON
- Liquified corn + 20 PPM DON

All samples were duplicated.

The DON contaminated solution was spiked. See details in Table 3.6.

Table 3.6 DON contaminated solution spiked volume table

DON concentration in batches (150 mL)	DON solution added (mL)
1 PPM	0.38 mL
5 PPM	1.9 mL
10 PPM	3.8 mL
20 PPM	7.6 mL

The Vomitoxin contribution to the COD for different batches were calculated. As noted in Table 3.7, there was a negligible COD contribution from DON for batches observed (less than 6 mg/L).

Table 3.7 Vomitoxin contribution to the COD

DON concentration in batches	COD contribution
1 PPM	0.28 mg/150 mL
5 PPM	1.38 mg/150 mL
10 PPM	2.75 mg/150 mL
20 PPM	5.5 mg/150 mL

3.8 BMP (Biomethane Potential) Design

Fifty milliliter samples of the mixtures were collected initially. The head space was flushed with oxygen-free nitrogen gas for a period of 60 s and capped tightly with rubber stoppers.

The bottles were then placed in their designated place in the Thermo Scientific shaker that was operated at 175 rpm and maintained at 37 ºC. Two blank bottles were prepared using ADS without liquified corn waste, and two control bottles were prepared using ADS and starch (see Table 3.8).All Don contaminated bottles were spiked with artificial DON(1, 5, 10 and 20 PPM).There was a negligible COD contribution from DON for batches observed (less than 6 mg/L).Substrates volume for all bottles were the same 5 mL.

Final samples were taken at the end of the batch experiments; the initial pH value for the mixed solution in each bottle was adjusted using a NaOH solution and the pH ranged from 7 to 7.3. Biogas production was measured daily using suitably sized glass syringes in the range of 20 to 100 mL. The methane content in the biogas was determined by injecting 0.5 mL of the biogas into a gas chromatograph model SRI 8610C (SRIGC, Torrance, California, United States) equipped with a thermal conductivity detector (TCD).

Table 3.8 Batches design for biomethane production

		Batch design for biomethane production from contaminated liquified corn			
Batch name	Substrate	F/M (g COD/g VSS)	DON concentration (PPM)	Sludge volume (ml)	Substrate volume (ml)
Blank	deionized water		0	145	5
Control	deionized water & starch	1	0	145	1.8 (g) + 5
Liquified corn	liquified corn	1	0	145	5
Liquified corn & DON	liquified corn	1	1	145	5
Liquified corn & DON	liquified corn	1	5	145	5
Liquified corn & DON	liquified corn	1	10	145	5
Liquified corn & DON	liquified corn	1	20	145	5

3.9 Analytical methods

Biogas production was measured by glass syringes on a daily basis. Cumulative volumes of biogas and methane produced are presented in Figures 4.21 and 4.22. TCOD, SCOD, NH3-N, and PO43- were measured according to HACH methods and testing kits (TNT plus 872, TNTplus 870, TNT AmVer, and COD high range digestion vials, respectively). (HACH Chemical Co, Loveland, Colorado, United States). In addition, the TSS and VSS concentrations were measured using standard methods (APHA, 1995), while soluble parameters were analyzed after filtering the samples using 0.45 μm filter paper. Tables 4.14 and 4.15 list the sample characteristics at both the initial and final steps. All of the steps were done three times.

3.10 Carrier media

3.10.1 Zeolite

Zeolite particles with an average diameter (dm) of 425–825 μm were used in the reactors. Zeolite was obtained from Bear River Zeolite Inc. (Preston, Idaho, United States). Zeolite characteristics were determined as follows: total porosity (ψ_T) of 61% (44% external and 17% internal); a dry bulk particle density (ρ_{mt}) of 885 kg $/m^3$; and a true particle density of (ρ_{mt}) of 2360 kg $/m^3$.

3.10.2 Plastic

The plastic medium was provided by Greenfield Global (Toronto, Ontario, Canada), and it had the following characteristics: size ranging from 425 to 825 μm, a total porosity (ψ_T) of 47% (40% external and 7% internal); a dry bulk particle density (ρ_{mt}) of 719 kg/m^3; and a true particle density (ρ_{mt}) of 1363 kg $/m^3$.

3.11 Analytical methods

3.11.1 General

3.11.1.1 Reagents

Except where otherwise stated, all chemicals used were of laboratory grade and were obtained from HACH (London, Ontario, United States) and VWR International LLC (Mississauga, Ontario, Canada).

3.11.2 Gravimetric analysis

The TSS/VSS and TS/VS determination was based on Standard Method 2540 G (APHA, 1998). Liquid samples were filtered using 1.2 μm filter paper. The filter paper was dried at 550°C for 15 min before using. The filter paper was dried in oven for 50–60 min at 105°C. The retained mass of the filter paper was measured for the difference between the weights of filter paper after drying and before sample filtration. This procedure yielded the amount of total suspended solids (TSS). The filter paper was then ignited at 550 ºC in the oven for 15–20 min. The mass lost as a result of the ignition indicates the amount of volatile suspended solid (VSS). The TS/VS were prepared in a similar manner as the TSS and VSS, except that the samples were not filtered.

3.11.3 Chemical and electrochemical analysis

3.11.3.1 pH

For all of the reactors, the pH was measured directly after sampling using a pH electrode and the Oaklon pH meter (Standard Methods for the Examination of Water and Wastewater [APHA et al., 2005]). The electrode was calibrated for pH buffers 4 and 7 on a daily basis. The electrode was rinsed, blotted dry, and placed into a beaker, the sample was directly discharged into the beaker, and the reading was recorded. The pH value was used as an indicator of the reactor's performance and internal environmental conditions.

3.11.3.2 Alkalinity

The following HACH kits were used for alkalinity: Alkalinity (Total) TNT plus, range (25–400 mg/L $CaCO_3$). The samples were filtered through 0.45 μm filter paper and diluted with distilled water (DW) if needed, in accordance with the HACH TNT870 (HACH Odyssey DR/2500) procedure.

3.11.3.3 Nitrogen ammonia

HACH kits for the Nitrogen-Ammonia Reagent Set were used for the sample. The sample was filtered through 0.45 μm filter paper and diluted with DW if needed, following the HACH procedure.

3.11.3.4 COD (Chemical Oxygen Demand)

High range HACH kits (0–1,500 mg/L) were used. For TCOD, samples were analyzed as is, and for SCOD, samples were filtrated through 0.45 μm filter paper and diluted with DW if needed, following the HACH procedure.

3.11.3.5 Total volatile fatty acid (VFAs)

HACH kits TNT872 (50–2500 mg/L) were used. Samples were filtrated through 0.45 μm filter paper and diluted with DW if needed, following the HACH procedure.

3.12 Biofilm analysis

3.12.1 Biofilm thickness measurement

Biomass attached to media periodically and were taken from the reactor. The biofilm analysis samples were sent to the Integrated Sustainable and Environmental Services (ISES) at York University for biofilm thickness measurement. The biofilm thickness results have been presented in Appendix .

3.12.2 Scanning electron microscopy

Scanning electron microscopy (SEM) tests also were performed by external provider.

3.13 Gas production

The biogas produced in the anaerobic column was measured by a wet tip gas meter (Wet-tip Gas Meter Company, Nashville, TN, USA) connected to the top of the anaerobic column (see Figure 3.3).

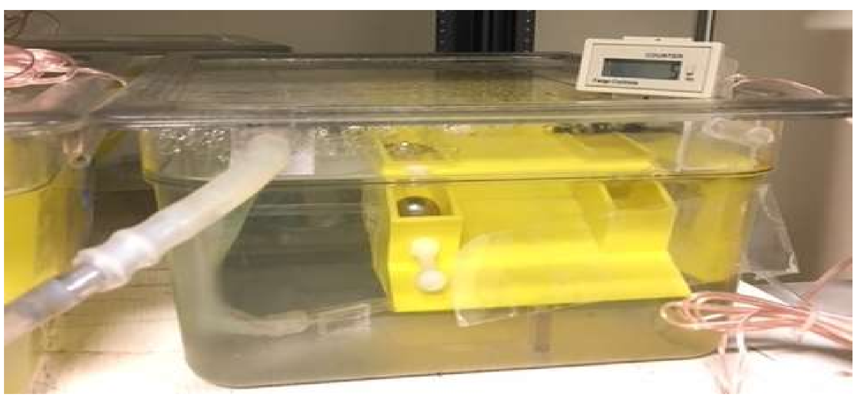


Figure 3.3 Wet tip gas meter.

3.13.1 Gas composition

The methane content in the biogas was determined by injecting 0.5 ml of the biogas into a gas chromatograph model SRI 8610C (SRIGC, Torrance, California, United States) equipped with a thermal conductivity detector (TCD) (see Figure 3.4).

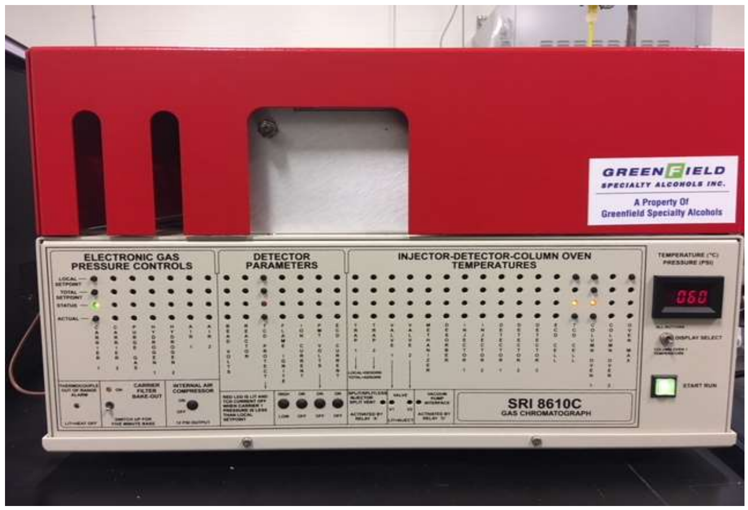


Figure 3.4 Gas chromatograph SRI 8610C and gas analysis experiment

3.14 Reactors operational parameters

The AnFBRs were operated in a batch, in semi-continuous and continuous modes. For the semi-continuous and continuous modes the reactors were fed daily at pre-determined time intervals, with a specific amount of synthetic waste. The digestate was removed to maintain a constant volume in the reactor. The organic loading rate (OLR) was determined according to Equation (3.1).

$$OLR = \frac{TCOD_{feed} * Q_{feed}}{V_{reactor}} \text{ ....Equation (3.1)}$$

Where:

$TCOD_{feed}$ Concentration of feed (g/L),

Q_{feed} is the rate of feed (L/day),

V reactor is the volume of reactor (l)

The retention time is related to the flow rate and the volume of the reactors and is associated with the microbial growth rate, which depends on the process temperature, OLR, and substrate composition. Two significant types of retention time are discussed.

The SRT is defined as the average time that bacteria (solids) have spent in a digester, and Hydraulic Retention Time (HRT) which is defined by the volume of a reactor with the flow rate (Ekama GA,1983).

The Hydraulic Retention Time (HRT) of the digester is expressed in equation (3.2) where:

$$HRT = \frac{V_{reactor}}{Q_{feed}} \quad \text{……….} \qquad \text{Equation (3.2)}$$

Where:

V reactor is the working volume of each reactor (ml),

Q_{feed} is the daily flow of material (substrate added) through the reactor (L/day),

The SRT is determined using equation 3.3:

$$SRT = (Vreactor \ * VSS_{inside\ reactor})/(Q_{eff} * \ VSS_{eff}) \quad \text{…………} \ \text{Equation (3.3)}$$

$$VSS_{inside\ reactor} = VSS_{attached\ on\ media} + VSS_{reactor\ sloution} \quad \text{………..} \ \text{Equation (3.4)}$$

3.15 Sources of error

The experiments done in this study may have been affected by both method-related and random errors. All the experiments were run in the presence of blanks. Standards were also run with other samples to ensure the accuracy of the machines' settings and results. All experiments were run three times, unless otherwise stated, to reduce human error. All graphs show the standard deviation as error bars. Those graphs in which error bars cannot be seen are hidden by the icons (with a standard deviation of less than 1%). Equipment such as pipettes, balance, and pH meter were calibrated before use.

CHAPTER 4

RESULTS AND DISCUSSION

4. Results and Discussion

4.1 Influence of particle properties on biofilm structure and operational parameters

The supporting carrier particles' characteristics, i.e., size, shape, density, porosity, roughness, and surface area, play an important role in the attachment and detachment rate of the biofilm as well as in the performance of the digestion process. In addition, the bioparticles can significantly affect capital investment and operational cost (Tang and Fan, 1989). Different carrier particles have already been tested in anoxic/anaerobic fluidized bed bioreactors such as sand, sepiolite, pumice stone, zeolite, lava rock, quartzite, alumina, resin, arlita, and kaolinite bead (Hao-Ran et al., 1983, Jeris, 1983; Rockey and Forster, 1983; Balaguer et al., 1997b, Chowdhury et al., 2009).

Based on a pilot plant experiment run by Greenfield Global (Chatham, Ontario, Canada), two different media of mineral (zeolite) and synthetic (high density polyethylene, HDPE as plastic) were run in the lab-scaled fluidized bed. This was done to aid in the comparison of the biomass attachment and the operational parameters in the treatment of simulated thin stillage.

4.1.1 Start-up period

The AnFBRs were inoculated with the secondary digester sludge from Stratford's Wastewater Treatment Plant (London, Ontario, Canada). The seed was pumped into the system and recirculated in the column for a day to transport and trap the bacteria from the bulk liquid on the medium's surface and its pores. The purpose of the start-up phase was to operate the zeolite and plastic media in a semi-continuous mode under similar operating conditions (same HRT) and identical feed composition to establish biofilm growth. During the start-up phase, the two reactors were fed in a semi-continuous mode and closely monitored for important parameters such as pH, alkalinity, volatile fatty acid (VFA), and chemical oxygen demand (COD) removal. Both reactors showed very similar trends of COD removal during the first 20 days of semi-continuous operation, which could be attributed to the high rate of biomass attachment to the media evident from the concentration of suspended solids of 1150 ± 75 mg/L in the effluent and the complete degradation of COD that was fed. There was limited change in the pH in either the zeolite or the plastic medium reactors.

Within a period of three months, most of the particles in both columns were coated with almost the same amount of attached biomass, with average concentrations of 10.2 ± 2.6 mg VSS/g and 10.9 ± 1.7 mg VSS/g media in the zeolite and plastic columns, respectively. The porous media are expected to be more conducive for biomass attachment compared to smooth particles. However, in the start-up phase of a fluidized bed bioreactor, fluidization hydrodynamics (velocities) are less favorable due to the brittleness and high shear forces created by particle attrition. During the steady state, however, porosity has a negligible effect on the bio-particle surface area and performance.

The steady-state pH value of 8 ± 0.45 for the zeolite medium were very similar to 8 ± 0.25 found in the plastic medium reactor. The values were within the optimum pH range of 6.5–8.3 reported in many studies of simulated thin stillage (Jun, et al., 2009). A pH of 7.8 ± 0.2 was maintained in the bioreactor during the experiment.

Total VFAs, measuring less than 1000 mg/L, was observed between Day 0 and Day 90 for both the zeolite and plastic media reactors, which could be attributed to the different pH and alkalinity values in the start-up of the reactor. This would indicate that the methanogens were not influenced by such fluctuations in VFA levels and reactor conditions, and that the established microbial community was able to withstand some variations in reactor conditions during the start-up period without major changes in methanogens profiles. Wagner et al. (2011) recognized that an increase of VFAs was observed when feeding occurred during semi-continuous feeding period. Between Day 60 and Day 70, the organic loading rate (OLR) was increased from 2 to 2.5 g COD/L/day for the reactor with plastic medium; this loading could have been more than of the methanogenesis bacteria's capability and, consequently, VFAs accumulated. The VFAs would gradually increase to levels of 760 to 935 mg/L.

COD removal rates in both reactors also showed similar trends during most of the start-up phase (Figure 4.1 and Figure 4.2). A COD removal of 96% ± 2% was achieved in a semi-continuous mode during a steady state condition in both reactors. COD removal in the reactor with the plastic medium showed instability when the OLR was increased to 2.5 kg COD/m^3/day, and its removal dropped to around 70%. Hence, the feeding rate was then resumed at OLR 2 kg COD/m^3/day. As a result, COD removal increased to 97%. In the reactor containing the plastic medium, bicarbonate alkalinity gradually increased from 9,100 mg/L to 13,600 mg/L between Day 35 and Day 70. After this, the values stabilized and reached a steady-state value of 12,600 ± 450 mg/L between Day 74 and Day 90. This was an indication of the high buffering capacity in the reactor and low VFAs effluent concentrations.

A steady state was assumed to be achieved when the changes in the operational parameters of the reactors were within ±5% of the average value for three consecutive feeding cycles. The zeolite medium reactor also showed a gradual increase of alkalinity from 7,700 to 10,300 mg/L between Day 31 and Day 55. Following this, a decline occurred and a steady-state value of 8,100 ± 280 mg/L was achieved from Day 71 to Day 90. This rise in alkalinity could also be attributed to the buffer capacity introduced into the feed by the sodium bicarbonate. To enhance the buffering capacity and to maintain the optimum targeted pH value of 7.8 ± 0.2, more alkalinity, in the form of $NaHCO_3$, was added to the influent for both zeolite and plastic media reactors from Day 31 to Day 55 and Day 35 to Day 70, respectively.

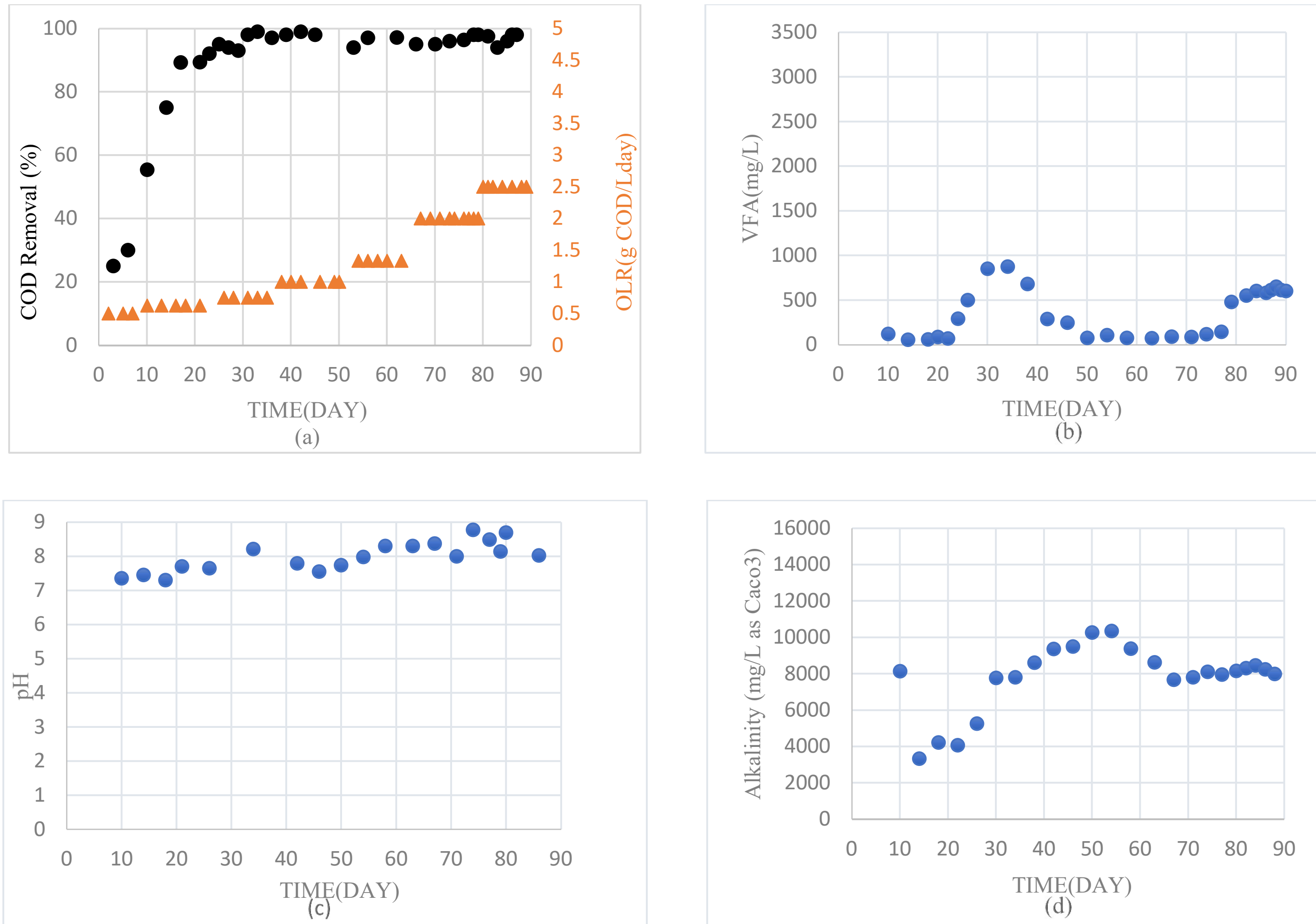


Figure 4.1 Variation of pH, COD removal, OLR, and alkalinity with time during the start-up phase for the zeolite media reactor.

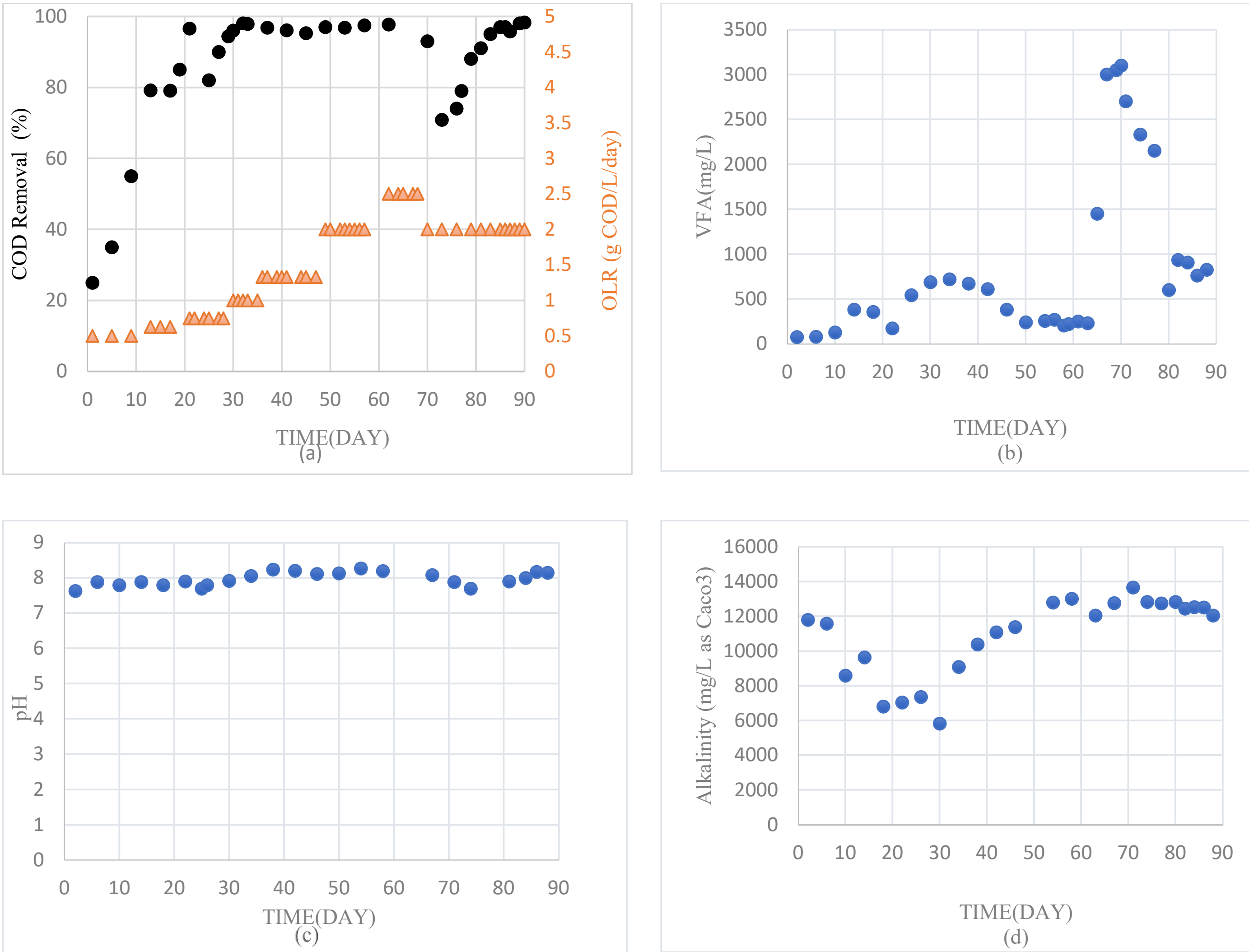


Figure 4.2 Variation of pH, COD removal, OLR, and alkalinity by change in time during the start-up phase for plastic media reactor.

4.1.2 Operational parameters for continuously fed Reactors

4.1.2.1 Media characteristic and biomass yield

The biomass yield was calculated as the sum of the net change in the attached biomass. Samples of the media from the reactors were taken at OLR operational stage and were then sent for biofilm analysis at the external provider. (Integrated Sustainable and Environmental Services (ISES, York University, Toronto, Ontario, Canada)). As is seen in

Figures 4.3, Z1–Z5, the biofilm thickness for the zeolite medium increased from 100 to 370 µm, while in Figure 4.4 P1–P5, for the plastic medium, the increase was from 40 to 200 µm. However, the final biofilm analysis demonstrated detachment. For the zeolite medium, the biomass attachment decreased from 33.6 to 23.6 mg VSS/g of media when the biofilm thickness declined from 370 to 300 µm (Figure 4.3). Detachment for the plastic medium was also observed and it showed a reduction from 19.7 to 14.7 mg VSS/g of media when the biofilm thickness declined from 200 to 140 µm (Figure 4.4).

The final phase in the biofilm's growth included: detachment; effect of shedding of cells; changes in the environment; grazing (bacteria consumption of the outer surface of the biofilm); sloughing (large patch loss); erosion (liquid shear stress of the biofilm surface); and abrasion (collision of particles). Similar results were observed by other researchers (Bryers and Charachlis, 1990).

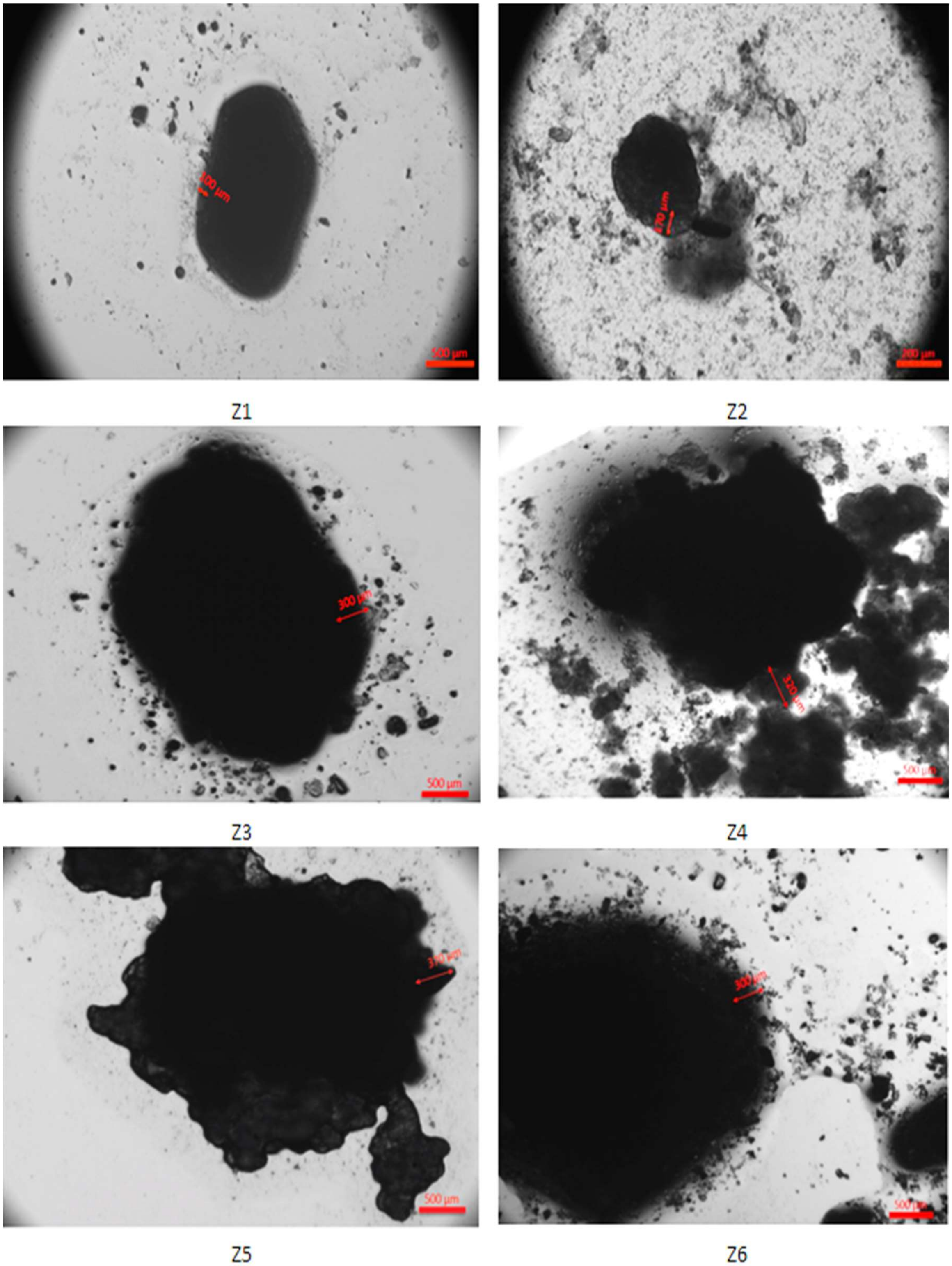


Figure 4.3 Picture of attached biomass on zeolite by Scanning electron microscope (SEM)

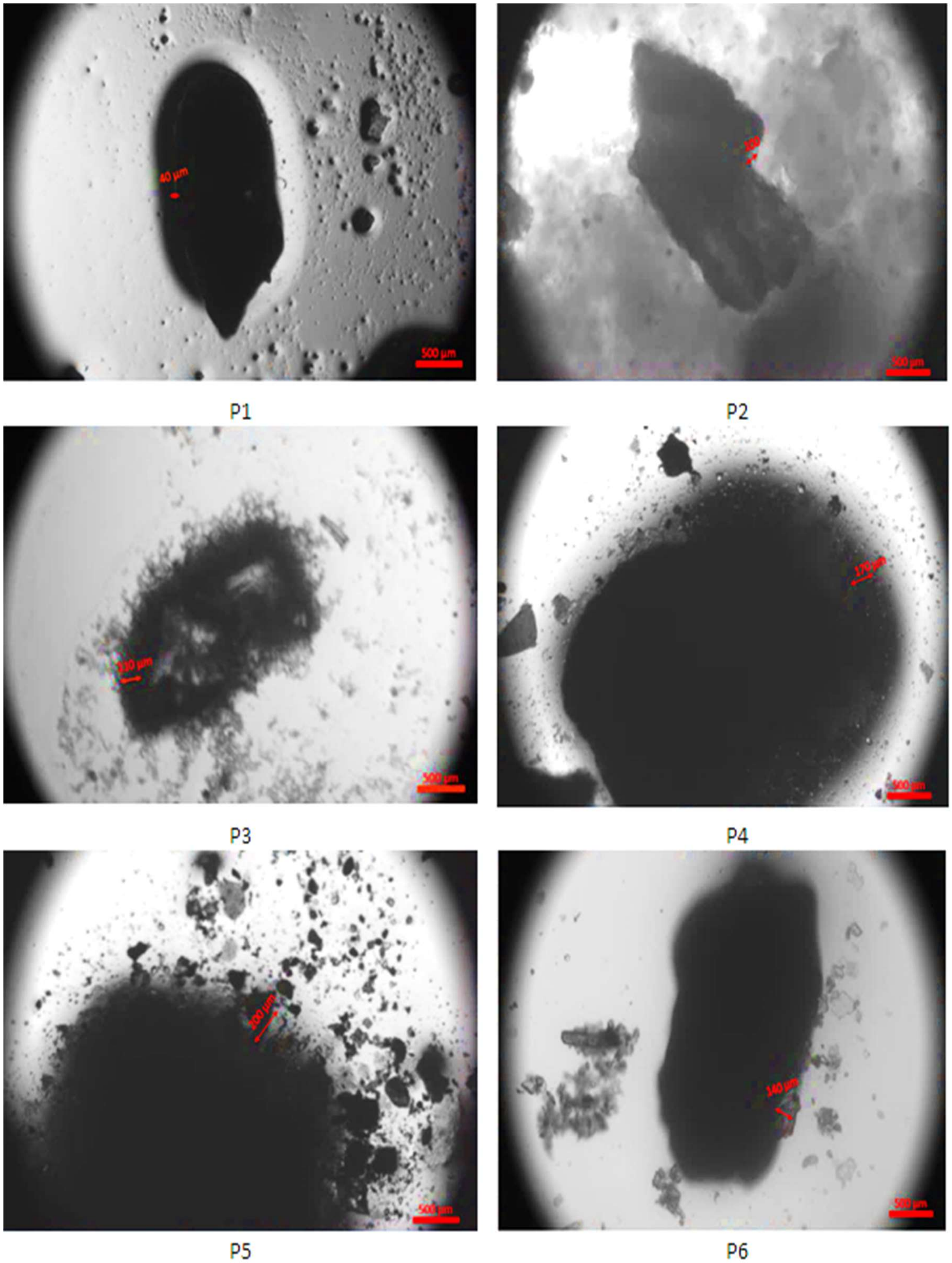


Figure 4.4 Picture of attached biomass on plastic media by Scanning electron microscope (SEM)

The most important aspect of AnFBRs is the formation and ty of the biomass film on the carrier medium. Biofilm growth mainly relies on the carrier medium characteristics, such as particle size, sphericity, porosity, density, and specific surface area (SSA) (Eldyasti et al., 2012). Based on the external provider reports, the average attached biomass per gram of zeolite medium sharply increased from 10.2, to 33.6 mg VSS/g media, while a gradual increase of 10.9, to19.7 mg VSS/g media was reported for the plastic medium in the period of operation (Figure 4.5 and Figure 4.6).

The zeolite medium reactor showed a capacity to function with increased OLR from 20 to more than 25 g COD/L/day. The hydraulic retention time (HRT) of 1.6 days and the biofilm attachment of 33.6 mg VSS/g media were observed. The plastic medium could not operate with more than 20 g COD/L/day with an HRT of 2 days and a biofilm attachment of 19.7 mg VSS/g media under stable conditions. The reactor with plastic medium is not suitable for the treatment of simulated thin stillage. The zeolite medium could reach stable conditions with 25 g COD/L/day, which could be attributed to a more attached biomass on the media.

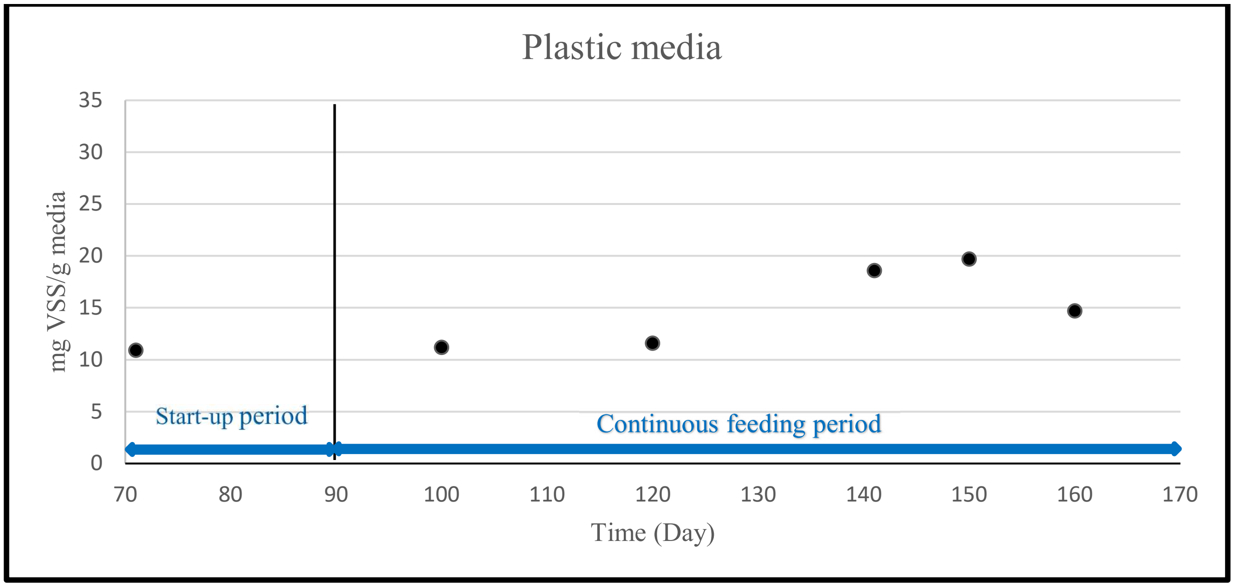


Figure 4.5 Plastic media's average attached VSS inventory.

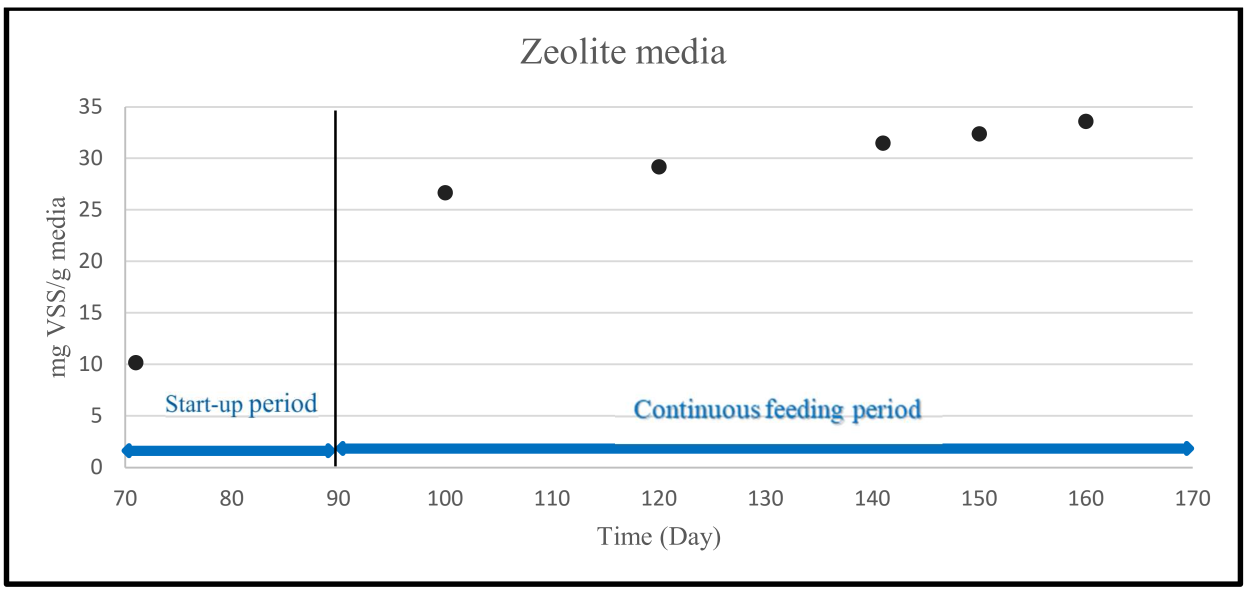


Figure 4.6 The zeolite media's average attached VSS inventory.

4.1.2.2 Process evaluation

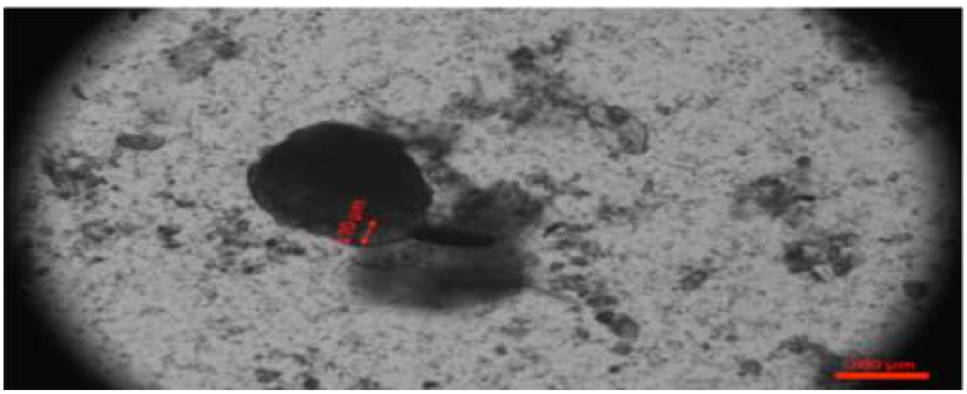
Both reactors were started in a semi-continuous mode during the start-up period and then were switched to the continuous mode after. In order to ensure the attainment of steady-state conditions in the reactors, the COD removal percentage and VFAs were measured. The coefficient of variation (COV) for COD removal percentage in the start-up period has been presented earlier (Figures 4.1.a, and 4.2.a). Also, variation of the VFAs' level in the effluent was within the standard deviation (SD) of less than 10% for the start-up period before changing the reactors into the continuous mode.

Both reactors were subjected to a loading of 5 g COD/L/day in the continuous mode at the beginning. Following this, the loading was increased to 10, 16, 20, and 25 g COD/L/day. Based on different HRT results for both reactors (presented in the following sections), the zeolite medium reactor showed more effective operation and better results than the plastic medium in terms of operational parameters. On the other hand, the maintenance and replacement cost of natural zeolite could be significant considering the different replacement frequencies involved, i.e., every 1 year, 5 years, and 10 years, as reported by Eldyasti et al. (2012).

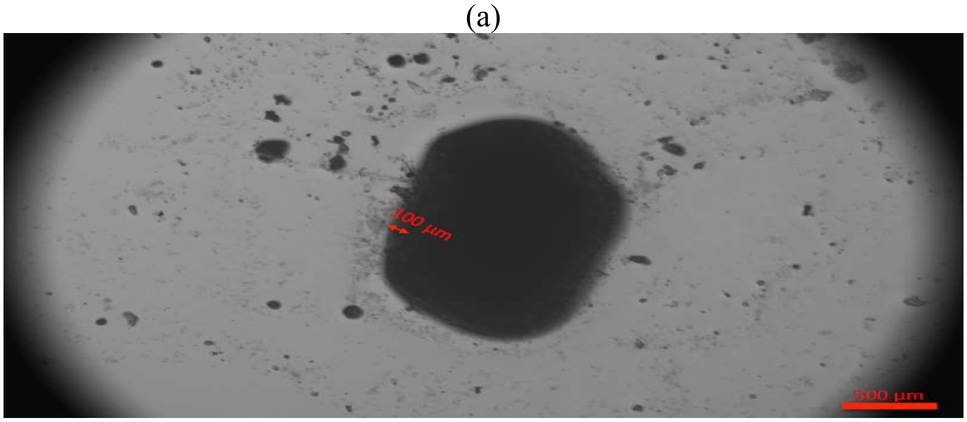
4.1.2.2.1 HRT of 8 days

After the system had been run for a time approximately up to that of the longer HRT (40 days), in semi-continuous mode, the reactors were fed with OLR 5 g COD/L/day with an HRT of 8 days. Based on the system's configuration and previous studies (Andalib et al., 2012), operation changed to continuous mode with HRT of 8 days. The feed flow rate was 250 mL/d for a period of almost one month. The feed was composed of 40 g COD/L_{feed} and 4 mL/L_{feed} macro-elements and trace elements, respectively.

The biofilm thickness was 170 µm and 100 µm for zeolite and plastic media, respectively (Figure 4.4.1). Attached biomass average results showed significant differences, from 10.2 to 26.7 mg VSS/g media for zeolite. A slight increase of biomass, from 10.9 to 11.2 mg VSS/g media for the plastic medium was observed. According to the attachment biomass average results and the effluent VSS value on Day 119 (470 mg/L and 580 mg/L for zeolite and plastic media), the SRT has been calculated. The steady-state SRT based on VSS were 76 days and 26 days for zeolite and plastic media, respectively. The longer SRT for the zeolite confirmed the improvement in the biofilm attachment.



(a)



(b)

Figure 4.4.1. Picture of attached biomass on zeolite (a)and plastic (b)media by Scanning electron microscope (SEM) at HRT 8 days.

To enhance the buffering capacity and to maintain the optimum targeted pH value of 7.0 ± 0.2, 13.5 g/L of $NaHCO_3$ was added. During the operation with the HRT of 8 days, limited changes were observed in the reactors' effluent pH (~8.45 and ~8.25 for the zeolite and plastic media, respectively). During this period, all the operational parameters remained unchanged compared with the start-up phase, confirming that the system was stable under continuous mode.

As shown in Figure 4.7(b), for the plastic medium reactor, the concentration of alkalinity decreased from 11,200 to 8,920 mg/L between Day 90 and Day 110, while at the same time, the concentration of the VFAs also decreased from 2,900 mg/L to 2,450 mg/L. However, between Days of 90 to 93 VFAs went up to 3500 mg/L duo to operational problem (Recirculation pump was broken).

Alkalinity for the zeolite medium reactor showed an increase from ~8,000 to ~9,800 mg/L, which could be attributed to the generation of alkalinity and the buffer capacity introduced into the feed by the sodium bicarbonate.

The theoretical methane yield at standard temperature and pressure (STP, 0 ºC, and 1 atm) is 0.35 mL/mg COD digested, which corresponds to 0.4 mL/mg COD digested at the operational temperature of 37 ºC (Metcalf & Eddy, 2003).

The zeolite medium showed a COD removal of 89% and methane production of approximately 3.3 L/day, while 72% COD removal and 2.5 L/day were reported for the plastic medium.

For the zeolite medium reactor, the methane yield approached a maximum value of 368 mL CH_4/g COD removed, while the overall COD removal efficiency was 94%. For the plastic medium reactor, the methane yield approached a maximum value of 306 mL CH_4/g COD removed, while the overall COD removal efficiency was 91%; this result was in accordance with those of previous studies (Andalib et al., 2014).

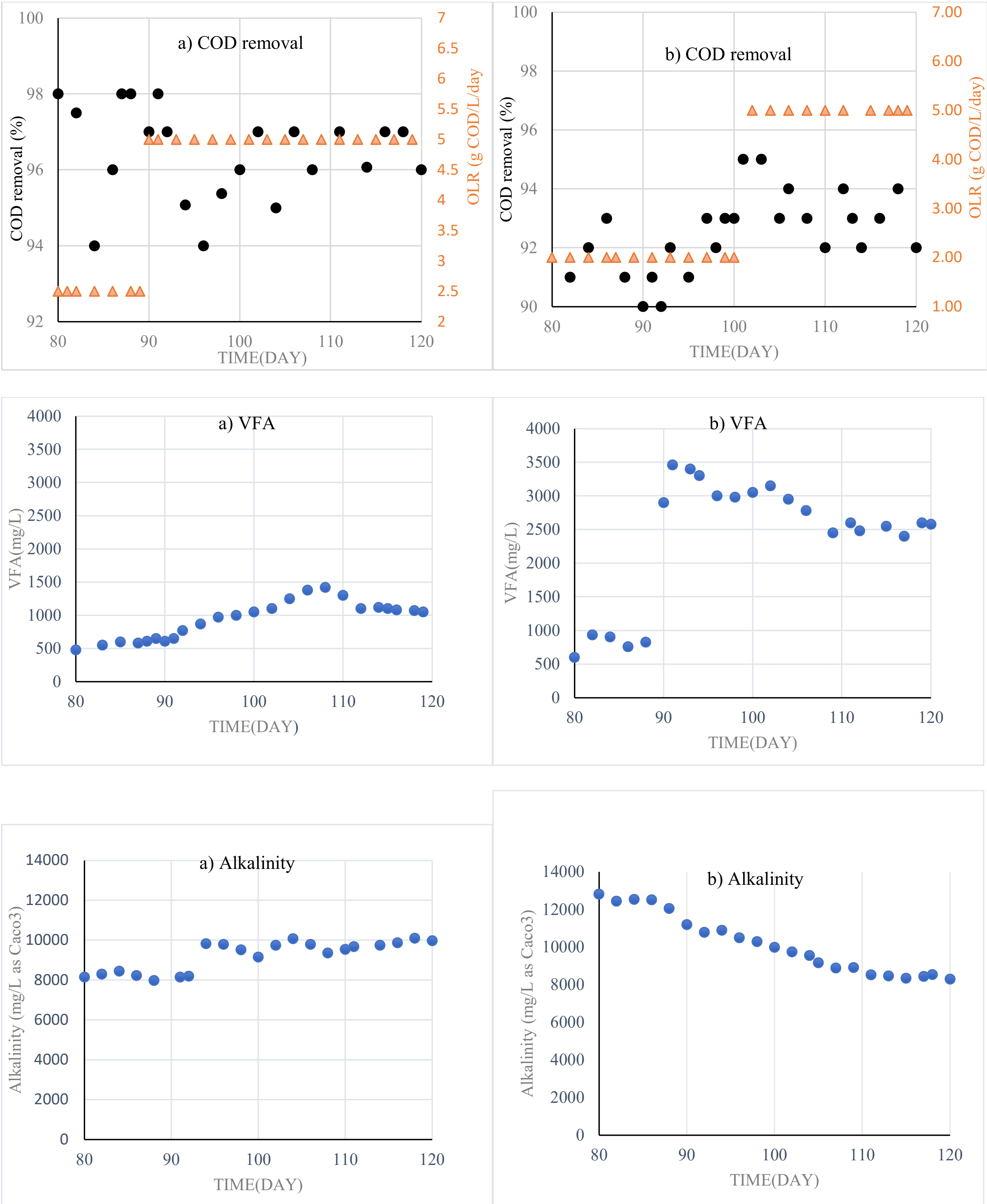


Figure 4.7 Variation of COD removal, OLR, VFAs, and bi-carbonate alkalinity with time during the operation at HRT = 8 days: (a) Zeolite media reactor; (b) Plastic media reactor.

Other operational parameters for the steady-state condition are presented in Table 4.2.

Table 4.2 Steady-state operation parameters for zeolite and plastic media reactors (HRT of 8 days)

	Unit	Zeolite media	Plastic media
Volumetric biogas production	mL/d	6,994 ± 320	5,380 ± 250
Volumetric methane production	mL/d	3,287 ± 180	2,530 ± 140
$COD_{removal}$	%	89 ± 3	72 ± 3
VFAs	mg/L	1,070 ± 20	2,620 ± 115
Alkalinity	mg $CaCO_3$/L	9,800 ± 280	8,250 ± 100
pH	-	8.45 ± 0.15	8.25 ± 0.1

4.1.2.2.2 HRT of 4 days

A shorter HRT is desirable, as it is directly related to the reduction of capital cost and the increase of process efficiency. Both reactors exhibited stable operation at HRT of 8 days. To compare the performance of this study with that of Andalib et al (2014), in this phase, HRT was reduced to 4 days. The reactors were fed with OLR 10 g COD/L/day; the feed flow rate was 500 mL/d; the feed was composed of 40 g COD/L_{feed} and 4 mL/L_{feed} macro-elements and trace elements, respectively. To enhance the buffering capacity and to maintain the optimum targeted pH value of 7.0 ± 0.2 (based on the first phase results [HRT of 8 days]), 11.5 g/L of $NaHCO_3$ was added to the system.

Biofilm thickness showed an increase of around 76 % for the zeolite medium, from 170 to 300 μm. For the plastic medium, biofilm thickness increased from 100 to 110 μm. This was 10 % higher than the value reported earlier, with HRT of 8 days. The average attached biomass for the zeolite medium analysis showed an increase from 26.7 to 29.2 mg VSS/g media, while the plastic medium showed a slight increase from 11.2 to 11.6 mg VSS/g media. That may be attributed to the total porosity and specific surface area of the zeolite. For the zeolite medium reactor, the methane yield approached a maximum value of 390 mL CH_4/g COD removed, while the overall COD removal efficiency of 92% was achieved; this result was in accordance with previous work (Nasr et al., 2012). For the plastic medium reactor, the methane yield for the reactor approached a maximum value of 360 mL CH_4/g COD removed, while the overall removal efficiency for the COD was 87%; similar results have been reported by Nasr et al. (2012).

Estimated SRT for the zeolite media showed an increase by more than 15% from Days 76 to 87. While SRT for the plastic media was increased from 26 to 27 days. The effluent VSS values of 420 ± 20 mg/L and 300 ± 15 mg/L for the zeolite and plastic were observed on Day 130 until Day 140, respectively. The increase in SRT for the zeolite media might be attributed to more biomass attachment compared with slightly increase in biomass for the plastic media. As the SRT increases so does the treatment efficiency because the microbial population has more time to develop and mature. The active biomass retained within the system is closely related to an anaerobic digester's treatment efficiency Uyanik et al., (2002).

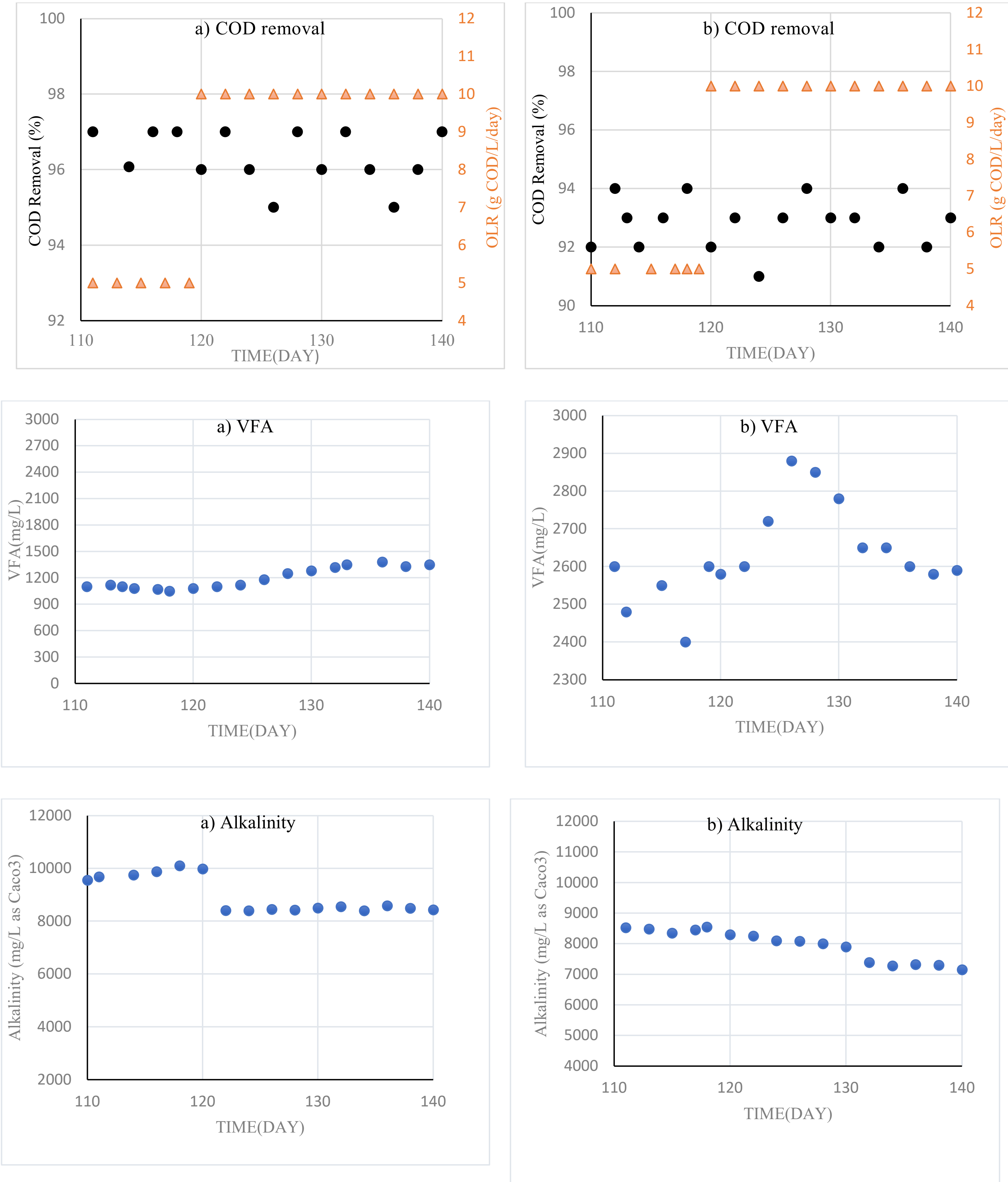


Figure 4.8 Variation of COD removal, OLR, VFAs, and bi-carbonate alkalinity with time during the operation at HRT = 4 days: (a) Zeolite media reactor; (b) Plastic media reactor.

Other operational parameters for the steady-state conditions are presented in Table 4.3.

Table 4.3 HRT of 4 days with steady-state operation parameters for zeolite and plastic media reactors

	Unit	Zeolite media	Plastic media
Volumetric biogas production	mL/d	14,432 ± 580	13,520 ± 380
Volumetric methane production	mL /d	6,780 ± 420	6,350 ± 410
$COD_{removal}$	%	92±2	87 ± 3
VFAs	mg/L	1,380 ± 95	2,580 ± 85
Alkalinity	$mgCaCO_3/L$	8,300 ± 310	7,050 ± 250
pH	-	8.35 ± 0.10	8.15 ± 0.15

No significant change was observed in COD removal compared with an HRT of 8 days. The VFAs for the zeolite medium had slight increase from 1,070 to 1,380 mg/L, while for the plastic medium reactor, no appreciable changes were observed, and the values were 2,620 and 2,580 mg/L, respectively (Figure 4.8). A reduction in alkalinity was observed from 9,800 to 8,300 mg/L and 8,250 to 7,050 mg/L for the zeolite and plastic media, respectively (Figure 4.8).

At the HRT of 8 days, the reactors' effluent pH was slightly higher than that with the lower HRTs of 4 days, with 8.45, 8.35, for the zeolite and 8.25, and 8.15 for the plastic media reactors, respectively. This may have been due to the reduction from 13.5 g/L to 11.5 g/L,in $NaHCO_3$ in the feed solution.

4.1.2.2.3 HRT of 2.5 days

Based on the results and system stability at HRT of 4 days, it was decided to decrease the HRT while progressively increasing the OLR. In this phase, the HRT was reduced to 2.5 days. The reactors were fed with OLR 16 g COD/L/day. The feed flow rate was 800 mL/d. The feed composition was 40 g COD/L_{feed} and 4 mL/L_{feed} macro-elements and trace elements, respectively. No other conditions were changed compared with the previous step. To enhance the buffering capacity and to maintain the optimum targeted pH value of 7.0 ± 0.2 and the system's pervious performance, 10 g/L of $NaHCO_3$ was added.

The biofilm thickness analysis results on Day 145 showed an increase of greater than 75% for the zeolite with 320 µm, while for plastic media an increase of 70 % biofilm thickness with 170 µm was observed. For the average attached biomass for the zeolite medium, the analysis demonstrated a gradual increase from 29.2 to 31.5 mg VSS/g media, while the plastic medium showed an increase from 11.6 to 18.6 mg VSS/g media. This may be attributed to the total porosity and specific surface area as well as biofilm formation on the plastic media. Similar results were observed elsewhere, and porosity was determined as an important factor in the adhesion of microorganisms (A.P et al., 2001).

The average VSS in the effluent for both reactors between Day 135 and Day 140 was 320 ± 10 and 230 ± 8 mg/L for the zeolite and plastic media, respectively. This decrease in VSS may be attributed to the synthetic feed and an increase in the biomass attachment, compared with the trials using an HRT of 4 days. Similar results have been reported by Wang et al. (2014).

The pH in both reactors decreased slightly, which may be due to the decline in buffering capacity due to the reduced level of $NaHCO_3$, from 11.5 g/L to 10 g/L. No considerable variation in VFAs concentration for the zeolite medium reactor was observed, and the value just changed from 1,380 to 1,495 mg/L. The plastic medium showed a decline in VFAs, and VFAs decreased from 2,580 mg/L to 2,350 mg/L and then remained unchanged. This could be attributed to the extra biomass generation on the media, which may have consumed all of the VFAs (Figure 4.9).

For the zeolite medium reactor, the methane yield approached a maximum value of 390 mL CH_4/g COD removed, while achieving overall COD removal efficiency of 95%; For the plastic medium reactor, the methane yield for the reactor approached a maximum value of 381 mL CH4/g COD removed, while achieving an overall COD removal efficiency of 92%. Similar results were obtained elsewhere. Nasr et al. (2014) reported methane yields based on COD removed of 321, 333, and 317 mL CH4/g COD removed for the methanogenic batches.

Estimated SRT for the zeolite media showed more than 3 % increase from 87 to 90 days. SRT for the plastic media also had more than 29% increase and reached 35 days. Increasing the SRT allows slow growing bacteria to become more enriched and increases the diversity of the biological community Clara et al., (2005). Increasing the diversity of microorganisms also increases the physiological capabilities of the wastewater treatment technology. The average VSS in the effluent for both reactors between Days 145 and 150 was 320 ± 10 and 230 ± 8 mg/L for the zeolite and plastic media, respectively. This may be attributed to feeding synthetic feed and increase in the biomass attachment.

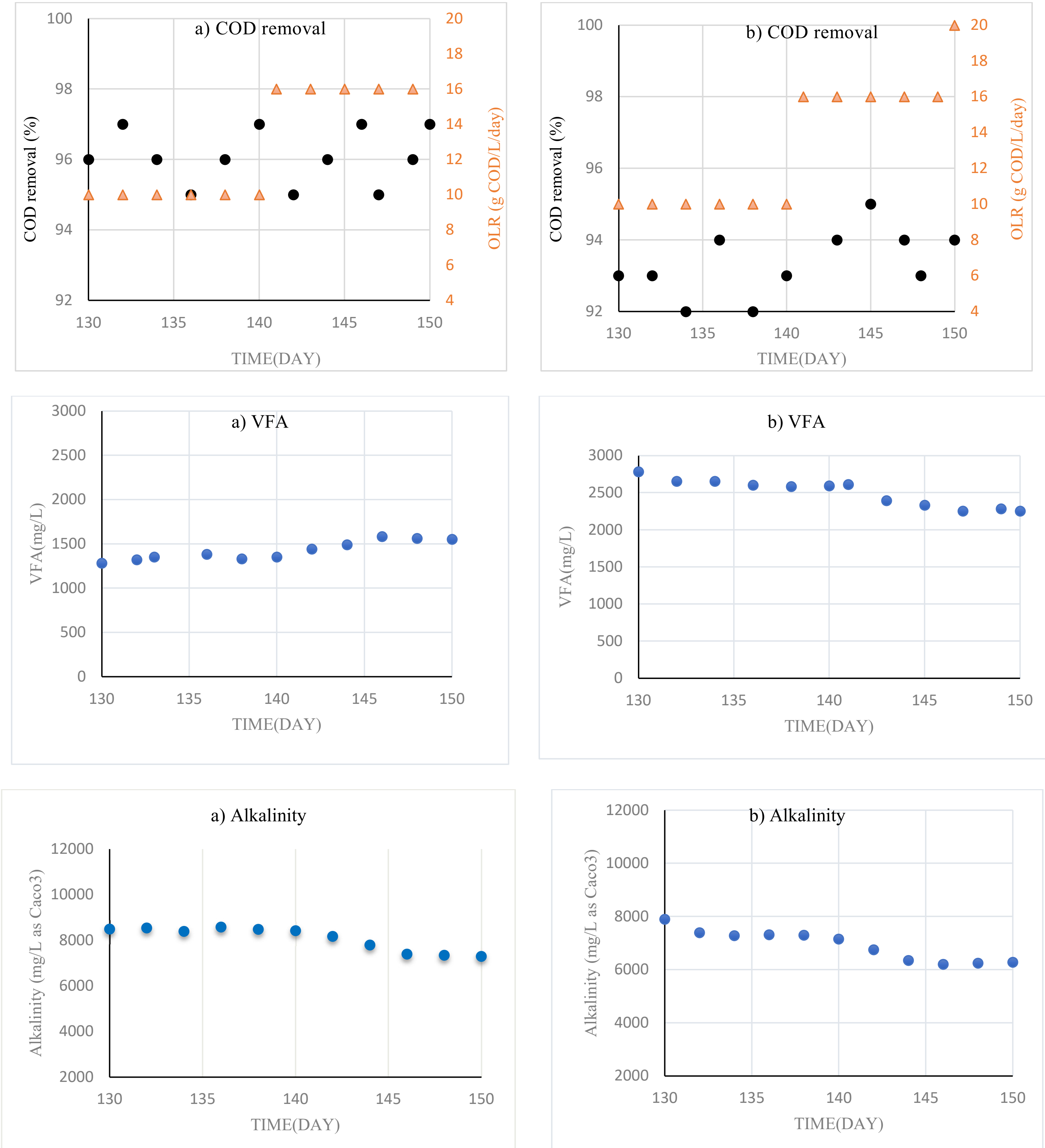


Figure 4.9 Variation of COD removal, OLR, VFAs, and bi-carbonate alkalinity with time during the operation at HRT = 2.5 days: (a) Zeolite media reactor; (b) Plastic media reactor.

Other operational parameters for steady-state conditions are presented in Table 4.4.

Table 4.4 HRT of 2.5 days with steady-state operation parameters for zeolite and plastic media reactors

	Unit	Zeolite media	Plastic media
Volumetric biogas production	mL/d	24,016 ± 790	22,280 ± 630
Volumetric methane production	mL/d	11,280 ± 560	10,450 ± 400
$COD_{removal}$	%	95 ± 2	92 ± 2
VFAs	mg/L	1,495 ± 80	2,350 ± 130
Alkalinity	$mgCaCO_3/L$	7,745 ± 435	6,365 ± 198
pH	-	8.15 ± 0.10	8.05 ± 0.15

4.1.2.2.4 HRT of 2 days

Following the positive results from the previous phase of the study, the HRT was reduced to 2 days. The reactors were fed with OLR 20 g COD/L/day and the feed flow rate was 1000 mL/d. Feed composition was 40 g COD/L_{feed} and 4 mL/L_{feed} macro-elements and trace elements, respectively. Based on the results from the previous step and the stability in pH and alkalinity values, this time around, 9 g/L $NaHCO_3$ was added for buffering.

Biofilm thickness showed an increase of 30 µm for both zeolite and plastic media, compared with the previous phase (HRT of 2.5 days). The average attached biomass showed a gradual increase from 31.5 to 32.4 and 18.6 to 19.7 mg VSS/g media on Day 150 for the zeolite and plastic media, respectively.

The VSS values for both reactors decreased and reached a steady state at 210 ± 15 mg/L and 170 ± 10 mg/L between Day 140 and Day 150 for the zeolite and plastic media, respectively. The drop in concentrations might be the result of the increased biomass attachment on the media and decreased VSS value in the effluent.

The pH in both reactors decreased from 10 g/L to 9 g/L of $NaHCO_3$, which may be due to the reduction in buffering capacity, for the zeolite media, VFAs decreased from 1,495 mg/L and reached the steady-state value of 1,265 ± 220 mg/L on Day 160. Plastic media also demonstrated a decrease from 2,350 and reached the steady sate at 1,490 ± 260 mg/L on Day 160 (Figure 4.10).

For the zeolite media reactor, the methane yield for the reactor approached the maximum value of 388 mL CH_4/g COD removed, while achieving an overall COD removal efficiency of 96%. For the plastic media reactor, the methane yield for the reactor approached a maximum value of 387 mL CH_4/g COD removed, while achieving an overall COD removal efficiency of 94%. Similar results were observed elsewhere, and the influent OLR was held initially at 6.11 g of COD/L/d with the constant HRT of 2.0 days (Sowmeyan, 2008). Estimated SRT for the zeolite media showed an increase from 90 days at HRT of 2.5 days to 97 days. Plastic media also showed an increase from 35 days to 40 days, even though feed flow rate increased from 800 ml/day to 1000 ml/day. That can be attributed to increased biomass attachment on the media and decreased VSS value in the effluents. A long SRT protects against a loss in digestion efficiency caused by fluctuations in temperature, potential inhibitory compounds, and slowly degradable compounds.VSS value for both reactors decreased and reached a steady state at 210±15 mg/L and 170±10 mg/L between Days 155 and 160 for the zeolite and plastic media, respectively.

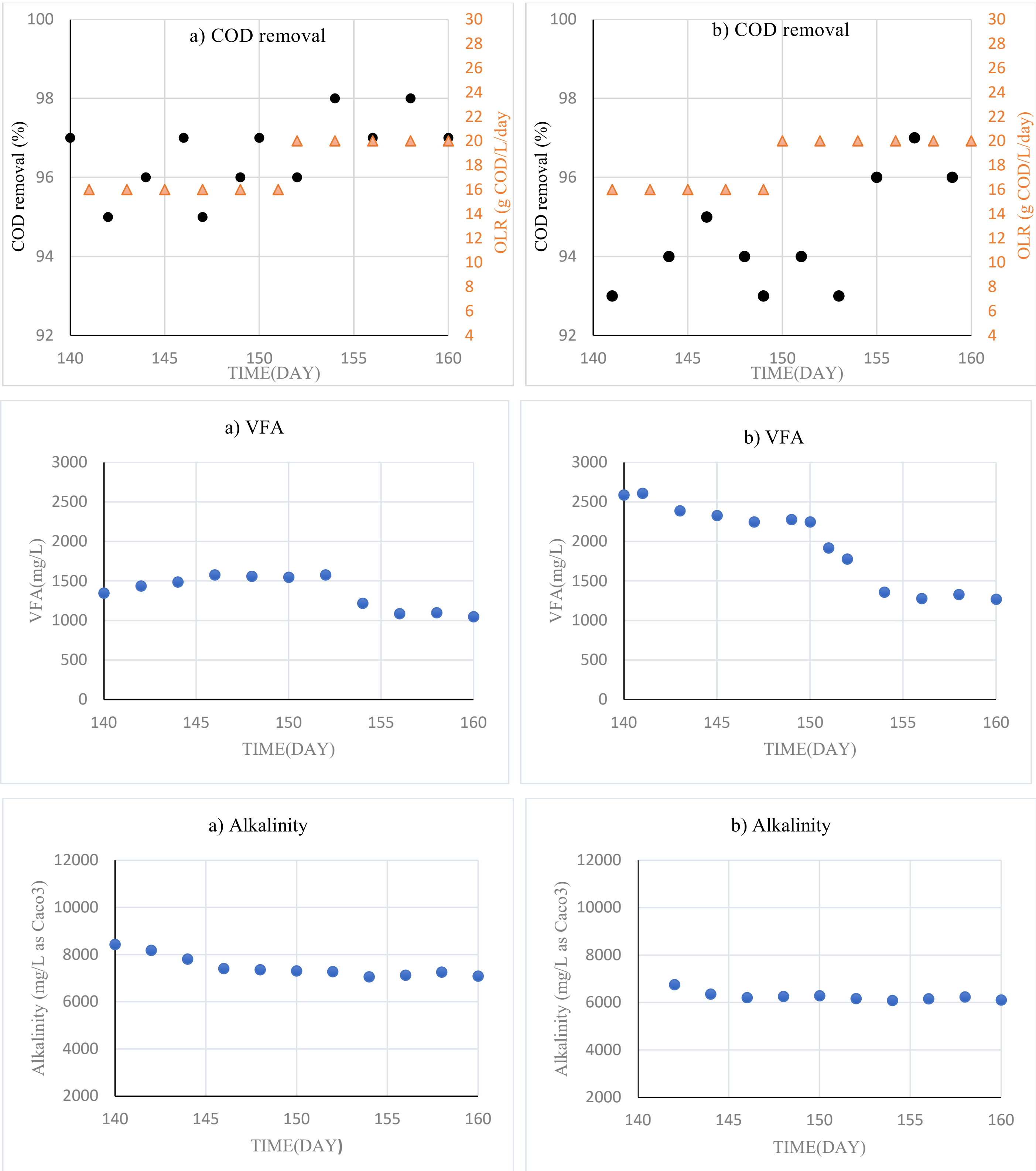


Figure 4.10 Variation of COD removal, OLR, VFAs, and bi-carbonate alkalinity by change in time during the operation at HRT = 2 days: (a) Zeolite media reactor; (b) Plastic media reactor.

Other operational parameters for the steady-state condition are presented in Table 4.4.

Table 4.5 HRT of 2 days with steady-state operation parameters for zeolite and plastic media reactors

	Unit	Zeolite media	Plastic media
Volumetric biogas production	mL/d	29,430 ± 1050	28,970 ± 870
Volumetric methane production	mL/d	14,810 ± 480	16,370 ± 510
$COD_{removal}$	%	96 ± 3	94 ± 2
VFAs	mg/L	1,265 ± 220	1,490 ± 260
Alkalinity	$mgCaCO_3/L$	7,150 ± 90	6,145 ± 55
pH	-	8.00 ± 0.15	7.90 ± 0.10

4.1.2.2.5 HRT of 1.6 days

The HRT was further reduced to 1.6 days to identify the maximum capacity of the systems. Reactors were fed with OLR of 25 g COD/L/day at a feed flow rate of 1,250 mL/d. The feed composition was kept the same with 40 g COD/L_{feed} and 4 mL/L_{feed} macro-elements and trace elements, respectively. To enhance the buffering capacity and to maintain the optimum targeted pH value of 7.0 ± 0.2 and the system's pervious performance, the addition of 9 g/L (as $NaHCO_3$) was kept constant.

Biofilm thickness showed an increase of 20 µm for the zeolite, from 350 to 370 µm, while some detachment happened for the plastic media and biofilm thickness declined from 200 µm to 140 µm compared with the results using an HRT of 2 days. This may be attributed to both abrasion and sloughing, which may have impacted biofilm detachment. For the zeolite media reactor, average attached biomass showed a gradual increase from 32.4 to

33.6 mg VSS/g media. A decline for the average attached biomass for the plastic media was observed and attachment decreased from 19.7 mg to 14.7 mg VSS/g media. The VSS value sharply increased from 170 mg/L to 4,150 mg/L. This can be attributed to biomass detachment from the plastic media.

For the zeolite media reactor, the methane yield for the reactor approached a maximum value of 383 mL CH_4/g COD removed, while achieving an overall COD removal efficiency of 98%. The methane yield for the plastic reactor approached a maximum value of 313 mL CH_4/g COD removed, while achieving overall COD removal efficiency of 89%.

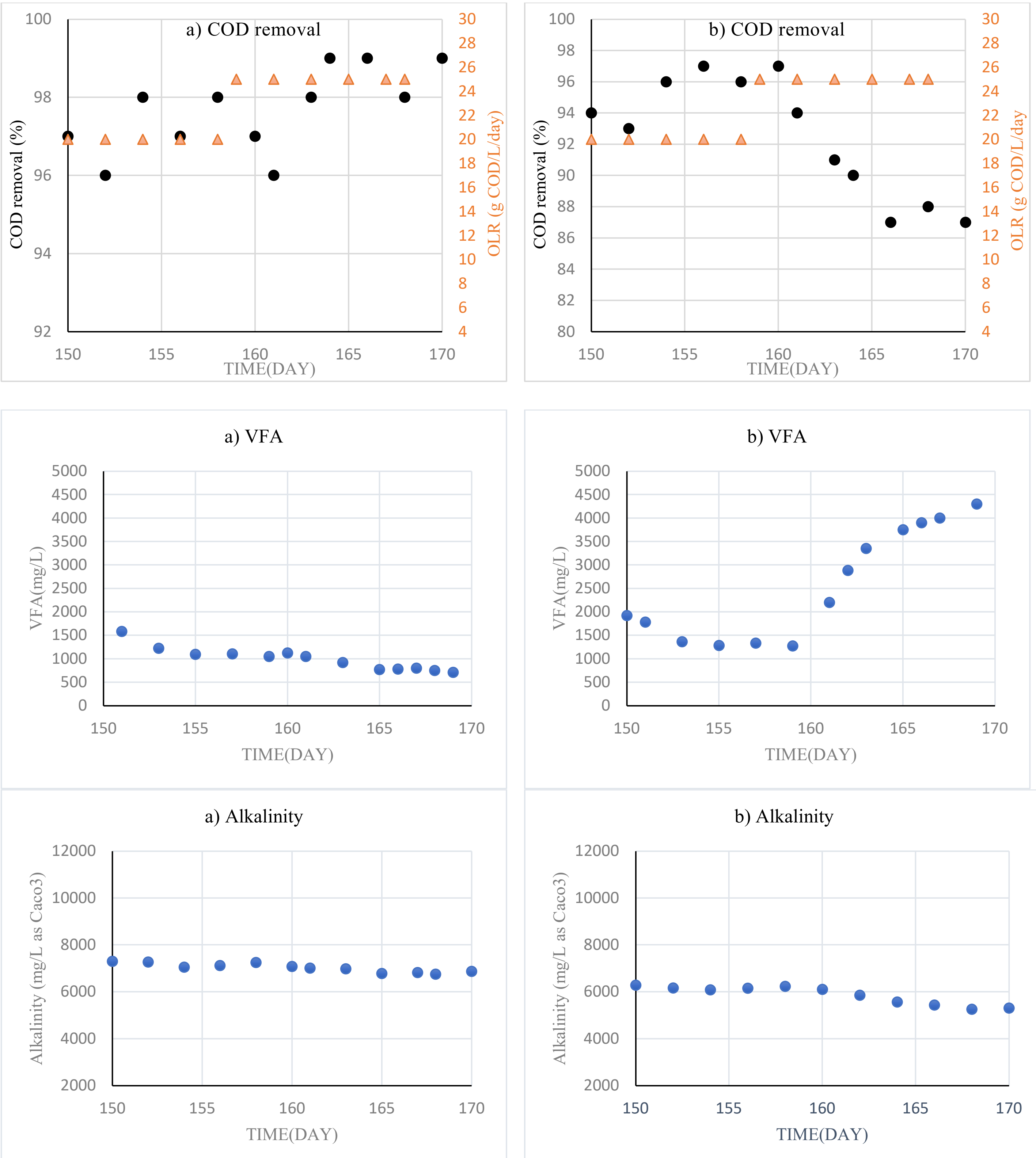


Figure 4.11 Variation of COD removal, OLR, VFAs, and bi-carbonate alkalinity by change in time during the operation at HRT = 1.6 days: (a) Zeolite media reactor; (b) Plastic media reactor.

Other operational parameters for the steady-state condition are presented in Table 4.6.

Table 4.6 HRT of 1.6 days with steady-state operation parameters for zeolite and plastic media rectors

	Unit	Zeolite media	Plastic media
Volumetric biogas production	mL/d	35,405 ± 1280	28,020 ± 760
Volumetric methane production	mL/d	18,665 ± 680	14,120 ± 290
$COD_{removal}$	%	98 ± 3	89 ± 2
VFAs	mg/L	860 ± 140	3,485 ± 380
Alkalinity	$mgCaCO_3/L$	6,870 ± 100	5,480 ± 215
pH	-	7.95 ± 0.10	7.35 ± 0.10

The level of the VFAs for the zeolite media reactor gradually decreased from 1,265 mg/L and reached a steady-state condition at 860 ± 140 mg/L, indicating stable digestion performance. On the other hand, the VFAs in the plastic media reactor showed an increase and reached 3,485 ± 380 mg/L. This can be attributed to the biomass detachment and reduction in the biofilm thickness.

The pH in the zeolite reactor did not show a considerable change and was stable at 7.95 ± 0.10. A decline of the pH in the plastic media was observed and the value reached to 7.35 ± 0.10 under steady-state condition. This may be the effect of biomass detachment and an increase in the level of VFAs in the reactor. Other operational parameters for the steady-state condition are presented in Table 4.6.

SRTs of 76, 87, 90, 97, and 111 days were calculated for the zeolite media. SRTs showed a continuous increase from 76 to 87, 90, 97, and 111 days, which can be attributed to the biomass attachment from 26.7 to 29.2, 31.5, 32.4, and 33.6 mg VSS/g media, respectively. However, Q_{eff}s was increased from 0.25 to 0.5, 0.8, 1, and 1.25 L/day, respectively.

SRTs of 26, 27, 35, and 40 days were observed for the plastic media. SRT increased from 26 to 27 days, by small increasing in biomass attachment from 11.2 to 11.6 mg VSS/g media. However, Q_{eff}s increased from 250 mL/day to 500 mL/day. SRT again showed an increase from 27 to 35 days, because biomass attachment per gram media increased slightly from 11.6 to 18.6 mg VSS/g media, even though Q_{eff}s was increased from 250 mg/L to 800 mg/L. Reactor demonstrated another step of SRT increase from 35 to 40 days, while biomass attachment increased from 18.6 and 19.7 mg VSS/g media. Meanwhile, Q_{eff}s was increased from 800 to 1000 mL/day. VSS value in the effluent was around 230 and 170 mg/L, respectively. Detachment happened when Q_{eff}s was 1.25 L/day, biomass attachment decreased from 19.7 to 14.7mg VSS/g media, and VSS in the effluent was around 2000 mg/L. Overall SRT for the MX, MB, NZ, and LR reactors was estimated on 18, 42, 50, and 24 days, respectively (Eldyasti et al., 2012)

Overall operational parameters are presented in Table 4.6.

Table 4.7 Zeolite and plastic media's operational parameters.

Media	Zeolite	Plastic	Zeolite	Plastic	Zeolite	Plastic	Zeolite	Plastic	Zeolite	Plastic
Biofilm thickness (µm)	170	100	300	110	320	170	350	200	370	140
Avg. attached biomass (mg VSS/g media)	26.7 ± 1.7	11.2 ± 0.4	29.2 ± 3.2	11.6 ± 2 .0	31.5 ± 2.9	18.6 ± 5.4	32.4 ± 6.2	19.7 ± 1.6	33.6 ± 4.2	14.7 ± 3.4
Influent flow, Qin (L/d)	0.25	0.25	0.5	0.5	0.8	0.8	1	1	1.25	1.25
Average organic loading (g COD/L/day)	5	5	10	10	16	16	20	20	25	25
HRT (day)	8	8	4	4	2.5	2.5	2	2	1.6	1.6
Estimated SRT (d)	76	26	87	27	90	35	97	40	111	1
Volumetric biogas production	6994 ± 320	5,380 ± 250	14,432 ± 580	13,520 ± 380	24,016 ± 790	22,280 ± 630	29,430 ± 1050	28,970 ± 870	35,405 ± 1,280	28,020 ± 760
Volumetric methane production	3,287 ± 180	2,530 ± 140	6,780 ± 420	6,350 ± 410	11,280 ± 560	10,450 ± 400	14,810 ± 480	13,670 ± 510	18,665 ± 680	14,120 ± 290
COD removal	89 ± 3	72 ± 3	92 ± 2	87 ± 3	95 ± 2	92 ± 2	96 ± 2	94 ± 1	98 ± 1	89 ± 3
Total VFAs (mg/L)	1,070 ± 20	2,620 ± 115	1,380 ± 95	2,580 ± 85	1,495 ± 80	2,350 ± 130	1,265 ± 220	1,490 ± 260	860 ± 140	3,485 ± 380

In the present experiment, the effect of HRT on the performance of anaerobic digestion, gas production rate, treatment efficiency, and COD removal, was evaluated for both the zeolite and the plastic media. As mentioned above, both reactors started in a semi-continuous mode before being switched to a continuous mode. The zeolite and plastic media were switched to the continuous mode starting at an HRT of 8 days. The reactor operating with the zeolite media was successful at the HRT of 1.6 days, while the plastic media reactor was just able to handle the HRT of 2 days with OLR equal to 20 (g COD/L/day).

COD balance across all experimental conditions were closed at an average of 99 ± 2% thus confirming the reliability of the data. However, for plastic media reactor at HRT 1.6 days, the COD balance closed on average at 84 ± 3 %. The drop in COD mass balance might be the result of that complete a perfect COD mass balance in the detachment condition is difficult.

The Summary of COD balance for zeolite and plastic media reactors at different HRTs are presented at Table 4.7.

Table 4.8 Summary of COD balance for zeolite and plastic media reactors at different HRTs.

HRT (day)	8		4		2.5		2		1.6	
Media	Zeolite	Plastic	Zeolite	Plastic	Zeolite	Plastic	Zeolite	Plastic	Zeolite	Plastic
$COD_{influent}$ (g COD/day)	10	10	20	20	30	30	40	40	50	50
$COD_{effluent}$ (g COD/day)	1.15 ± 0.07	2.83 ± 0.12	1.52 ± 0.09	2.79 ± 0.11	1.61 ± 0.08	2.54 ± 0.13	1.37 ± 0.23	1.61 ± 0.21	0.93 ± 0.14	5.18 ± 0.82
Cumulative CH_4 (mL/day)	3,285 ± 180	2,530 ± 140	6,780 ± 420	6,350 ± 410	11,280 ± 560	10,450 ± 400	14,810 ± 480	13,670 ± 510	18,665 ± 680	14,120 ± 290
[a] CH_4 (g COD)	8.35 ± 0.45	6.40 ± 0.35	17.15 ± 1.07	16.05 ± 1.04	29.35 ± 1.42	26.45 ± 1.03	36.95 ± 1.21	37.65 ± 1.29	47.5 ± 1.72	35.25 ± 0.73
[b] COD balance (%)	94 ±3	91 ±3	95 ±2	91 ±3	96 ±2	94 ±2	94 ±3	92 ±2	95 ±3	81 ±3

[a] Based on 0.395 L CH4 /g COD

[b] COD balance (%) = [CH4 (g COD) + COD effluent (g COD)] * 100/ [COD influent (g COD)]

4.1.2.3 Fluidization energy cost

The 700 g of the zeolite media provided 25% fixed bed height for the reactor. The effective height of reactor was 113 cm. The fixed bed height (25%) was 28 cm. The zeolite media reactor's recirculation flow was 1,272 L/day, corresponding to 120 revolutions per minute (RPM), equal to 172,800 R/day. According to the pump's manual (BT600-2J Longer Peristaltic pump), 6 rotations are equal to one-Watt energy consumption. Thus, 28.8 KWH/day of energy consumption with a unit cost of 0.1 $/KWH, shows 288 cents/day or $2.88/day.

Approximately 350 g of HDPE plastic media was needed to provide 25% of the fixed bed height. Recirculation flow for the plastic media reactor was 640 L/day, which corresponds to 60 RPM for the pump and 86,400 R/day. Thus, the energy consumption is 14.4 KWH/day, which with a unit cost of 0.1 $/KWH, represents 144 cents/day or $ 1.44/day. The fluidization energy costs were found by other researchers to be the dominant component, accounting for 76–97 % of the total annualized cost (Eldyasti et al., 2012). Compared with $2.88/day for the zeolite media, the plastic media needed $1.44/day (50% energy was needed compared to the zeolite reactor). Similar results were observed elsewhere (Eldyasti et al., 2012).

4.1.2.4 Up-flow velocity

The up-flow velocity of the AnFBR was controlled by maintaining liquid recirculation flow. The zeolite media reactor operated with a recirculation flow rate at 1,272 L/d corresponding to 0.8 cm/s superficial velocity. This flow provided a 25% bed expansion in the reactor. The bed expansion of 25% in the plastic media reactor was equal to running at 680 L/d as the recirculation flow rate. Superficial velocity with 680 L/d recirculation flow rate corresponded to 0.4 cm/sec. The same recirculation flow is supported by results from other studies for zeolite (Andalib et al, 2014). The liquid from the top of the reactor was recycled and pumped back into the reactor from the bottom to maintain the up-flow velocity at 0.8 cm/s as an energy saving solution (Wang, 2015). An issue was faced with the plastic media floating to the top of the reactor (Figure 4.12), even though the reactor was running at a very low recirculation flow rate.

Figure 4.12 Plastic media floating.

Therefore, for the plastic media, the up-flow velocity was reduced to 0.15 cm/s.

4.2 Comparing mesophilic and thermophilic digestion in the treatment of simulated thin stillage employing AnFBRs with plastic media (HDPE)

In this section, the adaptation of stable mesophilic reactors to thermophilic temperature (55^0C) was studied by applying single-step or multi-step increase in the temperature. The HRT and OLR were kept constant and the temperature was increased either stepwise with an increment not exceeding 6 degrees, or directly in a single step up to the desired temperature of 55ºC. The main objective of this section is to compare mesophilic and thermophilic ADs during the treatment of simulated thin stillage employing AnFBRs with plastic media (HDPE), and the impact on biogas and methane production. Detailed factors affecting biogas production, methane content, reactors, and AD processes are listed based on the operational parameters. The use of different media in anaerobic digestion employing FBR is not original. However, very few studies have been published that report the use of different media in a fluidized bed for the treatment of wastewater. Artificial, MX and MB, (Maxi-Blast Inc., Canada) and natural particles, NZ and LR, (Bear River Zeolite Inc., Preston, Idaho, United States) were used (Eldyasti, 2013). HDPE as a plastic support with true particle density (ρ_{mt}) of 1,363 Kg/m^3 has not been used in FBR for the treatment of simulated thin stillage under thermophilic conditions. Thus, it is interesting to compare the biogas and methane content produced with different OLRs and HRTs. In this section, as well, the effect of the operating temperature, either mesophilic or thermophilic, on the kinetics of the process is examined by fitting the experimental data of the treating simulated thin stillage and kinetic models.

4.2.1 Acclimatization, start-up, and reactor's operation

The FBR reactor was inoculated with secondary digester sludge from Stratford's wastewater treatment plant (London, Ontario, Canada) with TSS and VSS concentrations of 28.9 and 16.8 mg/L, respectively. The seed was pumped into the system and recirculated in the column for a day to transport and trap the bacteria from the bulk liquid onto the media surface and the pores. The start-up phase was planned to operate in a semi-continuous mode for 25 days. The feeding was conducted once every 4 days. Following this, there was a switch to operate for 16 days in a continuous mode. The reactor was monitored over the course of time to allow for reaching a similar performance or baseline for the monitored parameters (pH, alkalinity, VFAs, COD removal, biogas, and methane production). Continuous operation with HRTs of 16, 6, and 4 days was carried out to evaluate and compare the mesophilic and thermophilic process configurations for the treatment of simulated thin stillage.

4.2.2 Operating conditions for mesophilic reactor in the start-up phase

After recirculation of the seed for a day, the reactor started to operate in a semi-continuous mode and then operated in a continuous condition with an HRT of 16 days. The reactor was fed with 200 mL feed of simulated thin stillage (composition, synthetic waste, and trace elements were same as mentioned in Table 4.1) used in the semi-continuous mode, corresponding to 25.2 g COD per feed.

The reactor was fed every 4 days. After four days, on Day 5, the reactor sample was analyzed. Table 4.8 shows that during the start-up (semi-continuous) mode, the pH inside the reactor was between 7.18 and 7.60. In each feeding cycle, a decline in pH was observed after adding simulated thin stillage. And then, with degradation of VFAs in the reactor, the pH went up again. As depicted in Table 4.8, on Day 5, the feeding day, the pH was 7.5.

While feeding the reactor, the pH went down to 7.22 and gradually increased to 7.52 on Day 9. While feeding the reactor, on Day 9, the pH declined to 7.18, measured on Day 10, and gradually increased to 7.4 on Day 13. The differences in the reactor pH may be attributed to the addition of the alkalinity ($NaHCO_3$) present in the simulated thin stillage (15 and 20 g/batch on Day 5 and Day 9, respectively). Therefore, the alkalinity addition had a positive impact on pH buffering, to avoid the inhibition of methanogenesis. On Day 25, as the last day for the semi-continuous phase, results of pH and alkalinity were 7.60 and 7,200 ± 175 mg/L, respectively. Similar results have been reported by Lee (2011). This study found that the methanogenesis was most efficient at a pH of 6.5–8.2 and the optimal pH was 7.0 (Lee, 2011).

Table 4.9 Variation of pH, COD, VFAs, biogas production of pH, COD, VFAs, and biogas production in the start-up phase

Day	Biogas production (ml)	CH_4 Gas content (%)	pH	T (˚C)	TCOD (mg/L)	SCOD (mg/L)	TVFAs (mg/L)	TSS (mg/L)	VSS (mg/L)	Alkalinity (mg/L) as $CaCO_3$	TVFAs/ Alkalinity
1	800	50	7.71	36.6	20,100 ± 520	110 ± 30	70 ± 15	27,560 ± 685	14,200 ± 355	6,150 ± 155	0.01
2	5,340		7.30	35.6							
3	4,600		7.40	36.0							
4	4,720		7.45	37.0							
5	3,820	51	7.50	36.0	18,500 ± 645	220 ± 25	115 ± 5	26,050 ± 675	13,000 ± 310	5,800 ± 150	0.02
6	5,680		7.22	37.0							
7	5,100		7.24	37.0							
8	4,660		7.27	36.0							
9	3,800	51	7.52	37.5	17,000 ± 760	560 ± 10	450 ± 5	25,800 ± 585	12,800 ± 385	4,800 ± 140	0.01
10	5,080		7.18	36.5							
11	4,660		7.20	37.5							
12	4,175		7.25	36.5							
13	3,300	53	7.40	37.0	16,200 ± 645	860 ± 45	800 ± 60	24,750 ± 620	12,550 ± 500	6,800 ± 200	0.11
14	5,600		7.30	37.0							
15	4,700		7.45	37.5							
16	4,200		7.50	37.5							
17	2,300	54	7.55	37.0	15,900 ± 560	750 ± 50	625 ± 35	24,367 ± 680	11,100 ± 445	5,500 ± 165	0.10
18	5,900		7.40	37.6							
19	5,100		7.45	37.1							
20	2,300		7.50	37.2							
21	2,200	53	7.55	37.3	15,200 ± 730	840 ± 75	785 ± 40	22,433 ± 450	10,500 ± 315	7,400 ± 260	0.10
22	5,200		7.52	37.1							
23	4,900		7.42	37.3							
24	3,300		7.59	37.2							
25	2,000	52	7.6	37.1	15,350 ± 680	860 ± 60	820 ± 45	22,230 ± 510	10,450 ± 255	7,200 ± 175	0.10

For total VFAs and SCOD, fluctuation from ~115 to ~820 mg/L and ~220 to ~860 mg/L, respectively, was observed between Day 5 and Day 25. This may be attributed to the fact that biomass on the media was not developed during the start-up period, and all VFAs were not consumed.

The results between Days 5 to Day 25 are presented in Table 4.8. On Day 25, after four days of feeding (on Day 21), the reactor sample was analyzed, and consistent biogas production was observed (~17.2 L to ~17.8 L). Feeding was changed to the continues mode on Day 26 with HRT of 16 days.

The reactor was fed in continuous mode for approximately 40 days. The feeding rate was 440 mL/day. The corresponding OLR was 1.2 g COD/L/day. The level of VFAs gradually decreased from ~800 to ~280 mg/L between Day 26 and Day 62 (Figure 4.13a). This may be attributed to the fact that biomass generation on the media resulted in consuming all the VFAs that were produced by the faster growing acid forming bacteria at the beginning of reactor operation.

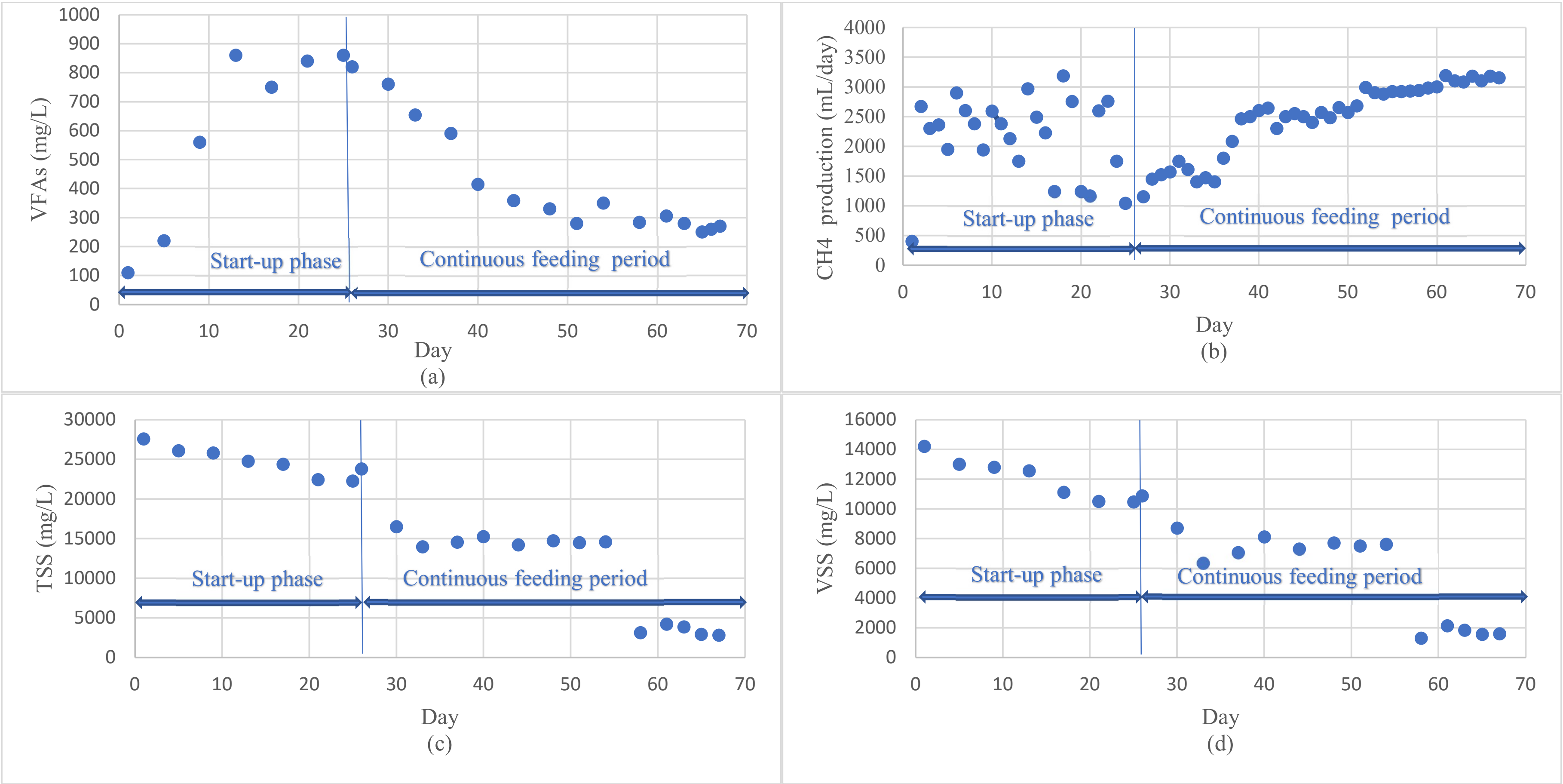


Figure 4.13 Variation of operational parameters for mesophilic reactor during the start-up phase.

The variation in methane production for the start-up phase on Day 52 until end of Day 62 was not significant. The variation reached values approximately similar to steady-state, at 2,965 ± 50 mL/day. Additionally, the methane yield for the reactor approached a maximum value of 390 mL CH_4/g COD removed, while achieving overall COD removal efficiency of 96%. Similar results have been reported by Andalib et al. (2012). Hafez (2009) also reported the methane yield for the biomethanator approached a maximum value of 426 mL CH_4/g COD, while achieving an overall COD removal efficiency of 94% in the CSTR. The variation of the effluent VFAs, TSS, VSS, and biogas production is presented in Figure 4.13.

The COD concentrations for the reactor showed a similar trend for the entire start-up phase, wherein initial COD concentrations for the reactor were approximately 19 g/L, which gradually dropped to approximately 735 mg/L on Day 52, corresponding to VFAs of ~300 mg/L (Figure 4.13a). VFAs and COD for the reactor entered a steady-state condition for the remainder of the start-up phase.

The TSS and VSS concentrations decreased gradually and reached steady-state values of 2,950 and 1,560 mg/L, respectively (Figure Figure 4.13c and Figure 4.13d). During the start-up phase, the TSS's reduction efficiency was 89%. This result was in accordance with those of previous studies Andalib et al. (2014). The AnFBR has been recently demonstrated for the digestion of primary sludges, as researched by Andalib et al. (2014), with the TSS destruction efficiency of 82% at the OLR of 9.5 g COD/L/day.

4.2.3 Operating conditions for mesophilic reactor in the continuous mode

Operational parameters such as OLR, HRT, pH, biogas, and CH4 production, VFAs/alkalinity ratio, COD removal, and C/N ratio were monitored for the mesophilic reactor. These parameters and their effects are examined in the following discussion. In the continuous mode, feed of the reactor was modified and the HRTs were at 16, 6, and 4 days, respectively. Accordingly, 40 g COD/L of concentration of simulated thin stillage was included. The reactor was operated until it was deemed to have reached a steady state for each HRT. All other operating conditions were the same as the continuous mode in the start-up phase.

4.2.3.1 HRT

The reactor was operated at HRTs of 16, 6, and 4 days. The reactor was monitored over the course of each HRT to allow it to reach a similar steady state performance or baseline for the monitored parameters (pH, alkalinity, VFAs, COD, biogas, CH4 production, TSS, VSS, and ammonia N). HRTs of 16, 4 days have been reported elsewhere for zeolite media (Eldyasti et al., 2014, Wang, 2015).

For the HRT of 16 days, from Day 67, the reactor was fed with OLR 2.4 g COD/L/day. The feed flow rate was 420 mL/d. The feed composition was 40 g COD/L $_{feed}$ and 4 mL/L $_{feed}$ macro-elements and trace element, respectively. To enhance the buffering capacity and to maintain the optimum targeted pH value of 7.0 ± 0.2, 13.5 g/L of $NaHCO_3$ was added. The reactor was monitored for 35 days until it showed stability in biogas production, VFAs' level, and COD removal. The change in pH was not significant. The reactor started to slightly decrease from 8.71 on Day 67 and reached a steady-state level of 8.62 ± 0.03, based on data collected on Day 67 until Day 102. This can be attributed to the addition of a load increase of the reactor from Day 56 that increased the acidity of the influent. This did not affect the reactor pH initially, but with the continuous addition of influent, it started to drop the pH from Day 83 until it reached 8.62 ± 0.03. The increase in the VFAs concentration at HRT of 16, from the concentration of 280 mg/L to 935 ± 70 mg/L, can be attributed to the acidity added with the influent of the reactor. However, the period that the reactor took before stabilizing was probably due to the shock received during the change in the OLR. For bicarbonate alkalinity, a gradual increase from 9,000 mg/L to 11,600 mg/L was observed between Day 67 and Day 84, after which the values stabilized to reach a steady-state value of 11,600 ± 150 mg/L between Day 84 and Day 102. This rise in alkalinity may also be attributed to the buffer capacity introduced into the feed by the sodium bicarbonate. The stable values of biogas and methane production were 11.6 and 5.83 L/day, respectively (Figure 4.14b). A COD removal of 96 ± 2 and VFAs level of 935 mg/L were observed (Figure 4.14c). However, at the beginning of each OLR increase, there existed a corresponding decrease in the removal efficiency. The drop in removal efficiency might be the result of the organic shock loads and the continuous feeding.

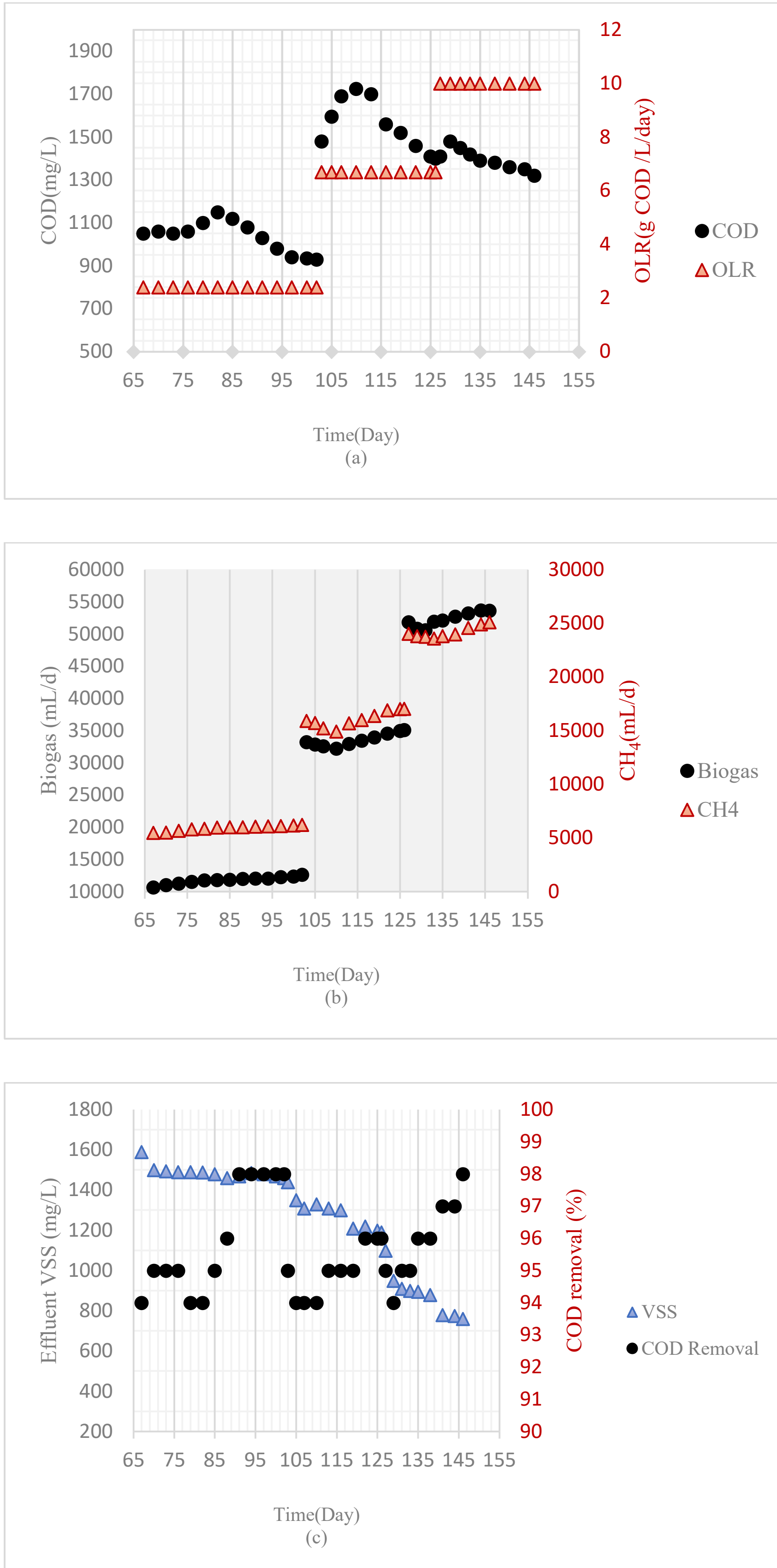


Figure 4.14 (a) Trend of COD (b) Methane and biogas production (c) Trend of VSS in the effluent.

The system responded by recovering shortly and it adapted to the new condition with time. Steady-state results for the three HRTs are presented in Table 4.9.

Table 4.10 Summary of operational parameters in the steady-state condition for mesophilic reactor at different HRTs

HRT = 16 days

	Unit	Mesophilic
Volumetric biogas production	mL/d	11,600 ± 1000
Volumetric methane production	mL/d	5830 ± 370
$COD_{removal}$	%	96 ± 2
VFAs	mg/L	935 ± 70
Alkalinity	$mgCaCO_3/L$	11,600 ± 150
pH	-	8.62 ± 0.03
Ammonia N	mg/L	420 ± 15
TSS	mg/L	1,705 ± 35
VSS	mg/L	1,490 ± 30
TVFAs/Alkalinity	-	0.08

HRT = 6 days

	Unit	Mesophilic
Volumetric biogas production	mL/d	33,605 ± 1430
Volumetric methane production	mL/d	15,950 ± 1060
$COD_{removal}$	%	95 ± 1
VFAs	mg/L	1,460 ± 150
Alkalinity	$mgCaCO_3/L$	9,200 ± 250
pH	-	8.25 ± 0.15
Ammonia N	mg/L	380 ± 25
TSS	mg/L	1,550 ± 45
VSS	mg/L	1,285 ± 75
TVFAs/Alkalinity	-	0.16

HRT = 4 days

	Unit	Mesophilic
Volumetric biogas production	mL/d	52,115 ± 1525
Volumetric methane production	mL/d	24,335 ± 750
$COD_{removal}$	%	96 ± 2
VFAs	mg/L	1,285 ± 50
Alkalinity	$mgCaCO_3/L$	7,200 ± 350
pH	-	8.08 ± 0.2
Ammonia N	mg/L	320 ± 45
TSS	mg/L	1,170 ± 130
VSS	mg/L	885 ± 85
TVFAs/Alkalinity	-	0.18

From Day 102 until Day 126, the reactor was operated at an HRT of 6 days according to an OLR of 6.7 g COD/L/day. The feed flow rate was 1,160 mL/d. The added $NaHCO_3$ was reduced to 10 g/L and, accordingly, alkalinity was gradually reduced for the reactor from 11,600 to 9,200 mg$CaCO_3$/L. All other conditions remained unchanged. For the HRT of 6 days, no major change in the reactor pH was observed until Day 112. The pH was observed to decline from 8.62 to 8.45 between Day 112 and Day 117. This may be attributed to a decreased buffer capacity in the influent from 11,600 to 9,200 mg/L. The pH declined to the lowest level of 8.25 on Day 120. The pH level for the HRT of 6 days reached the steady state value of 8.25 ± 0.15 for the time period between Day 120 and Day 126.

The VFAs concentration with the HRT of 6 days increased slightly from 935 mg/L on Day 102 to 985 mg/L on Day 112. This initial increase might be attributed to the increase of the OLR. Another increase in the concentration of VFAs occurred between Day 112 and Day 117. This rise can be attributed to the system's capabilities to handle organic shock loads and the continuous increase in the acidity of the influent. The concentration of 1,460 ± 10 mg/L of VFAs was measured for HRT of 6 in the steady-state condition from Day 117 to Day 126.

As shown in Table 4.9, when the HRT was 6 days, the decline in alkalinity in the effluent from Day 102 until Day 126 can be attributed to a decreased buffering capacity from 11,600 to 9,200 mg/L. In general, no significant change in the COD removal efficiency was observed during the entire run period with HRT of 6 days, suggesting that the microorganisms were able to take up the organic carbon. Moreover, as depicted in Table 4.9, the maximum methane yield for the HRT of 6 days in the system approached 360 mL CH4 /g COD removal with a methane content of 48% in the biogas produced.

The methane content of biogas should be fairly stable over time unless there is a problem with the reactor. The methane content, which ranged from 47% to 49%, was observed as stable during almost all the HRT period of 6 days. At the HRT of 6 days, based on simulated thin stillage feed, the TSS and VSS in the reactor were reduced by 9% and 14%, respectively, compared to the HRT of 16 days, which was from 1,705 to 1,551 mg/L and 1,490 to 1,285 mg/L, respectively (Table 4.9). The drop in concentrations might be the result of the increased biomass attachment.

The reactor was operated at an HRT of 4 days from Day 126 until Day 146. The concentration of COD in the feed was 40 g COD/L, similar to that for HRT of 16 days and 6 days. The feed flow rate was 1,750 mL/d. All other conditions remained unchanged from HRTs 16 and 6 days.

The amount of alkalinity added to the influent of HRT 4 days was decreased to 8 g/L (as $NaHCO_3$) on Day 127; accordingly, the pH of the reactor declined to 8.15 by Day 129. This could be attributed to the increase in the OLR to 10 g COD/L/day, due to the decrease in HRT. Although the pH of the reactor remained unchanged from Day 129 to Day 132, a further drop in the pH level was expected due to the continuous feed at an OLR equal to 10 g COD/L/day. To prevent this pH decline, the concentration of the added alkalinity was increased to 9 g/L (as $NaHCO_3$) on Day 132 and this slightly higher dosage continued for the remaining operation time. Michael (2003) observed that the pH value increased due to ammonia accumulation during the degradation of protein, while the accumulation of VFA, resulting from the degradation of organic matter (1 g of volatile acids produced per gram of volatile solids) decreased the pH value.

No noticeable change was observed in the level of pH (8.08 ± 0.2) from Day 132 to Day 146. During the operation of the reactor at an HRT of 4 days, the VFA concentration gradually increased from 1,460 mg/L starting on Day 129 and it reached a level of 1,490 mg/L after two days. This can be attributed to the increase of OLR. The concentration of VFA then reached a steady state of 1,285 mg/L on Day 132 and remained so until Day 146. To enhance the buffering capacity and to maintain the optimum targeted pH value of 7.2 ± 0.2, 8 g/L, NaHCO3 was added throughout the entire operation with the HRT of 4 days (as was done with the HRT of 6 days). As expected, alkalinity was gradually consumed and reached an approximate steady-state value of 7,200 ± 350 mg$CaCO_3$/L. The same results have been reported by Lee (2011), who carried out studies with thin stillage under mesophilic condition. The result showed that for the HRT at 20 days, the VFAs' concentration dramatically increased to 3,400 mg/ L, whereas alkalinity concentration decreased to 5000 mg/ L as $CaCO_3$.

Even though the COD removal showed a small decline (95% to 93%) from Day 127 to Day 129, due to the OLR increase, it increased again from Day 129 and remained at this level until Day 146. The maximum methane yield for the HRT of 4 days in the system approached 361 mL of CH_4/g COD removed with a methane content of 47% in the biogas produced.

Due to the acclimation of the organisms to the new condition and attachment on the media, the TSS/VSS concentration decreased gradually and reached the steady-state values of 1,170 and 885 mg/L, respectively. Compared to the HRT of 6 days, 25% and 31% decreases were observed for TSS and VSS mg/L, respectively. The TVFAs/Alkalinity ratio, during the operation for the reactor ratio, were 0.08, 0.16, and 0.18 for HRTs of 16, 6, and 4, respectively indicating favourable methanogenic conditions.

4.2.3.2 C/N ratio

The reactor was operated at an HRT of 4 days with an OLR of 10 g COD/L/day for the last 40 days. Different C/N ratios were tested. The ratio was changed by changing the urea (CH_4N_2O as nitrogen sources) concentration in the feed solution. The feed solution for the C/N ratio of 32, 25, and 16 was prepared by adding 0.27, 0.35, and 0.54 g/L urea, respectively. Andalib (2011) used 9.5 mL of acetic acid /L $_{Feed}$ (CH_3COOOH as carbon sources) for a C/N ratio of 32. A summary of the average values for biogas and methane production during steady-state operation for the reactor is presented in Table 4.11.

Table 4.11 Summary of biogas and methane production of the steady-state condition at different C/N ratios for the mesophilic reactor at an OLR of 10 g COD/ L

Reactor condition	C: N	Total gas production rate (mL/d)	Methane %	Methane production rate (mL/d)
Mesophilic	32	52,115 ± 1525	47 ±1	24,335 ± 750
	25	46,290 ± 1200	49 ±2	22,680 ± 670
	16	39,000 ± 850	51 ±1	19,890 ± 780

The reactor was run at an OLR of 10 g COD/L/day from Day 126 to Day 146. The feed composition was similar to that used for HRT of 16 days and 6 days. The C/N was 32. The biogas and methane production were monitored. From Day 126 until Day 134, the methane content showed a fluctuation of approximately 46%. This might be attributed to the increase of carbon source in OLR. When the C/N ratio is too high, methane content is not at an optimum level due to acidogenic bacterium rapidly consuming nitrogen, compared with methanogenic bacteria. It is necessary to maintain a proper C/N ratio of substrate in the desired range. Methane content in biogas reached a steady-state value of 47% ± 1% for the period between Day 134 and Day 146. The system approached 361 mL of CH4 /g COD removed.

The concentration of urea in the reactor influent was changed to 0.35 g/L (C/N = 25) on Day 146. All other conditions remained unchanged. The biogas and methane production rates at a C/N ratio of 32 started to decrease from 52,115 ± 1525 and 24,335 ± 750 mL/day, respectively. A decrease in system COD removal was observed and reached 92% in the steady-state condition. The system approached 352 mL CH_4/g COD removed. This can be attributed to the increase of nitrogen sources by the increase in urea concentration in the influent on Day 146. The biogas and methane production rate then reached the steady state values of 46,290 ± 1,200 and 22,680 ± 670 mL/day on Day 154 and remained unchanged until Day 165. However, the percentage of methane increased with the decreasing C/N ratio of 32 to 25, from 47% to 49%, respectively. The results indicate that the source of carbon appears to be important. It has been established that during the biomethanation process microorganisms utilize carbon 25–30 times more than nitrogen (Yadav, 2004).

The addition of 0.54 g/L of nitrogen in the form of urea with the influent from Day 166 decreased the influent C/N ratio from 25 to 16. As a result, the biogas production rate gradually declined. The steady-state results for the biogas and methane production rates were 39,000 ± 850 and 19,890 ± 780, respectively (Day 166 and Day 185). The COD removal decreased from 92% to 88% and the reactor showed a methane yield of 322 mL CH_4 /g COD removed. This may be attributed to the increase in nitrogen and lack of carbon source of influent composition. Since the source of carbon during biomethanation is vital for microorganisms, the lack of carbon source causes a decrease in the formation of acid. Alternatively, nitrogen accumulates in the form of ammonium ions (NH_4) that increase the pH, which adversely affects biogas production (Giovanna, 2016).These findings are consistent with the literature (Hills and Roberts, 1981; Angelidaki et al., 2003). The methane content decreased with the increasing carbon to nitrogen ratio under mesophilic conditions (i.e., 51% methane for C/N = 16 and 47% methane for C/N = 32).

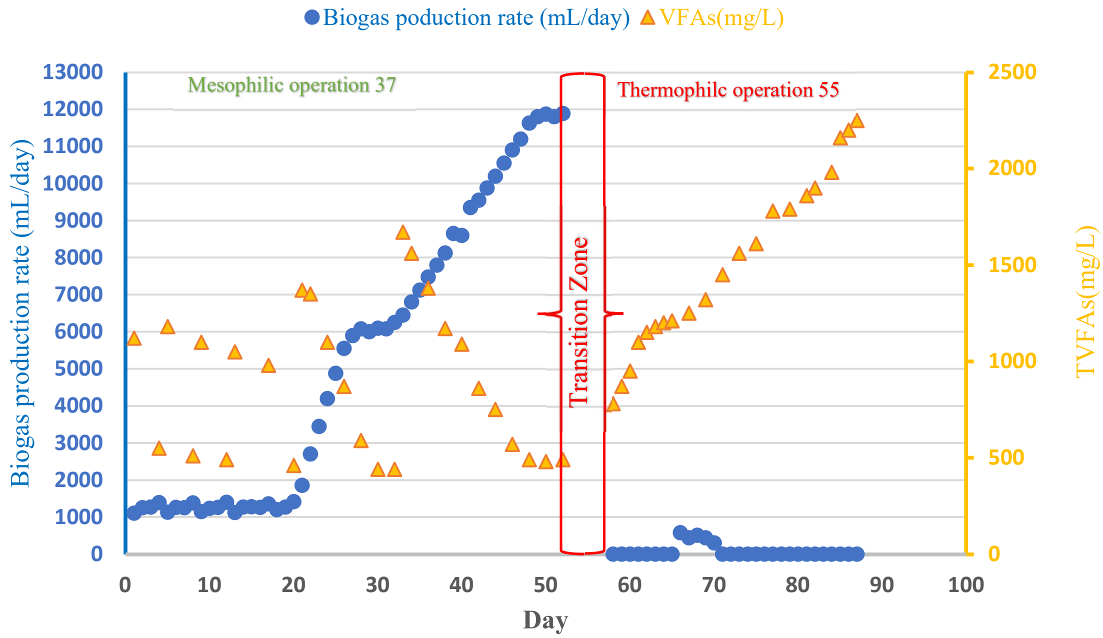
4.2.4 Operating conditions for thermophilic reactor

Attempts to secure thermophilic sludge were not successful; hence, mesophilic anaerobically digested sludge (ADS) was used. The ADS was collected from the secondary digester at Stratford's wastewater treatment plant (London, Ontario, Canada). To start the thermophilic AFBR using mesophilic sludge, two separate temperature change strategies were investigated: (1) multi-step, where temperature was increased from 37 ºC to 55 ºC in several steps over a 5-day period; and (2) a single–step, where the temperature was increased from 37 ºC to 55 ºC in a single step within a day.

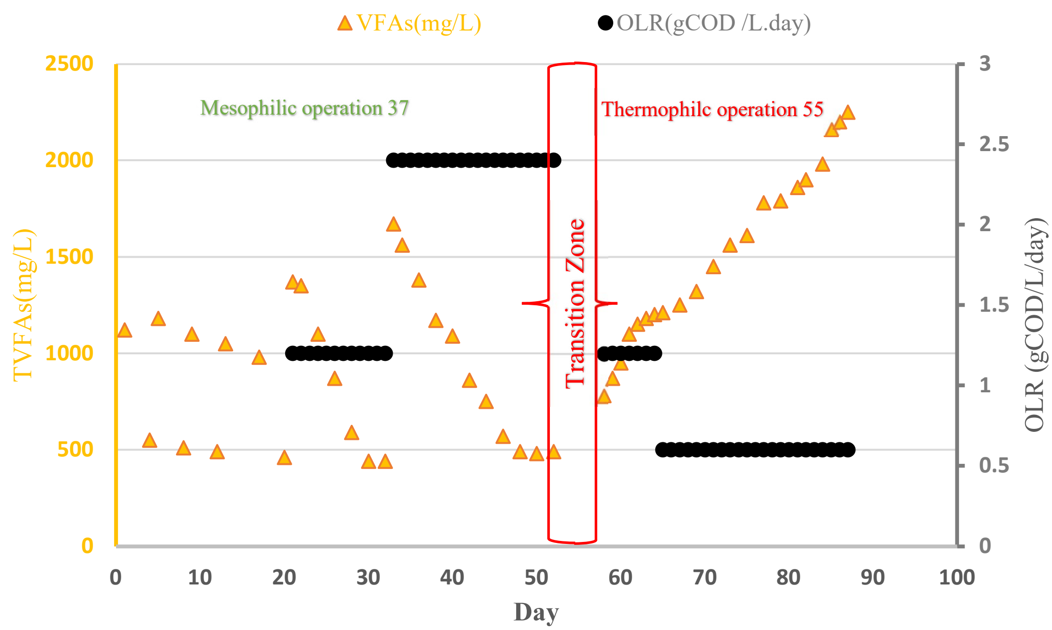
4.2.4.1 Multi-step strategy

One AnFBR started with mesophilic sludge and operated under mesophilic conditions for 52 days. These results are presented in Figure 4.15.The same procedure used for the mesophilic reactor (section 4.2.3) was applied in the multi-step strategy. The mesophilic reactor was operated in a semi-continuous and continuous mode accordingly, as was done earlier (section 4.2.3). During mesophilic conditions, the OLR was increased to 2.4 g COD/L/day. Starting from Day 52, the feeding was stopped, and the operating condition was switched from mesophilic to thermophilic using the multi-step strategy. The temperature was increased from 37 to 43, 43 to 45, 45 to 47, 47 to 50, and 50 to 55 ºC, respectively, over a five-day period. The feeding was resumed on Day 62 at half the previous OLR (1.2 g COD/L/day). The lower OLR was applied because of the lower shock to the thermophilic microbial community and let an adapted condition was achieved in the microbial community.

Soon after the resumption of feeding, VFAs levels were seen to increase to around 1500 mg/L. This may be due to the fact that adaptation of the microbial community under this new environment did not happen. Therefore, the OLR was further reduced to 0.6 g COD /L/day on Day 65 and maintained at that level until Day 87. However, the VFAs levels kept creeping up and reached a level of almost 2,200 mg/L by Day 87. While there was some gas production between Day 65 and Day 72, it became negligible after that date (Figure 4.15). The thermophilic AnFBR start-up was deemed unsuccessful and the reactor operation was discontinued after Day 87.



(a)



(b)

Figure 4.15 Multi-step increase strategy to obtain thermophilic seed from mesophilic sludge (a) Biogas production; (b) Trend of VFAs.

4.2.4.2 Single-step strategy

In the second run, the AnFBR was restarted with the mesophilic sludge. It was operated under mesophilic conditions for 35 days to reach stable operations at the OLR of 1.2 g COD/L/ day (Figure 4.16). During this period, the VFAs levels remained more or less stable, at 340 ± 20 mg/L.

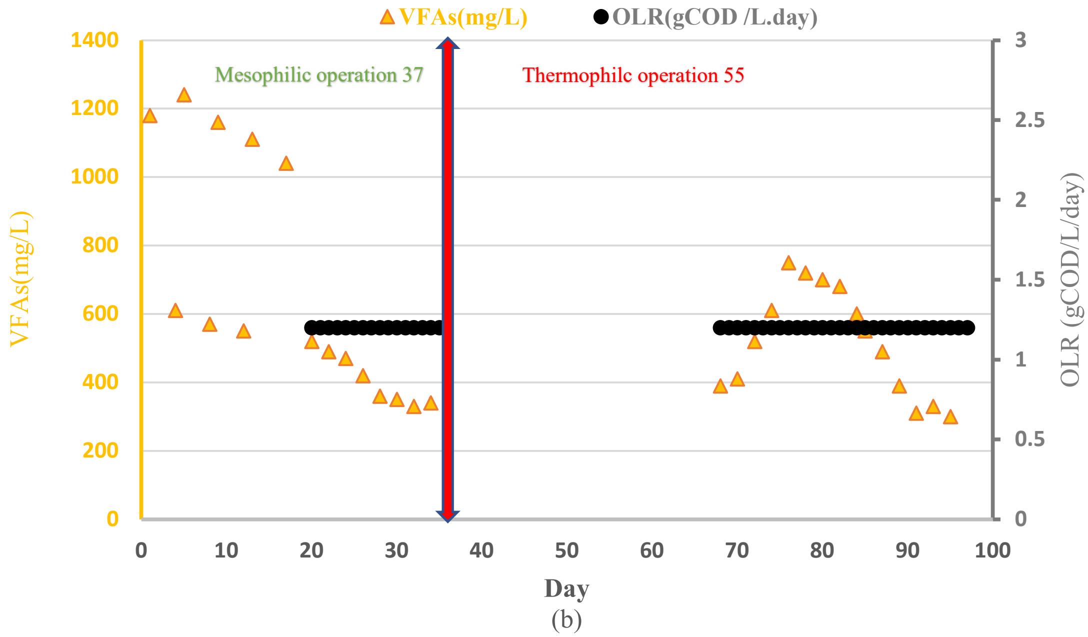


Figure 4.16.a Single-step increase strategy to obtain thermophilic seed from mesophilic sludge: (b) Trend of VFAs.

The temperature was then raised to 55 ºC in one day on Day 36. The reactor was not fed for almost 35 days to allow the micro-organism population to adapt (Bouskova et al. 2005). Feeding was resumed on Day 68 at the same OLR of 1.2 g COD/L/ day. The VFAs levels were seen to increase during the first week, reaching around 750 mg/L by Day 76, with negligible gas production. The increase in VFAs level might be the result of the shock loading that was introduced to the system after the adaptation period. The VFAs levels, however, steadily declined after reaching the level of 300 mg/L by Day 95 (Figure 4.17). No significant gas production was observed until Day 80. Gas production started on Day 81 and steadily increased to reach the rate of around 6,300 mL/day.

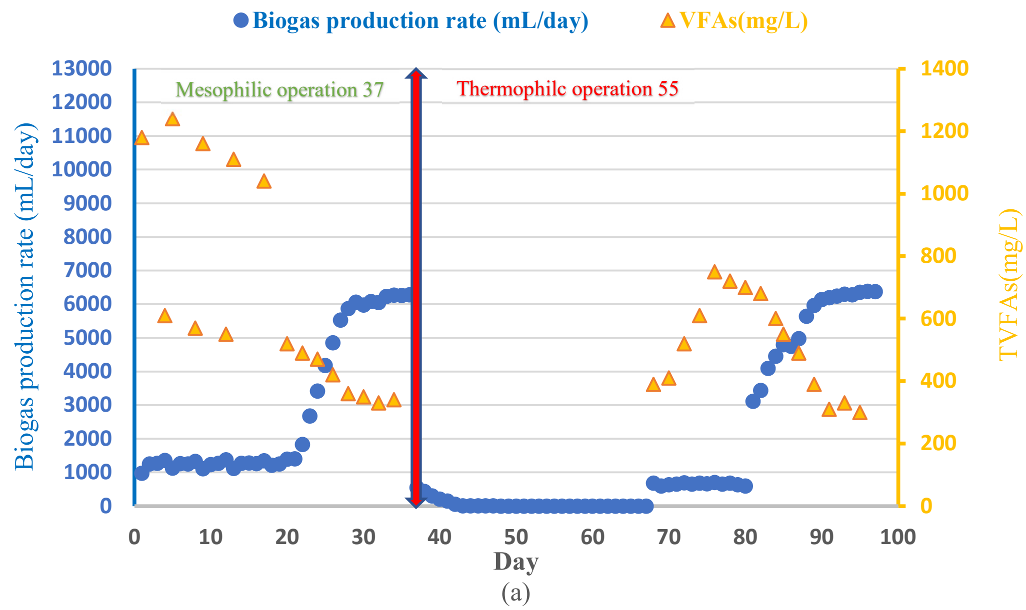


Figure 4.17.b Single step increase strategy to obtain thermophilic seed from mesophilic sludge: (a) Biogas production

Bouskova, et al. (2005) studied the one-step temperature increase in an anaerobic CSTR reactor. They reported that although the one-step temperature increase caused severe disturbances with all the process parameters, the system reached a new stable operation after only 30 days, indicating that this strategy may be better in changing from mesophilic to thermophilic operations in anaerobic digestion plants. Tian, et al. (2014) also showed that the thermophilic anaerobic digester can be rapidly started up from a steady-state mesophilic system by adopting the one-step temperature increase strategy.

4.2.4.3 HRT

Similar to the mesophilic reactor, the thermophilic reactor was operated with 16, 6, and 4 days of HRTs. The reactor was monitored for pH, alkalinity, VFAs, COD, biogas, CH_4, TSS, VSS, and ammonia.

For HRT of 16 days, from Day 105 in the thermophilic operation, the reactor was fed with OLR of 2.4 g COD/L/day. The feed flow rate was 420 mL/d and feed composition was 40 g COD/L_{feed} and 4 mL/L_{feed} macro-elements and trace element, respectively. To enhance the buffering capacity and to maintain the optimum targeted pH value of 7.0 ± 0.2, similar to the mesophilic reactor, 13.5 g/L of $NaHCO_3$ was added. The reactor was monitored for 35 days, from Day105 until it showed stable biogas production, VFAs level, and COD removal. For an HRT of 16, the pH of the reactor started to decrease from 8.78 on Day 105 and reached the steady-state level of 8.75 ± 0.04 based on the data collected from Day 105 until Day 140. This can be attributed to the addition of increased loading to the reactor from Day 106. This did not affect the reactor pH initially, but with the continuous addition, pH started to drop from Day 122. The thermophilic reactor at an HRT of 16 days had a pH of 8.75 under steady state condition, while the pH of the mesophilic reactor was 8.62.

An increase in the VFAs concentration at the HRT of 16, from a concentration of ~300 mg/L to 450 ± 40 mg/L, can be attributed to the acidity added with the influent of the reactor. However, the period the reactor took before stabilizing was probably due to the shock loading received during the change in the OLR. The thermophilic process reached a higher stabilization rate of organic matter because it showed lower VFAs values that contributed to the COD. The level of VFAs stabilized as the activities of microorganisms came into balance.

A gradual increase in alkalinity, from 7,800 mg/L to 8,170 mg/L, was observed between Day 106 and Day 122, after which the values stabilized and reached a steady-state value of 8,550 ± 100 mg/L between Day 122 and Day 140. This rise in alkalinity may also be attributed to the buffer capacity introduced into the feed by the sodium bicarbonate.

Stable values were recorded for biogas and methane production during steady-state operation between Day 122 and Day 140, of 12.15 and 6.22 L/day, respectively. Biogas and methane production rates were higher in the thermophilic reactor than the mesophilic one by approximately 10% and 7%, respectively. As expected, the methane content in the biogas was seen as being almost the same 50% and 51%, respectively for the mesophilic and thermophilic reactors.

Moreover, as depicted in Table 4.11, the maximum methane yield for the HRT of 16 days in the system approached 382 mL CH_4/g COD removed with the methane content of 51% in the biogas produced. The methane yield increased from 361 mL CH_4/g COD in the mesophilic operation to 382 mL CH_4/g COD under the thermophilic condition. In addition, methane content remained approximately the same in both reactors.

Table 4.12 Summary of operational parameters in the steady-state condition for thermophilic reactor at different HRTs

HRT = 16 days		
	Unit	Thermophilic
Volumetric biogas production	mL/d	12,150 ± 1400
Volumetric methane production	mL/d	6,220 ± 260
$COD_{removal}$	%	97 ± 1
VFAs	mg/L	450 ± 40
Alkalinity	$mgCaCO_3/L$	8,550 ± 100
pH	-	8.75 ± 0.04
Ammonia N	mg/L	540 ± 20
TSS	mg/L	1,830 ± 75
VSS	mg/L	785 ± 35
TVFAs/Alkalinity	-	0.05

HRT = 6 days		
	Unit	Thermophilic
Volumetric biogas production	mL/d	34,935 ± 1610
Volumetric methane production	mL/d	17,800 ± 890
$COD_{removal}$	%	96 ± 1
VFAs	mg/L	1,175 ± 80
Alkalinity	$mgCaCO_3/L$	7,250 ± 100
pH	-	8.45 ± 0.1
Ammonia N	mg/L	450 ± 30
TSS	mg/L	1,710 ± 85
VSS	mg/L	775 ± 45
TVFAs/Alkalinity	-	0.16

HRT = 4 days		
	Unit	Thermophilic
Volumetric biogas production	mL/d	53,310 ± 1,395
Volumetric methane production	mL/d	27,150 ± 1,425
$COD_{removal}$	%	97 ± 2
VFAs	mg/L	880 ± 40
Alkalinity	$mgCaCO_3/L$	6,750 ± 250
pH	-	8.35 ± 0.15
Ammonia N	mg/L	410 ± 20
TSS	mg/L	1,325 ± 30
VSS	mg/L	750 ± 25
TVFAs/Alkalinity	-	0.13

The COD removal of 97% ± 1% and a VFAs level of 450 mg/L were observed. However, at the beginning of each OLR increase, there existed a corresponding decrease in removal efficiency. However, the system recovered shortly and adapted to the new condition over time. The TSS and VSS on Day 106 were 2,250 and 1,100 mg/L, respectively, and the system was considered to be under steady state condition; these values decreased to 1,830 ± 75 mg/L and 785 ± 35 mg/L, respectively by the end of operation Day 140. The decline in concentrations might be the result of the biomass attachment and systems dilution due to simulated thin stillage feeding.

At an HRT equal to 16 days, the VFAs/Alkalinity ratio was around 0.05. However, the optimum range of VFAs to alkalinity ratio for methane production in anaerobic digesters ranges from 0.3 to 0.4 to prevent process failure (Barggman et al., 1996). The increase in alkalinity added to 8,550 mg/L allowed the ratio to decline to 0.05. Steady-state results for three HRTs are presented in Table 4.12.

Table 4.13 Operational parameters in the steady-state condition for thermophilic reactor at HRT 16 days.

HRT = 16 days		
	Unit	Thermophilic
Volumetric biogas production	mL/d	12,150 ± 1400
Volumetric methane production	mL/d	6,220 ± 260
COD $_{removal}$	%	97 ± 1
VFAs	mg/L	450 ± 40
Alkalinity	$mgCaCO_3/L$	8,550 ± 100
pH	-	8.75 ± 0.04
Ammonia N	mg/L	540 ± 20
TSS	mg/L	1,830 ± 75
VSS	mg/L	785 ± 35
TVFAs/Alkalinity	-	0.05

From Day 141 until Day 165, the reactor was operated at the HRT of 6 days with an OLR of 6.7 g COD/L/day. The feed flow rate was 1,160 mL/d and $NaHCO_3$ was reduced to 10 g/L. Accordingly, the alkalinity was gradually reduced for the reactor from 8,550 to 7,250 mg $CaCO_3$/L. All other conditions remained unchanged. Results of the operation under the described changes are presented in Table 4.13.

Table 4.14 Operational parameters in the steady-state condition for thermophilic reactor at HRT 6 days.

HRT = 6 days		
	Unit	Thermophilic
Volumetric biogas production	mL/d	34,935 ± 1610
Volumetric methane production	mL/d	17,800 ± 890
$COD_{removal}$	%	96 ± 1
VFAs	mg/L	1,175 ± 80
Alkalinity	mg$CaCO_3$/L	7,250 ± 100
pH	-	8.45 ± 0.1
Ammonia N	mg/L	450 ± 30
TSS	mg/L	1,710 ± 85
VSS	mg/L	775 ± 45
TVFAs/Alkalinity	-	0.16

For an HRT of 6 days, no major change in pH was observed until Day 152. The pH was then observed to decline from 8.75 to 8.54 between Day 142 and Day 149. This can be attributed to a decrease in buffer capacity in the influent from 13,500 to 10,000 mg/L. The pH declined to the lowest level of 8.45 on Day 152. The pH level for an HRT of 6 days reached a steady state of 8.45 ± 0.10 for the time period of Day 153 until Day 165.

The VFAs concentration for the HRT of 6 days increased slightly from 450 mg/L on Day 142 to 785 mg/L on Day 149. Decreasing HRT usually leads to VFAs accumulation, while a longer than optimal HRT results in insufficient utilization of digester components. Another increase in the concentration of VFAs occurred between Day 149 and Day 152 that can be attributed to the continuous increase in the acidity of the influent. A concentration of 1,175 ± 80 mg/L of VFAs was measured for HRT 6 under the steady-state condition from Day 153 to Day 165.

As shown in Table 4.11.2, the decline in alkalinity in the effluent of HRT 6 from Day 142 until Day 165 can be attributed to a decrease in buffering capacity from 13,500 to 10,000 mg/L. In general, no significant change in the COD removal efficiency was observed in the entire period for an HRT of 6 days, suggesting that microorganisms were able to take up the organic carbon.

Moreover, the maximum methane yield for the HRT of 6 days in the system approached 399 mL CH_4/g COD removed with a methane content of 51% in the biogas produced. A methane content concentration ranging from 50% to 52% was observed while the reactor was operated with an HRT of 6 days. The methane yield increased from 361 mL/g COD in the mesophilic operation to 399 mL/g COD under the thermophilic condition. In addition, the methane content showed a slight increase from 48% under the mesophilic to 51% in the thermophilic condition. This was consistent with other reports that the methane content in thermophilic digesters, in general, is slightly higher than that in the mesophilic digesters (Rodríguez et al., 2013). At the HRT of 6 days, no significant TSS/VSS variation was observed. The steady-state TSS/VSS concentrations of 1,710 mg/L and 775 mg/L were observed. The slight decline can be attributed to the SWW in the influent and the biomass attachment.

Following this, the reactor was operated at HRT of 4 days from Day 166 until Day 185. The concentration of COD in feed was 40 g COD/L in the reactor influent, similar to that for HRT of 16 and 6 days. The feed flow rate was 1,750 mL/d. All other conditions remained unchanged.

Similar to the mesophilic reactor, the amount of alkalinity added to the influent of HRT 4 was reduced to 8 g/L (as $NaHCO_3$) on Day 166. Accordingly, the pH of the reactor declined to 8.40 by Day 170. Although the pH of the reactor remained unchanged from Day 170 to Day 175, a slight drop in pH level was expected due to continuous feeding at OLR, which was equal to 10 g COD/L/day. This pH was 8.35. Further to this, no noticeable change was observed in the level of pH and it seemed that the total generated and added alkalinity was able to stabilize the pH level. The steady-state pH of 8.35 ± 0.15 was reached on Day 175 and remained unchanged until Day 185.

During the operation of the reactor at HRT of 4 days, no significant changes was observed in the VFAs concentration between Day 166 and Day 170. Then, a gradual decrease occurred until reaching the steady-state concentration of 880 ± 40 from Day 170 to Day 185. The decrease in the concentration of VFAs after Day 170 can be attributed to the increase in the activity of bacteria and consequent reduction in the acetate concentration. Due to the VFAs decrease to approximately 880 mg/L, the VFAs to alkalinity ratio became stable at approximately 0.13. This was consistent with other reports indicating that the VFAs to alkalinity ratios were stable at 0.19 for the mesophilic and at 0.16 for the thermophilic digesters, except for the early stage of the operation (Zábranská, 2000).

The COD removal showed a small change from Day 170 until Day175 that was from 96% to 94% due to the OLR increase. Then, the COD value decreased to 940 ± 35 from Day 176 until Day 181. The COD value showed no shift until Day 185 and then it maintained a steady-state value of 915 ± 65 mg/L by Day 185 (Figure 4.18).

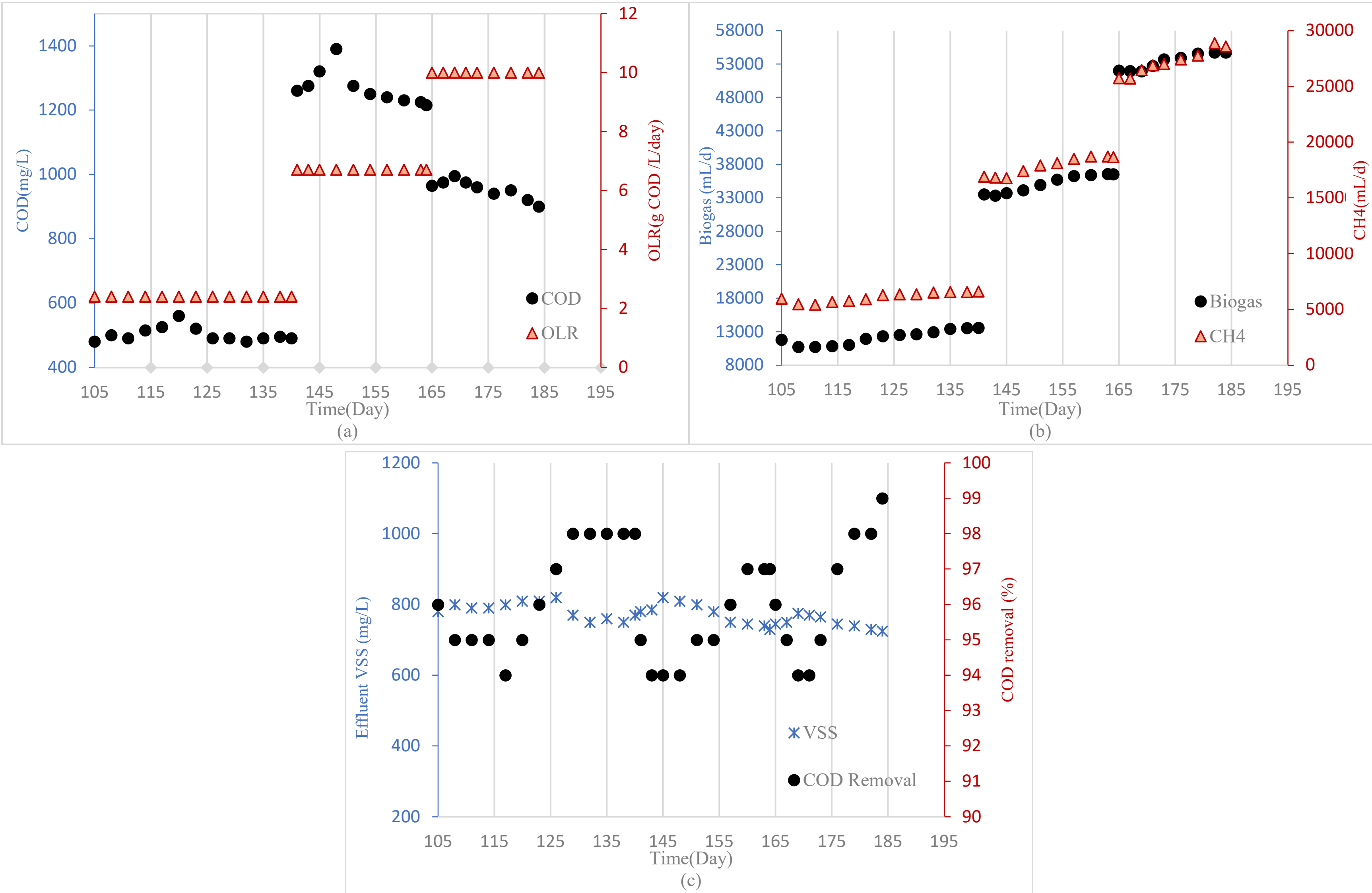


Figure 4.18 (a) Trend of COD; (b) Methane and biogas production; (c) Trend of VSS in the effluent.

Moreover, as depicted in Table 4.11, the maximum methane yield for the HRT 4 days in the system approached 400 mL CH_4 g COD removal with a methane content of 51 % in the biogas produced. For the methane content, like with the HRT of 6 days, the range was from 50% to 52% and remained stable during the entire period of operation with the HRT 4 days. The methane yield increased from 361 CH_4 mL/g COD removed in the mesophilic operation to 400 CH_4 mL/g COD removed under the thermophilic condition. Suhartini (2014) similarly reported that decrease in CH_4 yield of around 20% was reached under thermophilic conditions, compared with the corresponding mesophilic conditions. In addition, the methane content showed a slight increase from 47% under the mesophilic condition to 51% under the thermophilic condition.

The TSS and VSS on Day 175 were 1,450 and 765 mg/L, respectively, and the system was considered to be under steady state conditions. These values decreased to 1,325 ± 35 mg/L and 750 ± 15 mg/L, respectively by the end of operation Day 185. The drop in concentrations might be the result of the acclimation of the organisms to the new conditions and attachment on the media, and also contributed to feeding the simulated thin stillage to the reactor.

4.2.4.4 C/N ratio

The reactor was subjected to an OLR of 10 g COD/L/day with an HRT of 4 days with a varying C/N ratios. The ratio changed by changing the urea (CH_4N_2O) concentration in the feed solution. The C/N ratios of 32, 25, and 16 were prepared by adding 0.27, 0.35, and 0.54 g/L urea to the synthetic feed, 9.5 mL/L of acetic acid.

The reactor was run at an OLR of 10 g COD/L/day from Day 166 to Day 185. The feed composition was similar to that used for the HRT of 16 and 6 days, respectively. Similar to the mesophilic operation, the C/N ratio was 32. Between Day 166 and Day 174, the methane content showed a small fluctuation. Methane content of approximately 49% ± 1% was observed. However, that could be attributed to the increasing the carbon content of the OLR. The methane content in the biogas reached a steady-state value of 51% ± 2% (Day 174 to Day 185). The system approached 400 mL CH_4/g COD removed with a methane content of 51% in the biogas produced (Table 4.12).

Table 4.15 Summary of biogas and methane production under steady-state condition at different C/N ratios for the thermophilic reactor at an OLR of 10 g COD/L

Reactor condition	C: N	Total gas production rate (mL/d)	Methane %	Methane production rate (mL/d)
Thermophilic	32	53,310 ± 1395	51 ± 2	27,150 ± 1,425
	25	47,275 ± 1,350	53 ± 2	25,055 ± 1,250
	16	40,050 ± 980	56 ± 1	22,430 ± 880

The ratio of C/N was changed to 25 by adding 0.35 g/L of urea on Day 186. All other conditions remained unchanged. Biogas and corresponding methane production rates started to decrease with time from 53,310 ± 1,395 and 27,150 ± 1,425 mL/day, respectively. A decrease in COD removal was observed, and it reached 93% under the steady-state condition. The system showed a slight decline in methane production (384 mL CH_4/g COD removed), which can be attributed to the increase of nitrogen source. The biogas and methane production rates then reached a steady state of 47,275 ± 1,350 and 25,055 ± 1,250 mL/day, respectively on Day 205.

An addition of 0.54 g/L of nitrogen in the form of urea was introduced to the influent from Day 206 and decreased the influent C/N ratio from 25 to 16. With this decreased ratio, the biogas production rate gradually declined. The steady state biogas and methane production rates between Day 206 and Day 225 were 40,050 ± 980 and 22,430 ± 880 mL/day, respectively (Table 4.12). The COD removal decreased from 93% to 89%, and the reactor showed 360 mL CH_4/g COD removed. As can be seen (Table 4.12), the methane content decreased slightly with the increasing carbon to nitrogen ratio under the thermophilic conditions (e.g., 56% methane for C/N = 16, and 51% methane for C/N = 32). Similar results were obtained By Angelidaki et al. (2003), who reported improved methane yield with a C/N ratio in the range of 25–32, using manure as the substrate.

The biogas production, the major product of anaerobic digestion, increased by more than 30%, from 40.05 L/day to 53.31 L/day, when the C/N ratio changed from 16 to 32. The methane production rate also showed an increase by more than 20% from 22.43 L/day to 27.15 L/day for the C/N ratios of 16 and 32, respectively. Similar observations have been reported by other researchers (Giovanna Guarino et al., 2016).

4.2.4.5 Kinetic analysis

Analyzing the kinetics in the mesophilic and thermophilic conditions is fundamental for the correct performance of the process. The kinetic equations developed by Bernd (2006) have been used to determine the maximum methane yield (L/g COD removed), the ratio of biogas yield to the maximum biogas yield (%), and the substrate degradation constant at different OLRs for mesophilic and thermophilic anaerobic digestion of potato wastes. Details of the calculation have been given in Appendix 5. These equations were applied to the mesophilic and thermophilic anaerobic digestion of simulated thin stillage in this study. The equation for determining the maximum methane yield is Equation 4.2.1.

$$\mathrm{OLR} = \frac{k * C_0}{G/(G_0 - G)} \qquad \text{Equation 4.2.1}$$

The values of k and G_0 are specific parameters for different substrates used. The reaction rate constant k results from the plot of $G/(G_0 - G)$ against 1/OLR. With the slope of k derived from $k * C_0 = 5.1392$ g/L/day, and setting $C_0 = 40$ g COD/L, $k = 0.128$ day^{-1} (Figure 4.19).

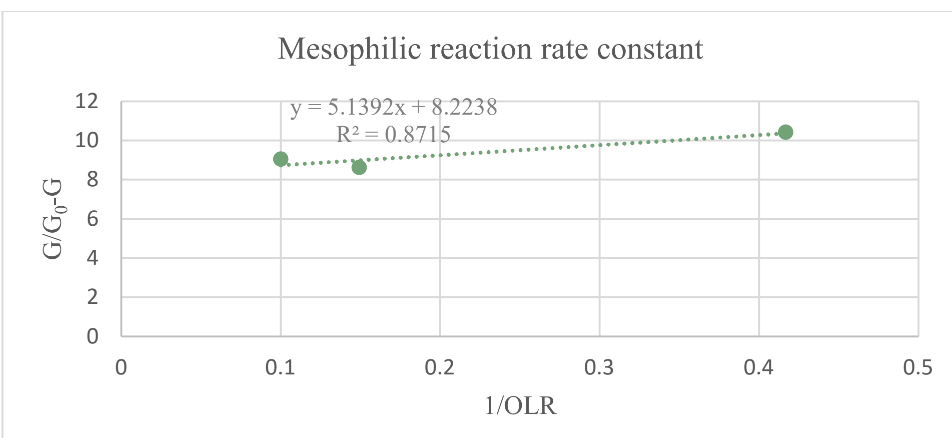


Figure 4.19 Mesophilic: Linear regression analysis of the reciprocal of OLRs of 2.4, 6.7, and 10 g COD /L/day versus $G/(G_0 - G)$ ($C_0 = 40$ g COD/ L, Slope: $k * C_0$ g /L/day).

The reaction rate constant observed was low with a k value of 0.128 day^{-1}. The lower reaction rate constant can correspond to the lower growth rate of mesophilic bacteria. Moreover, based on the lower k, the mesophilic reactor requires higher HRT (Figure 4.20). Similar results were observed by Li et al. (2018), who carried out a study using batch reactors for vegetable crop residues under mesophilic condition. They reported that the k value of different kinetic equations varied within the range of 0.094 to 0.167 day^{-1} under mesophilic conditions (36 ± 1 °C).

The plot of $G/(G_0 - G)$ against 1/OLR for the thermophilic reactor and the slope of k were shown and calculated using $k * C_0 = 7.1328$ g /L/ day. Setting $C_0 = 40$ g COD /L, k is calculated as k = 0.178 day^{-1} (Figure 4.20).

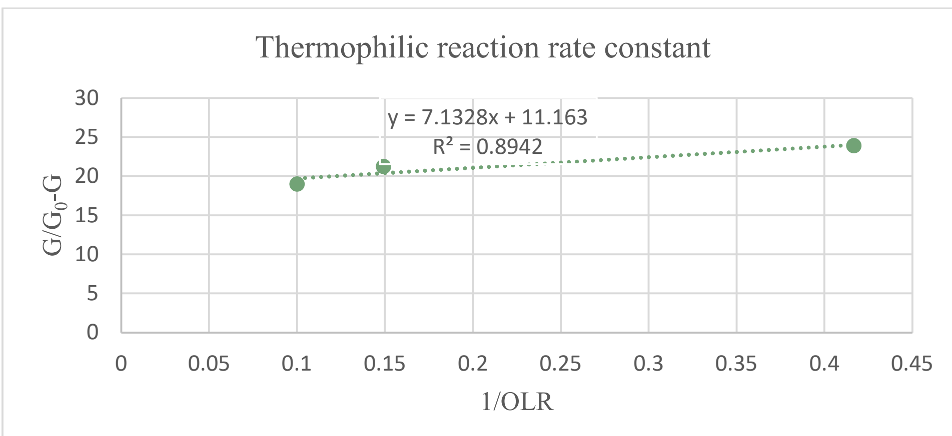


Figure 4.21 Thermophilic: Linear regression analysis of the reciprocal of OLRs of 2.4, 6.7, and 10 g COD /L/day versus $G/(G_0 - G)$ ($C_0 = 40$ g COD/L, Slope: $k * C_0$ g /L/ day).

Results of the kinetic analyses showed that the substrate degradation constant was 0.128 /day under mesophilic condition, while 0.178 /day was observed for the thermophilic condition. Due to a greater reaction rate and accelerated conversion ability of thermophilic bacteria, volatile fatty acids are observed as being always lower and utilized in a shorter time. A graph of absolute proportion p (G/G_0) for different values of HRT and k (Figure 4.21) indicates that the HRT decreased with the increase of k. Based on decreasing HRT with an increase in k (Linke, 2006), a thermophilic reactor required less HRT.

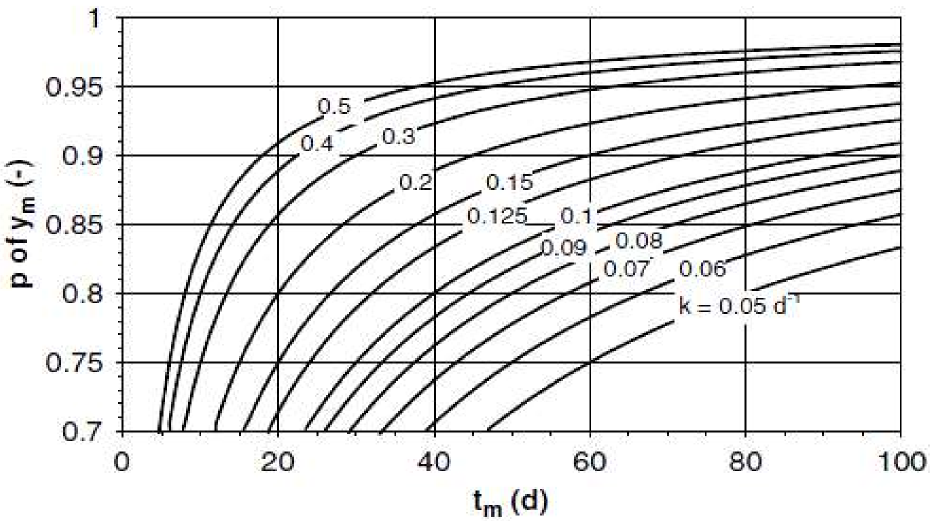


Figure 4.21 Absolute proportion p of y_m for different values of HRT and k. (y_m: maximum methane yield) (Linke, 2006).

Thermophilic digestion of simulated thin stillage showed performance advantages over the mesophilic condition, including a higher specific methane production and fewer HRTs. Table 4.16 shows that the methane production and content were approximately at least 10% more for the thermophilic reactor compared with the mesophilic reactor at different HRTs. The finding and results in stronger cohesive properties and consistent with the literature that the methane content of biogas under the thermophilic steady state was 74%, which was higher than that under the mesophilic conditions, i.e., 62 % (Tian, 2014). In another study, Rodríguez et al. (2013) reported the accumulated volume of methane per reactor volume was 7.31 m^3 in the mesophilic reactor and 9.26 m^3 in the thermophilic one, which was done in CSTR.

Table 4.13 illustrate that, at the same OLR, significant differences in biogas production were not observed. However, methane production for the thermophilic reactor was notably more than the mesophilic reactor. The higher methane production is connected with the higher degradation efficiency and the improvement of the energetic balance of the process. It is important to note that digestion temperature is crucial, since thermophilic reactors produce more biogas and removed more solids than mesophilic reactors in similar conditions (Coelho, 2011). As can be seen in Table 4.16, the methane content in the biogas for the thermophilic reactor was higher than the mesophilic in AnFBR with the plastic media.

Table 4.16 Average values of performance and stability indicators for mesophilic and thermophilic reactors

OLR/HRT	(g COD /L. day/day)	Mesophilic			Thermophilic		
		2.4/16	6.7/6	10/4	2.4/16	6.7/6	10/4
Volumetric biogas production	ml/day	11,600 ± 1000	33,605 ± 1430	52,115 ± 1525	12,150 ± 1400	34,935 ± 1610	53,310 ± 1395
Volumetric methane production	ml/day	5,830 ± 370	15,950 ± 1060	24,335 ± 750	6,220 ± 260	17,800 ± 890	27,150 ± 1425
Methane content in biogas	%	48 ± 2	49 ± 3	47 ± 2	53 ± 2	54 ± 2	51±3
Total VFA	mg/l	935 ± 70	1,460 ± 150	1285 ± 50	450 ± 40	1,175 ± 80	880 ± 40
$COD_{removal}$	%	96 ± 2	95 ± 1	96 ± 2	97 ± 1	96 ± 2	97 ± 2
pH	-	8.62 ± 0.03	8.25 ± 0.15	8.08 ± 0.2	8.75 ± 0.04	8.45 ± 0.1	8.35 ± 0.15
TVFAs/Alkalinity	-	0.08	0.16	0.18	0.05	0.16	0.13

4.3 Impact of vomitoxin on biological methane production kinetics from synthetic contaminated liquified corn using mesophilic anaerobic digestion

This part of the study investigated the effects of vomitoxin on biogas and methane production. The anaerobic digestion was conducted in batch reactors. A comparison was made of the results in the present study and similar literature studies operated in batch systems. Based on the Greenfield Global by-product and DON contaminated corn across Ontario in the past few years, this study considered the possibilities to manage liquified corn contaminated with vomitoxin for the production of biogas.

Batch anaerobic studies were conducted in bottles with a liquid volume of 100 mL and a head space volume of 50 mL. Eight bottles were spiked with 1, 5, 10, and 20 ppm of vomitoxin solution (duplicated). The initial characteristics of all the samples are presented in Table 4.17.

Table 4.17 Initial characteristics for liquified corn batches

Reactor	Parameter						
	TCOD (mg/L)	SCOD (mg/L)	TSS (mg/L)	VSS (mg/L)	PH	NH3-N (mg/L)	PO_4^{3-} (mg/L)
Blank	19,630 ± 475	335 ± 9	24,265 ± 575	13,165 ± 515	7.35 ± 0.01	510 ± 18	1,505 ± 30
Control	31,753 ± 2215	300 ± 15	35,965 ± 1270	22,250 ± 1055	7.12 ± 0.03	498 ± 5	1,570 ± 100
Liquified Corn	33,860 ± 810	11,295 ± 150	37,120 ± 1820	21,850 ± 1155	7.05 ± 0.12	490 ± 0	1,545 ± 25
Liquified Corn + 1 PPM DON	34,320 ± 725	11,093 ± 25	36,190 ± 695	20,875 ± 895	7.03 ± 0.03	485±35	1,550 ± 30
Liquified Corn + 5 PPM DON	34,910 ± 1927	11,653 ± 23	37,060 ± 405	21,810 ± 315	7.01 ± 0.03	510 ± 10	1,640 ± 50
Liquified Corn + 10 PPM DON	34,140 ± 805	10,265 ± 130	35,690 ± 900	21,375 ± 585	7.02 ± 0.04	530 ± 20	1,640 ± 30
Liquified Corn + 20 PPM DON	34,500 ± 993	10,133 ± 61	35,650 ± 1165	21,250 ± 730	7.04 ± 0.05	505 ± 10	1,535 ± 50

4.3.1 Biogas and methane production:

Most of the biogas production was achieved after the period of 33 days of anaerobic digestion at 37 °C (following the norm VDI 4630, 2006, daily biogas production was less than 1% of the total volume produced up to that time). The accumulated biogas production on Day 33 reached 1175, 1129, 1206, 1168, and 1154 mL for liquified corn and the contaminated samples were characterized by 1, 5, 10, and 20 ppm DON, respectively. Different DON concentration did not significantly affect the organic matter of conversion into biogas, with a level of less than 2% (Figure 4.22). Different DON content did not affect biogas production, as no statistical differences were detected. Even when a very high concentration of DON was considered (20 ppm) no adverse effect on the biogas production was detected. The results were consistent with other findings (Goux et al., 2010; De Gelder et al., 2017).

Concerning methane production for liquified corn and contaminated samples with 1, 5, 10, and 20 ppm, DON was 458, 443, 456, 453, and 459 mL, respectively. There were no significant differences among all the contaminated and non-contaminated samples (Figure 4.23). Surprisingly, the reactor with highest contamination (20 ppm) and the one without DON yielded nearly the same methane production. This is attributed to the fact that the culture only utilizes liquified corn and is not affected by the presence of DON. These results are in line with previous studies in which neither aflatoxin B1 (AFB1) (Salati et al., 2014) nor DON (Goux et al., 2010) negatively affected methane production.

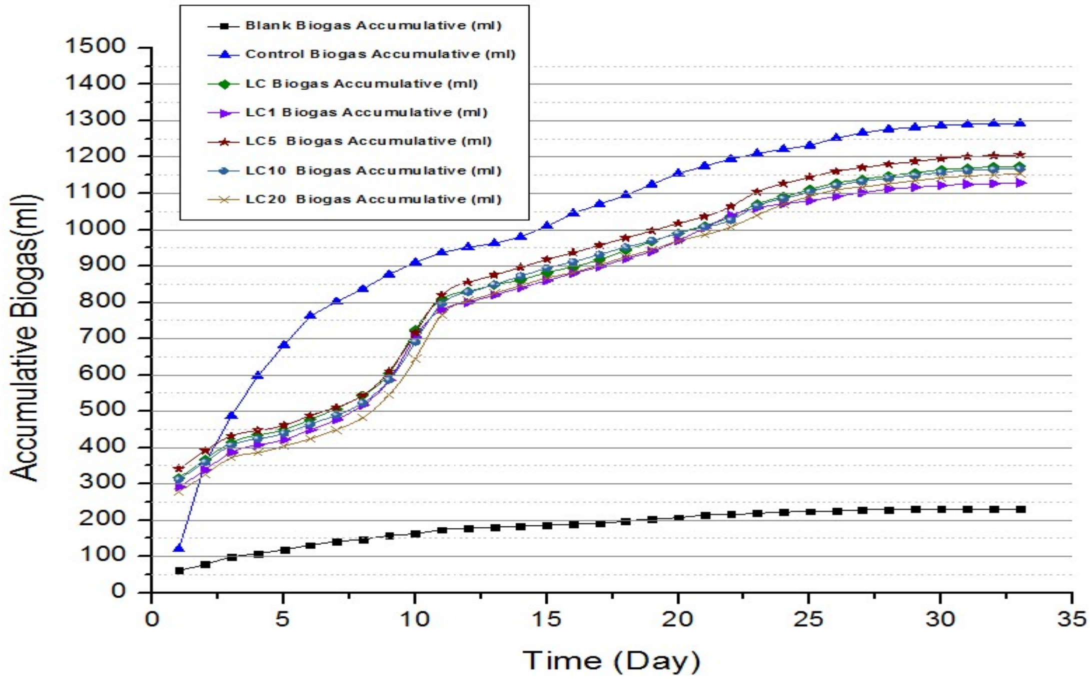


Figure 4.22 Biogas accumulative for batches.

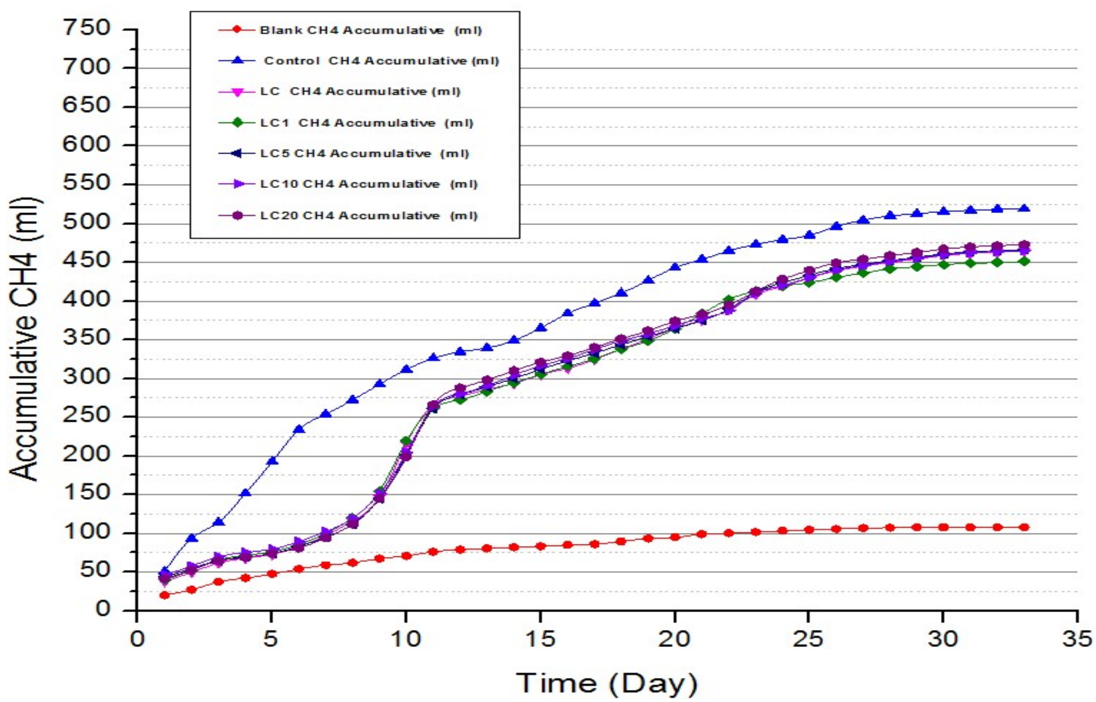


Figure 4.23 CH4 accumulative for batches.

Final samples were analyzed at the end of the batch experiment results are presented in Table 4.17.

Table 4.18 Final characteristics for liquified corn batches

Reactor	Parameter						
	TCOD (mg/L)	SCOD (mg/L)	TSS (mg/L)	VSS (mg/L)	PH	NH_3-N (mg/L)	PO_4^{3-} (mg/L)
Blank	16,830 ± 675	311 ± 37	22,633 ± 257	10,317 ± 266	7.06 ± 0.02	795 ± 105	1,583 ± 15
Control	18,305 ± 773	844 ± 389	24,583 ± 659	12,150 ± 384	7.02 ± 0.03	661 ± 12	1,627 ± 39
Liquified corn	18,230 ± 307	971 ± 85	24,817 ± 690	12,050 ± 597	7.13 ± 0.02	855 ± 23	1,647 ± 33
Liquified corn + 1 PPM DON	18,343 ± 562	1,055 ± 64	25,333 ± 714	12,100 ± 608	7.15 ± 0.04	934 ± 33	1,627 ± 39
Liquified corn + 5 PPM DON	18,400 ± 382	1,127 ± 188	24,850 ± 920	12,000 ± 625	7.11 ± 0.01	907 ± 17	1,722 ± 33
Liquified corn + 10 PPM DON	18,924 ± 1010	1,136 ± 237	24,917 ± 406	11,725 ± 258	7.10 ± 0.01	905 ± 27	1,745 ± 94
Liquified corn + 20 PPM DON	17,793 ± 577	1,065 ± 140	24,167± 666	11,450 ± 187	7.08 ± 0.02	856 ± 22	1,628 ± 36

4.3.2 COD balance

The COD mass balance for the biomethane tests was calculated, after correcting for the volume of the gas produced by the blank, using the following equation:

$$\text{COD mass balance (\%)} = \frac{TCOD_{CH4} + TC \quad {}_{Final}}{TCOD_{Initial}} \qquad \text{Equation (4.3.1)}$$

The theoretical methane yields from acetic acid on a g COD basis was 395 mLCH_4/g COD removed at 37°C. The COD balance closure was calculated at more than 95% for blank and control samples based on biogas production and COD removal showing the reliability of data and procedure. However, for liquified corn samples, the COD balance closed on average at 73 ± 3 %. The drop in COD mass balance might be the result of that to complete a perfect COD mass balance is difficult. In accounting for the fates of COD in the anaerobic digestion process potential errors can arise in measuring COD when the concentration of suspended solids is high. The COD mass balance data are shown in Table 4.19.

Table 4.19 Final results for batch

Batch name	Substrate	So/Xo g COD/g VSS	COD added (g)	Vomitoxin conc. (PPM)	Net ΔCOD		Net CH_4		COD balance	Actual yield	Theoretical yield	LCH_4/ g $COD_{removed}$
					mg/L	mg	mL	mg COD	%	LCH_4/ g COD_{added}		
Blank	Deionized water				2,800	280	106	268	96			
Control	Deionized water & Starch	1	1.8	0	13,448	1,345	516	1,307	97	0.23	0.29	0.39
Liquified corn	Liquified corn	1	1.8	0	15,630	1,563	459	1,162	74	0.20	0.26	0.29
Liquified corn & 1 PPM DON	Liquified corn	1	1.8	1	15,977	1,598	443	1,122	70	0.19	0.25	0.26
Liquified corn & 5 PPM DON	Liquified corn	1	1.8	5	16,510	1,651	456	1,154	70	0.19	0.25	0.26
Liquified corn & 10 PPM DON	Liquified corn	1	1.8	10	15,216	1,522	454	1,149	76	0.19	0.25	0.28
Liquified corn & 20 PPM DON	Liquified corn	1	1.8	20	15,607	1,561	459	1,162	74	0.20	0.26	0.28

4.3.3 BMP (Biomethane Potential) parameter estimation model

4.3.3.1 First order kinetic model

Methane yields can be predicated using first-order kinetic models which are mostly applied to anaerobic digestion systems. The BMP parameter estimation model, as described by Gunaseelan (2004), was employed to estimate the coefficients G_o and k, where a first order rate was used to compare the extents and rates of biomass conversion into methane. This rate was described using the following equation:

$$G = G_o\,(1 - e^{-kt}) \qquad \text{Equation (4.3.2)}$$

Where:

G is the cumulative methane yield (mL/g COD removed) at time t; G_o is the theoretical methane yield in mL/g COD removed at the end of the fermentation period; and k is the methane production rate constant in d^{-1}.

4.3.3.2 Gompertz model

The modified Gompertz equation (Equation 4.3.3), as described by Chen et al. (2006), was employed with the parameters estimated using the solver function in Microsoft Excel 2013:

$$G\,(t) = G_o.\ \exp\,\{-\exp\,[\,\frac{R_{max}\cdot e}{Go}\,(\lambda - t) + 1]\} \qquad \text{Equation (4.3.3)}$$

Where:

$G(t)$ is the cumulative biogas potential (mL) at time (t); λ is the lag phase (d); G_0 is the biogas production potential (mL); R_m is the biogas production rate (mL/d); and e is exp (1), which is approximately 2.7.

4.3.4 First order kinetic model

Table 4.17 shows the kinetic data from the first order model. The determination coefficients (R^2) are also shown in the respective figures. The highest R^2 values (0.955–0.957) were calculated for the first order model. Such good performance ($R^2 > = 0.955$) demonstrates that the proposed first order kinetic model equation can accurately describe the accumulative methane curves. This result agreed with results obtained by Zahan et al. (2014), who did a study on food waste anaerobic digestion in batch reactors, suggested that the small deviations achieved between the measured and predicted value (almost equal to or less than 10%) indicate that the first order kinetic models have correctly predicted the performance of the anaerobic reactors.

Figure 4.24 depicts the results of the nonlinear fitting of measured methane yield for six different substrates.

Table 4.20 Summary of the kinetic study using first order model

Batch experiment	Vomitoxin conc.	G_0 ($_{max\ CH4}$)	K	R^2
	PPM	mL	Day^{-1}	
Liquified corn	0	458	0.041	0.957
Liquified corn & 1 PPM DON	1	443	0.037	0.956
Liquified corn & 5 PPM DON	5	456	0.036	0.957
Liquified corn & 10 PPM DON	10	453	0.042	0.957
Liquified corn & 20 PPM DON	20	459	0.041	0.955

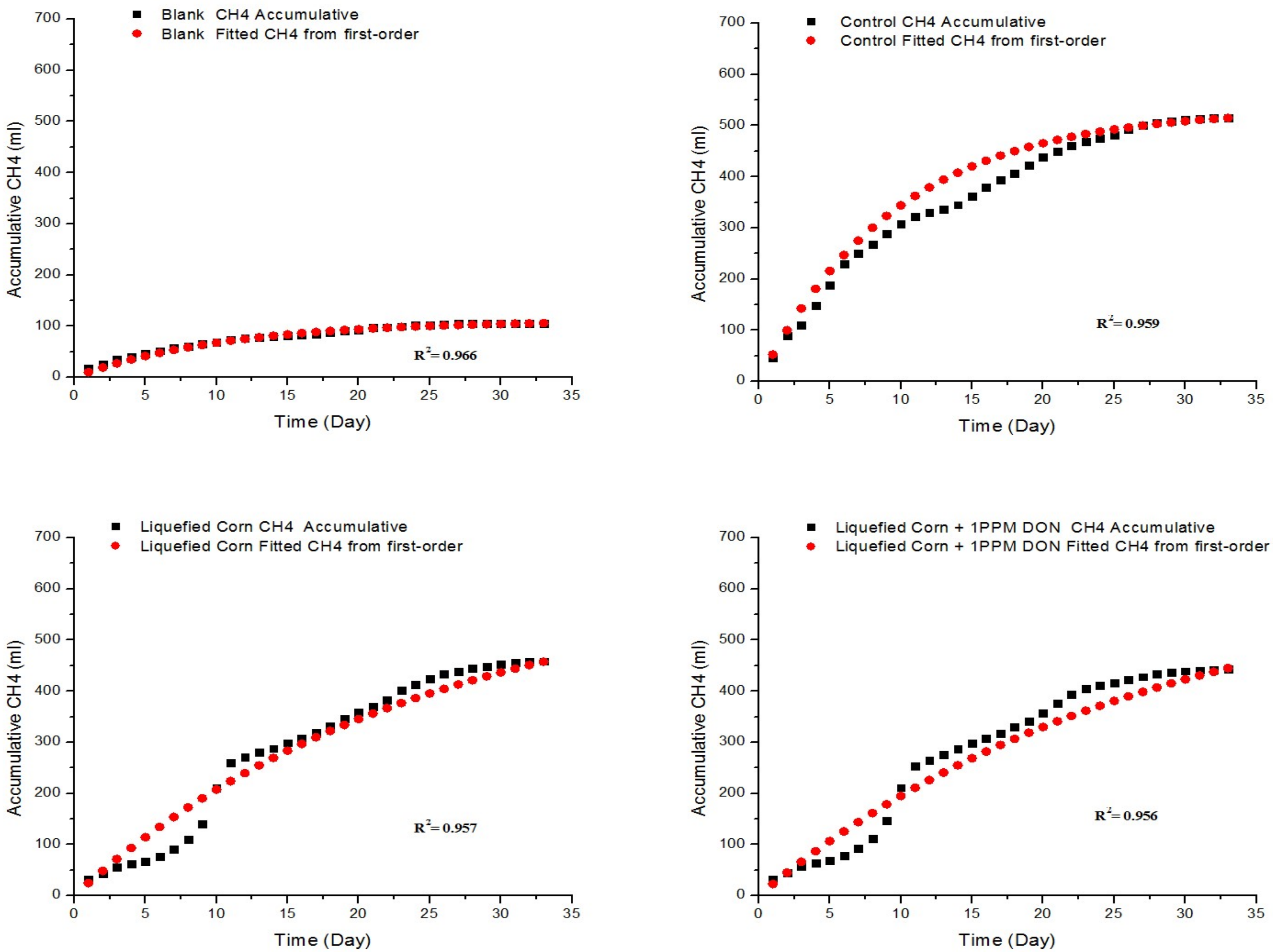
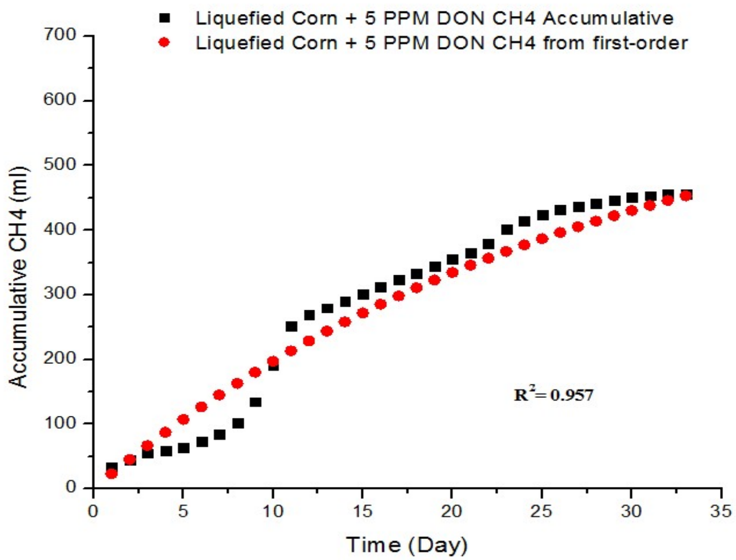
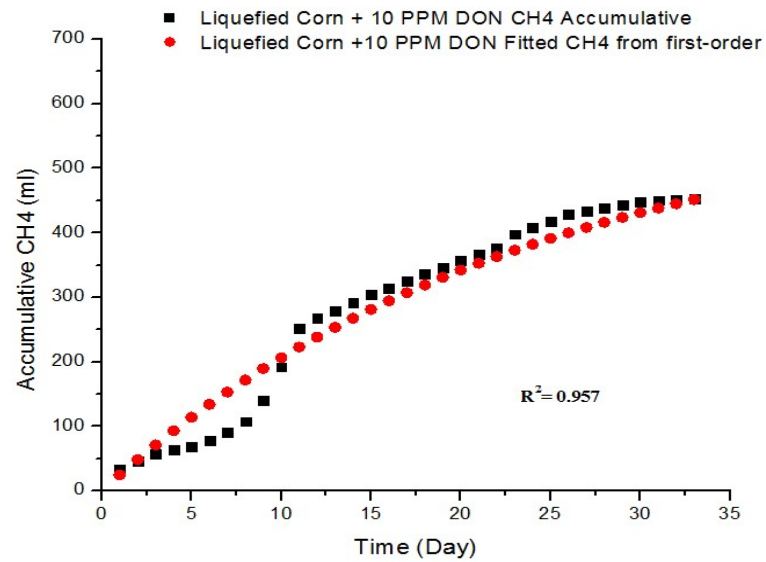


Figure 4.24 Methane accumulative plotted with methane predicted using first order kinetic model.

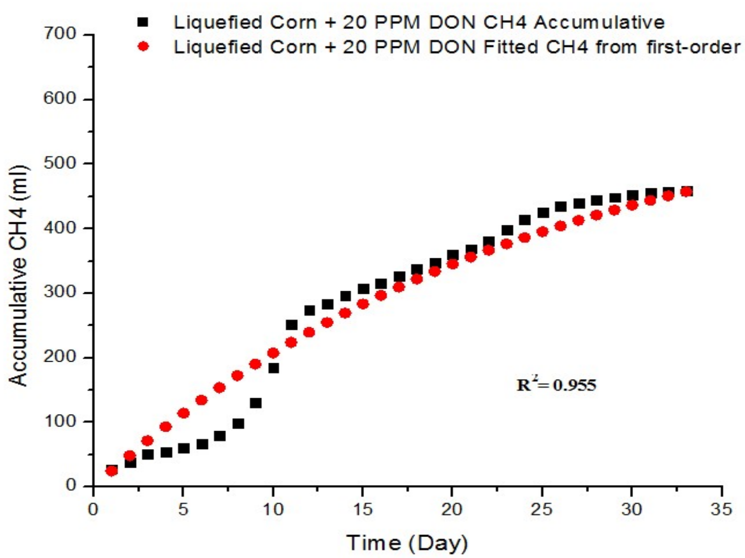
Continued



Liquefied Corn + 5 PPM DON CH4 Accumulative
Liquefied Corn + 5 PPM DON CH4 from first-order
Accumulative CH4 (ml)
Time (Day)
R^2= 0.957



Liquefied Corn + 10 PPM DON CH4 Accumulative
Liquefied Corn +10 PPM DON Fitted CH4 from first-order
Accumulative CH4 (ml)
Time (Day)
R^2= 0.957



Liquefied Corn + 20 PPM DON CH4 Accumulative
Liquefied Corn + 20 PPM DON Fitted CH4 from first-order
Accumulative CH4 (ml)
Time (Day)
R^2= 0.955

4.3.5 Gompertz modified model parameters

Table 4.21 presents a summary of the Gompertz rates data. The coefficient of determination R^2, was between 0.922 and 0.937. For the control batch, the lag phase was not observed since starch was the soluble substrate in the control batch. However, for all the liquified corn batches, the lag phase was 6 days and not affected by DON concentrations. Therefore, the existence of lag phase time for all the liquified corn batches may be one of the main reasons why the modified Gompertz model could perform better in its estimation. The maximum methane rate in the batches was 70 mL/day for liquified corn without DON. This could be because liquified corn is more easily degradable than other substrates, including DON. However, total methane production for the batches showed that DON of up to 20 ppm had no inhibitory effect on liquified corn digestion (Figure 4.25).

The same is supported by results from other studies, including Frauz et al. (2007), who carried out a study on contaminated grain in two batch reactors. They reported biogas and methane production were similar between the two reactors and all other process parameters were within normal range. They also reported no inhibitory effects on the anaerobic digestion process of cereals with 20,000 ppb DON compared to the control cereals. In another study (Goux et al., 2010), seven DON contaminated wheat flour samples were subjected to anaerobic digestion in triplicate lab-scale batch reactors, in which no significant effect was observed on the biogas quantity and quality.

Table 4.21 Summary of the kinetic study using Gompertz modified model

Batch experiment	Vomitoxin conc.	G_0 (max CH_4)	R_{max} (Maximum CH_4 prod.rate)	λ (Lag phase)	R^2
	PPM	mL	mL/d	Day	
Liquified corn	0	458	70	6	0.922
Liquified corn & 1 PPM DON	1	443	65	6	0.930
Liquified corn & 5 PPM DON	5	456	60	6	0.936
Liquified corn & 10 PPM DON	10	453	60	6	0.937
Liquified Corn & 20 PPM DON	20	459	67	6	0.929

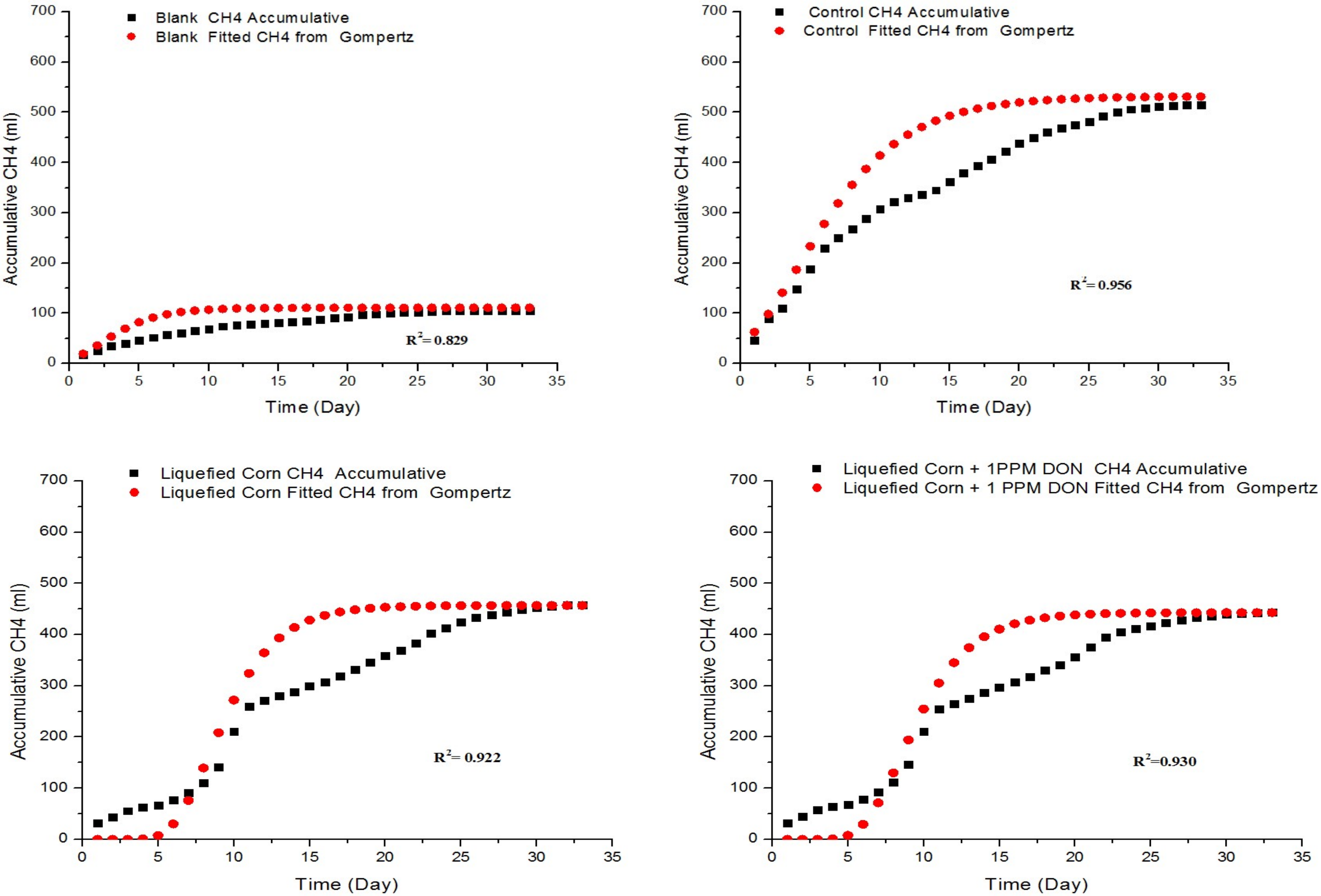
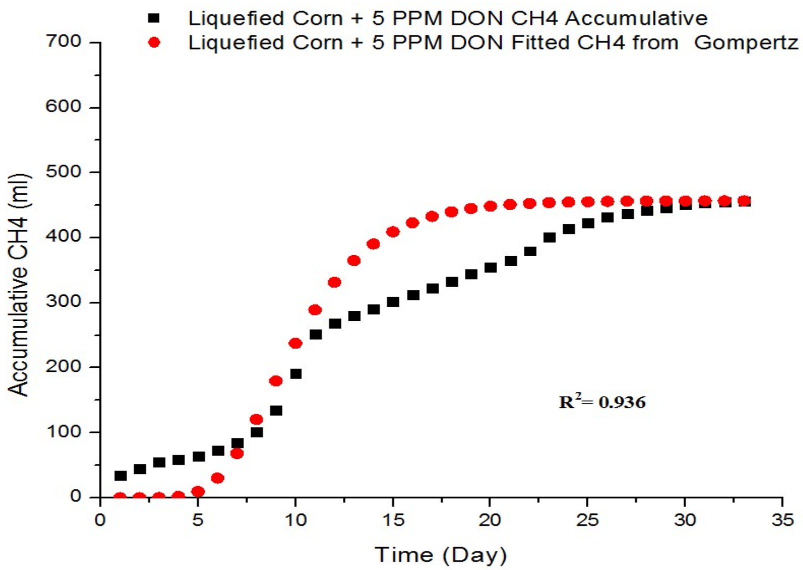
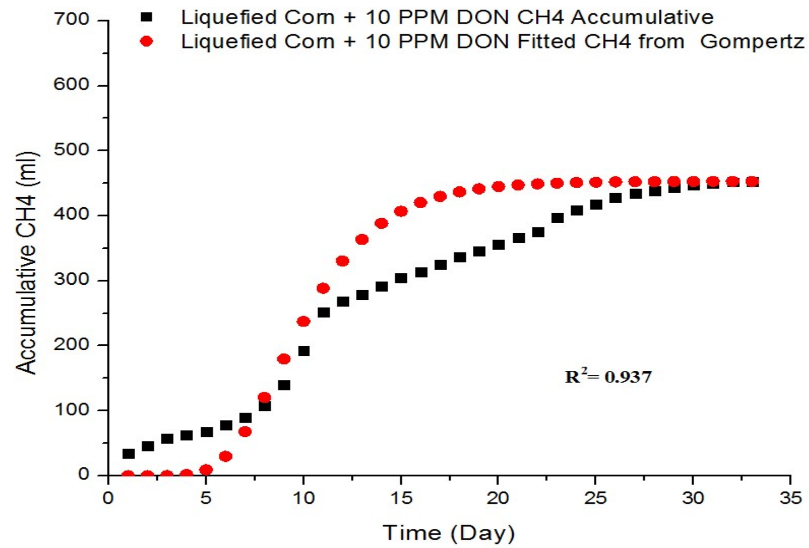


Figure 4.25 Methane accumulative plotted with methane predicted using the Gompertz modified model kinetic model.

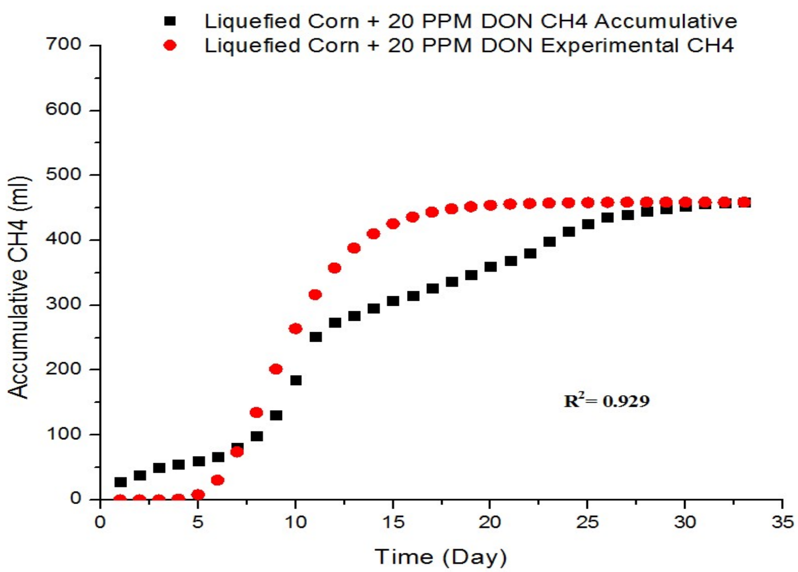
Continued



Liquefied Corn + 5 PPM DON CH4 Accumulative
Liquefied Corn + 5 PPM DON Fitted CH4 from Gompertz
Accumulative CH4 (ml)
Time (Day)
R^2= 0.936



Liquefied Corn + 10 PPM DON CH4 Accumulative
Liquefied Corn + 10 PPM DON Fitted CH4 from Gompertz
Accumulative CH4 (ml)
Time (Day)
R^2= 0.937



Liquefied Corn + 20 PPM DON CH4 Accumulative
Liquefied Corn + 20 PPM DON Experimental CH4
Accumulative CH4 (ml)
Time (Day)
R^2= 0.929

As depicted in Table 4.19, vomitoxin has not been reported on in the literature very widely. The impact of DON on the kinetic parameters of methane production in a mixed culture environment is not available in the literature. To the best knowledge of the author, no kinetic model has been used to describe the impact of DON on biomethane production from liquified corn wastes using mixed cultures.

Table 4.22 Vomitoxin for different substrates

	Inoculum	Temp.	substrate	Toxin type and Conc.	Degradation %	Reference
Batch	ADS	37	Maize	DON 4413 ± 1,300 µg/kg	70	De Gelder et al. 2017
Batch	Rumen fluid	40	Cellulose/Corn starch	DON 40mg/kg	90	Jeong et al. 2010
Batch	ADS	37	Artificially spiked commercial flour	DON 80000 µg/kg	100	Goux et al. 2010
CSTR	ADS	37	Contaminated corn plus pig slurry	Aflatoxin B1 110 µg/kg	42	Salati 2014
CSTR	ADS	37	Maize	Aflatoxin and fumonisins	12–95	Giorni 2018

4.3.6 Kinetic analysis

Table 4.20 and Table 4.21 summarize the fitting results of the dynamic models' parameters. Between the two kinetic models used in this experiment, the first order model presents a slightly lower difference between the predicted and measured methane yields (G_0) in comparison with the Gmopertz model. The modified Gompertz model with a lag phase (λ) of six days was calculated for all the liquified corn, contaminated and non-contaminated samples. The performance ($R^2 \geq 0.922$) of liquified corn in the Gompertz model demonstrated that the two proposed equations could accurately describe the variation of liquified corn methane yield curves. Furthermore, based on the kinetic analysis results (the difference between the predicted and measured methane yield values, and the correlation coefficient of nonlinear fitting), the first order model is recommended as the most suitable model for fitting liquified corn methane yield in our tests (Appendix 6).

4.3.7 Vomitoxin contaminated liquified corn inhibition effect on anaerobic digestion process

Table 4.21 presents the cumulative methane production for all the liquified corn and contaminated liquified corn samples. The liquified corn at 0 g/L vomitoxin produced around 458 mL of methane, while the sample with 1, 5, 10, and 20 ppm vomitoxin produced around 443, 456, 453, and 459 mL, respectively. Methane fractions in the biogas for all bottles were between 51% and 56%. Similar results were observed by De Gelder et al. (2018), who carried out a study on contaminated maize in batch reactors (R1, R2). They reported that the methane fraction in the biogas after 15 days of AD was 56% for bioreactor R1 and 55% for bioreactor R2. Neither biogas production, nor methane production for all different vomitoxin concentrations were severely hampered, which agrees with the report by Goux et al. (2010).

4.3.8 Vomitoxin concentrating during the digestion process

Table 4.23 illustrates that after 33 days of anaerobic digestion, the vomitoxin degradation was in the range of 24% to 52%. In the liquified corn reactor without artificial DON, a lower degradation of 24% was observed. It is probable that when the DON is free in the liquified corn and sludge it may have a slight inhibition effect on the microbial consortium, while when DON is bound to an organic matrix, the liquified corn, it is released at slower pace. Similar results were observed in a study by Salati (2014), which revealed 42% degradation for contaminated corn by Aflatoxin B1. Similar results have been reported in another study by Goux et al. (2010). A liquified corn sample was analyzed by HPLC and 66 ppm vomitoxin was detected. They reported that, concerning the DON, a concentration decreases rate greater than 25% was achieved after 15 days. In these studies, results indicated that at least 30 days of retention would be necessary to completely eliminate the DON toxin.

Table 4.23 Vomitoxin degradation during batch anaerobic mesophilic digestion of liquified corn

Batches name	Initial vomitoxin concentration	Final vomitoxin concentration detected after 33 days
	PPM	PPM
Liquified corn	4.38 ± 0.13	3.34 ± 0.11
Liquified corn & 1 PPM DON	5.34 ± 0.16	3.8 ± 0.14
Liquified corn & 5 PPM DON	9.69 ± 0.2	6.55 ± 0.21
Liquified corn & 10 PPM DON	14.2 ± 0.25	8.33 ± 0.33
Liquified corn & 20 PPM DON	23.42 ± 0.72	11.17 ± 0.56

4.3.9 Economic assessment of thin stillage in the corn-ethanol industry

The recovery of co-products and use contaminated liquified corn are one of the key drivers for the economic sustainability of ethanol manufacturing; DDGS can account for nearly 25% of the total revenue for some ethanol plants (Hill et al., 2006). DDGS and other co-products within the system also provide the greatest opportunity to decrease environmental impacts, through the displacement of other products.

Based on DON contaminated liquified corn digestion process, it can be deducted that vomitoxin contamination, even at a very high level of 20 ppm, does not harm the anaerobic digestion process of liquified corn, and the process may present a good alternative to incineration to treat such substrates since it allows the recovery of energy and nutrients. This evidence suggests a safe use of contaminated feedstock for biogas production, although studies conducted on the fate of mycotoxins in contaminated feedstocks for biogas production have shown that toxins are degraded at a different rate during the anaerobic fermentation process (Salati et al., 2014; De Gelder et al., 2017).

The natural gas use for the ethanol industry was reduced due to the methane produced in the anaerobic digester and the decrease in energy required for processing the thin stillage. Table 4.2 3 the methane production from thin stillage in the AD process.

Table 4.23 Methane production from thin stillage (Sayedin et al., 2019)

Methane Production	0.31 L CH_4/g TCOD
Thin Stillage COD	0.0689 kg COD/L thin stillage
COD Removal Rate	92.50%
Lower Heating Value (CH4)	35.8 kJ/L
Density (approximate)	1.0 kg/L
Methane Production	169.8 GJ/h

The methane in the biogas is used to supplement the natural gas use within the plant; this is subtracted from the natural gas required.

The economic analysis is dependent on the parameters of a specific plant and should be region specific. The market price of commodities has a significant influence on the economic stability of the corn ethanol process including the cost of corn, ethanol, DDGS, carbon dioxide, electricity, energy, and other feed co-products. However, for the conventional facility, the overall profitability is largely determined by the relationship between ethanol prices and corn prices (Wood et al., 2013).

An in-depth economic evaluation should include the costs for raw materials, labor, utilities, maintenance, plant overhead, administrative and financing costs. An important parameter is determining the total capital costs and operating costs for the facility. The annualized cost for the plant is a useful tool for predicting the yearly infrastructure costs based on the capital costs for the plant, the lifetime of the plant and the finance rate.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5. Conclusions and Recommendations

5.1 Conclusions

The study was conducted in three distinctly separate parts with their respective results presented in Chapter 4. The conclusions from each part are summarized and presented below.

5.1.1 Influence of particle properties on biofilm structure and operational parameters

The aim of this study was to investigate the effect of different media on anaerobic digestion of simulated thin stillage using fluidized bed reactors. Zeolite, a mineral media and plastic, a synthetic media were employed in two lab-scale fluidized bed reactors. Biofilm structure, reactor performance, and energy consumption were studied in FBR at different HRTs. Based on the results of the study:

A significant increase in the biofilm thickness was observed in the zeolite media. The attached biomass in the zeolite media and the plastic media were 33.6 and 19.7 mg VSS/g media, respectively. The zeolite showed a maximum biofilm thickness of 370 µm, compared with 200 µm that was observed in the plastic media. From an operational perspective, the zeolite media was more suitable for FBR, with less floating issues. The plastic media reactor showed the media floating on top of the reactor even at 50% of the zeolite media's superficial velocity. However, the maintenance and replacement cost of natural zeolite will be significantly higher than plastic media, considering different replacement frequencies. Furthermore, fluidization energy costs due to higher recirculation flow for zeolite was more than for the plastic media.

5.1.2 Comparing mesophilic and thermophilic digestion in the treatment of simulated thin stillage employing AnFBRs with plastic media (HDPE)

The aim of this study was to compare mesophilic and thermophilic digestion in treatment of thin stillage using fluidized bed reactors. Two 7-liter working volume FBRs were operated mesophilically (37±1˚C) and thermophilically (55 ±1˚C) over different hydraulic retention times (HRT).The plastic media with a diameter of (d_m) in the range of 600-2000 μm was employed as carrier media. Each experimental run continued over a six-month period. Based on the results of the study, the following conclusions can be drawn:

1. One-step temperature increase was proven to be a more successful strategy than the gradual increase of temperature in a multi-step approach.
2. The adaptation of mesophilic anaerobic digesters to the thermophilic conditions was successfully carried out under the constant loading rates in the AnFBR.
3. Thermophilic digestion of simulated thin stillage showed performance advantages over the mesophilic process, which included higher specific methane production and fewer HRTs. The methane production and content were a minimum of approximately 10% more in the thermophilic reactor than the mesophilic reactor at different HRTs.

4. As in the mesophilic condition, the methane content decreased with increasing carbon to nitrogen ratio for thermophilic conditions, i.e., 56% methane for C/N = 16 and 51% methane for C/N = 32 for the thermophilic condition. However, in biogas production, the major result of anaerobic digestion was markedly increased by more than 30% from 40.05 L/day to 53.31 L/day, by changing the ratio from 16 to 32. The methane production rate also increased by more than 20% from 22.43 L/day to 27.15 L/day for a ratio of 16 and 32, respectively.

5. For the given values of reaction rate constant k, theoretical methane yield G_0, and methane yield G in the reactor's performance, both the required hydraulic retention time HRT and the OLR can be calculated by means of a few parameters.

6. The maximum difference in the substrate degradation constant was observed when being compared with the methane generation. In this case, the values were 0.128 day^{-1} in the mesophilic process, instead of 0.178 day^{-1}, which assumed an increment of more than 39%.

5.1.3 Impact of vomitoxin on biological methane production kinetics from synthetic contaminated liquified corn using mesophilic anaerobic digestion

The aim of this study was to investigate the effects of vomitoxin on biological methane production kinetics from synthetic contaminated liquified corn using mesophilic anaerobic digestion. The anaerobic digestion evaluation conducted in batch reactors indicated that DON contamination, even at a high level of 20 ppm, did not harm the anaerobic digestion process of liquified corn and that the process may present a good alternative to incineration to treat such substrates, since it allows for the recovery of energy and nutrients. The cumulative methane production for all the liquified corn and contaminated liquified corn was not affected. Liquified corn at 0 g/L vomitoxin produced approximately 458 mL methane, while the sample with 1, 5, 10, and 20 ppm vomitoxin produced around 443, 456, 453, and 459 mL, respectively. Methane fractions in the biogas for all the bottles were between 51% and 56%. Neither biogas production, nor methane production for all different vomitoxin concentrations, were severely hampered.

5.2 Recommendations

The following suggestions are recommended as the possible research potential:

1. Mineral and synthetic carrier

- Different media with different densities and specific surface area (SSA) should be tested in the system in order to find the best carrier media for this technology with a low density, high SSA and low price.
- Control of the liquid recirculation is essential for minimization of the running costs

2. Mesophilic and thermophilic

- Thermophilic digestion of simulated thin stillage showed performance advantages over the mesophilic one, which included higher specific methane production and less HRTs. Therefore, thermophilic conditions are recommended, especially when working with high organic loading rate at lower HRT.
- The methane production and content were at minimum approximately 10% more in the thermophilic reactor than the mesophilic reactor at different HRTs.

3. Deoxynivalenol (DON) contaminated corn effect

- In this study, we show that mycotoxin contaminated corn can be safely treated through anaerobic digestion into methane and digestated.

- The production of biogas is an interesting alternative use of contaminated corn without having an impact on methane production. The information presented could be helpful for researchers in selecting different DON concentrations in the contaminated corn, noting their effect on the digestion process.

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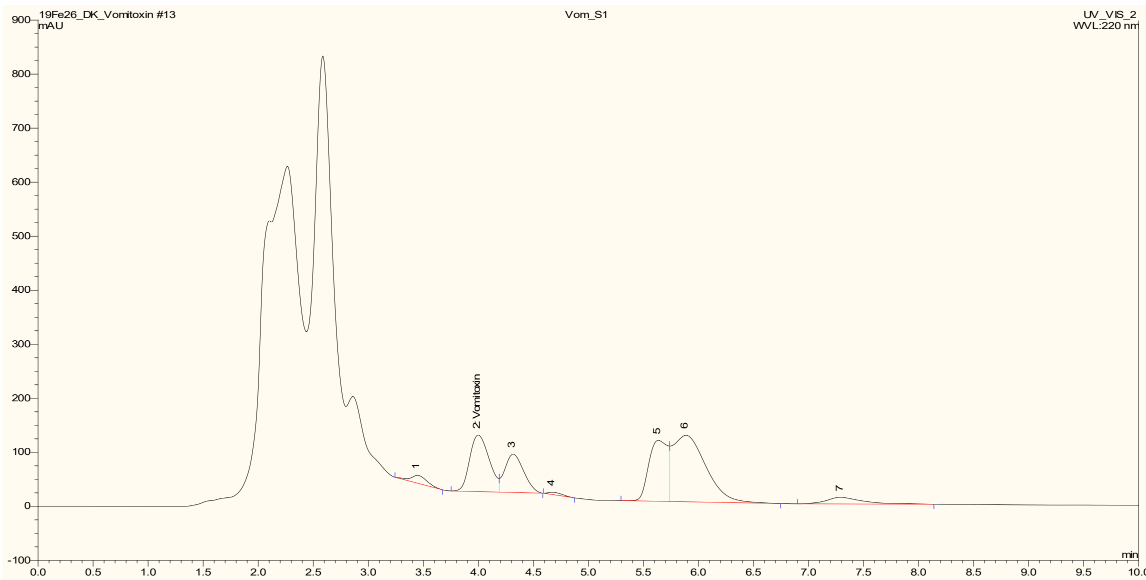
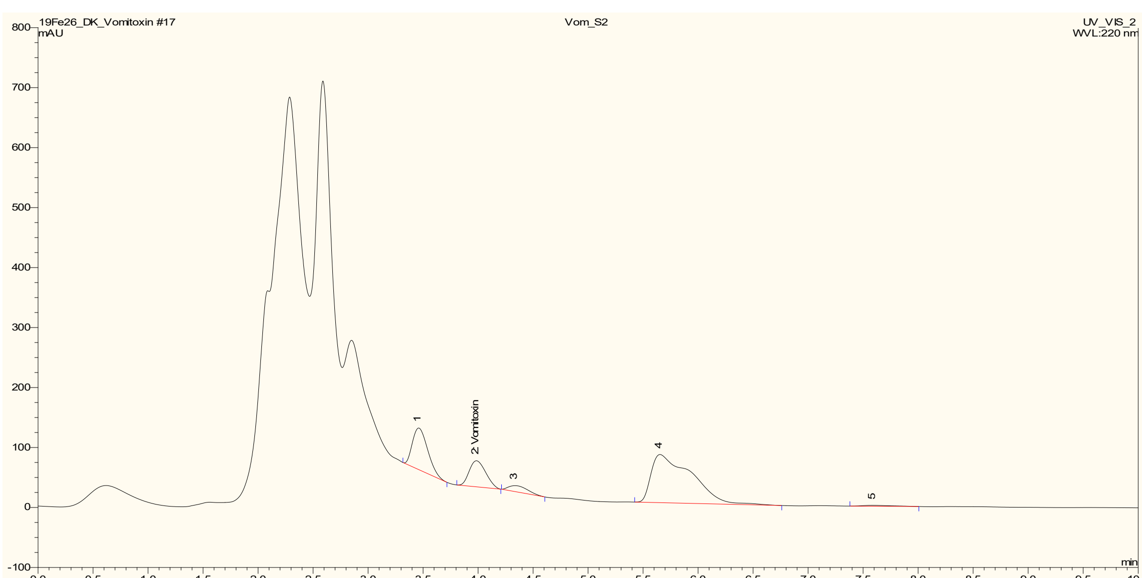
APPENDICES

Appendix 1

Vomitoxin concentration in the final samples (HPLC)

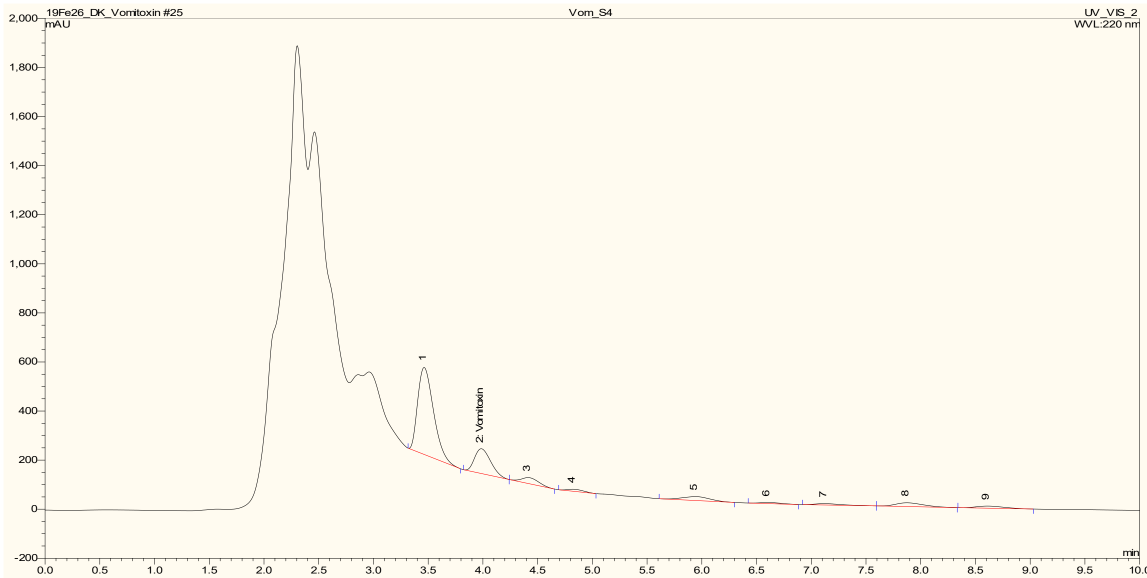


Vomitoxin concentration final results (20 PPM Batch)

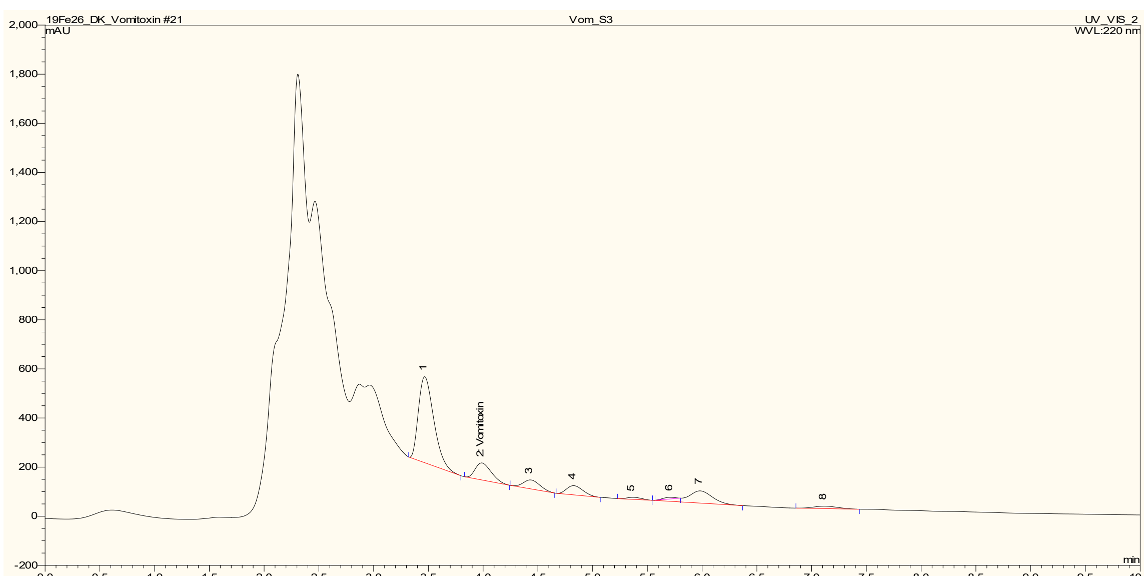


Vomitoxin concentration final results (10 PPM Batch)

Cont.



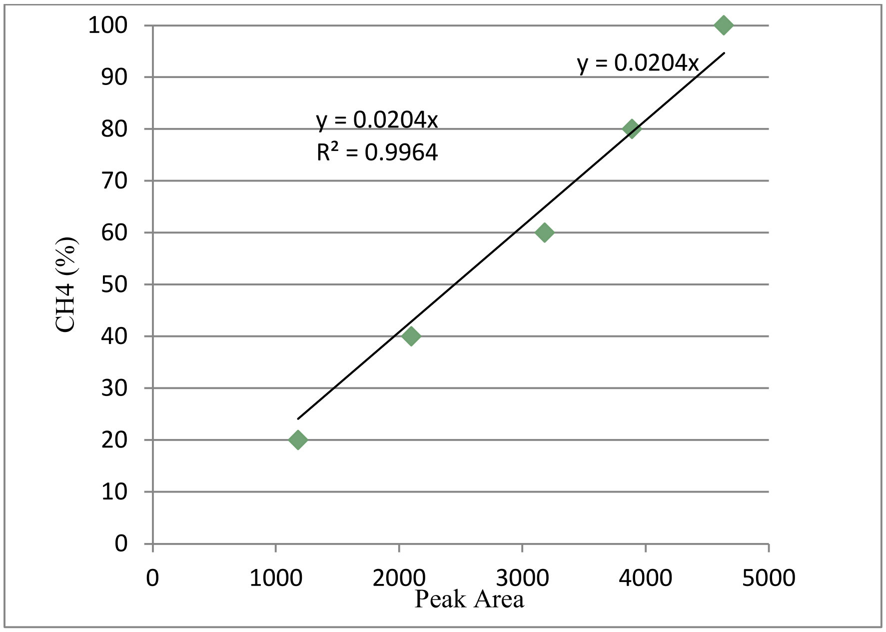
Vomitoxin concentration final results (5 PPM Batch)



Vomitoxin concentration final results (1 PPM Batch)

Appendix 2

GC-TCD Calibration



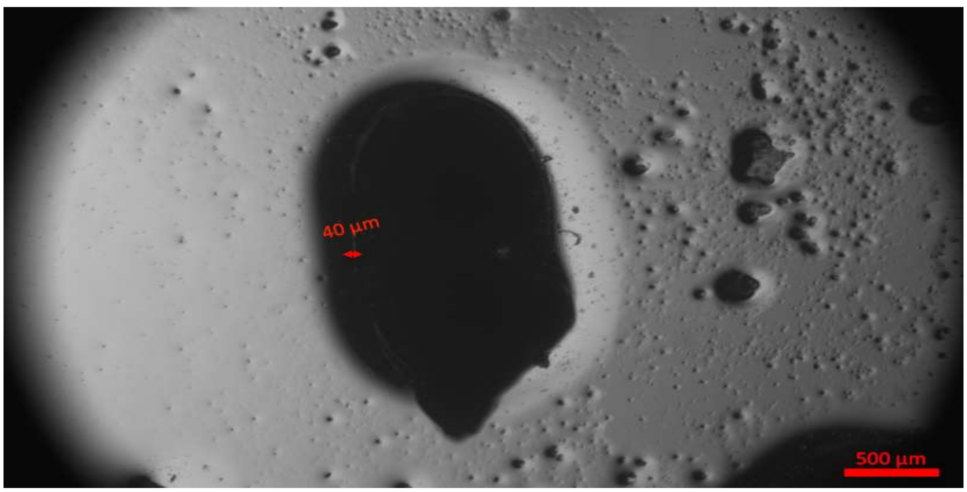
TCD calibration graph

Appendix 3

Biofilm measurement

Biofilm measurements

Sample #	Date	Sample label	Attached VSS (mgVSS/g media)	Biofilm thickness (µm)
Sample 1	04/12/2017	FBR New 45⁰ Bottom sliding port-7" out	11.2±0.4	40

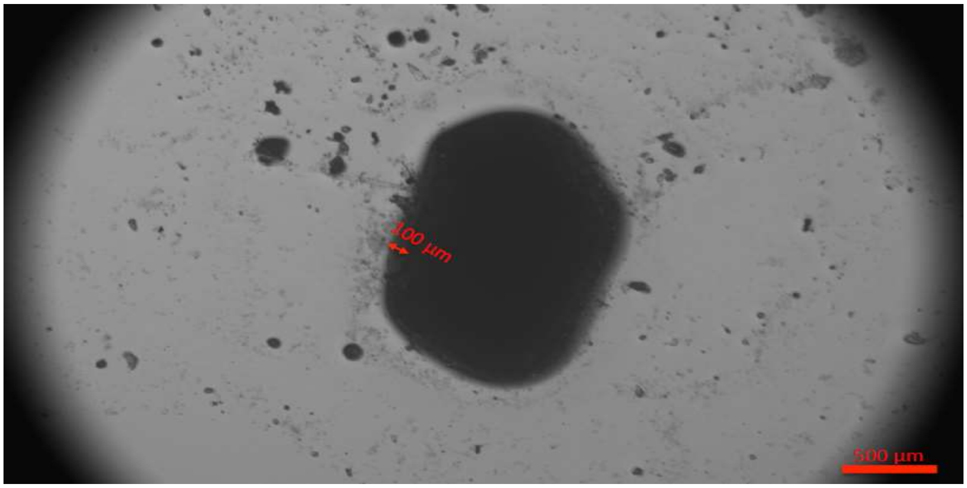


Biofilm measurements

Sample #	Attached VSS (mgVSS/g media)	Biofilm thickness (µm)
Sample 1	10.2±2.6	100

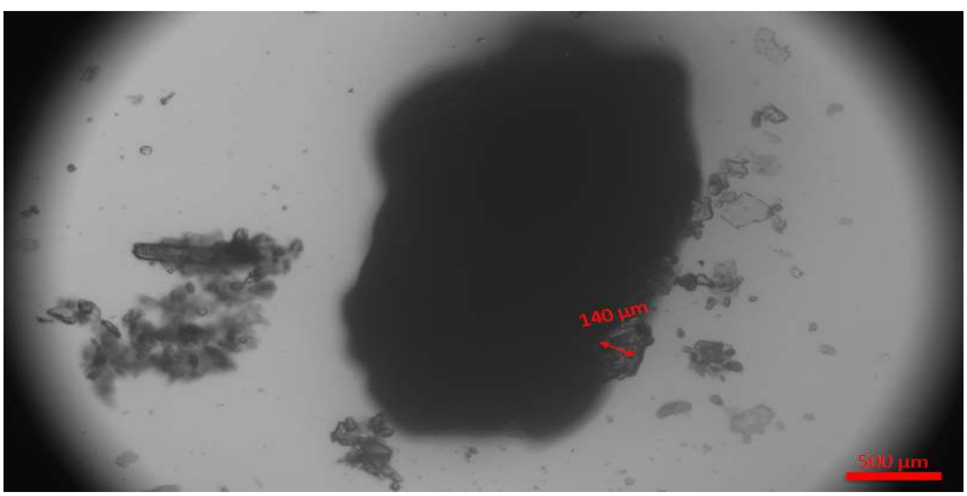
Biofilm measurement for zolite and plastic media

Cont.



Biofilm measurements

Sample #	Date	Sample label	Attached VSS (mgVSS/g media)	Biofilm thickness (µm)
Sample 1	18/12/2017	FBR	19.7±1.6	140

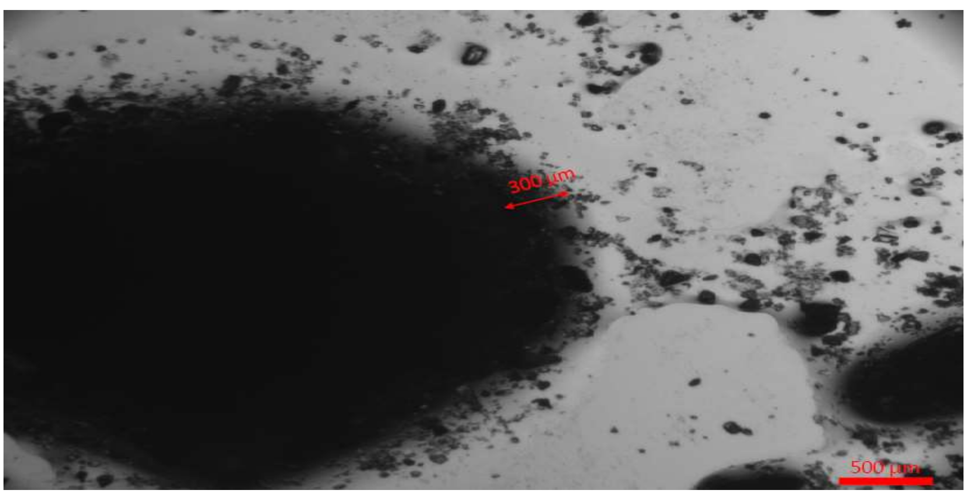


Biofilm measurement for plastic media

Cont.

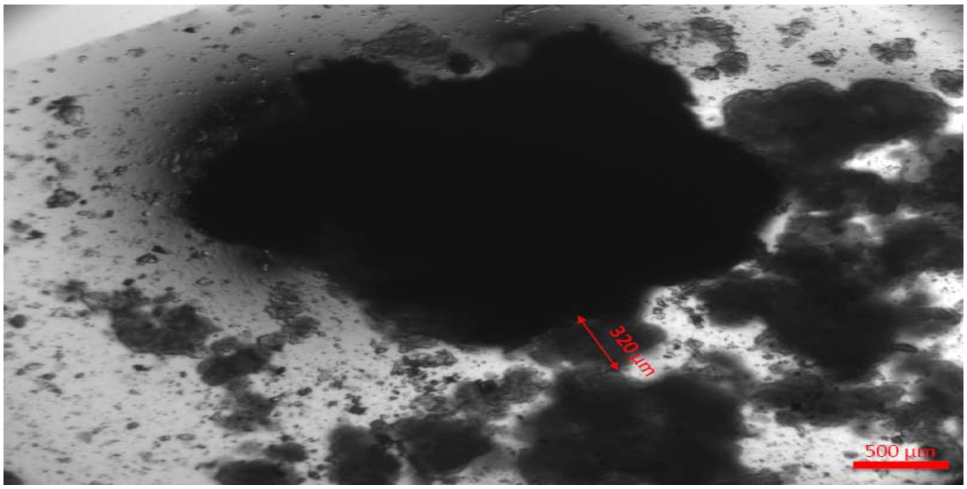
Table 1: average attached biomass per gram media for the U of W's media

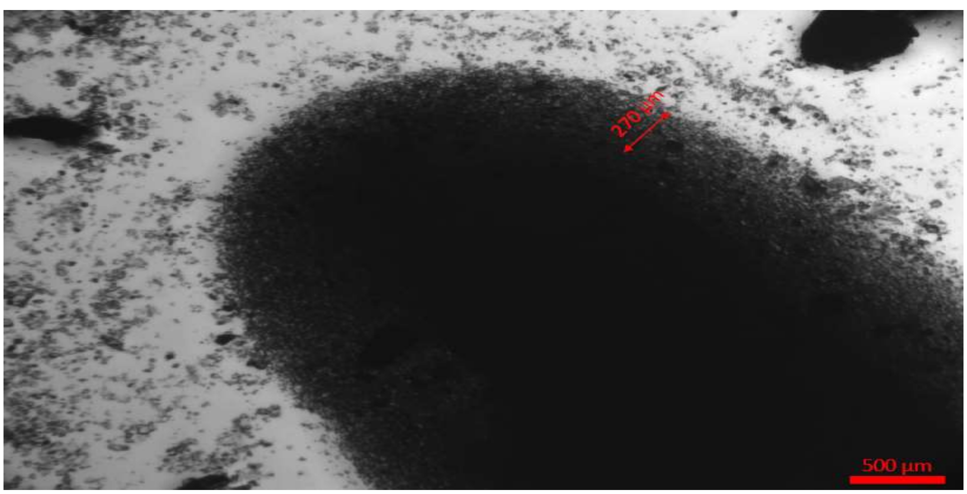
Sample #	Attached biomass per gram media (mgVSS/g) "Avg±SD"	Biofilm Thickness
Sample 1	32.4±6.2	**320 µm**
Sample 2	29.2±3.2	**300 µm**
Sample 3	24.3±2.2	**270 µm**
Sample 4	11.6±2.0	**100 µm**
Sample 5	14.7±3.4	**200 µm**
Sample 6	18.6.3±5.4	**170 µm**



Biofilm measurement for zolite media

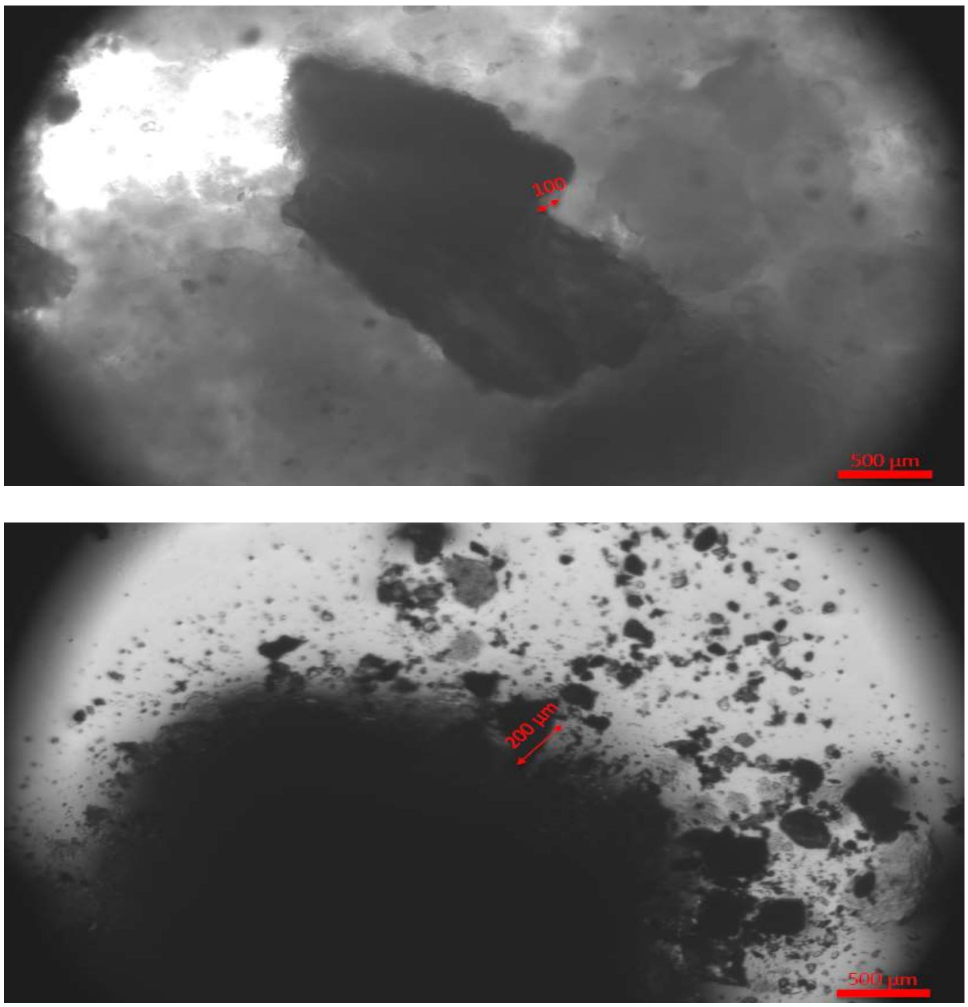
Cont.





Biofilm measurement for zolite media

Cont.



Biofilm measurement for plastic media

Cont.

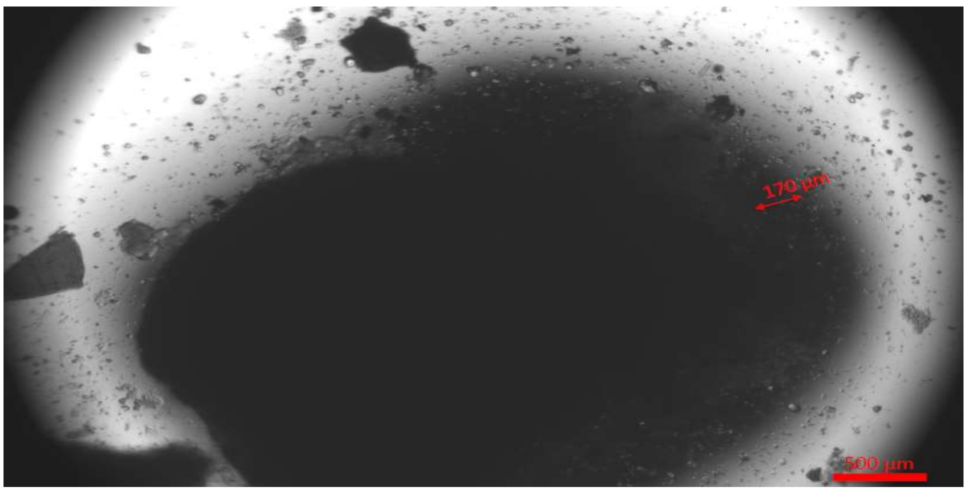
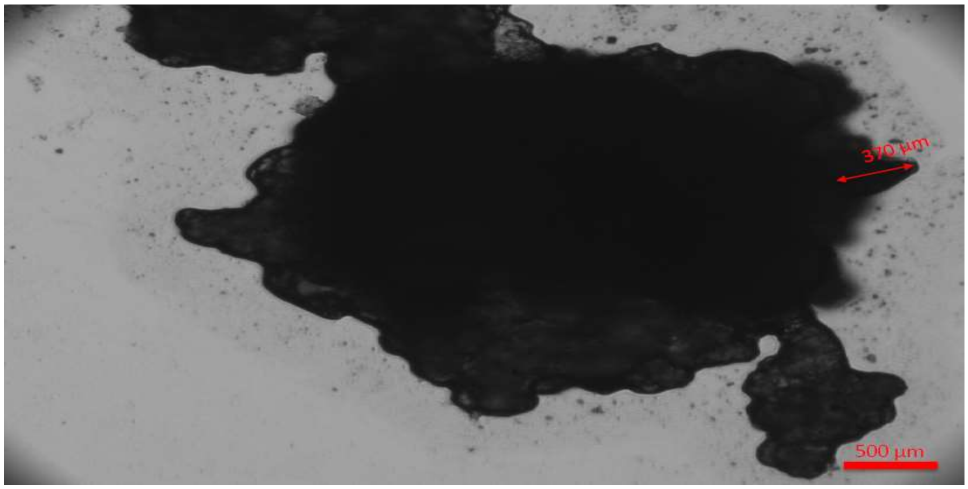


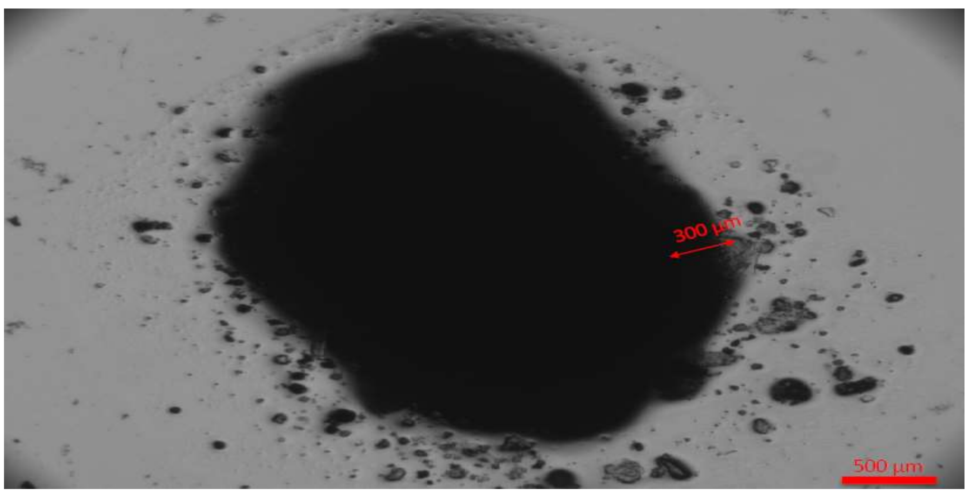
Table 1: average attached biomass per gram media for the U of W's media

Sample #	Attached biomass per gram media (mgVSS/g) "Avg±SD"	Biofilm Thickness
Sample 1	31.5±2.9	**370 µm**
Sample 2	23.6±5.2	**300 µm**

Biofilm measurement for plastic media

Cont.

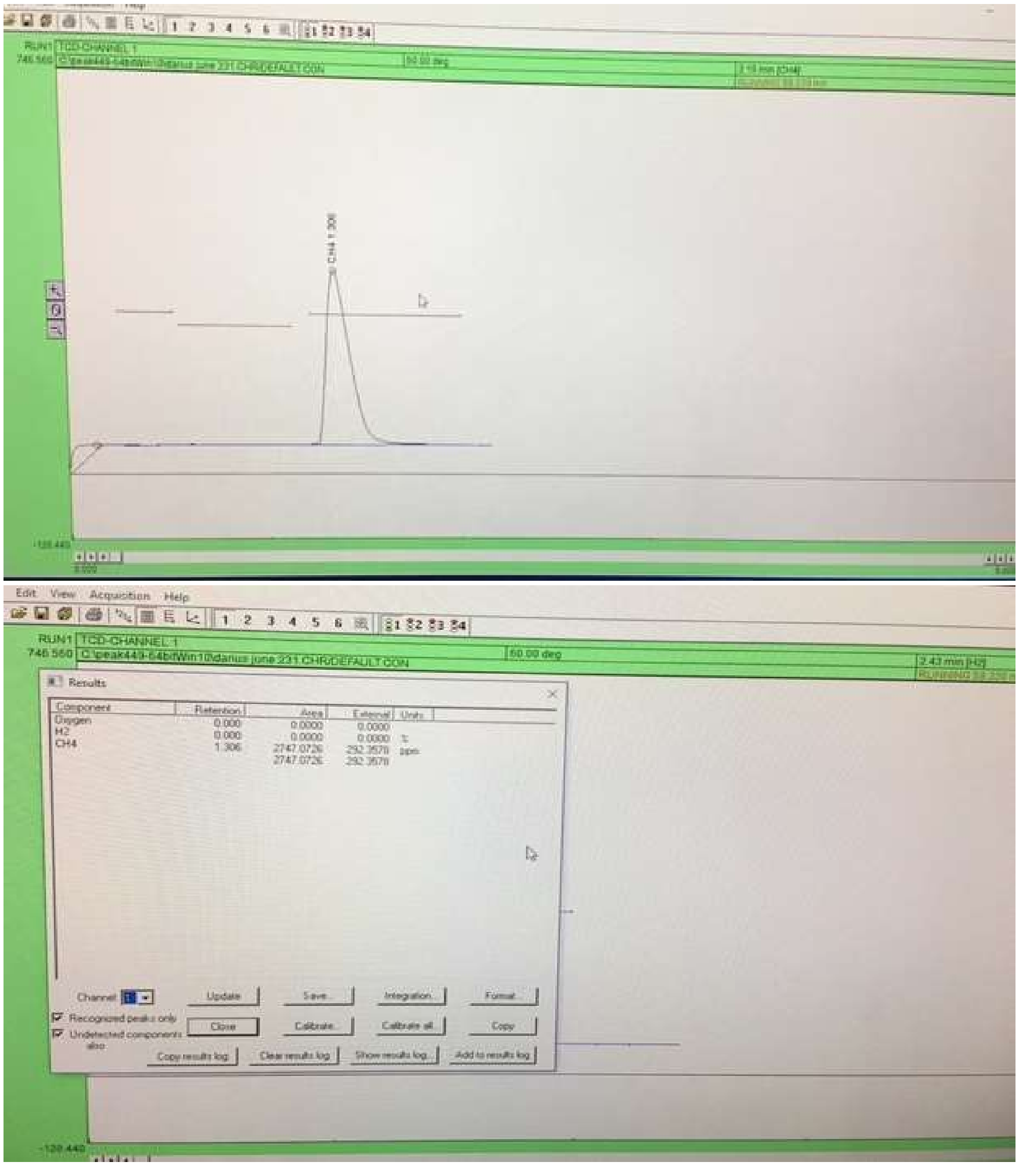




Biofilm measurement for zolite media

Appendix 4

Gas analysis results from GC SRI 8610C



Gas analysis results from GC SRI 8610C

Appendix 5

Development of the kinetic model (Temperature)

The mass balance equation with equal mass flow of input and output Q (mass of biogas is neglected) can be written as:

$V_R \frac{dc}{dt} = Q * c_0 - Q * c + V_R * r(c)$ Equation (4.2.1)

Where:

V_R Volume of the reactor (L)
Q flow of the input (L/ d)
c_0 COD concentration of the input (mg /L)
c COD concentration of the output (mg /L)
r(c) COD removal rate as function of c (mg/L/day)

The COD removal rate r(c) as a function of c is expressed as first order kinetic with:

$- \frac{dc}{dt} = r(c) = -k * c$

Where: k first order reaction rate constant (L/day)
By combining Equations 4.2.1 and 4.2.2 with $V_R = Q * HRT$ in the steady state for

$V_R * (\frac{dc}{dt}) = 0$

we obtain:

Cont.

HRT = $\frac{1}{k}$ ($\frac{c_0}{c}$ - 1)

The overall correlation between substrate concentration c and biogas yield y at time t is shown in the following equation:

The biodegradable fraction of the complex organic substrate is disintegrated to biogas according to Equation 4.2.4:

$$\frac{c_0 - c(t)}{c_0} = \frac{G(t)}{G_0} \qquad \text{Equation (4.2.4)}$$

Where;

G(t) COD removal methane yield (L/g)

G_0 COD removal methane yield (L/g) (theoretical)

Replacing $\frac{c_0}{c}$ in Equation (4.2.3) and Equation (4.2.4):

$$\text{HRT} = \frac{1}{k} * \left(\frac{G(t)}{G_0 - G(t)}\right)$$

For dimensioning the f size of AnFBRs, both the OLR and the HRT are the most applied parameters in practice:

OLR = c_0/HRT

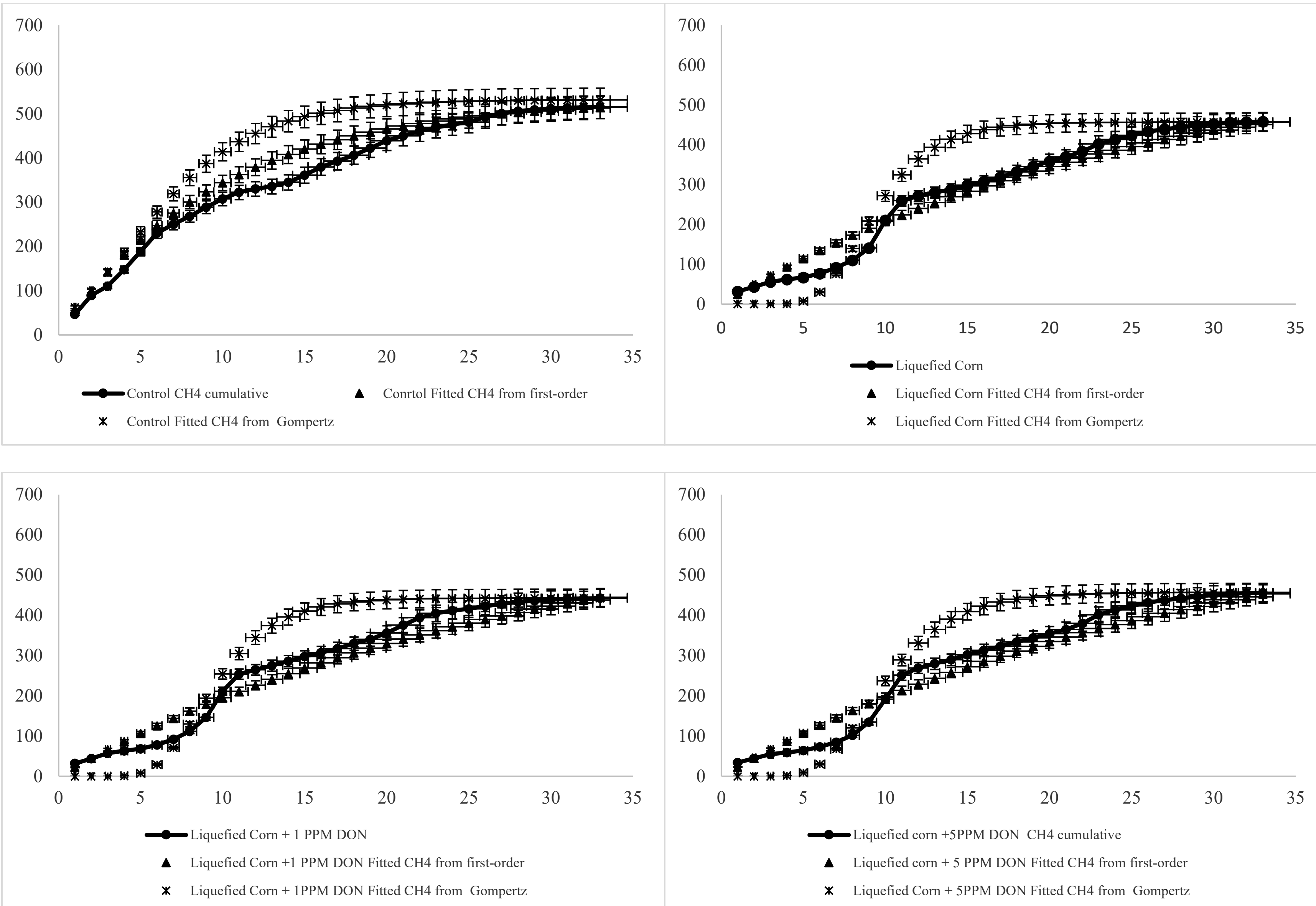
Replacing HRT with c_0/OLR in Equation (4.2.5):

Equation 4.2.5 can be written as:

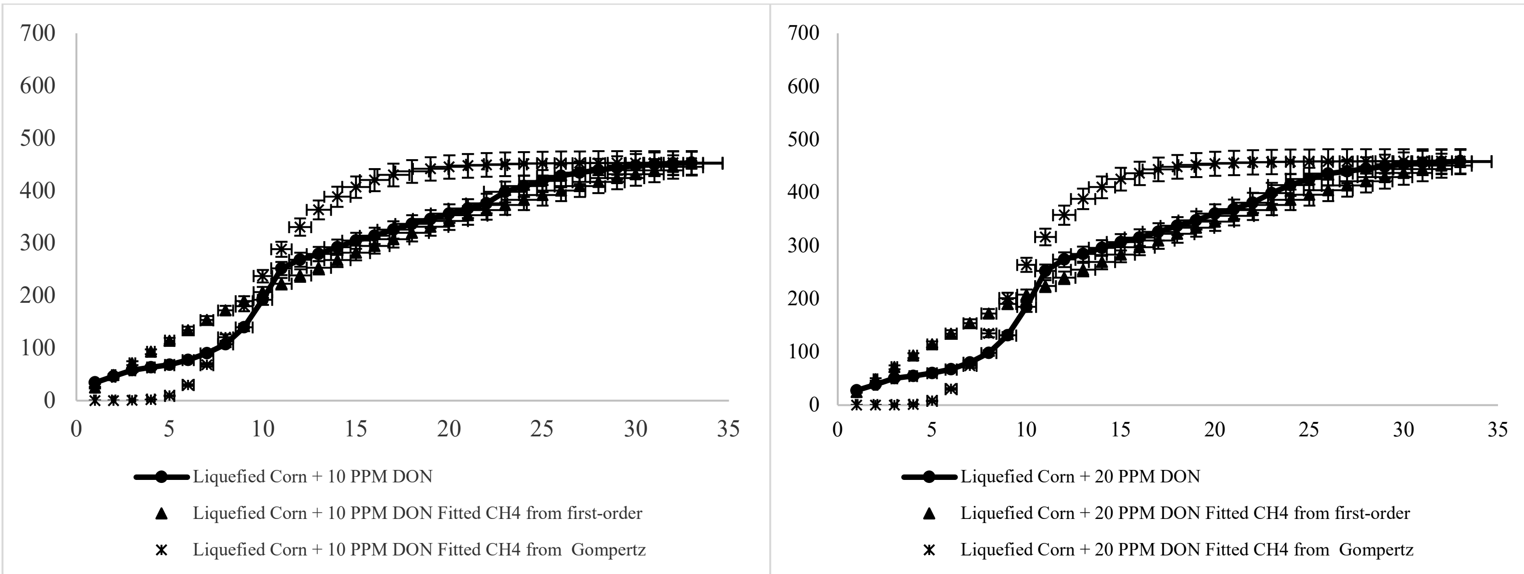
$$\text{OLR} = \frac{k * c_0}{G/(G_0 - G)}$$

Appendix 6

Methane accumulative plotted with methane predicted using first order and Gompertz modified kinetic models.



Cont.


700
600
500
400
300
200
100
0
0 5 10 15 20 25 30 35
Liquefied Corn + 10 PPM DON
Liquefied Corn + 10 PPM DON Fitted CH4 from first-order
Liquefied Corn + 10 PPM DON Fitted CH4 from Gompertz
700
600
500
400
300
200
100
0
0 5 10 15 20 25 30 35
Liquefied Corn + 20 PPM DON
Liquefied Corn + 20 PPM DON Fitted CH4 from first-order
Liquefied Corn + 20 PPM DON Fitted CH4 from Gompertz

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