LEBANESE AMERICAN UNIVERSITY

Batch Anaerobic Co-digestion of Agro-industrial Wastewater Produced in the Upper Litani Basin Lebanon

By

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A thesis

Submitted in partial fulfillment of the requirements

for the degree of Master of Science in Civil and Environmental Engineering

School of Engineering

December 2022

LAU
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Lebanese American University

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Program: Masters of science in Civil and environmental engineering

Department: Civil engineering

School: school of engineering

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I dedicate this work

to my loving parents Deeb Hashem and Doha Baderdine

who have always been a big support

ACKOWLEDGMENT

I am very grateful to my advisor Dr. Mahmoud Wazne for his guidance, counsel, and direction. I would also like to thank Dr. Moustapha Harb for his counsel and insight he provided during this project. I extend my gratitude and appreciation to the lab part timers Mr. Hussein Rayshouni and Mrs. Olga El-Kik for all of the information, knowledge and help. Also, I extend my gratitude to the graduate students Mr. Mohamad Abdallah and Mr. Akram Nawfal. Special thanks to the LAU labs and lab supervisors that provided the necessary equipment and materials to complete this project.

Batch Anaerobic

Co-digestion of Agro-industrial Wastewater Produced in the Upper Litani Basin Lebanon

Reem Deeb Hashem

ABSTRACT

The Litani River in Lebanon is subjected to many pollution sources originating from the agro-industries including dairy farms and slaughterhouses. This pollution is leading to a severe degradation in water quality. The aim of this research is to mitigate pollution resulting from the discharge of wastewaters originating from dairy farm and poultry slaughter house industries. The study included characterization of wastewaters samples collected from the upper Litani basin followed by mono-digestion and co-digestion experiments. The digested samples included cheese whey wastewater (CWW) and poultry slaughterhouse wastewater (PSW). The study was conducted in 5-L lab-scale anaerobic digesters at an organic loading rate of 1.1 g.COD/L.day for 58 days. Treatment combinations included CWW (R1), PSW (R2), 75:25 CWW:PSW (R3), 25:75 CWW:PSW (R4), and 50:50 CWW:PSW (R5). The test results indicated that R4 with 25:75 CWW:PSW combination yielded the best treatment outcome. A kinetic assessment was conducted for the five reactors to evaluate the disgestion kinetic parameters and lag time. The study showed that the logistic function model yielded the best fit for the cumulative methane data.

Keywords: Co-digestion, Mono-digestion, Anaerobic Digestion, Organic Waste, Methane Release

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LIST OF ABBREVIATIONS

AD……………………………………………………….… Anaerobic Digestion

AIC……………………………………………………… Akaike's Information Criterion

BMP……………………………………………………..… Bio Methane Potential

COD……………………………………………………..… Chemical Oxygen Demand

CSH…………………………………………………...… Chicken Slaughter house waste

GC……………………………………………………..… Gas Chromatography

IC……………………………………………………...… Ion Chromatography

rMSPE……………………………………………….…. Root Mean Square Prediction Error

RSS ……………………………………………………..…. Residual sum of squares

sCOD…………………………………………………… Soluble Chemical Oxygen Demand

TSS……………………………………………………..… Total Suspended Solids

TVFAs………………………………………………..… Total Volatile Fatty Acids

VSS……………………………………………………..… Volatile Suspended Solids

CHAPTER ONE

INTRODUCTION

1.1 Background

The Litani River is located in the Beqaa valley in Lebanon. It is one of the largest sources of fresh water supplies in Lebanon reaching 170 Km in length with an annual water capacity of 750 Mm^3. The Litani basin extends over 20% of Lebanon’s area of which 80% of the basin is located in the Bekaa valley. The quantity of water discharged from the basin is equivalent to more than 40% of the total amount of running water in the internal rivers.

It is a vital supply of water in southern Lebanon. The river begins west of Baalbek in the rich Beqaa Valley and drains into the Mediterranean Sea north of Tyre. The Litani River, is Lebanon's longest river. It originates and flows wholly within Lebanon's boundaries. It is a key source of water, irrigation, and hydroelectricity in Southern Lebanon as well as the rest of the country.

The Litani river water resources have been used for human services since the 1960s. The River provides electricity for Lebanon, irrigation and domestic water for South Lebanon and soon for the capital Beirut. Many families rely on the Litani River for income since they use it for a variety of recreational activities during the summer, as well as irrigation. Many hydroelectric and electric projects have been built along the Litani River's banks. The Lebanese government built a dam that is connected to an 185MW hydroelectric power plant. Qaraoun Lake, a contaminated man-made lake, was formed as a result of the dam's construction.

A $55 million loan from the World Bank was granted in 2016 to alleviate wastewater and agricultural runoff near the lake and river. The fund's issue is that it did not allocate a greater amount of money to agricultural runoff. 80 % of farm fields in Bekaa and 20% in south Lebanon are irrigated by the Litani River.

Many agricultural initiatives, including as the Joun and Al-Qasmieh projects, have been implemented in the area. Fertilizers and pesticides are used by farmers, contaminating the river with toxins. Farmers, on the other hand, are affected by the water they use to irrigate their crops since it is contaminated with pesticides and contains silt, gravel, and sand.

The River is beset by a slew of environmental issues. It has grown extremely polluted as a result of decades of neglect and mismanagement. Industrial pollution from factories and slaughterhouses, untreated sewage, chemicals from agricultural runoffs, and municipal garbage dumping are the chief factors to the Litani River's degradation. The contamination has reached a point where it is visible to the naked eye, posing major health risks to those who consume the tainted water. It is currently suffering from widespread pollution and has become a threat to public health as pollution is propagating to other compartments. A significant fraction of the pollution load is originating from the agro-industries in the upper basin which discharge their untreated wastewater into the river and its tributaries.

However, untreated sewage and industrial wastes are the primary source of water contamination in Lebanon. "The Lebanese government has spent more than $1.5 billion in loans in the last 20 years to put in place a water sanitation system," Jad Chaaban, an economist and social activist, told Al-Monitor.

On the Litani River's national day, thousands of people volunteered to clean up the river. This happened after activists launched a massive internet campaign called "Together to Save the Litani River." Bulldozers cleaned accumulated sands and sludge in the river from local sand quarries. Thousands of volunteers engaged online and then onsite to fish out trash.

1.1.1 Aim and Objectives

The aim of this research is to mitigate pollution resulting from the discharge of untreated wastewaters originating from dairy farm and poultry slaughter house industries by converting the agro-industry wastewater to innocuous material and energy rich biogas via anaerobic digestion.

The objectives of the study are:

1) Collect agro-industry wastewater samples from different locations in the upper Litani basin
2) Characterize the collected wastewater samples
3) Conduct mono and co-digestions experiments using the collected samples
4) Assess treatment results using methane yield
5) Use kinetic models to fit experimental data and assess kinetic parameters and lag time

1.1.2 Scope of Work

This study is limited to investigating the anaerobic digestion of only two types of agro-industry wastewater. The digestion was conducted in continuous stirred-tank reactors (CSTR) operated in batch mode.

1.2 Literature Review

1.2.1 Importance of Anaerobic Digestion

The fast population growth, the depletion of traditional energy sources, and the increase in energy costs encouraged many countries to search for new and renewable energy sources (Abouelenien et al., 2014). Some of the identified renewable energy sources are energy rich agro-industry wastewater. On the other hand, the dischrage of agro-industry wastewater is responsible for significnat environmental problems and health risks, such as contamination of surface and groundwater (Cole et al., 1999). The costs associated with treatment and disposal of these wastes account for 40–60% of total operation costs in wastewater treatment (Pilli et al., 2011). Consequently, researches sought different treatment options in order to produce energy from organic wastes using methods such as aneorobic digestion. It is a biological process where micro-organisms transform organic matter into a biogas mixture of methane and carbon dioxide (Wilkie, 2008). The reason researchers are investing more time and effort on the anaerobic digestion treatment rather than the aerobic digestion one is because the former has advantages such as low sludge production, waste stabilization, biogas production and low nutrient requirements (Rubio et al., 2019). In recent years, the use of anaerobic digestion has increased all across the European countries, because of the legislative tools that intended to increase the biogas production in different economic sectors. The European Union is obligated to reduce the greenhouse gases emissions because of the Kyoto Protocol (Comino et al., 2010).

Anaerobic digestion of agro-industry waste is a research topic of great interest in the field of biofuel production from biomass waste. It is a valuable alternative technology for waste management. In recent years, numerous studies devoted significant effort to basic research to further develop anaerobic biotechnology for food waste treatment and methane production (Komilis et al., 2017). Food waste is an easily available resource of biomass and is a great waste stream that contains a large amount of bioenergy. Food waste is generated through the steps of food supply chain from harvest to storage to transportation then to processing or cooking and eating (Uçkun et al., 2014; Xu et al., 2018). It has been considered a very well-implemented technology in treating streams of waste water due to its obvious economic and environmental benefits (Chen et al., 2014; Yanget al., 2015). From the economic perspective, this study showed that high-value added applications (for example, vehicle-use fuels and generation of on-grid power by methane gas) caused an increase in the economic benefit of the anaerobic digestion process. More researchers are focusing on anaerobic digestion of food waste and have implemented numerous studies from lab scale experiments to pilot scale applications (Xu et al., 2018).

Anaerobic biotechnology has been used to digest varied organic wastes, such as animal manure, sewage sludge, and foodwaste. The main reason why food waste is the most valued substrate in the anaerobic digestion experiments is because it contains large quantities of biodegradable organics and it could be easily converted to biogas by biochemical reactions of microorganisms, which results in high methane production and high organic removal rates (Zhang et al., 2015).

Anaerobic digestion connects food waste to energy and other resources. Methane in biogas could be an alternative to coal and oil consumption with lesser emissions whereas digested substrate is rich in nutrients, which could be used as organic fertilizer (Xu et al., 2018; Zhang et al., 2015).

In summary, converting organic wastes into energy-rich methane through anaerobic digestion is a sustainable solution to the current energy and environmental problems. It also has the ability to recover energy from waste biomass such as sewage sludge, animal manure, and foodwaste (Kim et al., 2019).

1.2.2 Process of Anaerobic Digestion

The Anaerobic digestion process involves acetogens bacteria that forms the acetic acid and methanogens archaea that forms the methane. These organisms feed on the substrates and convert it into intermediate molecules including acetic acid, sugars, and hydrogen, before finally being converted to methane. In an anaerobic system the majority of the chemical energy found within the starting material mixture is released by methanogenic organisms as biogas (Fergusen and Mah, 2006). The substrates digested are made up of large organic polymers. The process of breaking these chains and dissolving the smaller molecules into solution is called hydrolysis. Therefore, hydrolysis is the necessary first step in anaerobic digestion. Through hydrolysis the complex organic molecules are broken down into simple sugars, amino acids, and fatty acids (Sleat and Mah, 2006). Hydrolysis is followed by acidogenesis, acetogenesis, and methanogenesis, respectively.

Acetic acid, butyric acid, and propionic acid are the major volatile fatty acids produced during anaerobic digestion processes. Volatile fatty acids are one of the important indicators for assessing anaerobic digestion performance, because they are related to changes in alkalinity, pH, and the activity of methanogens (Abouelenien et al., 2014). Methane is also considered as an indicator for the efficiency of organics utilization (Zhao et al., 2019). The type of reactors used in this study are batch reactors. A batch system is operated by feeding the reactor only once at the start of the process. It is then sealed and monitored for the duration of the experiment until the full degradation of all the biodegradable material present in the medium (Aboudi et al., 2015).

1.2.2 Mono Digestion Problems

Many anaerobic mono-digestion industries of food waste are operated with a relatively low efficiency in terms of methane production and acid fermentation (acidogenesis) because of the unbalanced nutrient profiles , product inhibition, and the accumulation of volatile fatty acids (Li et al., 2018; Mata-Alvarez et al., 2014; Wu et al., 2016; Tiehm et al., 2001; Dai et al., 2016). Anaerobic mono-digestion of waste activated sludge WAS streams achieved only about 30-50% conversion of organic substrates to bio-methane gas. Hydrolysis of the sludge was the limiting step that slowed down the organics utilization (Gosset et al., 1982; Zhang et al., 2014). The WAS streams in most WWTPs usually have low VS/TS ratio ranging from 30% to 60%. This shows an insufficient organic content which negatively impacts the anaerobic mono digestion performance and removes the economic advantage (Zhao et al., 2019). Finally, problems encountered in high-solids composition waste include long retention time and mass transfer limitation, whereas problems connected with wet substrates include high water consumption,expensive processing of the down stream digestate and demand for larger digester volume (Zhang et al., 2017).

1.2.3 Co-digestion

A promising strategy to overcome the limitations of mono-digestion is co-digestion, which can improve the properties of the feedstock mixture (Fonoll et al., 2015). Anaerobic co-digestion caused an increase in the efficiency of methane production via optimization of the digestion process (Sarker et al., 2014; Wang et al., 2012). Certain food waste combinations in the co-digestion processes showed higher biogas production and better quality of biogas than the mono digestion process. It also gave better results in terms of system stability, bioenergy, and solids reduction. The toxicity of the inhibitory compounds is lowered due to dilution when using co-digestion (Anjum et al., 2012; Anjum et al., 2016; Serrano et al., 2013). It was reported recently that co-digestion of waste sludge with other streams enhances the production of methane which can be used as a renewable energy source (Hallaji et al., 2019).

Co-digestion can improve the performance of an anaerobic digestion process by adjusting the carbon to nitrogen ratio, increasing the buffering capacity, balancing the nutrient profiles and diluting the concentrations of inhibitors and toxic compounds (Kim et al., 2017). This should be accomplished carefully by selecting suitable co-substrates and determining their optimal mixing ratios to achieve a successful co-digestion .Also, to optimize this process best possible blended substrates and seed should be used to utilize complementary characteristics and synergy without causing inhibition (Sensai et al., 2014).The results in general show that the co-digestion a promising technology to effectively manage waste and to increase their potential as alternative energy sources (Kim et al., 2019).

1.2.4 Organic Loading Rate

The organic loading rate (OLR) is defined as the application of particulate and soluble organic matter as pounds of COD per unit volume per unit time. OLR is an important parameter for the anaerobic digestion process to assess the stability and performance of co-digestion process (Liu et al., 2012).

There is usually a range of typical organic loading rate in each AD process. Studies showed that co-digestion of municipal biomass waste and waste activated sludge can have stable operation when the OLRs are between 1.2 and 1.8 kg $VS(m^3d)^{-1}$ (Liu et al., 2012).
At some high OLR values the anaerobic digestion system could result in greater VFA accumulation, process inhibition and an increased risk of acidification (Liu et al., 2012). This could be due to the faster rates of acidogenic reactions for rapidly biodegradable substrates.

1.2.5 Mixing Ratios

The co-digestion process may be enhanced by trying different substrate mixing ratios. It may enhance the process by finding a ratio that gives better results than other mixing ratios (more biogas production, no inhibition, pH staying above 8, less accumulation of volatile fatty acids). Abouli et al. (2015) tested different mixed ratios of dried pellet of exhausted sugar beet with pig manure under batch anaerobic conditions and compared the results with the mono-digestion of each substrate.

On the other hand, Lin et al. (2011) fixed the organic loading and reported on the different mixing ratios of fruit and vegetable wastes and food wastes. When the mixing ratios of FVW: FW was 2:1 and 1:1, the operation and performance of the reactor was stable, and there was no accumulation of ammonia and volatile fatty acids. The use of 1:2 of FVW to FW caused an increase in the volatile fatty acids concentration. The values reached 1100–1200 mg/L and caused a slight inhibition in the methanogenesis process. After trying several mixing ratios. The optimum ratio appeared to be 1:1 for the co-digestion of FVW with FW. The methane gas production was 0.49 m^3 CH_4/ kg VS which was the highest in the study. There was no inhibition of the co-digestion process and the removal efficiency of soluble COD was 96.1%. It was shown that this mixing ratio gives higher biogas production, better removal efficiency and more stable anaerobic process.

Rahman et al. (2017) reported on co-digestion of poultry droppings and lignocellulosic-rich substrates. Five different mixing combination ratios were tested to achieve maximum biogas production. The different PD:LCS ratio mixtures were 100:0, 90:10, 70:30, 50:50, 30:70, 10:90 and 0:100.

The highest specific biogas production rate and the highest cumulative biogas productions were achieved at the ratio of 50:50. But the highest bio methane potential was obtained at the ratio of 70:30 for the first set (poultry droppings and wheat straw) (330.10 Nl kg^{-1} VS) and at the ratio of 50:50 for the second set (poultry droppings and meadow grass) (340.10 Nl kg^{-1} VS).

Similarly, the cumulative methane production increased from 70.5 mL CH_4/g VS to 76.0 mL CH4/g VS when the ratio of the aquaculture sludge /waste activated sludge was increased from 0% to 100 % (Wu and Song 2020).

El Gnaoui et al. (2020) reported on the use of olive mill waste water and food waste substrates. The ratios of olive mill waste water varied from 20 to 80 %. The results showed that digestion of food waste with olive mill waste can solve the olive mill effluent problems. The best results were attained when the ratio was 20% olive mill waste water, where the methane production was the highest (302.16 mLSTP CH_4).

Another study reported on the use of slaughterhouse wastewater with *Opuntia fícus-indica*. Four different mixing ratios were investigated at the mesophilic temperature of 38^{o}C. The ratios of slaughterhouse wastewater used were 100%, 75%, 25% and 0 %. The average methane contents and highest cumulative biogas yields were obtained at a mixing ratio of 25% *Opuntia fícus indica* and 75% slaughterhouse wastewater (Panizio et al., 2019).

Ripoll et al. (2020) reported optimal mixing conditions of 50:50% of sewage sludge and “sherry-wine” distillery wastewater, whereas Rubioet al. (2019) showed that ratios of 60:40 or 75:25 are recommended of two-phase olive-mill waste and cattle manure.

1.2.6 Kinetic Simulation Studies

Several mathematic model functions such as the first-order kinetic, Cone model, transfer function and logistic function have been used to delineate the digestion mechanisms of organic wastes in an anaerobic system. Kinetic models can determine the impact of factors that affect the anaerobic digestion process and predict the production of biogas, especially methane in order to prevent underestimation or overestimation of the methane production results. This estimation may lead to the definition of real criteria that may be of value in implementing the technology (Parra et al., 2017).

Selection of the appropriate model that can produce reliable prediction results is very important. The accuracy of models varies with the types of used substrates and operational conditions during the anaerobic digestion process. Therefore, for each anaerobic study the best fit model is the one that represents the measured values in the lab. By using the Cone model or the first-order kinetic, the cumulative methane yield and the hydrolysis rate of organic matter can be calculated. In addition to these two important parameters, the duration of the lag-phase is also an essential indicator that shows the efficiency of the AD process, which can be approximately calculated with the transfer function and logistic function (Zhen et al., 2016). These five models were all used and assessed in this study to explain the kinetics of methane production.

1.2.7 Summary

In the proposed research the anaerobic digestion will be conducted on different types of food wastes collected from different industries that discharge its effluents in the upper Litani basin in Lebanon. A comparison will be implemented between mono-digestion and co-digestion with different mixing ratios. The comparison will be in terms of substrate degradation and gas production under mesophilic conditions using batch mode. The objective is to enhance the digestion results in terms of system stability, bioenergy, and substrate reduction. As a result, less pollutants that are released into the Litani river by the industries. This practice could improve the water resources quality and provide better water for the residents in the Beqaa east Lebanon.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

The experimental setup consisted of six similar batch reactors with mixing agitator and a working volume of five Liters (figure 1). The reactors were placed in a water bath (Plexiglas) connected to a polystat water circulator at mesophilic temperature of 35°C (Figure 4). Gas bags were placed at the outlet of the reactors to collect the produced gases. Gas Chromatography (GC) (Agilent Technologies) was used to measure the percentages of gases collected from the different reactors (Figure 2). Ion Chromatography (IC) (Metrohm) was used to measure the composition of the samples collected from the reactors to determine the concentrations of volatile fatty acids (Acetic acid, propionic acid, and butyric acid).

A blender was used to homogenize the samples and obtain consistent results (Figure 8).
Hach DR3900 spectrophotometer (Figure 5) was used to measure COD, phosphate, sulfate, nitrate, ammonia, and the total volatile fatty acids as acetic acid. A Hach DRB200 Digester block (figure 9) was used need to test COD and TVFA as per Hach procedures. All reactors were purged with nitrogen gas for 20 minutes after each feeding cycle to ensure anaerobic conditions (Figure 7).

Figure 1: Six batch Reactors placed in water bath

Figure 2: Gas Chromatography machine (GC)

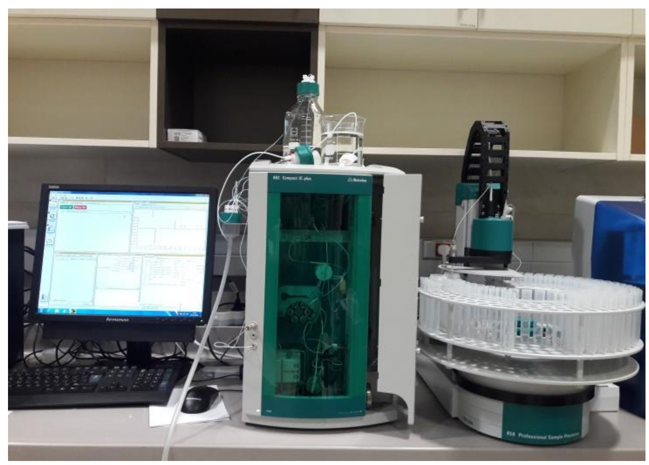

Figure 3: Ion Chromatography machine (IC)

Figure 4: Water Pump

Figure 5: Spectrophotometer

Figure 6: Condenser

Figure 7: Nitrogen Bottle

Figure 8: Blender

Figure 9: Digester Block

2.2 Methods

2.2.1 Step 1: Sampling and Characterization

2.2.1.1 Sampling

Food waste samples were collected from two different industries in the upper Litani basin. The first sample was collected from a chicken slaughter house whereas the second sample was collected from a dairy farm. The inoculum (seed) that was collected from an anaerobic digestion plant in Bkassine south Lebanon. The samples were blended (to ensure homogenization) and placed in the freezer at -20^{o}C until used.

Figure 10: Inoculum (seed) collection from Bkassine Anaerobic food waste Treatment Plant

2.2.1.2 Characterization

Hach methods were used for the characterization of the samples as listed in Table 1:

Table 1 Hach methods used and their functions

Hach Methods	Measurements
Method 8192	nitrate measurements
Method 10031	ammonia measurements
Method 10072	total nitrogen measurements
Method 10173	TOC measurements
Method 8000	COD measurements
Method 8051	sulfate measurements
Method 10242	TKN measurements
Methods (8158,8164)	total and volatile suspended solids
Method 10240	TVFA (total volatile fatty acids) measurements

2.2.2 Step 2 Mono and Co-digestion with Weekly Feeding of Two Food Wastes

The Theoretical Methane Production in each Reactor

Acetate to COD conversion:

$CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O$

Mass of $2O_2$ = 2x2x16 = 64 g

(COD)/(acetate) ratio = $\frac{64}{60}$ =1.067

Conversion between COD and CH_4:

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$$

$$1 \text{ mole of } CH_4 (\text{or } 22.4 \text{ L of } CH_4) \rightarrow \text{Needs } 64 \text{ g of COD} \Rightarrow$$

$$1 \text{ g of COD is equivalent to } 0.35 \text{ L } CH_4 \text{at standard conditions}$$

Five reactors were used in this study. Two liters of seed were added in each reactor. The seed was passed through a 1.8 mm sieve. The reactors were purged with nitrogen gas for 20 min with mixing rate speed at 200 rpm. After purging, the mixing speed in the reactors was decreased to 80 rpm and kept for 10 days to exhaust the substrate methane potential. The COD of the substrates was 85000 mg/L. The agitation was fixed at 150 rpm during operation. An acclimation cycle was implemented for 22 days followed by four feeding cycles.

OLR:

COD= 85 000 mg/L= 85 g/L

Volume = 2.25 liters → 0.25 Liter feed

85g/L ×0.25L= 21.25 g COD

21.25 g/2.25 liters=9.45 g/L/8 days = 1.18 g. COD /L. day

The five used reactors:

Reactor 1: 100% Whey: 2L seed+ 0.25 L Whey

Reactor 2: 100% Chicken slaughter house waste (CSH): 2L seed+ 0.25 L (CSH)

Reactor 3: 75%Whey+25% (CSH): 2L seed+ 0.1875 L Whey+ 0.0625 L (CSH)

Reactor 4: 25 %whey+75% (CSH): 2L seed + 0.0625 L Whey+0.1875 L (CSH)

Reactor 5: 50% whey+50% (CSH): 2L seed+ 0.125 Whey+0.125 (CSH)

The OLR is 1.1 g. COD /L day with 2 liters of seed and 0.25 Liters of feed. The total volume is 2.25 Liters. At the start of each feed cycle, 0.25 liters were removed from the reactor and were replaced by 0.25 liters of the new feed.

2.2.3 Step 3 Kinetic Simulation

Four kinetic models were used to fit the cumulative methane production during the acclimation phase of the five reactors over 22 days.

First order kinetic:

$B_{(t)} = \mathrm{fd}(1 - \exp(-\mathrm{Khyd}\ t)),\ t \geq 0$

Cone model:

$B_{(t)} = \frac{\mathrm{fd}}{1+(\mathrm{Khyd}\ t)\text{\^{}}{-n}} \quad t \geq 0$

Transfer Function:

$B_{(t)} = \mathrm{Pmax}\left(1 - \exp\left(\frac{-\mathrm{Rmax}\,(t-\lambda)}{\mathrm{Pmax}}\right)\right) \quad t \geq 0$

Logistic Function:

$B_{(t)} = \frac{\mathrm{Pmax}}{1+\exp\left(\frac{4\ \mathrm{Rmax}(\lambda - t)}{\mathrm{Pmax}}+2\right)} \quad t \geq 0$

The parameters of these equations were calculated using excel solver Tool pack and the least squares fitting method (Dinh et al., 2018).

B(t) is the specific methane yield at a given time (mL/g VS added); fd is the maximum methane potential (mL/g VS added); k_{hyd} is the hydrolysis rate constant (1/d); t is the digestion time (d); Rm is the maximum methane production rate (mL/g VS added.d); λ is the lag-phase (d); e is the exponential which is 2.71828; n is the shape factor; Rmax is the maximum rate of methane production ($mL.h^{-1}$); and Pmax is the maximum methane production (mL) (Zhen et al., 2016; Parra et al., 2016).

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Data of Food Waste Sample Characterization

Table 2:Characterization Table of collected food waste samples

Food Waste Sample	(Chicken slaughter house food waste)	Whey (waste from dairy farm)	Inoculum
pH	6.6	5.7	8.4
COD (mg/L)	86500	91300	29350
TOC (mg/L)	15829	29900	
NH3-N (mg/L)	16.9	26.8	245
Total PO_4^{3-} (mg/L)	127	2880	
SO_4^{2-} (mg/L)	82	176.6	
NO_3^- (mg/L)	25.8	8	
SCOD (mg/L)	16800	81500	433
TVFA (mg/L as acetic acid)	361	1501	
TS (mg/L)	49410	95930	
VS (mg/L)	46610	77050	
TSS (mg/L)			20316
VSS(mg/L)			16000
Carbohydrates (%TS)	6.2	33.1	
Proteins (%TS)	25.9	52.4	
Lipids (%TS)	63.6	0.4	
C/N ratio	21.8	33.4	

3.3 Mono and Co-digestion Experiments

3.3.1 Different Mixing Ratios

The five reactors used in this experiment are:

Reactor 1: 100% Whey : 2L seed+ 0.25 L Whey

Reactor 2: 100% (chicken slaughter house) CSH waste : 2L seed+ 0.25 L CSH

Reactor 3: 75%Whey+25% CSH : 2L seed+ 0.1875 L Whey+ 0.0625 L CSH

Reactor 4: 25 %whey+75% CSH : 2L seed + 0.0625 L Whey+0.1875 L CSH

Reactor 5: 50% whey+50% CSH: 2L seed+ 0.125 Whey+0.125 CSH

The OLR is 1.1 g. COD /L day and there is 2 liters of seed and 0.25 Liters of feed. Total volume is 2.25 Liters.

Each feed cycle 0.25 liters was removed from the reactor and a new feed was added.

3.3.2 Graph Results

3.3.2.1 pH

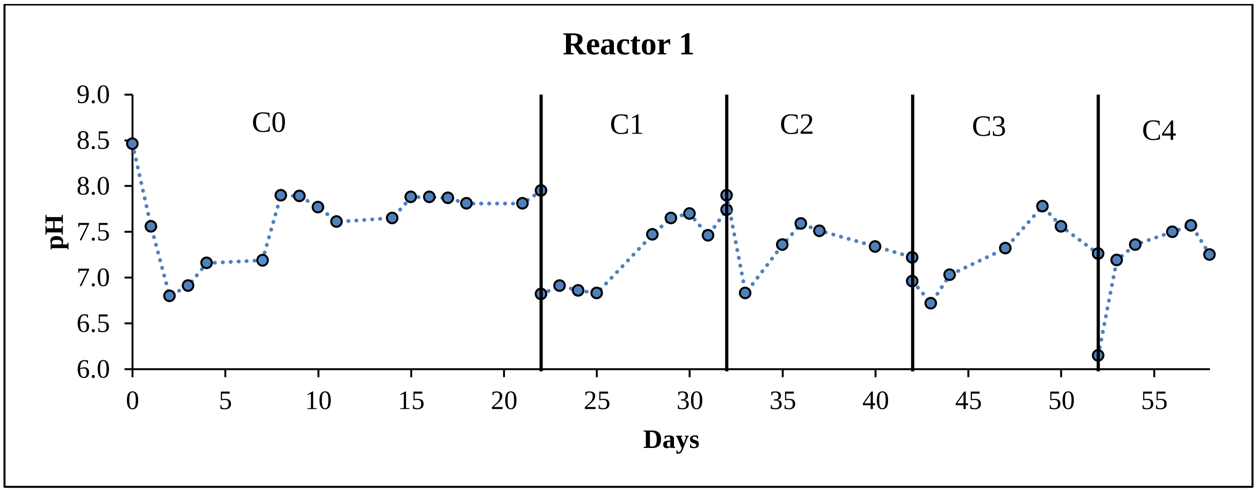

Figure 11 :pH values of reactor 1 (100% whey)

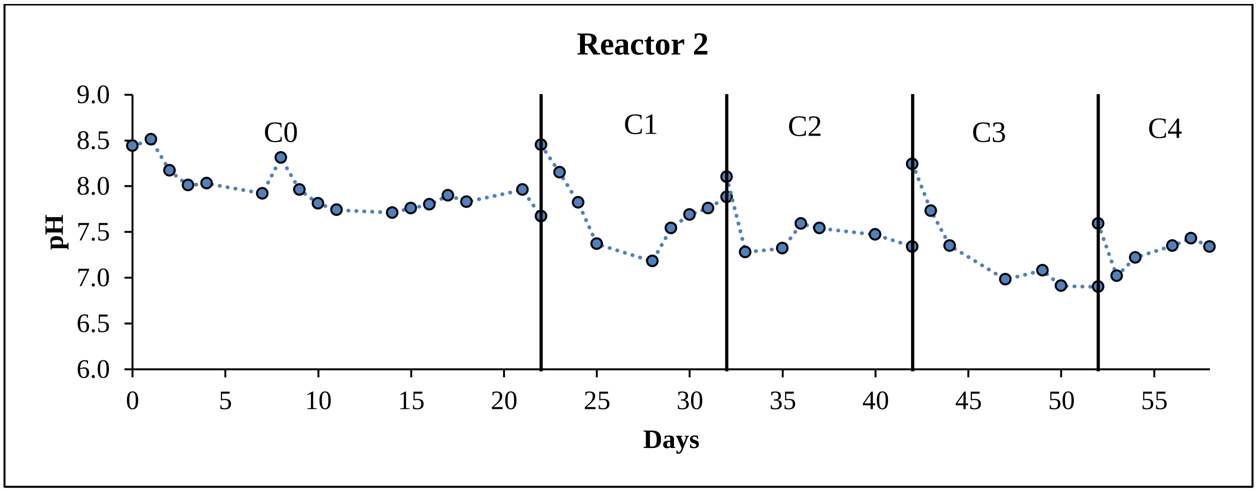

Figure 12: pH values of reactor 2 (100% CSH)

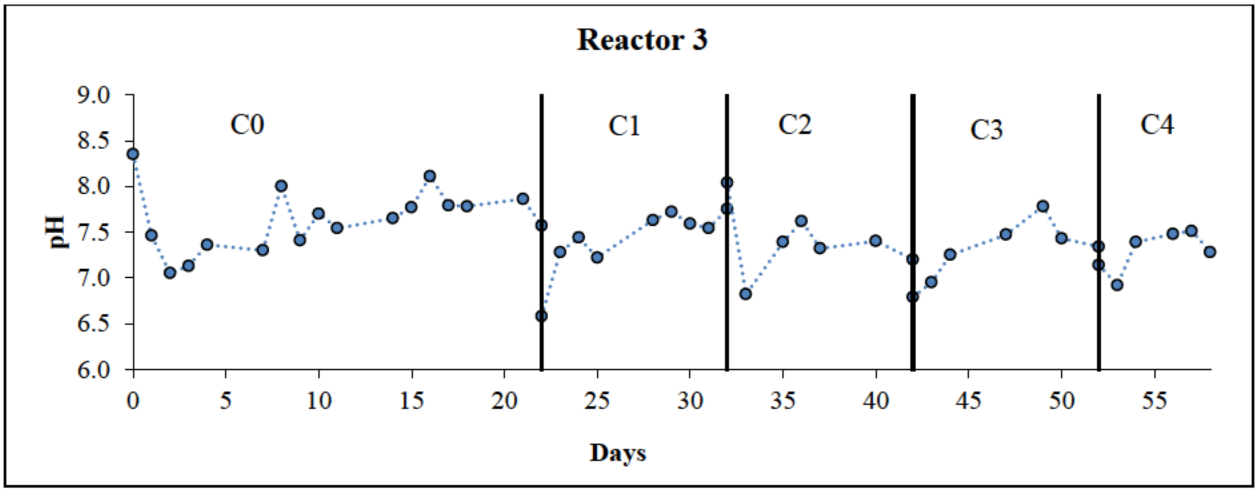

Figure 13: pH values of reactor 3 (75% whey+25% CSH)

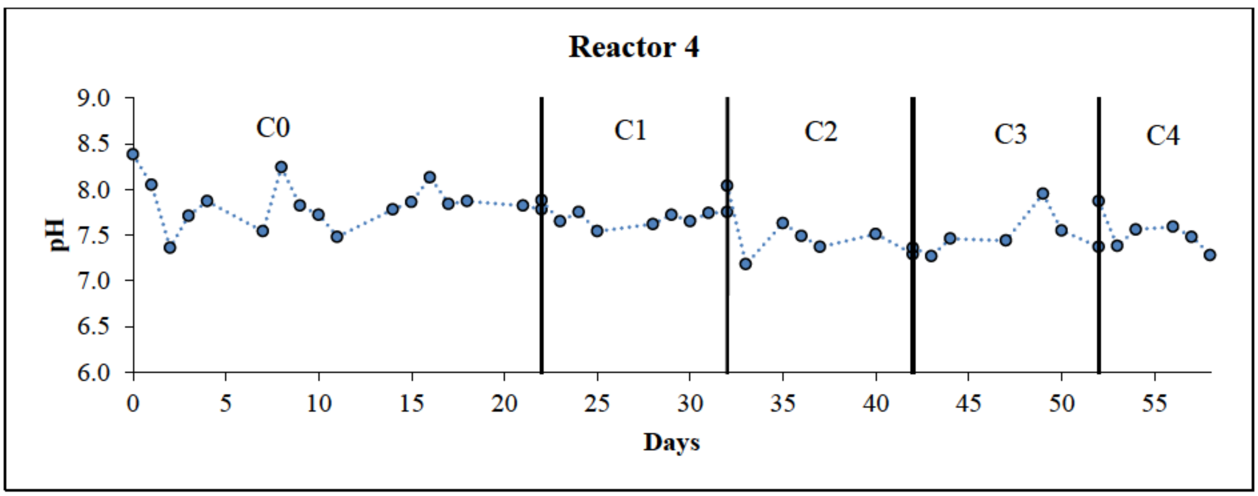

Figure 14: pH values of reactor 4 (25% whey+75% CSH)

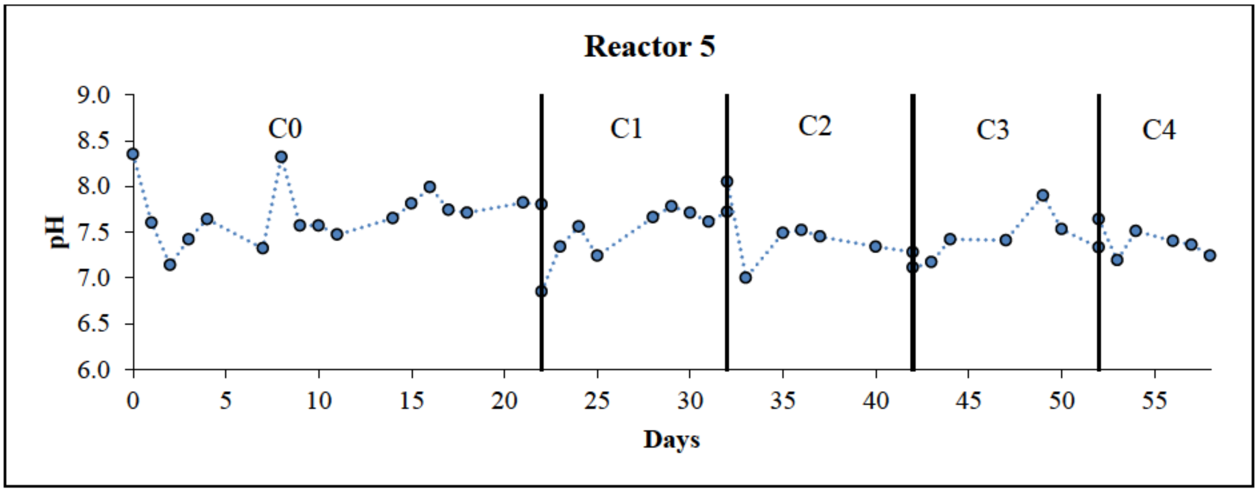

Figure 15: pH values of reactor 5 (50% whey+50% CSH)

3.3.2.2 TCOD and SCOD

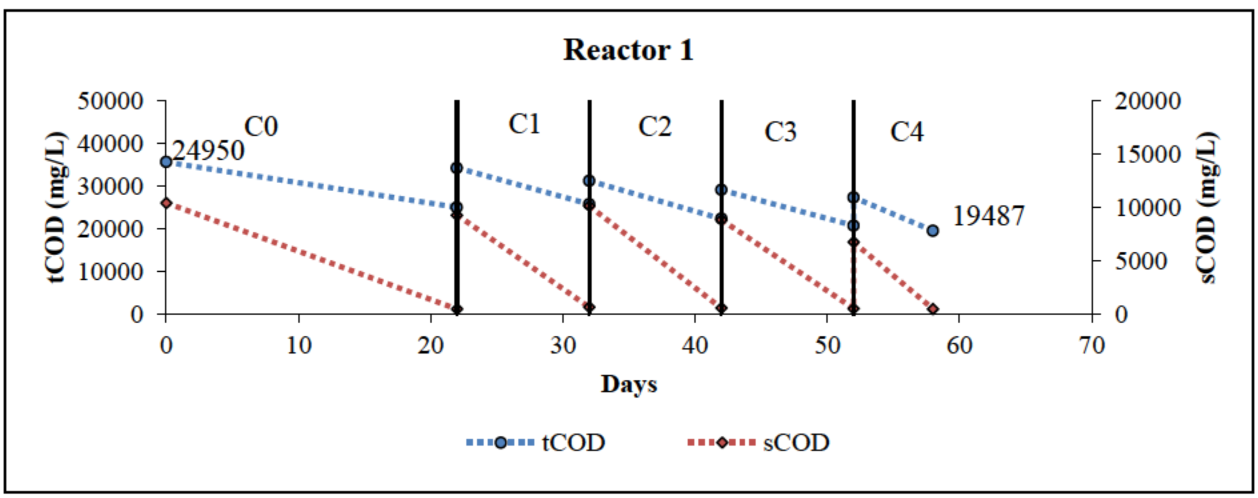

Figure 16: Total COD and soluble COD values of reactor 1 (100% whey)

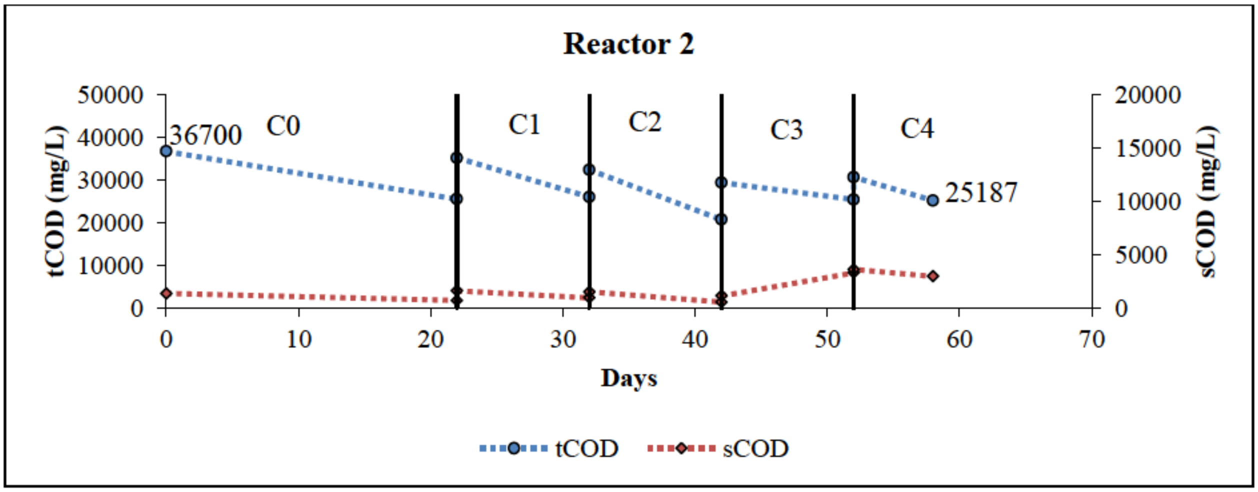

Figure 17: Total COD and soluble COD values of reactor 2 (100% CSH)

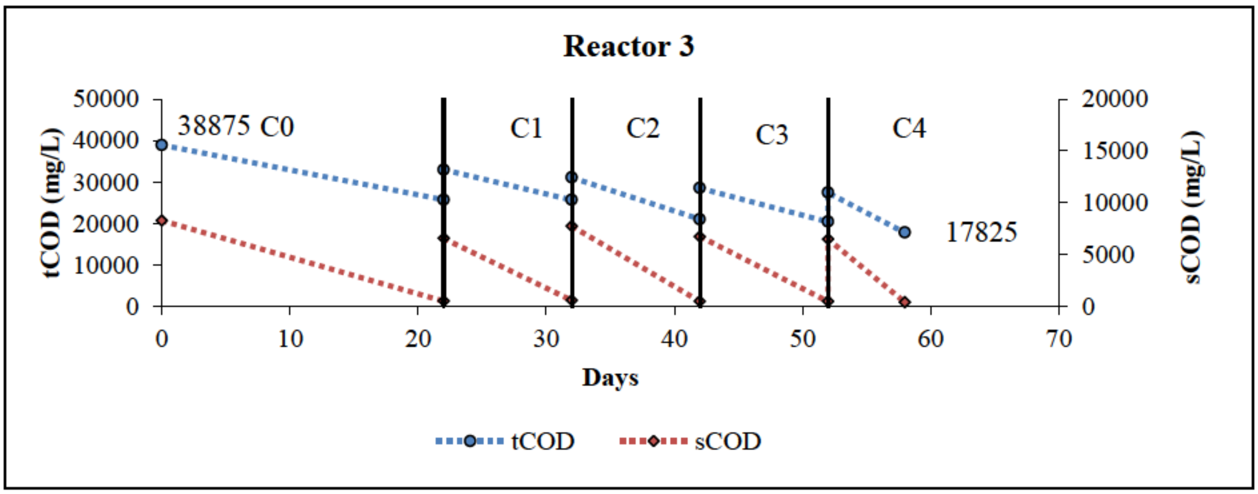

Figure 18: Total COD and soluble COD values of reactor 3 (75% whey+25% CSH)

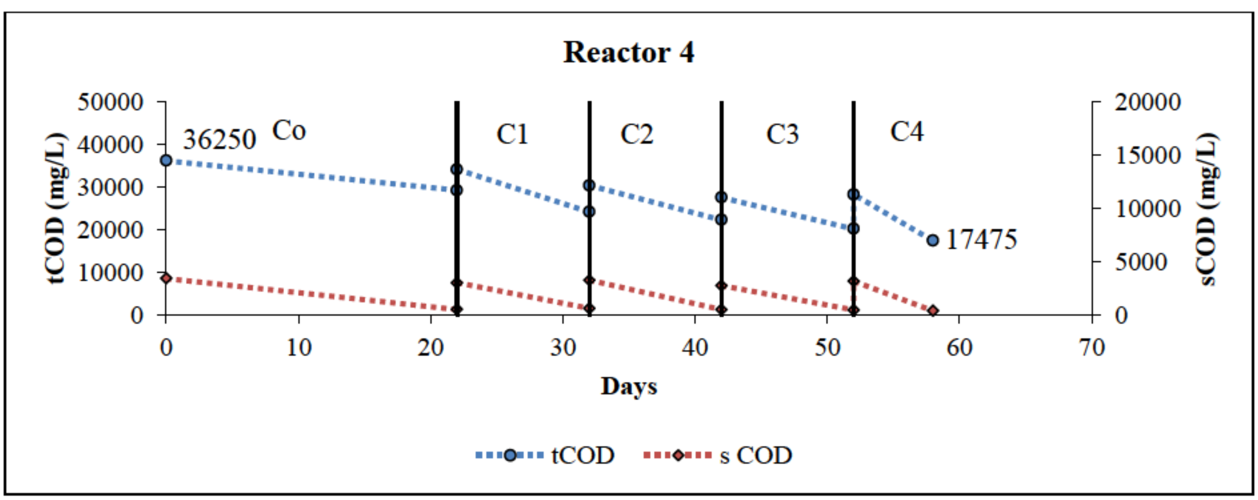

Figure 19: Total COD and soluble COD values of reactor 4(25% whey+75% CSH)

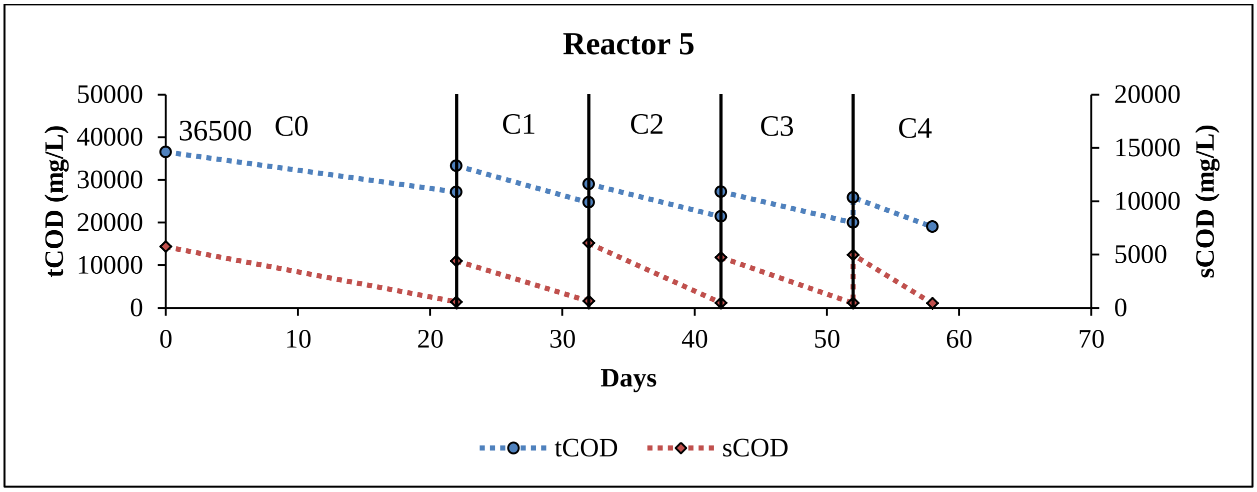

Figure 20: Total COD and soluble COD values of reactor 4(50% whey+50% CSH)

3.3.2.3 Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)

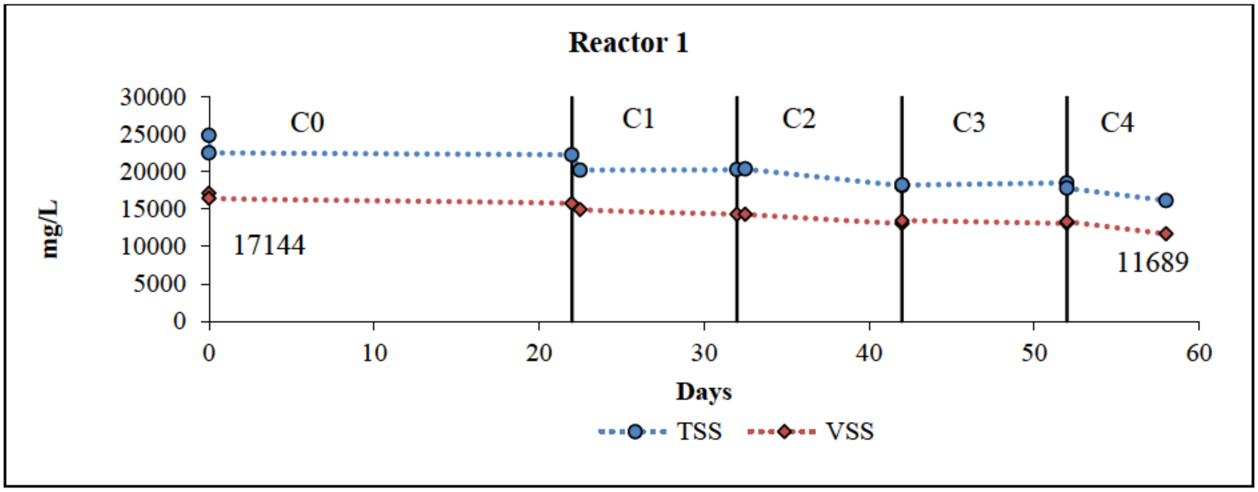

Figure 21: Total suspended and volatile suspended solids of reactor 1 (100% whey)

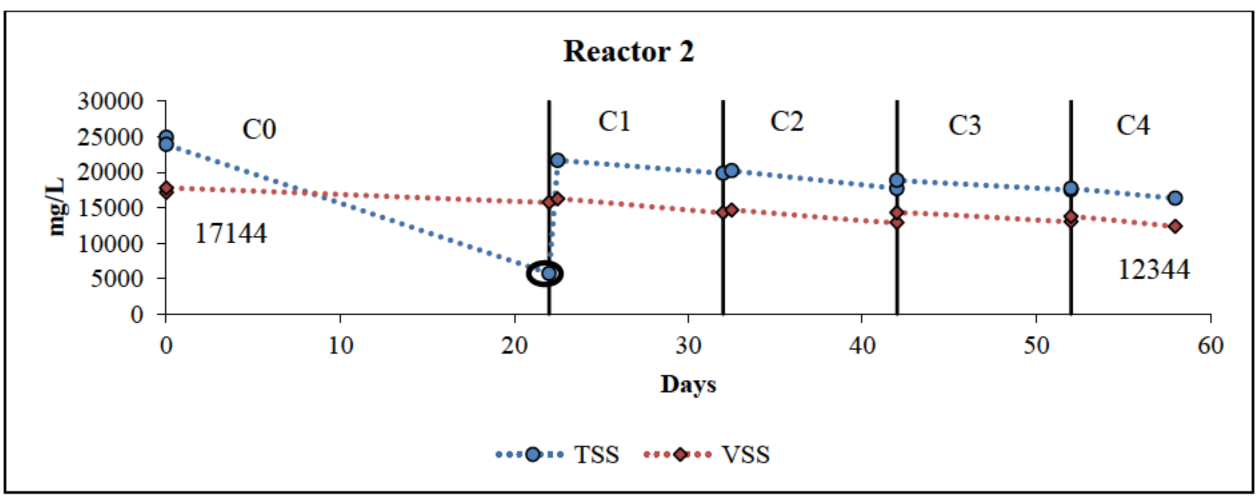

Figure 22: Total suspended and volatile suspended solids of reactor 2 (100% CSH)

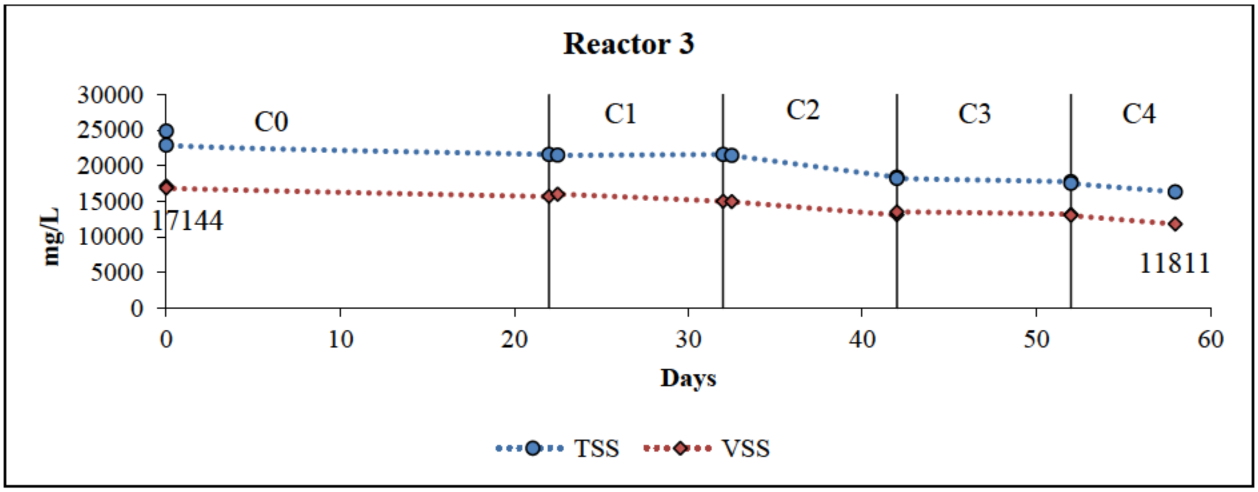

Figure 23: Total suspended and volatile suspended solids of reactor 3 (75% whey+25% CSH)

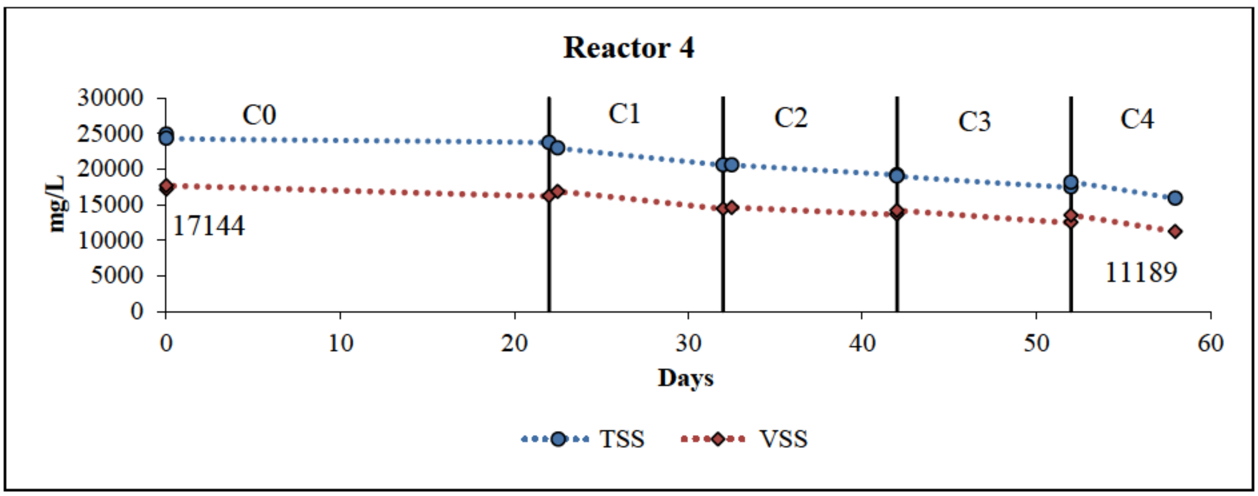

Figure 24: Total suspended and volatile suspended solids of reactor 4 (25% whey+75% CSH)

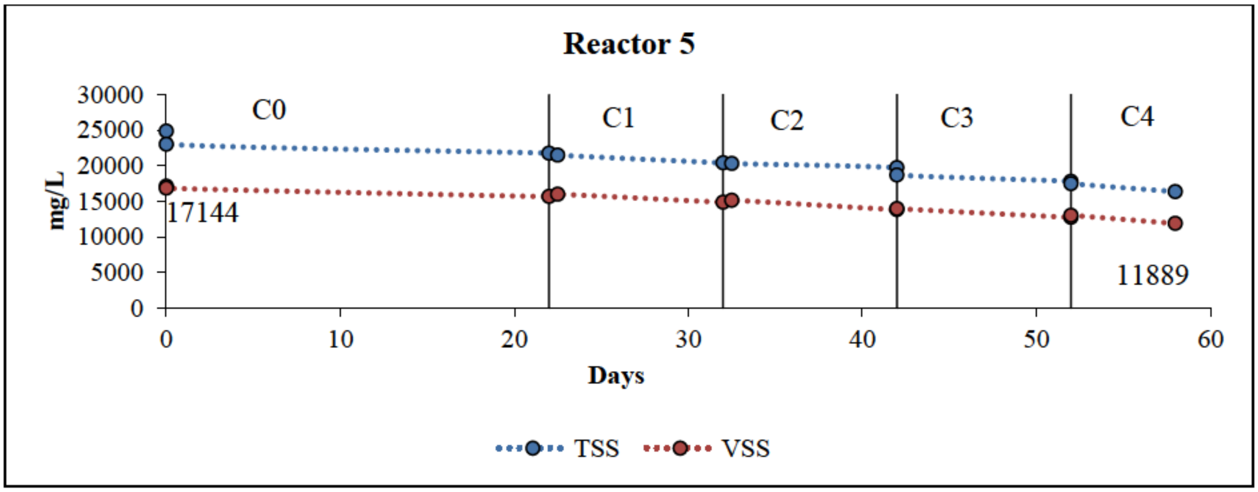

Figure 25: *Total suspended and volatile suspended solids of reactor 5 (50% whey+50% CSH)*

3.3.2.4 Volatile Fatty Acids (VFAs)

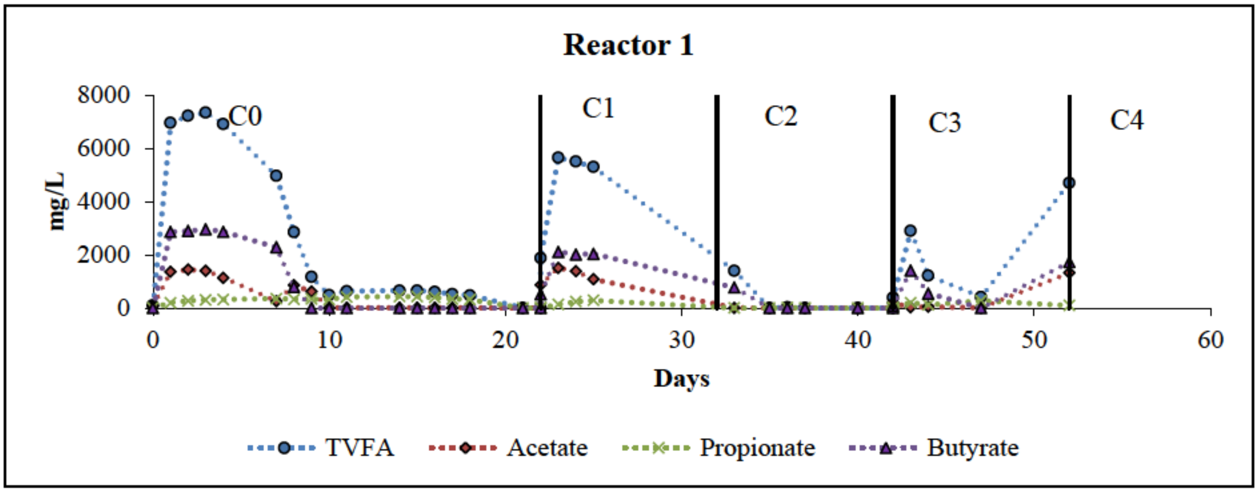

Figure 26: Volatile fatty acids of reactor 1(100% whey)

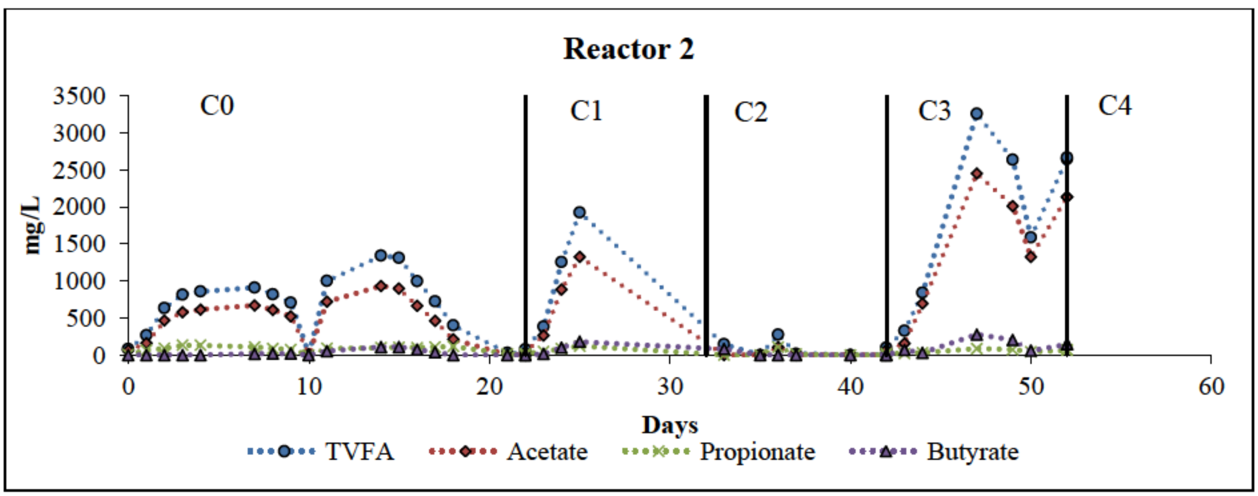

Figure 27: Volatile fatty acids of reactor 2(100% CSH)

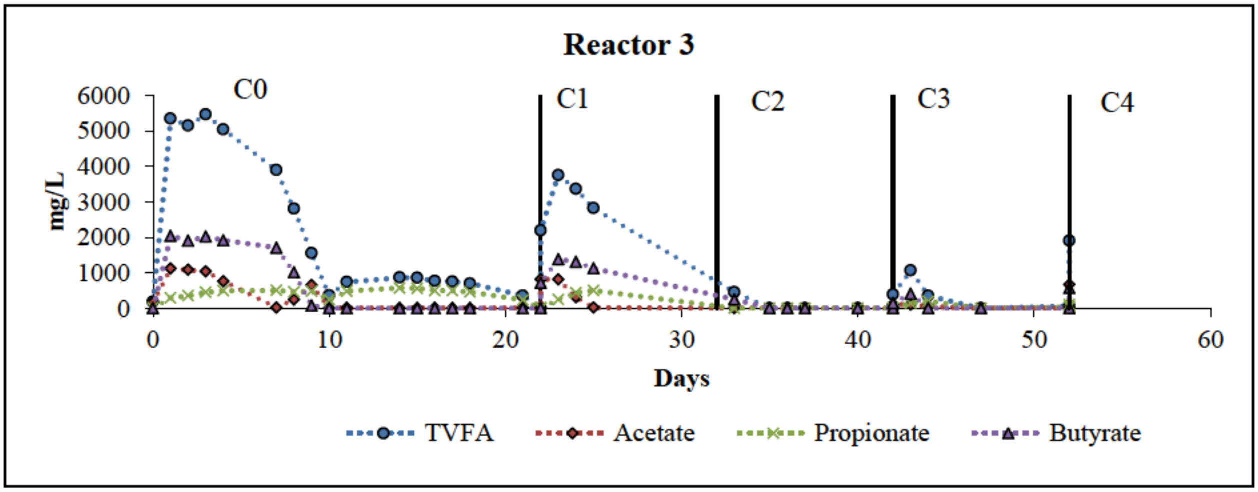

Figure 28: *Volatile fatty acids of reactor 3(75 % whey+25 % CSH)*

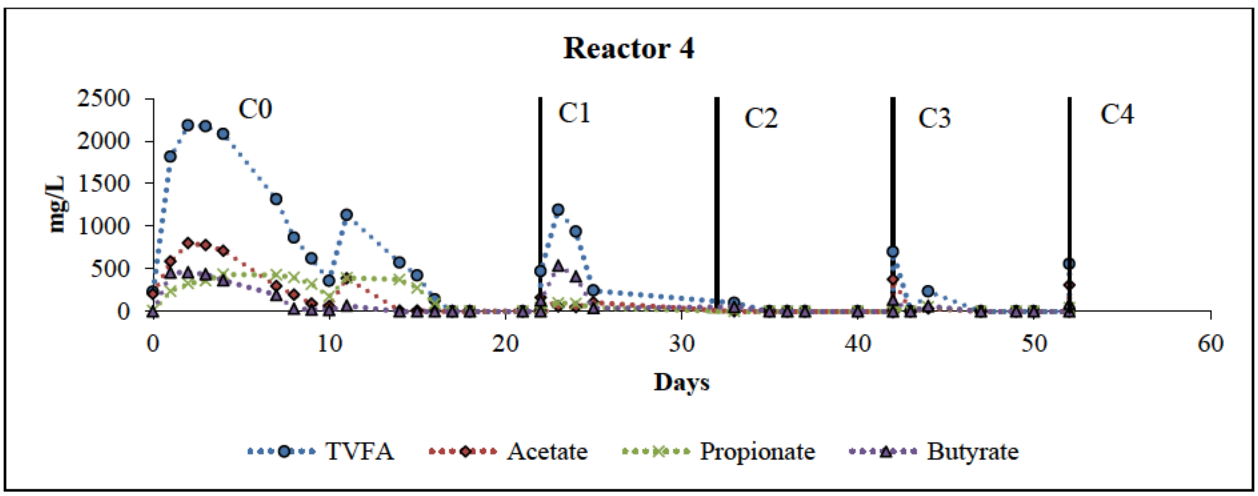

Figure 29: Volatile fatty acids of reactor 4(25 % whey+75 % CSH)

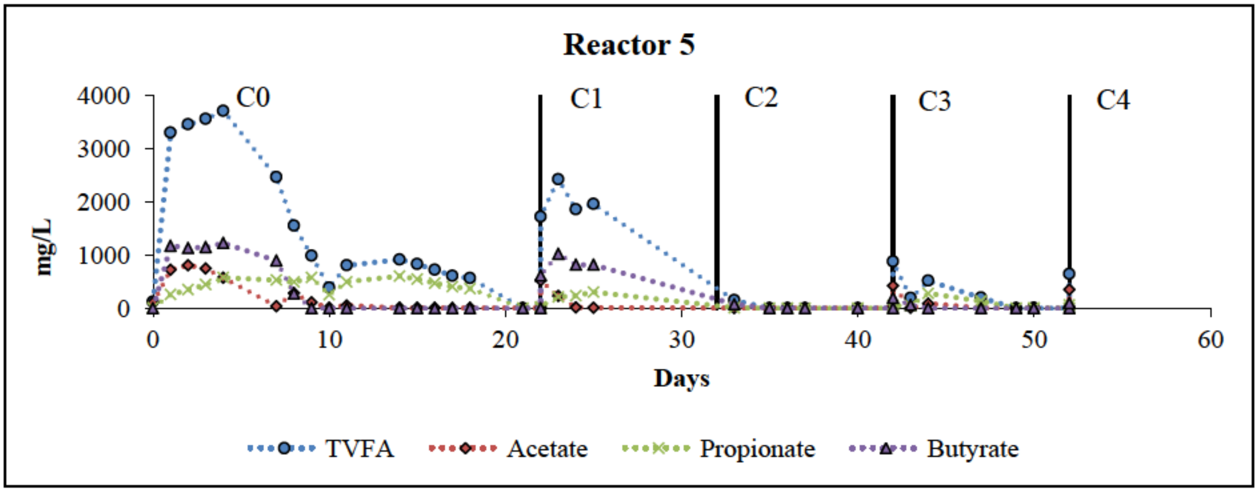

Figure 30: Volatile fatty acids of reactor 5(50 % whey+50 % CSH)

3.3.2.5 Gas Volume Rates

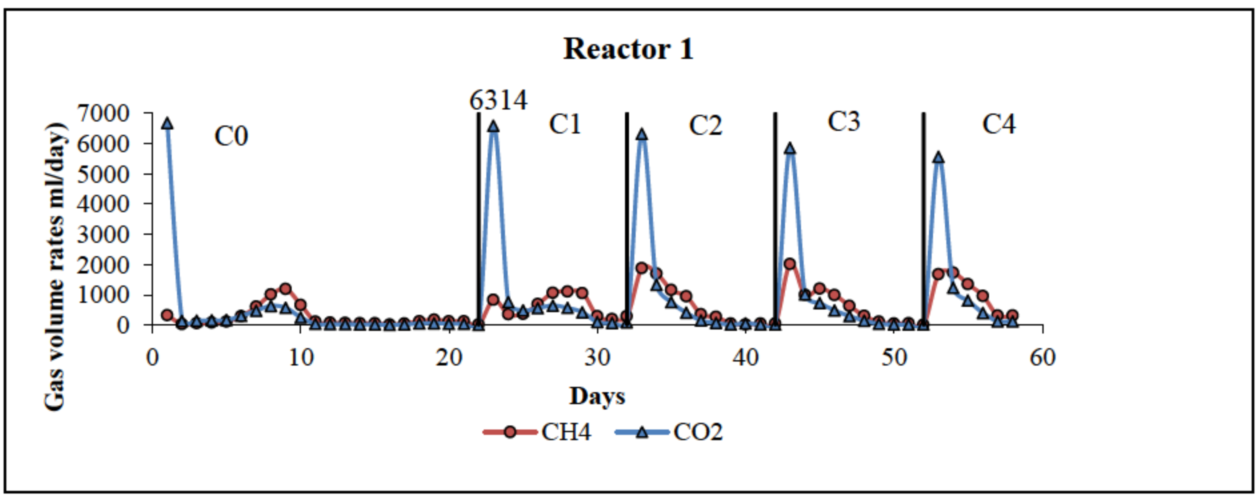

Figure 31: Gas rates of reactor 1(100 % whey)

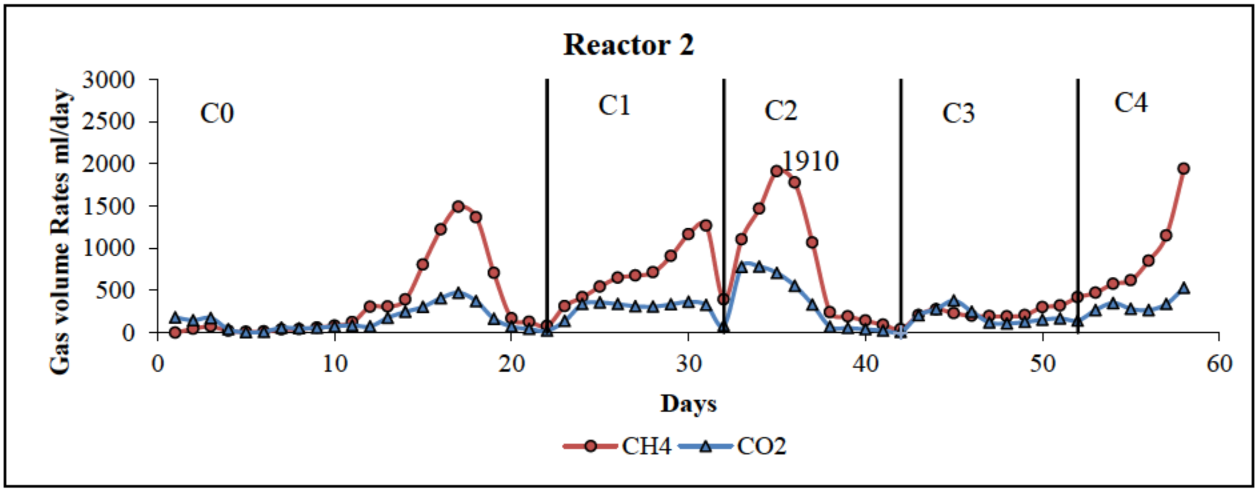

Figure 32: Gas rates of reactor 2(100 % CSH)

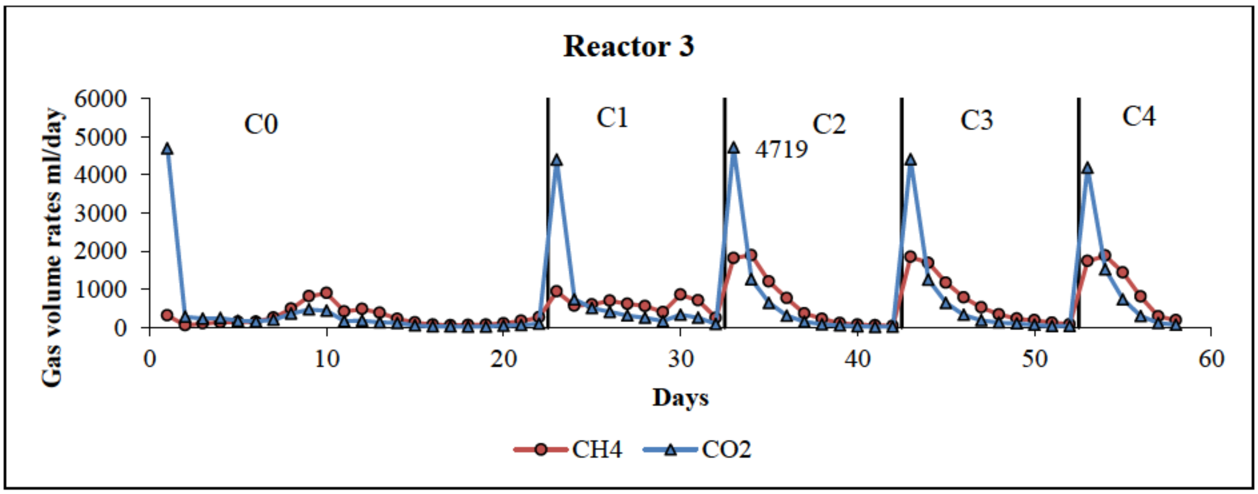

Figure 33: Gas rates of reactor 3(75 % whey+25 % CSH)

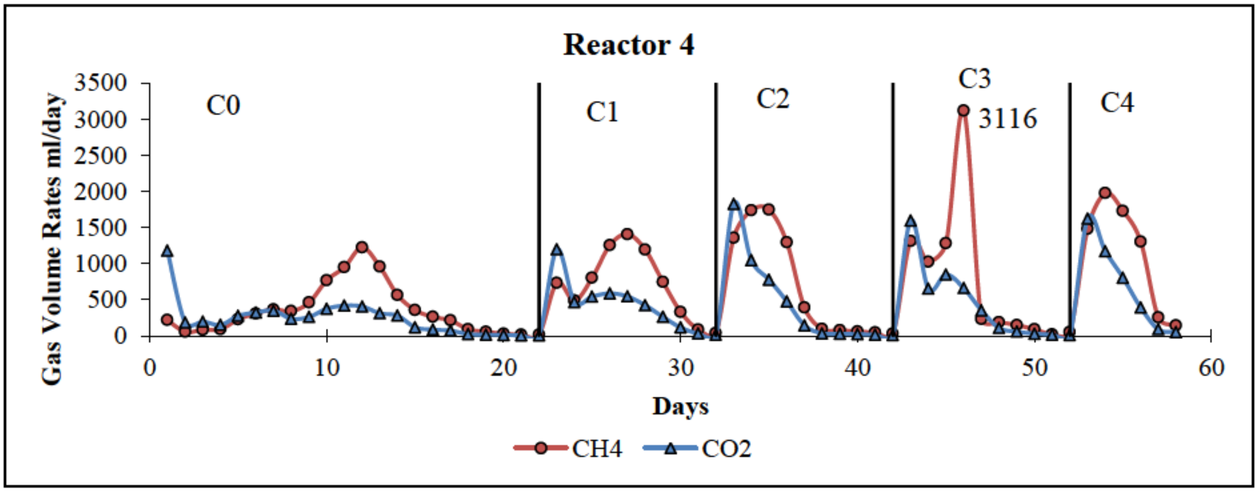

Figure 34: Gas rates of reactor 4(25 % whey+75 % CSH)

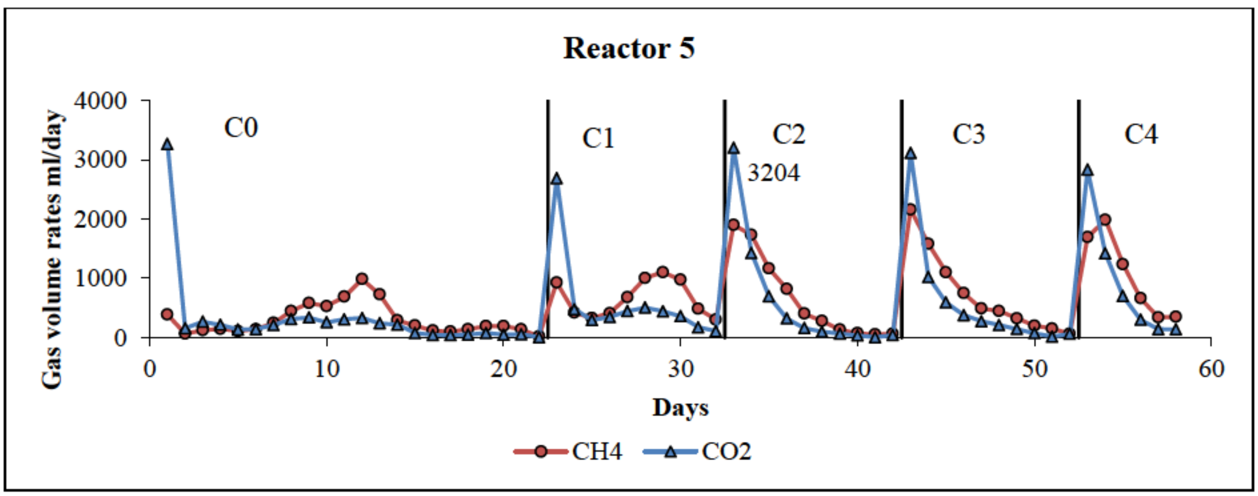

Figure 35: Gas rates of reactor 5(50 % whey+50 % CSH)

3.3.2.6 Cumulative Gas Production CH_4 (ml)

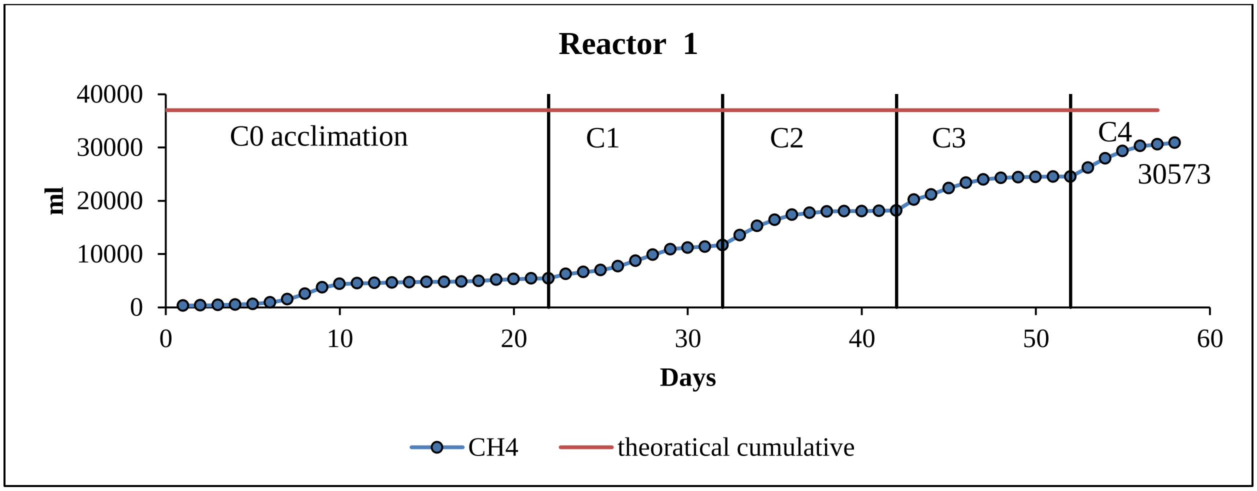

Figure 36: Cumulative gas production of reactor 1(100 % whey)

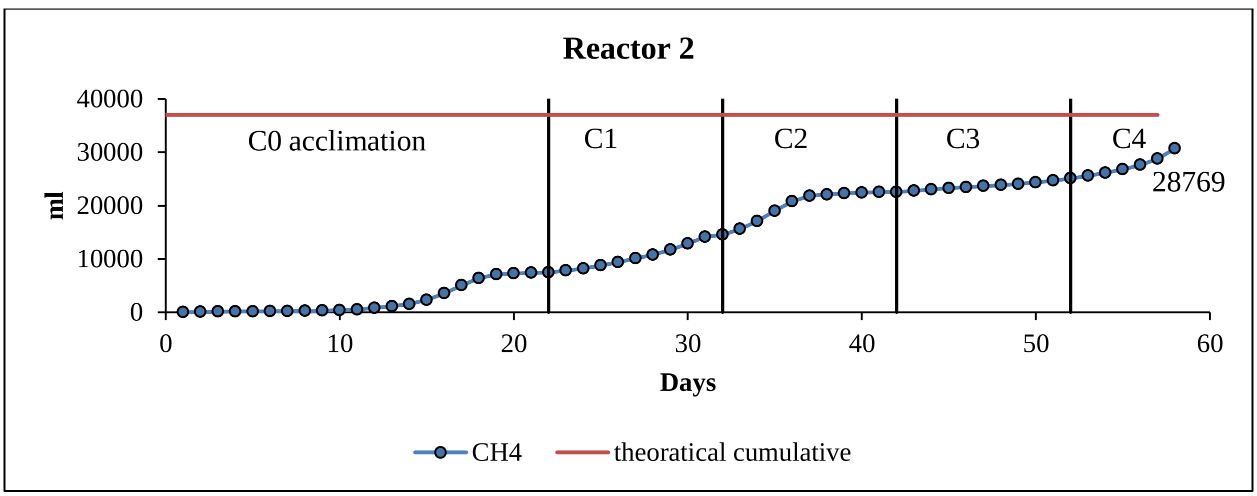

Figure 37: Cumulative gas production of reactor 2(100 % CSH)

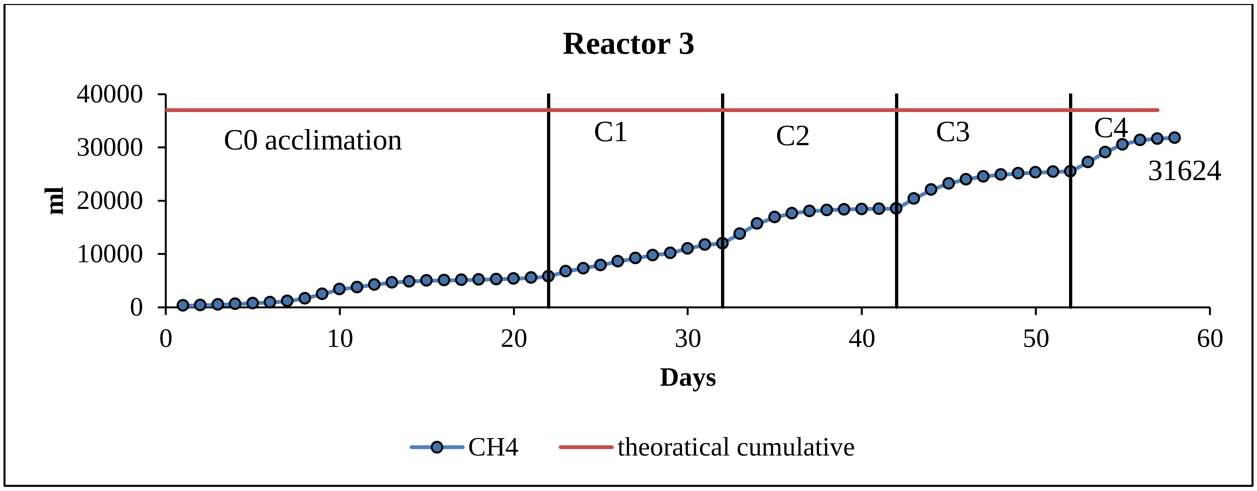

Figure 38: Cumulative gas production of reactor 3(75% whey +25% CSH)

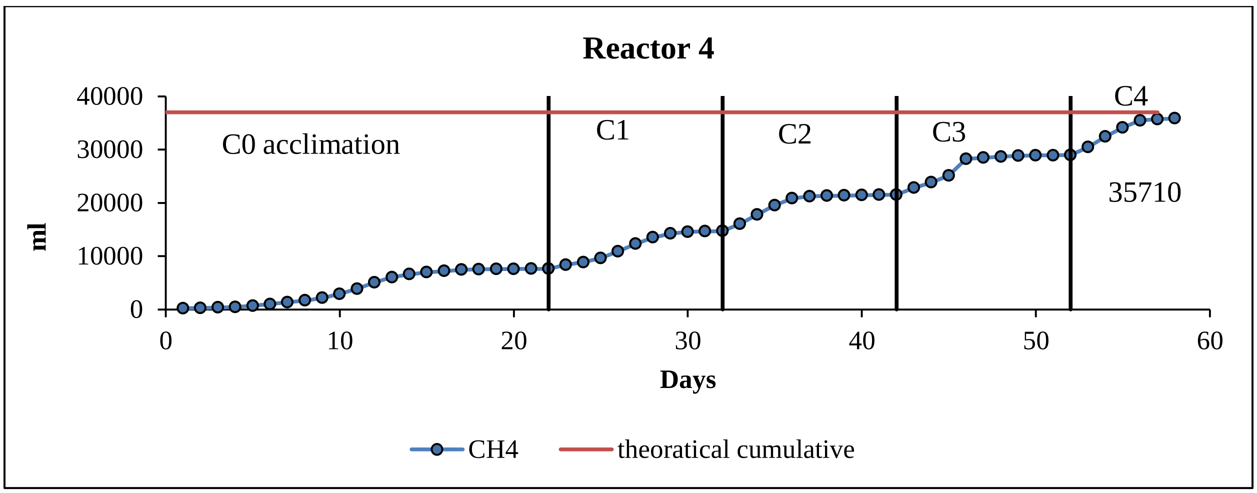

Figure 39: Cumulative gas production of reactor 3(25% whey +75% CSH)

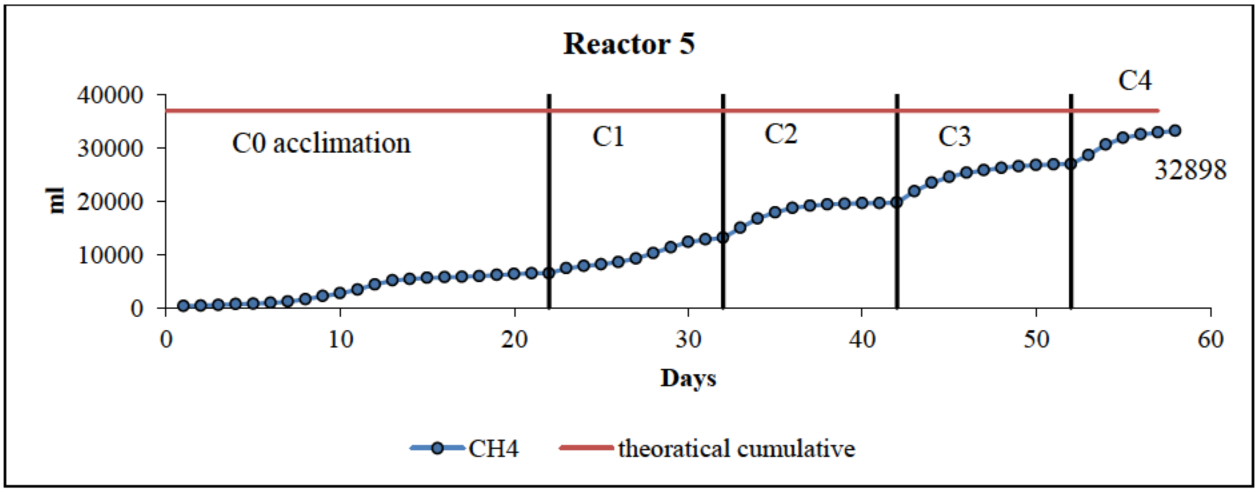

Figure 40: *Cumulative gas production of reactor 5 (50% whey +50% CSH)*

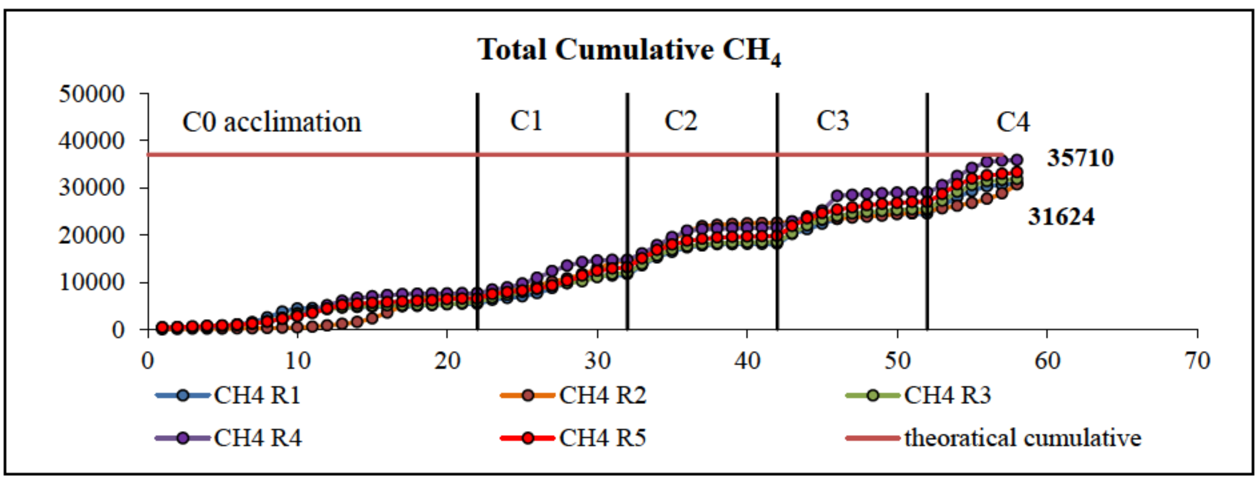

Figure 41: Total cumulative methane production of the 5 anaerobic reactors

3.3.2.7 Cumulative Gas Production CO_2 (ml)

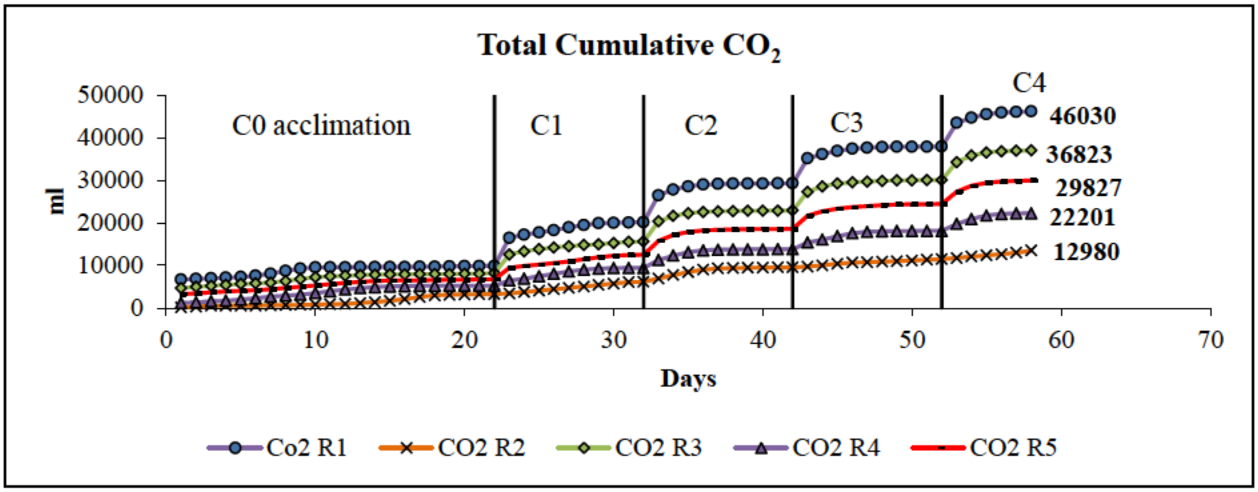

Figure 42: Total cumulative CO_2 production of the 5 anaerobic reactors

3.3.2.8 Cumulative Biogas Production (ml)

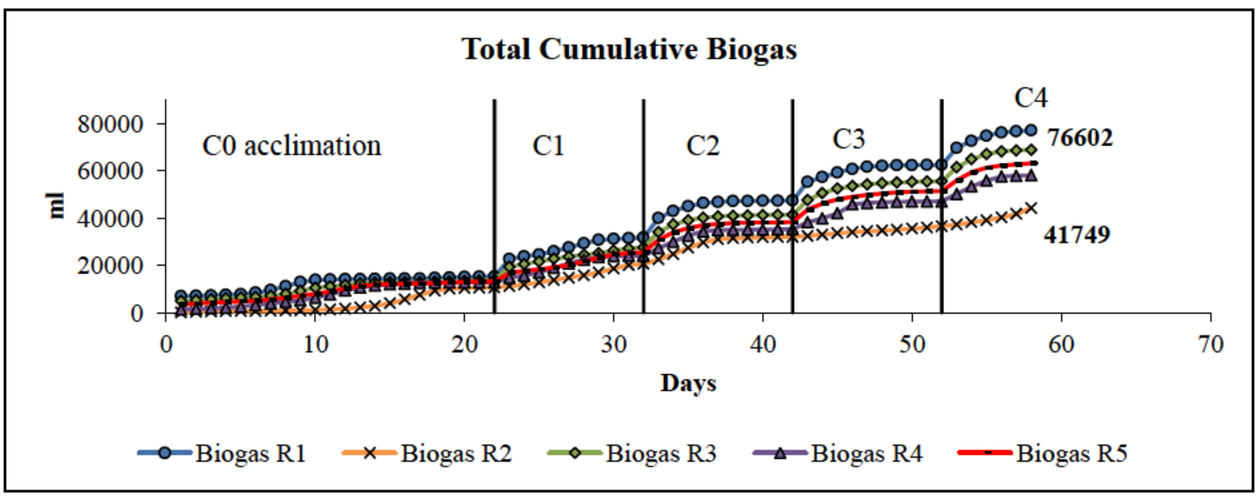

Figure 43: Total cumulative biogas production of the 5 anaerobic reactors

3.3.2.9 Cumulative VS Cumulative (×0.5, ×0.75, ×0.25)

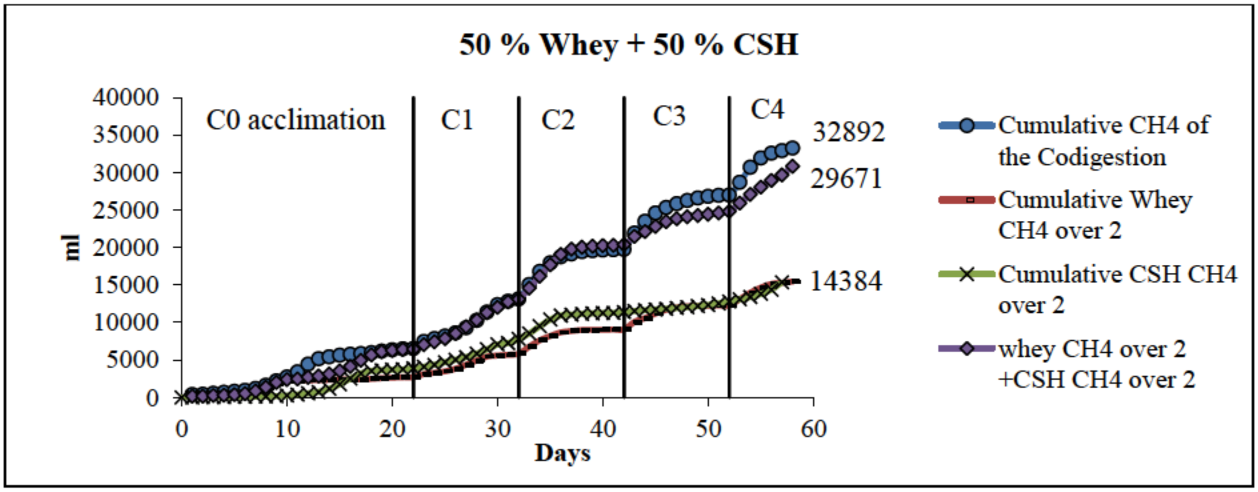

Figure 44: Whey CH_4 over 2 + chicken slaughter house CH_4 over 2

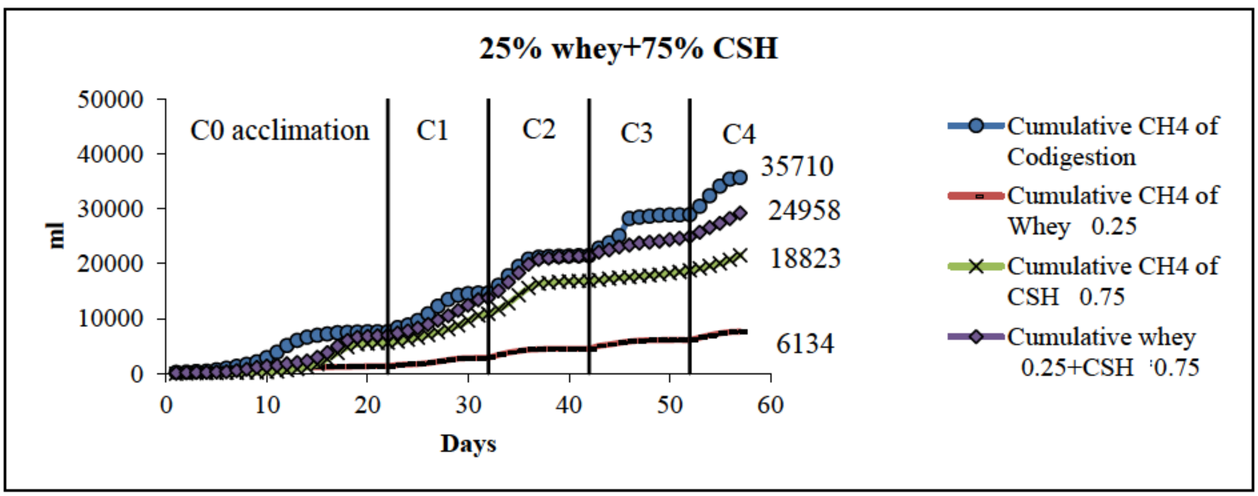

Figure 45: Whey × 0.25 + chicken slaughter house CH_4× 0.75

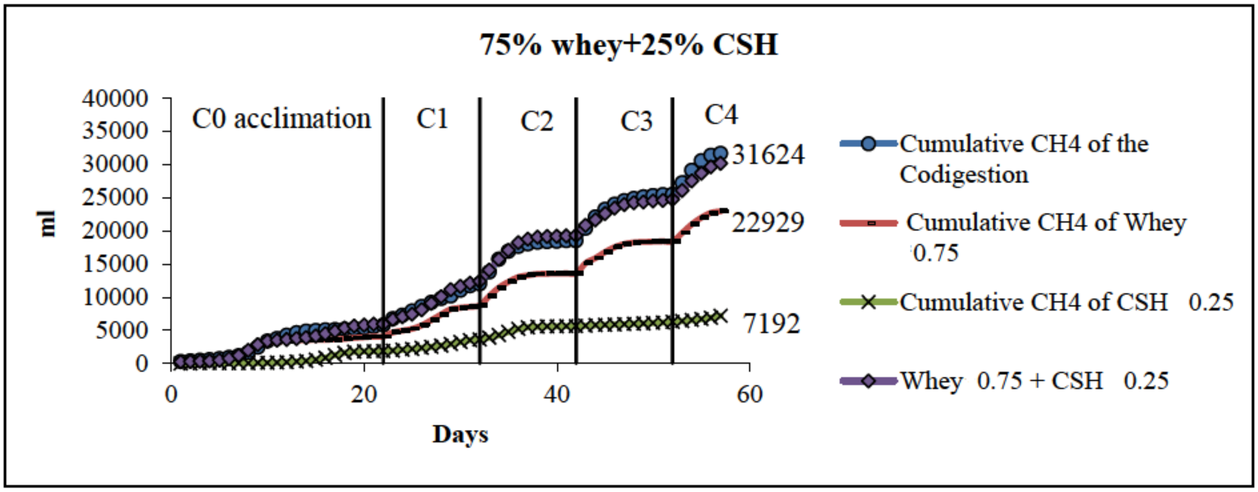

Figure 46: Whey ×0.75 +chicken slaughter house CH_4 × 0.25

3.3.3 Discussion

3.3.3.1 Substrate Characterization Discussion

Table 2 lists the characterization data for the Whey (dairy farm waste) and Chicken slaughter house food waste samples. Although the total COD levels of both samples were virtually identical, they differed in terms of other elements. The protein and carbohydrate composition of the Whey sample was predominantly 52.3 percent TS and 33.1 percent TS, respectively, with no lipid content (0.37 percent TS). The CSH sample, on the other hand, was largely made up of lipid fractions (63.6 percent TS) and had a low carbohydrate content (6.24 percent TS). Higher carbohydrate content has been linked to lower methane generation, while higher lipid content has been linked to increased methane production (Lu et al. 2017).

Furthermore, Nielfa et al. (2015) suggests that greater lipid content can contribute to higher SMP values when compared to protein and carbs, but that it may have slower hydrolysis kinetics. Due to greater long chain fatty acid synthesis, which can adsorb to microbial cells and limit their metabolic activity, high lipid concentration may hinder acidogenesis and methanogenesis (Cirne et al., 2007; Lesteur et al., 2010; Salminen et al., 2000). The pH of cheese whey, chicken slaughterhouse, and seed sludge, respectively, is 5.7, 6.57, and 8.35. The C/N ratios of the five reactors ranged from 33/1 to 21/1, with reactors R1 (100 percent whey) and R2 (100 percent CSH) having the highest C/N ratios.

3.3.3.2 Anaerobic Digestion Steps

To understand the graph results, it is important to explain the anaerobic digestion phases. In the hydrolysis phase, complex organic compounds (fats, carbohydrates and proteins) are decomposed into simpler components (amino acids and simpler sugars). The acidogenesis is where the acidogenic bacteria breaks down the products of hydrolysis into short-chain fatty acids. During acetogenesis, the third phase of biogas production, the products of the acidogenesis phase are converted to acetic acid, hydrogen, and carbon dioxide. The last step is the methanogenesis which are use the methanogenic microorganisms to form methane gas (Achinas et al., 2020).

3.3.3.3 pH

The values of pH varied between 8.5 and 6, indicating that the medium in the five reactors is almost neutral. However, in cycle 3 for R2, the pH declines slightly below 7 during the digesting period, as opposed to the other reactors' tendency of increasing above 7 and stabilizing. This could be linked to the build-up of VFAs in the reactor. In the other reactors, there was no significant acidification of the medium nor accumulation of volatile fatty acids that might inhibit the digestion

3.3.3.4 Total COD and Soluble COD

Food waste is composed of dissolved matter and particular matter. The total COD represents the dissolved and non-dissolved while the soluble COD represents the dissolved matter only. In each cycle, the tCOD and sCOD decrease is due to its transformation into VFAs and then CH_4. COD values were similar in the five reactors since the seed volume was 2 Liters in all reactors and the substrate volume was 0.25 L only. The 0.25 L will only affect the COD value slightly. Highest soluble COD was the whey (10405 mg/L). The reactor where whey is 75% is the second highest (8285 mg/L) since dairy farm waste is formed mainly of soluble wastes. On the other hand, chicken slaughter house (CSH) substrate contains particles and has very low soluble COD which was noticed in (CSH) reactor and in the reactor that contains 75% (CSH) (reactors 2 and 4). Peaks were formed in the graphs in every addition of new fresh waste. These increases at the beginning of each new feed were due to the addition of waste that includes COD. The acclimation phase was longer than the other phases, it had the highest lag time. The highest COD value in this run was 38875 mg/L in reactor 3 (75% whey+25% CSH).

3.3.3.5 Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)

There are two types of suspended solids: the total suspended solids and the volatile suspended solids. The VSS is composed of the biomass which is the bacteria responsible for the treatment. The TSS is composed of the VSS and the non-decomposed waste which are inert solids that do not react with anything. They are fixed solids and not biodegradable. The difference between total solids and volatile solids should always be fixed since the inert solids do not change as noticed in all the graphs. This constant difference represents the nonvolatile matter in the sample and it is constant throughout the days of the run. In the reactor 2 (chicken slaughter house), there was an experimental error in the measurement at the end of the first acclimation cycle were the TSS (5733 mg/L) was measured to be less than the VSS (21633 mg/L).

The curves were slightly decreasing because in each feed cycle 0.25 L of the effluent was removed and replaced with fresh waste. Solids were diluted and their concentration decreased with time in all reactors. TSS and VSS were almost the same in all the reactors since 2 liters of the same seed were added to all the reactors and only 0.25 L substrate was a different factor. In this run the highest TSS is 24889 mg/L and highest VSS is 17767 mg/L.

3.3.3.6 Volatile Fatty Acids (VFAs)

Carbohydrates and proteins are broken down into fatty acids, sugars and amino acids by the biomass during hydrolysis. Then, acidogenic bacteria convert amino acids, fatty acids, and sugars to organic acids, ketones, alcohols and acetate. Acetogenic bacteria then converts fatty acids and alcohols into H_2, CO_2 and acetate. These products are used by methanogenic archaea to produce methane.

Volatile fatty acids are products of the intermediate step of the treatment process which is the transformation of COD to methane. The fluctuation of the total volatile fatty acids measured as acetic acids was noticed in these five curves. Then the production of methane started and the volatile fatty acids were consumed and transformed into methane. The peak of methane production coincided with the minimum point of VFA. There was no accumulation of VFAs in the reactors and there was no inhibition of the process.

However, in cycle 3 for R2 the pH drops to below 7 as opposed to the behavior of the other reactors where the pH increases above 7 and stabilizes. This could be correlated to the accumulation of VFAs in the system, where there was a rise in VFA levels around the day six of operation compared to the other reactors where the VFAs level was nearly depleted. It was discovered that even when lipids have a high methane concentration, digestion blockage can occur (Cirne et al., 2007). This was linked to the accumulation of long-chain fatty acids on cell membranes as well as the liquefaction of complex lipids during hydrolysis (Cirne et al., 2006).

Reactor 1 (whey): Due to high soluble COD the whey reacted faster than other substrates and the acetogenesis products appeared in the first few days. High peaks appeared in the acclimation cycle (7218 mg/L TVFA) and the peak started to decrease gradually in each cycle due to the dilution and the decrease in the molecules that change to VFAs. The peaks might also be decreasing as the COD and VSS biomass was decreasing. The decrease was due to the consumption of the VFA by the biomass that changed into CO_2 and methane.

Reactor 2 (CSH) behaves in an opposite way. The acetogenesis reaction was delayed due to the presence of complex particles that were not dissolved and the high peak (3258 mg/L TVFA) was in the 4rth cycle.The peak increased gradually since the complex paricles started to degraded and turn into volatile fatty acids.

Reactor 3 (75 % whey +25% CSH), reactor 4 (25% CSH+75% whey) and reactor 5 (50% whey+50% CSH) had a similar behavoir as the whey reactor. The highest peaks were in the first acclimation cycle and the peaks started decreasing gradually from one cycle to another.

The VFAs in cycle 2 had problems in production and accumulation.

3.3.3.7 Gas Volume Rates

These curves represent the daily methane and carbon dioxide production in each reactor. Each black line represents the start of each feeding cycle. There was an increase in methane production then a decrease in each cycle which means that the entire methane has been produced and there was no more food waste to be degraded and converted to CH_4. In reactor 1 whey, the CO_2 peak at the beginning was above 6500 ml/day since whey has high soluble COD and the acetogenesis happened directly. It did not need time to break down the molecules to produce CO_2.

R1 (100 % whey) produced the most methane and peaked the earliest (at 9 days), but R2 (100% CSH) took the longest time to reach its peak (17 days). Peak methane productions in the co-digestion reactors fell between the two mono-digestion reactors, which were closer to each end based on the percent composition.

Daily methane productions revealed differences in the per-cycle development of methane output as well as differences between the reactors. The lipid content of the wastewater increased the lag time for daily peaks (Neves et al., 2008). With the exception of R2, which exhibited a lagging output in the third cycle, the highest daily peaks were detected much sooner as the cycles advanced. During the third feeding cycle, R2 performance declined, with no peaks visible, and a rising trend began during the fourth feeding cycle, indicating a significant lag period. This finding could indicate that R2's digestion of 100 percent CSH was inhibited during the latter feeding cycles.

3.3.3.8 Cumulative Gas Production CH_4 (ml)

Cumulative methane production curves show how much methane has been produced throughout the run and allow to track the performance of the reaction. In all reactors the methane started from a negligible value and started to increase gradually through the acetogenesis and methanogenesis processes until it reaches a plateau were no more biogas can be produced. A plateau shows that the bacteria consumed all the food waste and there is no more methane to be produced. Usually methane is analyzed more than CO_2 because it is flammable and can be used as an energy source.

The biogas production was monitored for all reactors on a daily basis. The acclimation cycle ended after 22 days, at which the cumulative methane yield for R1, R2, R3, R4 and R5 was 259, 397, 283, 395 and 328 mL/gCOD$_{added}$. R2 and R4 showed the highest methane yields. R1 exhibited the lowest yield among the different reactors.

By the end of the 22-day period, all reactors exceeded 80% of their methane potentials and reached a plateau. The fast production of biogas by the reactor fed with whey could be attributed to the readily biodegradable matter of the waste, as it contained mostly soluble material, while that of the CSH mainly constituted complex particulate matter which led to its longer lag time (Zahan et al., 2018).

Reactor 4 (25% whey+75% CSH) produced the highest value of CH_4 (35853 ml) while reactor 2 (CSH) was the one that produced the lowest amount (30707 ml).This shows that mixing CSH with whey increased its biogas production. CSH was approaching its bio methane potential because it contained much more lipids than the other reactors. Lipids produce VFAs and methane much faster than others. This could also be any additives like steroids that enhance the growth of the chicken. With the lipids from CSH substrate and the fast rate caused by whey, reactor 2 gave the highest value of methane among the 5 reactors.

For the 2nd feeding cycle, where R2 had a higher production, this could be attributed to residual substrates from the previous cycle. However, the difference became significant in the 3rd and 4th cycles due to the inhibition occurring in R2. On the other hand, R1 consistently had the lowest amount of produced methane compared to the co-digestion reactors and R2, yet it produced methane with the lowest lag times.

Whey waste in our study mainly consisted of carbohydrates and proteins which are relatively easier to biodegrade than lipids. The presence of easily biodegradable carbohydrates (such as lactose and glucose) and proteins, in addition to their high solubility, may have resulted in facilitated hydrolysis which was reflected in the lower lag time (Zahan et al., 2018).

3.3.3.9 Cumulative Gas Production CO_2 (ml)

Acetogenesis is the process of decomposing volatile fatty acids and alcohol to produce CO_2 gas which comes before the production of methane. Reactor 1 (Whey) has the highest CO_2 production (46155 ml) since it contains the highest value of acetic acid concentration. The lowest CO_2 production is Reactor 2 (CSH) (13512 ml).

3.3.3.10 Cumulative Biogas Production (ml)

The biogas is the summation of the methane and carbon dioxide volumes produced. The highest biogas volume is reactor 1 (whey) 77045 ml and the lowest biogas volume is reactor 2 (CSH) 44219 ml.

3.3.3.11 Cumulative VS Cumulative (×0.5, ×0.75,×0.25)

Figures 44, 45, and 46 were constructed to observe how different ratios of co-digestion affected the methane production. In figure 44: Reactor 4 (50% Whey + 50% CSH): the summation of cumulative CH_4 of whey over 2 and cumulative CH_4 of CSH over 2 was 29671 ml while the cumulative CH_4 of the co-digestion is 32892 ml. There is no big difference between the two numbers and these numbers showed that the co-digestion of whey and CSH with a 50:50 ratio slightly enhanced the methane production.

In figure 45, the summation of cumulative CH_4 of whey ×0.25 and cumulative CH_4 of CSH×0.75 was 29219 ml while the cumulative CH_4 of the co-digestion was 35710 ml at day 57. There was an increase in the co-digestion curve which showed that the co-digestion of whey and CSH with a 25:75 ratio have enhanced the methane production.

In figure 46: the summation of cumulative CH_4 of whey ×0.75 and cumulative CH_4 of CSH×0.25 was 29598 ml while the cumulative CH_4 of the co-digestion was 31624 ml. There was no big difference between the two numbers. These numbers showed that the co-digestion of whey and CSH with a 75:25 ratio slightly enhanced the methane production.

However, mixing whey and CSH with (25:75) and (75:25) ratios produced more methane than digesting whey alone and digesting CSH alone. This was due to the fact that lipids and large molecules from CSH substrate decomposed to give high volumes of methane and the fast rate caused by whey along with lipids and proteins of dairy farm wastes forms a combination better than digesting each substance alone.

3.4 Kinetic Study

3.4.1 Kinetic Study Graphs

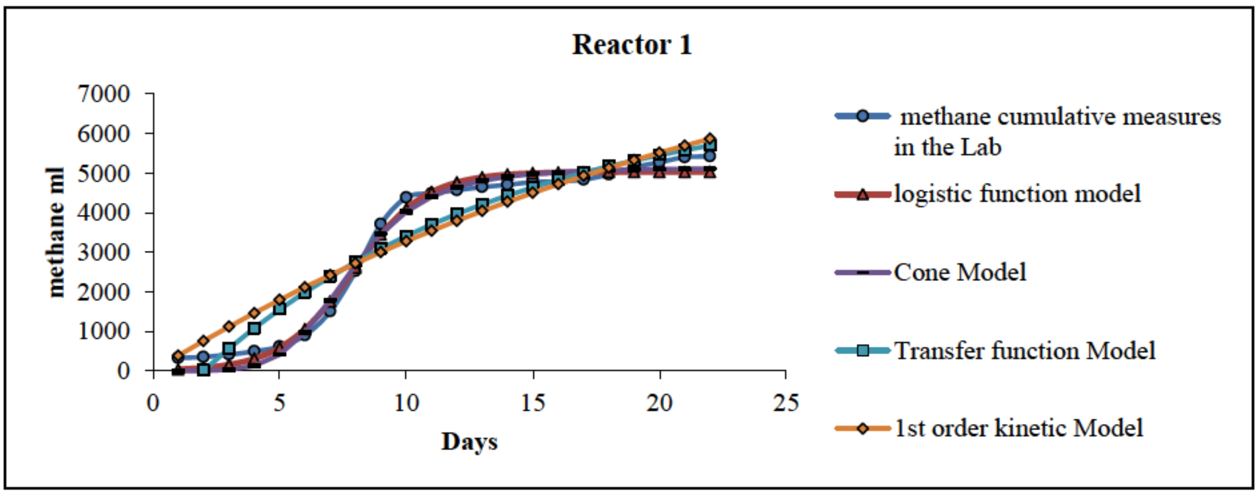

Figure 47: Cumulative methane production using different models for reactor 1 (100 % whey) during acclimation cycle

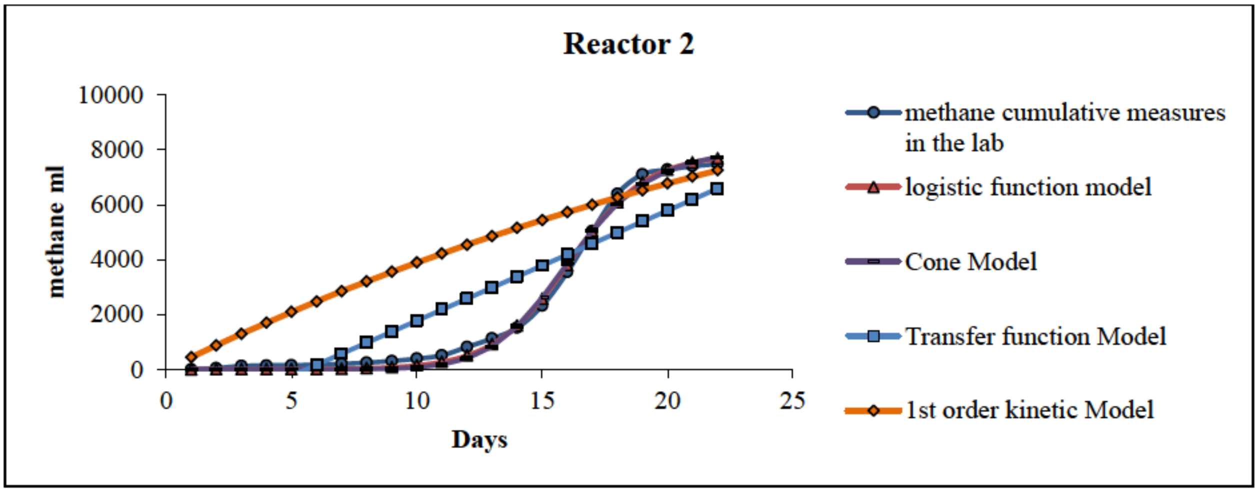

Figure 48: Cumulative methane production using different models for reactor 2 (100 % CSH) during acclimation cycle

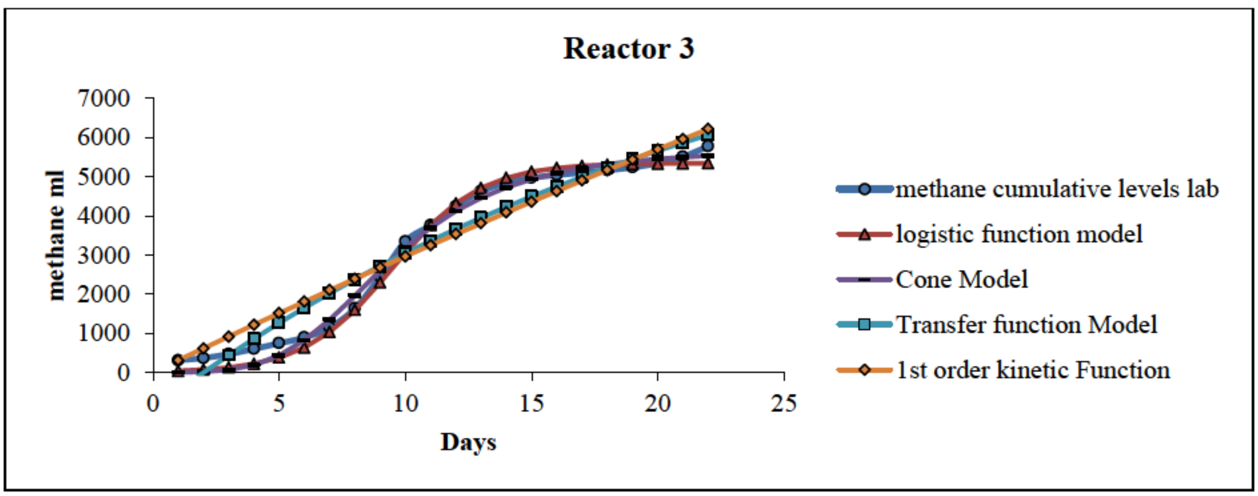

Figure 49: Cumulative methane production using different models for reactor 3 (75 % whey+25% CSH) during acclimation cycle

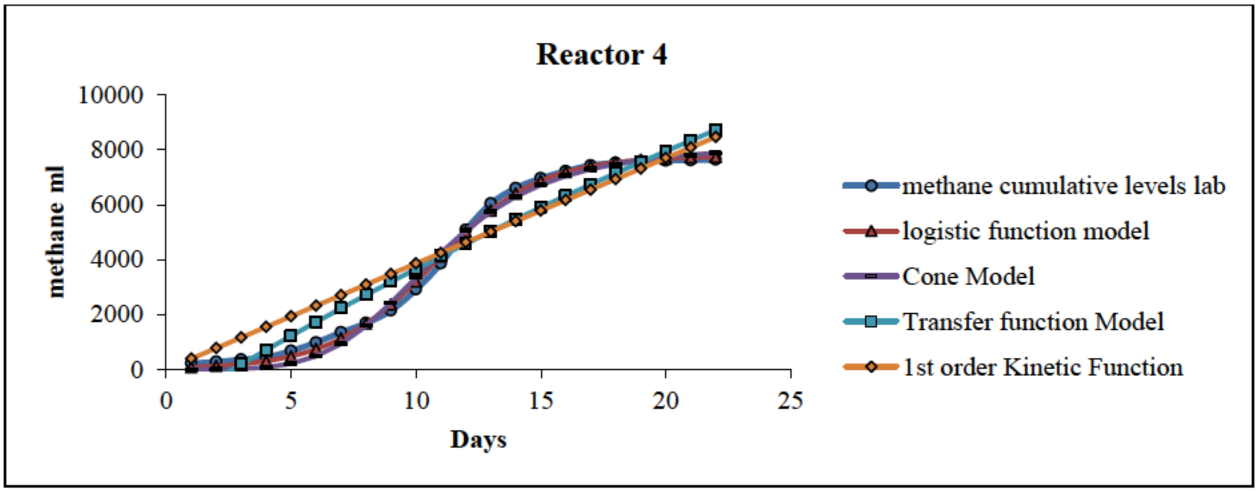

Figure 50: Cumulative methane production using different models for reactor 4 (75 % whey+25% CSH) during acclimation cycle

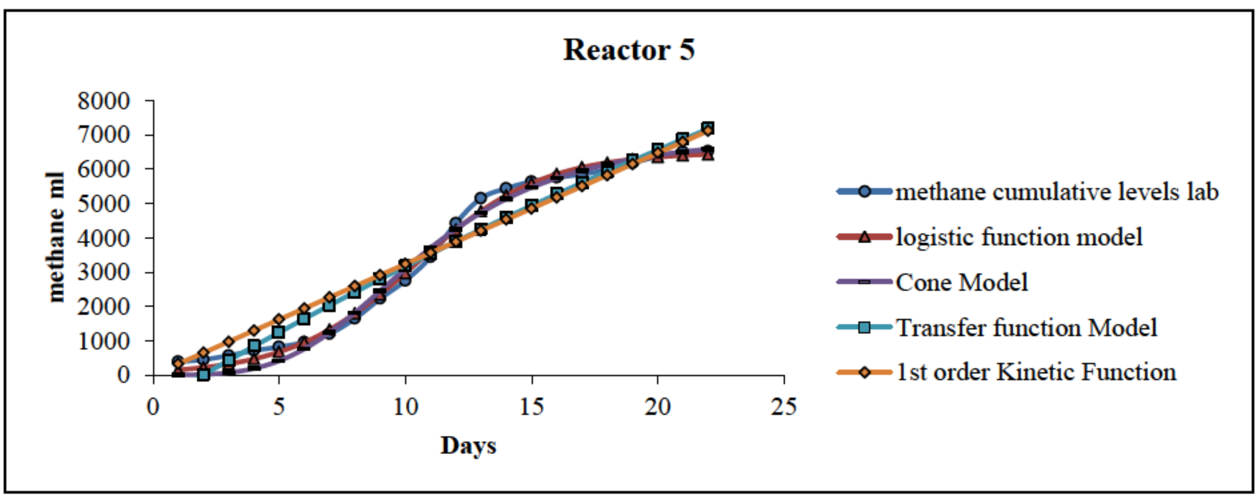

Figure 51: Cumulative methane production using different models for reactor 5 (50 % whey+50% CSH) during acclimation cycle

Table 3: Parameters of first order and Cone models

		Percentages (Chicken slaughter house waste: Whey)				
Model	Parameters	100% whey	100% CSH	75% whey+25% CSH	25% whey+75% CSH	50% whey+50% CSH
first order kinetic	B measured (ml/g VS added)	5417.97	7476.47	5773.76	7631.35	6521.82
	fd (ml/g VS added)	10323.87	15000	38236.41	870866.95	40822592
	Khyd (/d)	3.8×10^{-2}	0.030	8×10^{-3}	4.4×10^{-4}	7.92×10^{-6}
	B predicted (ml/g VS added)	5865.42	7247.22	6217.09	8458.71	7113.52
	Diff (%)	8.25	3.06	7.67	10.84	9.07
	RSS	9571090.44	123128527	6905504.14	19454845.62	8440997.40
	rMSPE	659.58	2365.74	560.25	940.37	619.42
	AIC	295.98	352.18	288.80	311.58	293.21
Cone	fd (ml/g VS added)	5131.44	8084.96	5738.71	8153.49	7058.93
	Khyd (/d)	0.12	0.061	0.11	0.09	0.09
	n	5.37	9.99	3.92	4.71	3.64
	B predicted (ml/g VS added)	5110.98	7732.72	5537.34	7877.81	6582.18
	Diff (%)	5.67	3.42	4.09	3.23	0.92
	RSS	1302566.76	1251862.63	1037920.97	1821386.17	1700503.25
	rMSPE	243.32	238.54	217.21	287.73	278.02
	AIC	255.50	254.63	250.51	262.88	261.37

Table 4: Parameters of transfer and logistic models

		Percentages (Chicken slaughter house waste: Whey)				
Model	Parameters	100% whey	100% CSH	75% whey+25% CSH	25% whey+75% CSH	50% whey+50% CSH
Logistic function	Pmax	5028.97	7838.97	5344.67	7769.79	6490.45
	Rmax	879.33	1277.82	764.66	922.45	645.34
	λ	5.03	13.03	6	6.54	5.39
	B predicted (ml/gVS added)	5028.71	7675.21	5340.51	7732.84	6426.13
	Diff (%)	7.18	2.66	7.50	1.33	1.46
	RSS	1168906	825452.9	1096052	582687.2	723825.4
	rMSPE	230.50	193.70	223.20	162.74	181.38
	AIC	253.12	245.46	251.71	237.81	242.57
Transfer function	Pmax	7055.77	4.99E+08	10663.28	30382.76	25051.38
	Rmax	580.44	400.99	448.56	530.72	421.99
	λ	1.98	5.58	2.01	2.66	1.98
	B predicted (ml/gVS added)	5696.2	6583.62	6064.19	8711.15	7169.96
	Diff (%)	5.13	11.94	5.03	14.15	9.94
	RSS	7044018	36304732	5147822	13044581	6505760
	rMSPE	565.84	1284.61	483.73	770.02	543.79
	AIC	292.63	328.71	285.74	306.19	290.88

3.4.2 Kinetic Study Discussion

Kinetic models are useful for quantifying, predicting, and understanding synergistic biogas production gains. Digestion rates, lag durations, and output rates are among the key characteristics derived from these rate-limiting models (Emebu et al. 2022). Determining the kinetic factors and parameters that could affect the process, is a first step towards designing a full scale plant. Several parameters were calculated and compared to discover which model is the best fit to the experimental data in this anaerobic digestion process. The calculated parameters are Akaike's Information Criterion (AIC) , Root Mean Square Prediction Error (rMSPE), RSS (residual sum of squares) and the percentage difference in between measured and estimated methane production volumes.

The rMSPE parameter represents the deviation between measured and estimated values. Root mean square prediction error (rMSPE) was calculated as mentioned by (Wang et al. 2011).

rMSPE= $\sqrt{\sum_{n=1}^{n} \frac{(PVi-Mvi)^2}{n}}$

Pvi is estimated value of methane volume by the model functions,
Mvi is measured value of methane volume in the lab, and n is number of data points.

AIC was developed based on information technology to compare models. AIC balances the change of how good the model fits vs. the number of the model parameters (Motulsky and Christopoulos, 2003).

AIC= n ln($\frac{RSS}{n}$) +2 (N+1) + $\frac{2(N+1)(N+2)}{(n-N-2)}$

where RSS is residual sum of squares, n is number of data points, and N is number of model parameters.

The model with the lowest value of AIC is most likely to be correct (Motulsky and Christopoulos, 2003). In this experiment, the lowest value of AIC is the logistic function (AIC (R1)=253.12, AIC (R2)=245.46, AIC (R3)=251.71, AIC (R4)=237.8059, AIC (R5)=242.57), which appears to be the best fit of this experimental data. The model that gave the highest AIC was the first order kinetic model which appeared to be the least model that fit these experimental data (AIC (R1)= 295.98, AIC (R2)= 352.18, AIC (R3)= 288.80, AIC (R4)= 311.58, AIC (R5)= 293.21). The transfer function model has close AIC values to the first order kinetic model which indicates that it is not a good fit to these experimental data too.

The rMSPE also represents the deviation between measured and estimated values, the lower the value of this parameter the better the model fits the experimental data. The lowest value of rMSPE is the logistic function (rMSPE (R1)= 230.504, rMSPE (R2)= 193.70, rMSPE (R3)= 223.20, rMSPE (R4)= 162.74, rMSPE (R5)= 181.38), which appears to be the best fit of this experimental data.

The model that gave the highest rMSPE was the first order kinetic model which appeared to be the least model that fit these experimental data (rMSPE (R1)= 659.58, rMSPE (R2)= 2365.74, rMSPE (R3)= 560.25, rMSPE (R4)= 940.37, rMSPE (R5)= 619.42).

The transfer function model has close rMSPE numbers to the first order kinetic model which indicates that it is not a good fit to the data too.
The cone model has low values of AIC and rMSPE close to the logistic function values, this indicates that these two models also represents a suitable fit for the experimental data measured in this anaerobic digestion batch.

To fit the biogas data and determine the rate-limiting constant k, the first order model was used. During acclimation, however, this model provided a poor match, with r2 values ranging from 0.729 to 0.969 for all reactors. The first-order model only accounts for the exponential phase of biogas generation, leaving out the lag phase, which could explain the low r2 values found. (Emebu et al., 2022; Zahan et al., 2018). These findings show that adding whey waste in various amounts can help with digestion because of its relatively easily biodegradable structure (Zahan et al., 2018).

The percentage difference between the measured and the estimated methane volumes were calculated. All the calculated values appear to be less than 10 %. This parameter represents the percentage difference between the measured and estimated for the highest point only, therefore this parameter does not accurately represent the whole set of data.
In the reactors when whey samples are present, the fit is better. This suggests that the addition of whey waste in reactors, particularly those containing chicken slaughterhouse waste, may aid anaerobic degradation.

3.5 Quantitate Co-digestion Synergy

3.5.1 Co-digestion Synergy Bar graph

Synergy is defined as two or more organizations, drugs, or other agents interacting or cooperating to produce a combined effect greater than the sum of their individual effects.

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$$

$$1 \text{ mole of } CH_4 (\text{or } 22.4 \text{ L of } CH_4) \rightarrow \text{Needs } 64 \text{ g of COD} \Rightarrow$$

$$1 \text{ g of COD is equivalent to } 0.35 \text{ L } CH_4 \text{at standard conditions}$$

Efficiency $= \frac{\text{CH4 measured}}{\text{Initially characterized tCOD (mg/L)x0.25(L)x0.35 L/g}}$ (represents the experimental numbers)

weighted Efficiency $= \frac{\text{CH4 weighted measured (expected)}}{\text{Initially characterized tCOD (mg/L)x0.25(L)x0.35 L/g}}$ (represents the theoretical numbers)

Efficiency/weighted Efficiency

when the efficiency is higher than weighted efficiency it means that mixing the substances is better and it indicates synergism:

efficiency /weighted efficiency>1

when the weighted efficiency is higher than the efficiency it indicates that mixing did not improve the methane production:

efficiency/weighted efficiency<1

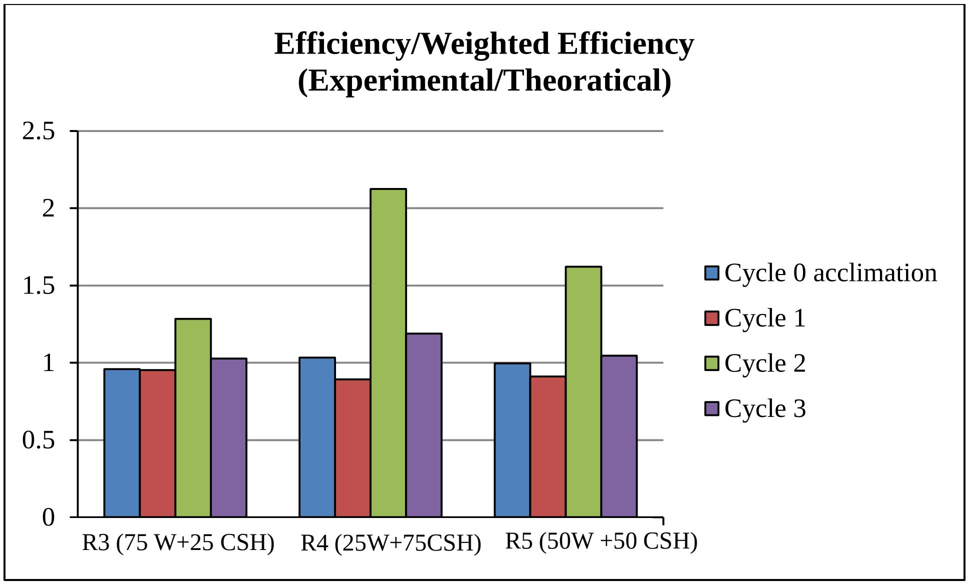

Figure 47: Efficiency/Weighted Efficiency

3.5.2 Co-digestion Synergy Discussion

Synergy is defined as two or more organizations, drugs, or other agents interacting or cooperating to produce a combined effect greater than the sum of their individual effects.

The treatment efficiency for mixed substrates was compared to the corresponding independent weighted efficiency for each substrate to examine the synergistic effects of co-digestion. Figure 82 shows the relationship between co-digestion efficiency and weighted co-digestion efficiency, which was used to see if there were any synergistic effects in the experiment with respect to X=1. A value that plots above X=1 shows synergism, while one that plots below the line implies reduced efficiency.

When the efficiency is higher than weighted efficiency it means that mixing the substances is better and it indicates synergism: efficiency /weighted efficiency>1.

when the weighted efficiency is higher than the efficiency it indicates that mixing did not improve the methane production: efficiency/weighted efficiency<1.

In the first two cycles, no substantial synergism was seen (C0 and C1). R3 and R5 showed strong synergism in cycle two (C2). However, when compared to the other reactors, R4 showed the most synergism, being the furthest over the line in cycles 3 and 4. The modest inhibition in R2 in the second cycle (C2) may have influenced the synergism observed.

CHAPTER FOUR

CONCLUSION AND RECOMMENDATIONS

The major goal of this research was to compare between the mono-digestion and the co-digestion results of methane production in the batch anaerobic digestion process while trying three different mixing ratios. This was done with chicken slaughter house waste and Whey (dairy farm waste). Mono-digestion and co-digestion was performed with weekly feeding cycles under the OLR of 1.1 g.COD/L. day with three different ratios of (50:50, 75:25, 25:75).

The co-digestion of the whey waste and chicken slaughter house waste feeds resulted in improved methane yield, likely due to their complementary characteristics which aided in balancing the anaerobic digestion process.

The best combination ratio appeared to be (25% whey + 75% CSH) which produced 35853 ml at the end of day 58. Which is larger than the 100 % whey (30890 ml) and larger than the 100 % CSH (30707 ml).So the best way to produce methane with an OLR of 1.1 g. COD /L .day is from a (25% whey + 75% CSH).

As for the kinetic study that was done using four model functions, the logistic function model appeared to be the best fit for the experimental data followed by the cone model. The transfer function and first kinetic model function were not a good fit for these data according to the AIC and rMSPE calculated paremters. Determining the kinetic factors and parameters that could affect the process, is a first step towards designing a full scale plant.

Reactor 4 (25% whey + 75% CSH) has also showed the most synergism in the efficiency study, being the furthest over the line in cycles 3 and 4.

Further studies could also focus on these two food wastes and try other organic loading rates and other different mixing   that might produce more biogas volumes and better results.

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APPENDICES

APPENDIX A: RAW DATA

Raw data of the Mono and Co-digestion with weekly feeding of the two food wastes (whey dairy farm waste and chicken slaughter house waste). These tables show the pH, COD, suspended solids, volatile fatty acids gas volume rates and cumulative gas volumes of the five reactors used in this run.

Table A1: PH values in the five reactors

Day	R1 pH	R2 pH	R3 pH	R4 pH	R5 pH
0	8.44	8.35	8.38	8.35	8.35
1	8.51	7.46	8.05	7.60	7.60
2	8.17	7.05	7.36	7.14	7.14
3	8.01	7.13	7.71	7.42	7.42
4	8.03	7.36	7.87	7.64	7.64
7	7.92	7.30	7.54	7.32	7.32
8	8.31	8.00	8.24	8.32	8.32
9	7.96	7.41	7.82	7.57	7.57
10	7.81	7.70	7.72	7.57	7.57
11	7.74	7.54	7.48	7.47	7.47
14	7.71	7.65	7.78	7.65	7.65
15	7.76	7.77	7.86	7.81	7.81
16	7.80	8.11	8.13	7.99	7.99
17	7.90	7.79	7.84	7.74	7.74
18	7.83	7.78	7.87	7.71	7.71
21	7.96	7.86	7.82	7.82	7.82
22	7.67	7.57	7.78	7.80	7.80
22	8.45	6.58	7.88	6.85	6.85
23	8.15	7.28	7.65	7.34	7.34
24	7.82	7.44	7.75	7.56	7.56
25	7.37	7.22	7.54	7.24	7.24
28	7.18	7.63	7.62	7.66	7.66
29	7.54	7.72	7.72	7.78	7.78
30	7.69	7.59	7.65	7.71	7.71
31	7.76	7.54	7.74	7.61	7.61

32	7.88	7.75	7.75	7.72	7.72
32	8.1	8.04	8.04	8.05	8.05
33	7.28	6.82	7.18	7.00	7.00
35	7.32	7.39	7.63	7.49	7.49
36	7.59	7.62	7.49	7.52	7.52
37	7.54	7.32	7.37	7.45	7.45
40	7.47	7.40	7.51	7.34	7.34
42	7.34	7.20	7.29	7.28	7.28
42	8.24	6.79	7.36	7.11	7.11
43	7.73	6.95	7.27	7.17	7.17
44	7.35	7.25	7.46	7.42	7.42
47	6.98	7.47	7.44	7.41	7.41
49	7.08	7.78	7.95	7.90	7.90
50	6.91	7.43	7.55	7.53	7.53
52	6.90	7.34	7.37	7.33	7.33
52	6.15	7.59	7.14	7.87	7.64
53	7.19	7.02	6.92	7.38	7.19
54	7.36	7.22	7.39	7.56	7.51
56	7.50	7.35	7.48	7.59	7.40
57	7.57	7.43	7.51	7.48	7.36
58	7.25	7.34	7.28	7.28	7.24

Table A2: Total COD and Soluble COD:

	Reactor 1 (mg/L)		Reactor 2 (mg/L)		Reactor 3 (mg/L)		Reactor 4 (mg/L)		Reactor 5 (mg/L)	
days	tCOD	sCOD	tCOD	sCOD	tCOD	sCOD	tCOD	sCOD	tCOD	sCOD
0	29350	432.5	29350	432.5	29350	432.5	29350	432.5	29350	432.5
0	35575	10405	36700	1370	38875	8285	36250	3445	36500	5735
22	24950	449	25525	706	25700	536	29325	535	27150	546
22	34200	9260	35150	1620	32950	6575	34200	3050	33275	4400
32	25700	626	26000	964.5	25675	606	24250	649	24675	625
32	31200	10120	32375	1510	31050	7740	30425	3285	29000	6065
42	22275	544	20725	560	21000	511	22375	519	21400	461
42	29050	8805	29375	1135	28500	6735	27625	2780	27225	4730
52	20675	491	25425	3305	20450	499	20275	486	19950	444
52	27300	6720	30650	3610	27425	6495	28350	3190	25875	4975
58	19487.5	462.8	25187.5	2966.6	17825	434.9	17475	418.8	19000	422.9

Table A3:Total and Volatile Suspended Solids

	Reactor 1		Reactor 2		Reactor 3		Reactor 4		Reactor 5	
Day	TSS	VSS	TSS	VSS	TSS	VSS	TSS	VSS	TSS	VSS
0	24889	17144	24889	17144.4	24889	17144.4	24889	17144	24889	17144
0	22533	16450	23867	17766.7	22889	16844.4	24278	17633	23022	16844
22	22283	15767	5733.3	15722.2	21589	15677.8	23689	16167	21778	15667
22.5	20217	14950	21633	16244.4	21478	16000	22956	16811	21500	16000
32	20278	14322	19822	14266.7	21589	15011.1	20544	14389	20400	14889
32.5	20367	14300	20167	14655.6	21433	14950	20544	14578	20311	15156
42	18156	13078	17678	12888.9	18378	13100	19144	13600	19733	13822
42	18223	13474	18822	14277.8	18222	13511.1	18967	14133	18711	13956
52	18511	13100	17478	13022.2	17750	13183.3	17433	12467	17822	12756
52	17804	13326	17689	13733.3	17522	13011.1	18122	13456	17500	13022
58	16167	11689	16300	12344.4	16322	11811.1	15856	11189	16367	11889

Table A4: Volatile Fatty Acids

	Reactor 1				Reactor 2			
Day	Acetate	Propionate	Butyrate	Total VFA	Acetate	Propionate	Butyrate	Total VFA
0	77.37	7.66	0	94.36	64.91	6.88	0	79.84
1	1359.68	198.00	2857.88	6955.18	157.11	65.37	0	266.82
2	1440.13	270.88	2894.56	7218.07	464.81	90.08	0	633.38
3	1400.02	302.94	2957.35	7337.83	576.36	132.47	0	816.73
4	1136.23	316.23	2866.65	6910.59	614.09	132.94	0	857.82
7	254.42	356.27	2282.64	4964.60	669.87	107.44	17.73	911.29
8	854.49	328.53	793.32	2854.23	608.37	86.85	20.94	820.21
9	618.92	332.84	0	1164.83	522.17	74.08	20.15	707.27
10	5.78	317.40	0	485.46	7.93	0	0	8.49
11	5.10	414.79	0	631.79	720.08	90.11	51.57	1000.42
14	4.49	429.65	0	653.57	931.64	98.01	107.99	1341.42
15	4.86	431.11	0	656.17	895.33	103.36	108.63	1311.81
16	7.16	395.14	0	604.33	663.42	99.95	73.99	995.48
17	5.10	341.84	0	521.63	462.96	107.41	37.64	726.07
18	7.65	309.76	0	475.92	210.78	115.18	0	399.47
21	2.01	0	0	2.15	5.48	16.78	0	31.22
22	1.61	0	0	1.72	1.52	0	0	1.63
22	863.31	10.58	517.38	1881.36	44.68	18.61	0	75.92
23	1508.47	131.40	2104.43	5642.53	263.95	47.45	15.18	381.71
24	1383.29	238.53	2011.39	5501.03	884.96	83.76	99.91	1255.24
25	1082.43	285.97	2035.36	5294.38	1324.45	120.63	178.94	1925.00
33	4.80	0	766.65	1400.44	0	0	79.54	144.76
35	0	0	0	0	0	0	0	0
36	4.56	15.92	0.00	28.93	107.31	105.73	0	274.47
37	0	0	0	0	1.20	10.83	0	17.63
40	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0
42	191.81	12.10	91.52	390.08	68.42	17.51	0	99.65
43	25.21	199.04	1408.72	2891.40	155.80	23.24	68.51	326.52
44	45.37	116.56	544.32	1215.22	693.07	30.25	30.18	842.21
47	2.39	271.89	0.00	413.12	2449.53	87.28	277.81	3258.41
52	0	0	0	0	2135.55	63.61	145.47	2645.85

	Reactor 3				Reactor 4			
Day	Acetate	Propionate	Butyrate	Total VFA	Acetate	Propionate	Butyrate	Total VFA
0	159.48	8.28	0	183.16	205.17	7.81	0	231.34
1	1119.14	291.67	2038.07	5347.20	585.38	237.09	454.65	1811.84
2	1079.51	351.26	1900.54	5144.46	798.74	325.46	459.63	2182.64
3	1034.53	447.76	2018.54	5456.81	776.67	362.52	435.31	2170.74
4	758.51	494.99	1911.50	5037.98	711.76	435.41	363.76	2081.09
7	23.02	505.78	1705.00	3891.48	297.73	426.96	193.14	1314.81
8	244.18	464.96	1009.48	2800.64	194.28	396.97	31.22	864.13
9	654.29	476.87	72.45	1552.03	92.55	319.62	20.35	618.70
10	5.22	235.89	0	361.79	60.22	174.73	16.39	358.13
11	10.43	482.48	0	739.71	387.55	391.92	67.57	1129.47
14	4.61	570.27	0	866.04	8.38	373.11	0	572.35
15	5.22	561.84	0	853.97	7.41	273.14	0	420.38
16	7.16	500.45	0	763.34	2.55	90.40	0	139.23
17	4.85	494.94	0	752.55	0	0	0	0
18	3.52	457.99	0	695.35	0	0	0	0
21	7.35	229.22	0	353.98	1.53	0	0	1.63
22	1.72	0	0	1.84	2.66	0	0	2.85
22	814.18	17.70	710.09	2190.28	161.25	42.15	127.44	468.15
23	813.76	241.81	1383.17	3753.24	56.62	99.82	538.51	1191.40
24	310.71	436.54	1303.43	3363.87	48.57	89.24	411.29	935.29
25	16.09	497.31	1125.67	2816.87	104.35	42.70	36.78	243.09
33	3.12	0	248.68	455.94	0	0	54.62	99.41
35	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0
42	105.38	14.33	141.83	392.53	374.26	31.21	137.51	697.87
43	101.54	139.81	408.24	1062.77	0	0	0	0
44	54.49	192.99	0	349.73	35.28	53.50	62.77	232.79
47	0	0	0	0	0	0	0	0
52	6.57	37.48	0	63.64	0	0	0	0

Day	Reactor 5			
	Acetate	Propionate	Butyrate	Total VFA
0	101.81	8.13	0	121.22
1	722.20	257.89	1174.61	3299.96
2	808.47	349.23	1132.35	3453.28
3	743.32	443.85	1148.09	3555.11
4	570.07	574.75	1226.03	3709.24
7	37.37	528.15	893.36	2463.42
8	282.52	491.54	278.99	1552.29
9	106.38	577.94	0	986.52
10	8.05	251.33	0	388.13
11	51.83	495.69	0	803.96
14	5.22	603.32	0	916.60
15	5.58	544.99	0	828.92
16	6.07	471.93	0	719.11
17	3.76	401.29	0	609.98
18	4.61	370.35	0	564.17
21	1.17	0	0	1.26
22	2.22	0	0	2.38
22	521.37	27.56	615.40	1719.51
23	228.97	209.69	1022.90	2423.32
24	13.88	237.25	816.66	1859.42
25	9.15	303.85	817.37	1956.24
33	2.40	7.32	74.27	148.79
36	0	0	0	0
37	0	0	0	0
40	0	0	0	0
42	0	0	0	0
42	424.19	47.77	192.14	875.72
43	5.52	50.31	60.85	192.64
44	87.38	279.62	0	515.72
47	1.64	130.27	0	198.46
52	0	0	0	0

Table A5: Gas volume Rates

Day	Reactor 1 Daily Biogas (ml)			Reactor 2 Daily Biogas (ml)		
	CH4	CO2	Total Biogas	CH4	CO2	Total Biogas
1	326.69	6673.30	7000	0	180	180
2	27.92	138.07	166	45.92	148.07	194
3	57.69	146.30	204	73.48	174.51	248
4	83.48	152.51	236	20.92	39.07	60
5	119.01	165.68	284.68	10.00	5.033	15.03
6	288.38	304.24	592.63	13.71	11.85	25.56
7	606.73	477.94	1084.68	36.24	59.15	95.40
8	1010.09	635.90	1646	41.56	50.43	92
9	1190.35	567.64	1758	61.23	58.76	120
10	666.78	255.21	922	83.08	75.91	159
11	110.02	36.97	147	122.42	87.57	210
12	80.54	26.05	106.60	304.95	74.86	379.82
13	75.40	25.25	100.65	307.52	177.79	485.32
14	59.66	21.07	80.74	391.54	247.30	638.85
15	65.99	22.01	88	805.67	306.32	1112
16	14.98	5.01	20	1221.27	408.72	1630
17	50.88	21.11	72	1488.13	472.87	1961
18	129.13	54.86	184	1363.21	372.79	1736
19	182.38	62.83	245.22	706.44	165.95	872.39
20	124.09	42.62	166.71	169.91	73.27	243.18
21	127.95	38.10	166.06	129.38	43.03	172.41
22	19.71	5.28	25	79.83	24.16	104
23	827.42	6577.57	7405	313.04	138.95	452
24	358.79	759.20	1118	418.18	337.81	756
25	370.47	484.52	855	545.19	356.80	902
26	697.02	559.89	1256.91	648.45	337.98	986.44
27	1061.84	646.12	1707.97	675.15	313.35	988.51
28	1110.71	564.39	1675.11	714.09	308.95	1023.04
29	1059.34	420.65	1480	905.01	334.98	1240
30	291.21	102.78	394	1162.88	365.11	1528
31	195.27	66.72	262	1266.36	326.63	1593
32	288.14	95.85	384	390.72	87.27	478
33	1874.94	6314.05	8189	1103.93	780.06	1884
34	1697.70	1329.89	3027.59	1468.25	782.98	2251.24
35	1156.93	759.46	1916.40	1909.56	705.19	2614.75
36	945.29	404.70	1350	1774.84	555.15	2330
37	359.21	153.78	513	1064.14	332.85	1397
38	270.24	67.93	338.18	242.65	71.45	314.10

39	52.27	16.16	68.44	192.38	56.28	248.66
40	18.20	51.17	69.37	142.09	41.12	183.22
41	48	18	66	91.95	25.76	117.71
42	48	18	66	41.61	10.67	52.28
43	2013.91	5856.08	7870	208.51	207.48	416
44	1001.91	1008.08	2010	277.11	279.88	557
45	1206.41	725.57	1931.98	229.72	379.37	609.10
46	995.05	483.92	1478.98	199.66	250	449.66
47	635.24	297.78	933.03	194.49	120.73	315.23
48	302.67	144.51	447.19	191.98	109.81	301.80
49	109.06	43.74	152.80	207.94	126.25	334.19
50	51.35	18.64	70	301.40	154.59	456
51	62.27	14	76.27	321.91	169.91	491.82
52	12.65	14	26.65	417.66	144.51	562.17
53	1678.21	5559.78	7238	473.71	268.28	742
54	1730.28	1233.71	2964	578.86	351.13	930
55	1347.78	810.99	2158.77	619.69	282.30	901.99
56	962.75	400.47	1363.22	849.79	268.21	1118.01
57	314.65	129.35	444	1149.28	340.71	1490
58	316.87	125.12	442	1938.62	531.37	2470

	Reactor 3 Daily Biogas (ml)			Reactor 4 Daily Biogas (ml)		
Day	CH4	CO2	Total Biogas	CH4	CO2	Total Biogas
1	310.34	4689.65	5000	217.74	1177.25	1395
2	56.16	277.83	334	54.81	182.18	237
3	100.33	229.66	330	81.67	195.32	277
4	132.71	244.28	377	89.97	150.02	240
5	144.65	172.88	317.54	227.11	274.78	501.90
6	147.51	157.33	304.84	307.82	321.38	629.21
7	254.66	200.95	455.61	364.34	347.53	711.88
8	487.87	362.12	850	340.31	235.69	576
9	813.60	472.39	1286	459.01	259.99	719
10	897.49	436.51	1334	766.45	373.54	1140
11	414.07	165.92	580	949.38	416.61	1366
12	481.05	175.29	656.35	1221.47	405.37	1626.84
13	382.59	129.51	512.11	955.96	310.72	1266.69
14	221.68	115.85	337.53	565.05	277.40	842.46
15	126.41	49.58	176	355.03	116.96	472
16	70.41	27.58	98	261.23	84.76	346
17	57.24	27.75	85	211.56	69.43	281
18	64.24	18.75	83	89.75	22.24	112
19	72.84	21.31	94.15	55.84	14.62	70.46
20	106.66	47.42	154.08	28.81	10.86	39.67
21	168.51	60.24	228.76	12.99	4.86	17.86
22	262.65	97.34	360	14.96	3.03	18
23	940.67	4395.32	5336	729.85	1195.14	1925
24	566.42	733.57	1300	486.09	465.90	952
25	602.20	502.79	1105	801.21	542.78	1344
26	698.10	408.52	1106.63	1252.24	586.18	1838.42
27	617.92	310.26	928.18	1405.47	543.60	1949.07
28	556.74	253.43	810.18	1190.96	421.53	1612.51
29	398.39	165.60	564	746.49	261.50	1008
30	855.45	330.54	1186	330.47	113.52	444
31	706.63	253.36	960	85.54	30.45	116
32	246.71	87.28	334	38.02	12.97	51
33	1815.65	4719.34	6535	1356.89	1827.11	3184
34	1893.19	1259.27	3152.47	1735.34	1045.13	2780.48
35	1199.99	643.53	1843.52	1743.31	776.19	2519.51
36	765.44	307.55	1073	1294.58	475.41	1770
37	363.81	146.18	510	389.10	142.89	532
38	224.25	78.96	303.21	93.34	33.65	127

39	109.01	50.57	159.59	76.53	27.46	104
40	71.83	30.35	102.19	59.71	21.28	81
41	55.60	15.17	70.77	42.87	15.12	58
42	38.23	20.99	59.22	26.06	8.93	35
43	1845.67	4406.32	6252	1312.83	1596.17	2909
44	1690.59	1252.41	2943	1021.84	652.15	1674
45	1171.32	643.40	1814.72	1282.19	847.96	2130.15
46	786.05	325.93	1111.99	3115.82	658.15	3773.97
47	522.67	183.61	706.28	229.51	352.34	581.86
48	340.28	125.91	466.19	187.34	108.35	295.69
49	229.42	100.37	329.80	147.25	56	203.25
50	186.19	69.80	256	87.95	29.04	117
51	124.02	33.91	157.93	19.33	15.00	34.33
52	80.03	40.02	120.06	50.31	7.34	57.66
53	1739.33	4192.66	5932	1481.18	1622.82	3104
54	1880.07	1519.92	3400	1973.65	1171.34	3145
55	1434.49	735.64	2170.13	1728.60	803.70	2532.30
56	804.93	299.93	1104.86	1304.52	391.16	1695.69
57	288.95	112.04	401	252.45	87.54	340
58	184.84	69.15	254	142.17	47.82	190

	Reactor 5 Daily Biogas (ml)		
Day	CH4	CO2	Total Biogas
1	385.26	3264.73	3650
2	62.72	153.27	216
3	119.18	264.81	384
4	143.90	211.09	355
5	106.06	133.56	239.62
6	138.48	138.13	276.61
7	244.65	208.11	452.75
8	445.38	310.61	756
9	579.18	336.81	916
10	526.52	253.47	780
11	686.73	306.26	993
12	984.61	328.02	1312.64
13	725.58	237.06	962.65
14	286.54	214.15	500.70
15	200.68	73.31	274
16	114.75	41.24	156
17	98.28	38.71	137
18	137.64	45.35	183
19	190.03	72.54	262.57
20	191.34	47.81	239.16
21	136.31	51.94	188.26
22	17.91	4.09	22
23	928.06	2685.93	3614
24	417.74	474.25	892
25	328.17	291.82	620
26	407.29	343.03	750.33
27	679.08	443.45	1122.54
28	1003.96	503.16	1507.12
29	1098.96	441.03	1540
30	972.75	357.24	1330
31	480.92	170.07	651
32	301.13	104.86	406
33	1900.28	3203.71	5104
34	1730.43	1424.37	3154.80
35	1162.31	692.87	1855.19
36	818.17	319.82	1138
37	401.17	156.82	558
38	276.59	94.14	370.73

39	129.55	62.59	192.14
40	70.59	32.52	103.11
41	49.58	2.15	51.74
42	63.83	39.42	103.25
43	2159.27	3112.72	5272
44	1577.22	1018.77	2596
45	1100.84	589.73	1690.58
46	745.71	376.47	1122.19
47	486.01	273.21	759.21
48	447.15	209.03	656.19
49	321.42	142.37	463.80
50	198.81	71.18	270
51	148.19	14.83	163.02
52	58.95	60.01	118.97
53	1692.79	2832.20	4525
54	1985.96	1416.03	3402
55	1235.77	696.68	1932.46
56	660.14	297.39	957.53
57	337.70	137.29	475
58	347.34	135.65	483

Table A6: Cumulative Gas Volumes:

Day	biogas 1	biogas 2	biogas 3	biogas 4	biogas 5
1	7000	180	5000	1395	3650
2	7166	374	5334	1632	3866
3	7370	622	5664	1909	4250
4	7606	682	6041	2149	4605
5	7890.68	697.03	6358.54	2650.90	4844.62
6	8483.31	722.60	6663.38	3280.11	5121.24
7	9568	818	7119	3992	5574
8	11214	910	7969	4568	6330
9	12972	1030	9255	5287	7246
10	13894	1189	10589	6427	8026
11	14041	1399	11169	7793	9019
12	14147.60	1778.82	11825.35	9419.84	10331.64
13	14248.25	2264.14	12337.46	10686.53	11294.29
14	14329	2903	12675	11529	11795
15	14417	4015	12851	12001	12069
16	14437	5645	12949	12347	12225
17	14509	7606	13034	12628	12362
18	14693	9342	13117	12740	12545
19	14938.22	10214.39	13211.15	12810.46	12807.57
20	15104.94	10457.58	13365.24	12850.14	13046.74
21	15271	10630	13594	12868	13235
22	15296	10734	13954	12886	13257
23	22701	11186	19290	14811	16871
24	23819	11942	20590	15763	17763
25	24674	12844	21695	17107	18383
26	25930.91	13830.44	22801.63	18945.42	19133.33
27	27638.89	14818.95	23729.81	20894.49	20255.87
28	29314	15842	24540	22507	21763
29	30794	17082	25104	23515	23303
30	31188	18610	26290	23959	24633
31	31450	20203	27250	24075	25284
32	31834	20681	27584	24126	25690
33	40023	22565	34119	27310	30794
34	43050.59	24816.24	37271.47	30090.48	33948.80
35	44967	27431	39115	32610	35804
36	46317	29761	40188	34380	36942
37	46830	31158	40698	34912	37500
38	47168.18	31472.10	41001.21	35039	37870.73
39	47236.62	31720.77	41160.80	35143	38062.88

40	47306	31904	41263	35224	38166
41	47372	32021.71	41333.77	35282	38217.74
42	47438	32074	41393	35317	38321
43	55308	32490	47645	38226	43593
44	57318	33047	50588	39900	46189
45	59249.98	33656.10	52402.72	42030.15	47879.58
46	60728.96	34105.76	53514.71	45804.13	49001.78
47	61662	34421	54221	46386	49761
48	62109.19	34722.80	54687.19	46681.69	50417.19
49	62262	35057	55017	46884.95	50881
50	62332	35513	55273	47001.95	51151
51	62408.27	36004.82	55430.93	47036.29	51314.02
52	62434.92	36567	55551	47093.95	51433
53	69672.92	37309	61483	50197.95	55958
54	72636.92	38239	64883	53342.95	59360
55	74795.70	39140.99	67053.13	55875.26	61292.46
56	76158.92	40259	68158	57570.95	62250
57	76602.92	41749	68559	57910.95	62725
58	77044.92	44219	68813	58100.95	63208

APPENDIX B: KINETIC STUDY

Raw data of the kinetic study that represents the cumulative methane production of the measured methane volumes in the lab and of five modeling functions of the acclimation phase for the five reactors within 22 days.

Table B1: Reactor 1: Cumulative methane for measured and estimated using logistic function

Day	Cumulative CH_4 measured	Cumulative CH_4 estimated logistic function
1	326.69	40.24
2	354.62	80.33
3	412.32	159.11
4	495.80	310.29
5	614.81	587.76
6	903.19	1057.73
7	1509.93	1755.01
8	2520.03	2609.85
9	3710.39	3443.17
10	4377.17	4092.44
11	4487.19	4515.52
12	4567.74	4760.03
13	4643.14	4891.65
14	4702.81	4959.78
15	4768.80	4994.35
16	4783.79	5011.71
17	4834.67	5020.38
18	4963.81	5024.69
19	5146.20	5026.84
20	5270.30	5027.91
21	5398.25	5028.44
22	5417.97	5028.71

Table B2: Reactor 1: cumulative methane for estimated cone function, transfer function and first order kinetic function

Day	Cumulative CH_4 estimated cone function	Cumulative CH_4 estimated transfer function	Cumulative CH_4 estimated first order kinetic function
1	0.08	-594.60	386.59
2	3.30	9.56	758.72
3	28.98	566.02	1116.91
4	132.94	1078.54	1461.68
5	415.36	1550.58	1793.54
6	974.01	1985.34	2112.98
7	1790.14	2385.76	2420.45
8	2684.26	2754.57	2716.42
9	3456.52	3094.25	3001.29
10	4023.68	3407.10	3275.50
11	4404.35	3695.25	3539.44
12	4650.15	3960.64	3793.50
13	4807.60	4205.07	4038.04
14	4909.25	4430.20	4273.43
15	4975.92	4637.55	4500.01
16	5020.45	4828.52	4718.09
17	5050.79	5004.41	4928.01
18	5071.84	5166.41	5130.07
19	5086.72	5315.62	5324.56
20	5097.41	5453.05	5511.77
21	5105.21	5579.62	5691.97
22	5110.98	5696.19	5865.42

Table B3: Reactor 2: Cumulative methane for measured and estimated using logistic function

Day	Cumulative CH_4 measured	Cumulative CH_4 estimated logistic function
1	0	0.41
2	45.92	0.79
3	119.40	1.53
4	140.33	2.93
5	150.33	5.63
6	164.04	10.80
7	200.28	20.71
8	241.84	39.67
9	303.08	75.79
10	386.16	144.19
11	508.58	272.17
12	813.54	506.26
13	1121.06	917.27
14	1512.61	1589.63
15	2318.29	2571.71
16	3539.57	3792.32
17	5027.69	5038.14
18	6390.90	6078.47
19	7097.35	6811.21
20	7267.26	7267.64
21	7396.64	7530.55
22	7476.47	7675.21

Table B4: Reactor 2: cumulative methane for estimated cone function, transfer function and first order kinetic function

Day	cumulative CH_4 estimated cone function	cumulative CH_4 estimated transfer function	cumulative CH_4 estimated first order kinetic function
1	6.70×10^{-9}	-1837.18	443.31
2	6.86×10^{-6}	-1436.19	873.53
3	3.9×10^{-4}	-1035.19	1291.03
4	7×10^{-3}	-634.20	1696.19
5	0.06	-233.21	2089.38
6	0.40	167.77	2470.94
7	1.88	568.77	2841.23
8	7.17	969.76	3200.58
9	23.25	1370.75	3549.30
10	66.33	1771.74	3887.72
11	169.82	2172.73	4216.14
12	393.89	2573.72	4534.85
13	827.48	2974.71	4844.14
14	1560.68	3375.70	5144.29
15	2610.49	3776.69	5435.57
16	3850.11	4177.68	5718.24
17	5053.29	4578.67	5992.56
18	6039.11	4979.66	6258.77
19	6752.69	5380.65	6517.11
20	7230.75	5781.64	6767.82
21	7538.22	6182.62	7011.12
22	7732.72	6583.61	7247.22

Table B5 : Reactor 3 : Cumulative methane for measured and estimated using logistic function

Day	Cumulative CH_4 measured	cumulative CH_4 estimated logistic function
1	310.34	41.04
2	366.51	72.32
3	466.84	126.84
4	599.55	220.76
5	744.21	379.17
6	891.72	637.10
7	1146.38	1033.95
8	1634.26	1594.28
9	2447.86	2296.50
10	3345.36	3055.99
11	3759.44	3757.06
12	4240.50	4315.69
13	4623.09	4710.91
14	4844.77	4967.60
15	4971.19	5125.16
16	5041.60	5218.56
17	5098.85	5272.77
18	5163.09	5303.86
19	5235.93	5321.57
20	5342.60	5331.61
21	5511.11	5337.29
22	5773.76	5340.51

Table B6: Reactor 3: Cumulative methane for estimated cone function, transfer function and first order kinetic function

Day	cumulative CH_4 estimated cone function	cumulative CH_4 estimated transfer function	cumulative CH_4 estimated first order kinetic function
1	0.85	-462.25	307.16
2	12.89	-3.95	611.86
3	62.76	435.46	914.12
4	189.75	856.78	1213.94
5	435.33	1260.74	1511.36
6	824.91	1648.06	1806.39
7	1349.29	2019.43	2099.04
8	1961.06	2375.50	2389.35
9	2592.73	2716.90	2677.33
10	3183.78	3044.24	2962.99
11	3697.42	3358.10	3246.35
12	4121.49	3659.02	3527.44
13	4460.31	3947.55	3806.27
14	4725.98	4224.19	4082.86
15	4932.43	4489.44	4357.23
16	5092.51	4743.76	4629.40
17	5216.89	4987.61	4899.38
18	5313.97	5221.41	5167.19
19	5390.24	5445.58	5432.85
20	5450.58	5660.51	5696.37
21	5498.69	5866.59	5957.78
22	5537.34	6064.19	6217.09

Table B7: Reactor 4: Cumulative methane for measured and estimated using logistic function

Day	Cumulative CH_4 measured	cumulative CH_4 estimated logistic function
1	217.74	75.13
2	272.55	120.09
3	354.22	191.29
4	444.20	303.03
5	671.32	475.94
6	979.15	737.76
7	1343.49	1121.48
8	1683.80	1657.72
9	2142.80	2359.37
10	2909.26	3202.40
11	3858.65	4117.42
12	5080.12	5007.25
13	6036.09	5784.81
14	6601.15	6403.24
15	6956.19	6859.32
16	7217.42	7177.27
17	7428.98	7390.33
18	7518.74	7529.34
19	7574.58	7618.47
20	7603.40	7674.98
21	7616.39	7710.55
22	7631.35	7732.84

Table B8 : Reactor 4: cumulative methane for estimated cone function, transfer function and first order kinetic function

Day	cumulative CH_4 estimated cone function	cumulative CH_4 estimated transfer function	cumulative CH_4 estimated first order kinetic function
1	0.10	-892.57	386.28
2	2.86	-351.00	772.39
3	19.36	181.19	1158.32
4	74.63	704.17	1544.09
5	210.13	1218.09	1929.69
6	479.52	1723.11	2315.11
7	933.16	2219.39	2700.36
8	1591.59	2707.07	3085.45
9	2422.19	3186.31	3470.36
10	3341.77	3657.25	3855.10
11	4249.46	4120.04	4239.67
12	5065.61	4574.81	4624.07
13	5750.11	5021.71	5008.30
14	6297.48	5460.87	5392.36
15	6722.37	5892.42	5776.25
16	7046.85	6316.51	6159.97
17	7292.92	6733.24	6543.52
18	7479.39	7142.76	6926.89
19	7621.16	7545.19	7310.11
20	7729.56	7940.65	7693.14
21	7813.03	8329.26	8076.01
22	7877.81	8711.15	8458.71

Table B9: Reactor 5: Cumulative methane for measured and estimated using logistic function

Day	Cumulative CH_4 measured	cumulative CH_4 estimated logistic function
1	385.26	149.44
2	447.98	219.96
3	567.17	322.07
4	711.07	468.03
5	817.14	672.94
6	955.622	953.34
7	1200.27	1323.99
8	1645.65	1792.11
9	2224.84	2350.44
10	2751.36	2972.65
11	3438.09	3615.73
12	4422.71	4230.62
13	5148.30	4776.34
14	5434.85	5229.55
15	5635.53	5585.63
16	5750.29	5853.41
17	5848.57	6048.21
18	5986.21	6186.53
19	6176.25	6283.07
20	6367.6	6349.65
21	6503.91	6395.17
22	6521.82	6426.13

Table B10: Reactor 5: cumulative methane for estimated cone function, transfer function and first order kinetic function

Day	cumulative CH_4 estimated cone function	cumulative CH_4 estimated transfer function	cumulative CH_4 estimated first order kinetic function
1	1.24	-418.76	323.36
2	15.58	6.68	646.73
3	67.78	425.03	970.09
4	190.02	836.38	1293.45
5	414.53	1240.87	1616.81
6	763.31	1638.60	1940.17
7	1237.81	2029.68	2263.52
8	1814.29	2414.23	2586.87
9	2449.42	2792.36	2910.22
10	3093.53	3164.18	3233.57
11	3704.12	3529.78	3556.91
12	4253.45	3889.27	3880.25
13	4728.97	4242.76	4203.59
14	5129.71	4590.35	4526.92
15	5461.55	4932.13	4850.26
16	5733.48	5268.20	5173.59
17	5955.15	5598.65	5496.92
18	6135.55	5923.59	5820.24
19	6282.53	6243.10	6143.56
20	6402.60	6557.27	6466.88
21	6501.06	6866.19	6790.20
22	6582.18	7169.96	7113.52

APPENDIX C: COD BALANCE

$$\mathrm{COD_{in}} = \mathrm{COD_{out}}$$
$$\mathrm{COD_{in}} = \mathrm{COD_0}$$
$$\mathrm{COD_{out}} = \mathrm{COD_f} + \mathrm{COD_{CH4}}$$
$$\mathrm{COD_{CH4}} = \frac{\mathrm{P.V}}{\mathrm{R.T}} \times 16 \times 4$$

COD_0: initial COD measured in the reactor accounting for the seed + old digestate / COD measured in the reactor at the starting point of the balance period.

COD_f : COD measured in the reactor at the end point of the balance period.

CH_4: COD value of the methane produced over the balance period.

Total COD consumed=Total COD value of methane produced

Table C1: Constants used in COD balance

R (L.atm/mol.K)	T (K)	P (atm)	Molar Mass CH4 (g/mol)	Total volume (L)
0.08205	308	1	16.04	2.25

Table C2:CH4 in Reactor 1

Day	Cycles	tCOD (mg/L)	tCOD (g)	ΔCOD	Cum. CH4 (mL)	Cum. CH4 (g)	Cum. CH4 (g COD)
0	1	35575	80.043	23.90	5417.97	3.43	13.75
22		24950	56.13				
22	2	34200	76.95	19.12	6260.26	3.97	15.89
32		25700	57.82				
32	3	31200	70.20	20.08	6470.82	4.10	16.42
42		22275	50.11				
42	4	29050	65.36	18.84	6390.56	4.05	16.22
52		20675	46.51				
52	5	27300	61.42	17.57	6350.55	4.03	16.12
58		19487.5	43.84				

Table C3: Error of each Cycle in Reactor 1

Day	Cycles	COD in	COD out	% Error
0 22	1	80.04	69.89	12.68
22 32	2	76.95	73.71	4.19
32 42	3	70.2	66.54	5.20
42 52	4	65.3625	62.74	4.01
52 58	5	61.425	59.96	2.36

Table C4: % Error in Reactor 1

Total consumed COD (g)	99.53
Total CH4 produced (ml)	30890.18
COD in	99.53
COD out	78.42
Error (%)	21.21

Table C5:CH4 in Reactor 2

Day	Cycles	tCOD(mg/L)	tCOD (g)	ΔCOD	Cum. CH4 (mL)	Cum. CH4 (g)
0 22	1	36700 25525	82.57 57.43	25.14	7476.48	4.74
22 32	2	35150 26000	79.08 58.50	20.58	7039.13	4.46
32 42	3	32375 20725	72.84 46.63	26.21	8031.44	5.09
42 52	4	29375 25425	66.09 57.20	8.88	2550.43	1.61
52 58	5	30650 25187.50	68.96 56.67	12.29	5609.97	3.56

Table C6: Error of each Cycle in Reactor 2

Day	Cycles	COD in	COD out	% Error
0 22	1	82.57	76.41	7.46
22 32	2	79.08	76.37	3.43
32 42	3	72.84	67.02	7.99
42 52	4	66.09	63.68	3.64
52 58	5	68.96	70.91	-2.83

Table C7: % Error in Reactor 2

Total consumed COD (g)	93.12
Total CH4 produced (ml)	30707.45
COD in	93.12
COD out	77.96
Error (%)	16.28

Table C8:CH4 in Reactor 3

Day	Cycles	tCOD(mg/L)	tCOD (g)	ΔCOD	Cum. CH4 (mL)	Cum. CH4 (g)	Cum. CH4 (g COD)
0 22	1	38875 25700	87.46 57.82	29.64	5773.76	3.66	14.65
22 32	2	32950 25675	74.13 57.76	16.36	6189.28	3.92	15.71
32 42	3	31050 21000	69.86 47.25	22.61	6537.03	4.14	16.59
42 52	4	28500 20450	64.12 46.01	18.11	6976.27	4.42	17.71
52 58	5	27425 17825	61.70 40.11	21.60	6332.62	4.01	16.07

Table C9: Error of each cycle in Reactor 3

Day	Cycles	COD in	COD out	% Error
0 22	1	87.46	72.48	17.13
22 32	2	74.13	73.48	0.88
32 42	3	69.86	63.84	8.61
42 52	4	64.12	63.72	0.62
52 58	5	61.71	56.18	8.95

Table C10: % Error in Reactor 3

Total consumed COD (g)	108.33
Total CH4 produced (ml)	31808.99
COD in	108.33
COD out	80.75
Error (%)	25.45

Table C11:CH4 in Reactor 4

Day	Cycles	tCOD(mg/L)	tCOD (g)	ΔCOD	Cum. CH4 (mL)	Cum. CH4 (g)	Cum. CH4 (g COD)
0 22	1	36250 29325	81.56 65.98	15.58	7631.35	4.84	19.37
22 32	2	34200 24250	76.95 54.56	22.38	7066.38	4.48	17.94
32 42	3	30425 22375	68.45 50.34	18.11	6817.77	4.32	17.30
42 52	4	27625 20275	62.15 45.61	16.53	7454.41	4.73	18.92
52 58	5	28350 17475	63.78 39.31	24.46	6882.59	4.36	17.47

Table C12: Error of each Cycle in Reactor 4

Day	Cycles	COD in	COD out	% Error
0 22	1	81.56	85.35	-4.65
22 32	2	76.95	72.50	5.77
32 42	3	68.45	67.65	1.17
42 52	4	62.15	64.54	-3.84
52 58	5	63.78	56.79	11

Table C13: % Error in Reactor 4

Total consumed COD (g)	97.08
Total CH4 produced (ml)	35852.52
COD in	97.08
COD out	91.02
Error (%)	6.24

Table C14:CH4 in Reactor 5

Day	Cycles	tCOD (mg/L)	tCOD (g)	ΔCOD	Cum. CH4 (mL)	Cum. CH4 (g)	Cum. CH4 (g COD)
0 22	1	36500 27150	82.12 61.08	21.03	6521.82	4.13	16.55
22 32	2	33275 24675	74.86 55.51	19.35	6618.10	4.20	16.80
32 42	3	29000 21400	65.25 48.15	17.10	6602.54	4.19	16.76
42 52	4	27225 19950	61.25 44.88	16.36	7243.62	4.59	18.39
52 58	5	25875 19000	58.21 42.75	15.46	6259.73	3.97	15.89

Table C15: Error of each Cycle in Reactor 5

Day	Cycles	COD in	COD out	% Error
0 22	1	82.12	77.64	5.45
22 32	2	74.86	72.32	3.40
32 42	3	65.25	64.91	0.51
42 52	4	61.25	63.27	-3.30
52 58	5	58.21	58.64	-0.72

Table C16: % Error in Reactor 5

Total consumed COD (g)	89.32
Total CH4 produced (ml)	33245.83
COD in	89.32
COD out	84.41
Error (%)	5.51

Including VSS Balance

Start VSS (mg) at end of acclimation cycle - Removed VSS (mg) at end of every cycle (acclimation + Cycle 1+Cycle 2+Cycle 3) = End VSS (mg) at end of last cycle (Cycle 4).

Table C17: COD Balance Including VSS Balance

	Acclimation Cycle (after feed)		Acclimation Cycle (end of feeding cycle)		
Characterization	tCOD (mg/L)	VSS (mg/L)	tCOD (mg/L)	VSS(mg/L)	CH4 (mL)
R1	35575	16450	24950	15766.67	5418
R2	36700	17766.67	25525	15722.23	7476
R3	38875	16844.45	25700	15677.78	5774
R4	36250	17633.34	29325	16166.67	7631
R5	36500	16844.45	27150	15666.67	6522
	Cycle 1 (after feed)		Cycle 1(end of feeding cycle)		
Characterization	tCOD (mg/L)	VSS (mg/L)	tCOD (mg/L)	VSS(mg/L)	CH4 (mL)
R1	34200	14950	25700	14322.23	6260
R2	35150	16244.45	26000	14266.67	7039
R3	32950	16000	25675	15011.12	6189
R4	34200	16811.12	24250	14388.89	7066
R5	33275	16000	24675	14888.89	6618
	Cycle 2 (after feed)		Cycle 2(end of feeding cycle)		
Characterization	tCOD (mg/L)	VSS (mg/L)	tCOD (mg/L)	VSS(mg/L)	CH4 (mL)
R1	31200	14300	22275	13077.78	6471
R2	32375	14655.56	20725	12888.89	8031
R3	31050	14950	21000	13100	6537
R4	30425	14577.78	22375	13600	6818
R5	29000	15155.56	21400	13822.23	6603
	Cycle 3 (after feed)		Cycle 3(end of feeding cycle)		
Characterization	tCOD (mg/L)	VSS (mg/L)	tCOD (mg/L)	VSS (mg/L)	CH4 (mL)
R1	29050	13474.07	20675	13100	6391
R2	29375	14277.78	25425	13022.23	2550
R3	28500	13511.12	20450	13183.34	6976
R4	27625	14133.34	20275	12466.67	7454
R5	27225	13955.56	19950	12755.56	7244
	Cycle 4 (after feed)		Cycle 4(end of feeding cycle)		
Characterization	tCOD (mg/L)	VSS (mg/L)	tCOD (mg/L)	VSS(mg/L)	CH4 (mL)
R1	27300	13325.92	19487.5	11688.89	6351
R2	30650	13733.34	25187.5	12344.45	5610
R3	27425	13011.12	17825	11811.12	6333
R4	28350	13455.56	17475	11188.89	6883

R5	25875	13022.23	19000	11888.89	6260

	Cycle 1					
Characterization	tCOD added(mg/L)	tCOD consumed(mg/L)	ΔVSS (mg)	Calculated CH4 (L)	Measured CH4 (L)	% Error
R1	9250	8500	-691.67	6.34	6.26	1.41
R2	9625	9150	-655.56	6.87	7.03	-2.31
R3	7250	7275	-2419.44	4.52	6.18	-36.73
R4	4875	9950	-41.67	7.81	7.06	9.57
R5	6125	8600	-2166.67	5.69	6.61	-16.19
	Cycle 2					
Characterization	tCOD added(mg/L)	tCOD consumed(mg/L)	ΔVSS (mg)	Calculated CH4 (L)	Measured CH4 (L)	% Error
R1	5500	8925	-780.56	6.64	6.47	2.55
R2	6375	11650	-466.67	8.94	8.03	10.19
R3	5375	10050	547.23	8.18	6.53	20.14
R4	6175	8050	-1822.22	5.43	6.81	-25.47
R5	4325	7600	-1322.22	5.32	6.60	-23.92
	Cycle 3					
Characterization	tCOD added(mg/L)	tCOD consumed(mg/L)	ΔVSS (mg)	Calculated CH4 (L)	Measured CH4 (L)	% Error
R1	6775	8375	-3319.44	4.94	6.39	-29.21
R2	8650	3950	-3522.22	1.36	2.55	-87.48
R3	7500	8050	-3462.5	4.61	6.97	-51.05
R4	5250	7350	-850	5.36	7.45	-38.92
R5	5825	7275	-1055.56	5.20	7.24	-39.18
	Cycle 4					
Characterization	tCOD added(mg/L)	tCOD consumed(mg/L)	ΔVSS (mg)	Calculated CH4 (L)	Measured CH4 (L)	% Error
R1	6625	7812.5	-100	6.10	6.35	-4.06
R2	5225	5462.5	-1730.56	3.44	5.61	-63.01
R3	6975	9600	-208.33	7.45	6.34	15.07
R4	8075	10875	-241.67	8.44	6.88	18.49
R5	5925	6875	-1238.89	4.79	6.26	-30.45

Appendix D: EFFICIENCY

Table D1: Total COD (mg/L)

Substrate	tCOD (mg/L)
Whey	92300
Tanmiya	86500
W+T 75:25	90850
W+T 25:75	89762.5
W+T 50:50	89400

Table D2: measured CH4 (L)

Reactors	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cumulative
R1	6.26	6.47	6.39	6.35	25.47
R2	7.03	8.03	2.55	5.61	23.23
R3	6.18	6.53	6.97	6.33	26.03
R4	7.06	6.81	7.45	6.88	28.22
R5	6.61	6.60	7.24	6.25	26.72

Table D3: weighted CH4 (L)

Reactors	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cumulative
R3	6.45	6.86	5.43	6.16	24.91
R4	6.84	7.64	3.51	5.79	23.79
R5	6.64	7.25	4.47	5.98	24.35

Table D4: Efficiency

Efficiency	Cycle 1	Cycle 2	Cycle 3	Cycle 4
R1	77.51	80.12	79.13	78.63
R2	93.00	106.11	33.69	74.12
R3	77.86	82.23	87.76	79.66
R4	89.97	86.80	94.91	87.63
R5	84.60	84.40	92.60	80.02

Table D5: weighted Efficiency

Reactors	Cycle 1	Cycle 2	Cycle 3	Cycle 4
R3	81.19	86.30	68.31	77.55
R4	87.14	97.28	44.69	73.78
R5	85.04	92.69	57.14	76.44

Table D6: efficiency/weighted efficiency

Ratio	Reactors	C0 acclimation	Cycle 1	Cycle 2	Cycle 3
W+T 75:25	R3	0.95	0.95	1.28	1.02
W+T 25:75	R4	1.03	0.89	2.12	1.18
W+T 50:50	R5	0.99	0.91	1.62	1.04