ANAEROBIC CO-DIGESTION WITH INDUSTRIAL WASTEWATER FOR BIOMETHANE PRODUCTION

Submitted in fulfilment of the requirements for the degree of Master of Engineering: Chemical Engineering in the Faculty of Engineering and The Built Environment at the Durban University of Technology

By

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ABSTRACT

The increasing demand for energy has led to the utilization of fossil fuels more abundantly as a quick alternative for generation of energy. The use of these sources of energy however as led to the generation of greenhouse gases which tend to cause climate change, thus affecting the ecosystem at large. Thus, there have been the search for alternative sources which cannot be depleted but do generate minimal greenhouse gases. One of such alternate sources is industrial wastewater which have shown to have high concentration of nutrients in the form of organic contents which can be converted by micro-organisms into energy, usually known as biogas, comprising majorly of CH_4, CO_2 and H_2. Another important factor is that industrial wastewaters are a renewable energy source which are continuously generated due to increasing urbanisation and population growth. In this study, the characteristics of three agro-industrial based wastewaters used shows their potential for application in anaerobic co-digestion". Anaerobic co-digestion method was utilized to harness the synergetic effect of both sewage sludge and agro-industrial wastewater as co-substrate for the generation of biomethane. The result of the effect of varying mix-ratio of the substrates on biomethane production of sugar wastewater and dairy wastewater indicated that mix-ratio of 1:1 for sewage sludge to sugar wastewater operated at 35^{o}C was suitable for optimum generation of biomethane of 1400.99 mL CH_4/g COD_{added} and COD reduction of 54%. The model generated using design expert was found to navigate the design space and could perfectly predict the yield of biomethane effectively for the sugar wastewater mix. The biomethane potential tests (BMP) experiment using varying inoculum-substrate ratio (ISR) showed that operating at mesophilic temperature of 25^{o}C with ISR of 1:2 and 2:1 for sugar wastewater and dairy wastewater respectively does increase the methane production within the first three (3) weeks. The kinetic models that best fit the anaerobic co-digestion for sugar wastewater was the first order model while the simplified Gompertz model favoured the dairy wastewater perfectly. The biomethane potential tests indicate significant increase the biomethane production and as well reduction in the volatile solid and chemical oxygen demand (COD) content. In conclusion, both sugar and dairy wastewater can be recommended as co-substrates for anaerobic digestion of sewage sludge for increased and improved biomethane production while simultaneously reducing their COD content at the same time.

DECLARATION

I**, Jeremiah Adedeji**, clearly state the following:

i. The research reported in this thesis, except where otherwise indicated, is my original work.

ii. No one has submitted this thesis for any degree or examination at another institution.

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Signed: J. Adedeji Date: 20 October 2020

DEDICATION

This study is dedicated to God and all who desire knowledge in renewable energy and environmental pollution control.

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NOMENCLATURE

AD	Anaerobic digestion
ANOVA	Analysis of variance
AcoD	Anaerobic co-digestion
AOX	Adsorbale organo halogens
BIS	British International Standard
BMP	Biochemical methane potential
BOD	Biological oxygen demand
BW	Brewery wastewater
CCD	Central composite design
$CH_4(g)$	Methane gas
C/N	Carbon to nitrogen ratio
COD	Chemical oxygen demand
D	Dairy
DO	Dissolved oxygen
DUT	Durban University of Technology
DWA	Department of Water Affairs
DW	Dairy wastewater
DWmix	Dairy wastewater mix
EU	European Union
FA	Free ammonia
GC	Gas chromatograph
HOA	Hydrogen oxidizing acetotrophs
HOM	Hydrogen oxidizing methanogens
HRT	Hydraulic retention time
$H_{2(g)}$	Hydrogen gas
H_2SO_4	Tetraoxosulphate (IV) acid
H_2S	Hydrogen sulphide
ISR	Inoculum to substrate ratio
KOH	Potassium hydroxide
KZN	KwaZulu-Natal
LCFA	Long chain fatty acids
MSW	Municipal solid waste

MWWTP	Municipal wastewater treatment plant
Mm	Maximum methane
NH_4^+	Ammonium ion
NH_3	Ammonia
NL	Normalized liters
NaOH	Sodium hydroxide
NRB	Nitrate-reducing bacteria
NRF	National Research Foundation
OCD	Optimal combined design
OP	Optimal design
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
RSM	Response surface method
SIR	Substrate to inoculum ratio
SW	Sugar wastewater
SRB	Sulphur reducing bacteria
SRT	Solid retention time
STP	Standard temperature and Pressure
sCOD	Soluble chemical oxygen demand
SA	South Africa
SS	Sewage sludge
TOC	Total organic carbon
TSS	Total suspended solids
TDS	Total dissolved solids
TS	Total solid
TC	Thermophilic condition
TKN	Total Kjeldahl Nitrogen
tCOD	Total chemical oxygen demand
UASB	Upflow anaerobic sludge blanket
UV/Vis spec	Ultraviolet visible spectrophotometer
VA	Volatile acids
VFA	Volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids

WAS	Waste activated sludge
WW	Wastewater
WWTP	Wastewater treatment plant

PREFACE

Research Outputs

Conference Participation:

Adedeji J.A and Chetty, M. 2018. Biomethane potential test for co-digestion efficiency of sewage sludge with industrial wastewater. 4th Global Change Conference, 3rd – 6th December, Polokwane, South Africa.

Adedeji J.A and Chetty, M. 2019. Optimal combined design for co-digestion of municipal sludge and industrial wastewater. 4th Interdisciplinary Research and Innovation Conference, 17th – 19th December, Durban, South Africa.

Adedeji J.A and Chetty, M. 2019. Anaerobic co-digestion of industrial wastewater with municipal sludge. 12th European Congress of Chemical Engineering, 16th -18th September, Florence, Italy.

Adedeji J.A and Chetty, M. 2019. Anaerobic co-digestion of sewage sludge with sugar wastewater for chemical oxygen demand reduction. The South African Institution of Chemical Engineers in collaboration with the Institute of Chemical Engineers, KwaZulu-Natal. 28th August, MUT, Umlazi, South Africa.

Book Chapter

Armah, E.K., Chetty, M., Kukwa, D., **Adedeji J.A.** 2020. Valorisation of lignocelluloses and microalgae. In Biomass. Intechopen 2020

Armah, E.K., Chetty, M., **Adedeji J.A,** Kukwa, D., Mutsvene, B., Shabangu, K.P, Babatunde, B. 2020. Emerging trends in wastewater treatment technologies: the current perspective. In Wastewater treatment. Intechopen 2020

CHAPTER 1

1.0 Background

Water is a useful compound to the existence of all living things and is a necessary resource for productivity to the manufacturing industries as well. It is a critical compound of any manufacturing industry for its sustenance in the production of products quality. According to a report by Oberholster and Ashton (2008) and WWF-SA (2017), it was stated that 60-70% of fresh water usage in South Africa is by the agricultural sector which is a subset of the manufacturing industries. The utilisation of large volumes of water by these industries for the production of their products result in the generation of large volumes of wastewater (Enitan *et al*. 2015, Ersahin *et al*. 2011, Liu and Haynes 2011).

Wastewater is generally defined as the water contaminated by human use (Templeton and Butler 2011). It is not only generated from manufacturing industries, but it can also result from domestic activities, commercial, sewer inflow or infiltration and storm water run-off. Yongabi (2010) indicated that domestic wastewater is mainly 99% water and 1% solids (this may not be an indication for industrial wastewater). This solid content of wastewater contains organic and inorganic matter, which are the main components that pose an adverse effect on the receiving environment or water bodies when discharged (Yongabi 2010). Organic matter in wastewater which are usually in the form of carbohydrates, fats, oils and proteins are measured in terms of chemical oxygen demand (COD), biological oxygen demand (BOD) and total suspended solid (TSS) while the inorganic matter is either suspended particles (plastics or paper) and metals either as sediment or in dissolved form (Templeton and Butler 2011).

Municipal wastewater treatment plants are designed for the treatment of wastewater from mainly domestic sources (such as residential) and commercial sources (Templeton and Butler 2011). In the treatment of domestic wastewater, sludge, usually referred to as waste activated sludge is generated in large quantities during the primary and secondary treatment processes (Qasim 2017). According to Wang *et al*. (2015), this sewage sludge if not adequately treated can result in secondary pollution if disposed to the environment because this sludge is an agglomeration of organic matter and microorganisms (Cieślik *et al*. 2015).

To mitigate pollution from the generated sewage sludge, various treatment processes have been applied. Among the treatment processes is the anaerobic digestion (AD) technique, which utilises microorganism(s) in degrading organic constituents of sewage sludge for the generation of energy. AD is a biochemical process which involves the breakdown of high molecular organic compounds into smaller ones before subsequent conversion into biogas by microorganisms in the absence of oxygen (Appels *et al*. 2011, Khan *et al*. 2016). AD perform a dual purpose of treating the sludge while generating renewable energy and solids, which can be utilized as fertilizers (Templeton and Butler, 2011). It is a method that produces energy while providing efficient means of treating wastes with minimal input (Bernardo *et al*. 2015).

AD is a proven method with wide application for sewage wastes, industrial organic wastes, municipal solid wastes, agricultural wastes, livestock manure and wastewaters for generation of biogas and biohydrogen (Appels *et al*. 2011, Deublein and Steinhauser, 2011, Ersahin *et al*. 2011, Khan *et al*. 2017, Mao *et al*. 2015). It has been applied on an industrial scale either at municipal wastewater treatment plants or on-site for industrial wastewater effluents with varying degree of success either for the generation of electricity or heat (Alatriste-Mondragón *et al*. 2006, Mao *et al.* 2015). However, the minimal yield of biogas has been recorded due to the instability of the process. The temperature of the reactor, the pH of the substrate and the nutrient availability are parameters reported to affect the stability of the process (Appels *et al*. 2011). The above parameters determine the rate of the process and the sustainability of the essential microorganisms by affecting the working mechanism of the organisms either by making them inactive or increasing their death rate (Appels *et al*. 2011).

Anaerobic co-digestion is one of the methods used to address the instability encountered in the AD process in recent years (Alatriste-Mondragón et al., 2006, Mata-Alvarez et al., 2014). Anaerobic co-digestion is the digestion of two or more substrates with different characteristics, to improve the yield of biogas, stabilise the process by providing balanced nutrients and moisture content, and simultaneously allowing high organic load rate which enhances the microbial activity (Hagos *et al*. 2017). Anaerobic co-digestion is done to mitigate the deficiency encountered during digestion of a single substrate with most studies directed to the use of food wastes and livestock manures as co-substrates for digestion. Various studies have shown improvement in process stability and yield of high biogas when anaerobic co-digestion is applied (Hagos *et al*. 2017, Hidalgo *et al*. 2018, Karray *et al*. 2017, Ondari, 2015, Owamah and Izinyon, 2015, Velásquez Piñas *et al*. 2018).

The AD process occurs in stages viz hydrolysis, acidogenesis, acetogenesis and methanogenesis and at each stage, different microorganisms operate on the by-products of the previous step (Ali Shah *et al.* 2014). Therefore, the understanding of the microbial community will aid the optimum operation of the reactor and consequently, the yield of biogas (Mata-Alvarez *et al*. 2014, Xu *et al.* 2017). Reason for this is that various microbes are more active at varying operating conditions. Also, kinetic studies have been used to predict the AD process, examine the relationship between operating parameters and to better understand the degradation process. Among various kinetics models that have been used for the anaerobic co-digestion study are the First-order kinetic, Monod, Contois, Haldane, Chen and Hashimoto, modified Gompertz and dual pooled first-order kinetic model (Xie *et al*. 2016).

1.1 Problem statement

The depletion and environmental impact of conventional fuel in the form of greenhouse gas emissions has led to the systematic shift on its dependence for the generation of energy into alternative means commonly known as renewable energy. AD is one of the processes by which renewable energy is generated from organic waste. Nonetheless, AD tends to be limited in efficiency in term of biogas yield when one readily degradable organic matter is utilised as the only substrate, hence, the need for anaerobic co-digestion (Mata-Alvarez *et al*. 2014). Likewise, various studies on co-digestion have proven to enhance the yield of biogas via the synergy of the co-substrates under certain conditions (Horváth *et al*. 2016). Above notwithstanding, investigation on co-digestion which are reviewed have been conducted on either agricultural biomass or domestic wastes with municipal sewage sludge, (Alatriste-Mondragón *et al*. 2006, Velásquez Piñas *et al*. 2018) with little studies conducted on co-digestion of industrial wastewater either with sewage sludge or other substrates. Therefore, this study considered investigating the efficiency of co-digesting various industrial wastewaters along with sewage sludge from municipal wastewater treatment in Durban on biomethane yield.

1.2 Aim and objectives of the study

This study aims to evaluate the yield of biomethane production from various industrial wastewaters using anaerobic co-digestion with sewage sludge.

To achieve the aim of this study, the following objectives were undertaken:

i. Characterisation of industrial wastewaters, inoculum and sewage sludge;
ii. Optimisation of co-digestion of sewage sludge with industrial wastewater;
iii. Evaluation of existing kinetics models for anaerobic co-digestion.

1.3 Research scope

This research study will provide distinctive knowledge of co-digesting municipal sewage sludge with industrial wastewater in separate investigations. This study will also aim to provide useful information for the reactor performance when co-digesting sewage sludge with industrial wastewater at different organic loading rates. Therefore, the scope of this research is limited to:

- **Biochemical methane potential (BMP) study**: Co-digestion of industrial wastewater with municipal sewage sludge at varying temperature (ambient, mesophilic and thermophilic) conditions to determine their methane potential. It will provide information on the industrial wastewater with optimum efficiency in biogas generation.
- **Bench-scale study**: Results obtained from the BMP tests (i.e. industrial wastewater with optimum efficiency) will be considered in varying inoculum to substrate ratio.
- **Kinetics study**: Data obtained from the bench scale study will be used to evaluate various kinetic models.

1.5 Thesis outline

Chapter 1: Presents an overview of the background of the study considering the enormous amount of industrial wastewater generated by society. It also describes the production of biogas from anaerobic digestion and the motivation for this study in the use of anaerobic co-digestion for improving the yield of biogas and the problem associated with anaerobic digestion.

Chapter 2: Focuses on the literature review of work that has been done by researchers on the characteristics of wastewater, anaerobic and anaerobic co-digestion process and factors affecting the process.

Chapter 3: Focuses on the methodology used to collect data and information in this study to fulfil the research objectives. and

Chapter 4: Discusses the results of the experimental work and analysis of data obtained.

Chapter 5: Presents the general conclusion(s) of research work and recommendation(s).

CHAPTER 2

LITERATURE REVIEW

2.0 Introduction

The search for renewable sources of energy is not a new issue in global trends and likewise, the need to conserve the environment from various forms of pollution. Pollution of the environment is an issue of debate because of the threat it poses to humans and the ecosystem at large. One of the primary sources of pollution to the water bodies and even the environment is wastewater because of the presence of high organic and inorganic compounds which can profoundly alter the quality of water bodies and the living organisms in it (Radwan *et al.* 2017). On the other hand, the characteristics of wastewater make it a potential source for the generation of renewable energy when appropriate technology is applied, and all operating conditions for maximisation of this characteristic are satisfied. In this chapter, the characteristics of wastewater from specific industries would be discussed with review related to the use of anaerobic and anaerobic co-digestion techniques in the treatment of the various wastewater for the generation of renewable energy.

2.1 Wastewater

The term wastewater is defined as water contaminated mainly by human use (Templeton and Butler 2011). It comes from multiple sources which include domestic, commercial or agricultural, industrial activities, sewer inflow or infiltration and storm run-off (Templeton and Butler 2011). Wastewater is generally 99.9% water and the rest solids (Yongabi 2010). Its significant constituents are indicated in Figure 2.1 as this is typical of domestic wastewater. Domestic wastewater, which is channelled to wastewater treatment plants, is discharged from the residential, commercial, institutional organisations and sometimes factories.

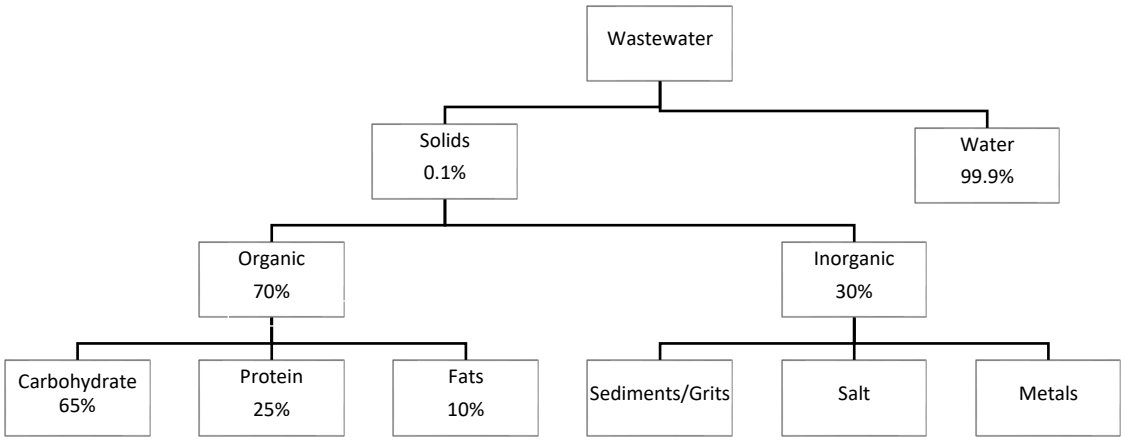

Figure 2.1: Physicochemical content of domestic wastewater (Templeton and Butler 2011, Yongabi 2010)

The characteristics of wastewater are usually determined via the chemical component and flow condition as this is used in the design of each wastewater treatment plant (Qasim 2017). The flow conditions of wastewater are based on the season and is mainly, a dry or wet season which will result in an inflow of storm run-offs. The organic and inorganic constituents of wastewater are used as an indication of the chemical quality of wastewater (Qasim 2017). The following parameters are usually considered when measuring the chemical characteristics of wastewater; biochemical oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), volatile solids (VS), total nitrogen (Karim et al. 2007), total phosphorus (Bachmann et al. 2015), pH and alkalinity (Von Sperling 2007). Their description and concentration in raw domestic sewage are given in Table 2.1. These concentrations are different for industrial wastewater streams because of their variability, as shown in Table 2.2. The results in Table 2.2 are not standard values for each industry as this was adapted from literature.

Tariq, Ali and Shah (2006) stated that industrial and agricultural wastewater effluents contain a high level of organic contaminants in the form of COD, BOD and TSS which are above the permissible limits and which pose a threat to underground water tables. Their study revealed that wastewater from various industries exhibits different characteristics. A research also supported the above statement carried out on three municipal wastewater treatment plants (WWTPs) in KwaZulu-Natal region, where it was seen that the degree of biodegradability of a

WWTP was affected because of the effluent from a textile industry as compared to others (Mhlanga and Brouckaert 2012).

Table 2. 1: Main chemical characteristics of domestic sewage (Von Sperling 2007; Qasim 2017)

Parameters	Description	Concentrations	
		Range	Typical
BOD_5, mg/L	It is the quantity of oxygen required to stabilise through biochemical processes, the carbonaceous organic matter. Measurement is done after five days and 20°C.	110-400	210
COD, mg/L	It is the quantity of oxygen required to chemically stabilise the carbonaceous organic matter.	200-780	400
TS, mg/L	TS is the organic and inorganic solids; suspended and dissolved solids of wastewater.	375-1800	730
Suspended	Total suspended solids (TSS) are the part of organic and inorganic solids that are filterable	120-360	230
Dissolved	Total dissolved solids (TDS) are the part of organic and inorganic solids that are non-filterable. It is usually considered to have a dimension of less than 10^{-3}µm.	250-800	500
Volatile	Volatile solids (VS) are combustible or organic compounds (at 550 ± 50°C) of TDS.	105-300	200
TOC, mg/L	Total organic carbon will be determined through the conversion of organic carbon into carbon dioxide. It is the measure of organic carbonaceous matter.	80-290	150
TN, mg/L	Total nitrogen that includes organic nitrogen, ammonia, nitrite and nitrate. It is an essential nutrient for microorganisms' growth in biological wastewater treatment. Organic nitrogen and ammonia together are called Total Kjeldahl Nitrogen (TKN)	20-85	40
TP, mg/L	Total phosphorus exists both in inorganic and organic forms. It is one of the important nutrients for wastewater treatment biologically	4-8	6
pH	It is the indication of acidity or basicity of wastewater. The value of 7 denotes neutrality	6.7-7.5	7.0
Alkalinity (at $CaCO_3$) mg/L	Indicator of the buffer capacity of the medium (resistance to variations in pH). It is due to the presence of bicarbonate, carbonate and hydroxyl ions in wastewater.	50-200	100

Table 2.2: Chemical characteristics of some raw industrial wastewaters (Enitan 2015, Kushwaha 2015, Ondari 2015)

Parameters	Brewery	Abattoir	Cane Sugar
BOD_5, mg/L	1609.34-3980.61	476-3850	350-2750
COD, mg/L	1096.41-8926.08	935-6600	1000-4340
TS, mg/L	1289.26-12248.13	----	----
TSS	530.67-3728.02	750-4400	760-800
VS	1832.82-4634.31	----	----
VSS	804.11-1278.43	660-5250	173-2190
Alkalinity	500-10000	----	----
pH	4.6-7.3	6.85-8.19	5-6.5
Nitrate, mg/L	1.14-11.55	24-250	----

2.1.1 Wastewater treatment

Domestic wastewater is treated in municipal WWTPs to meet the minimum standard before being discharged into large water bodies. The following are brief narratives of the processes carried out in WWTP:

Preliminary treatment: This process aims to remove large debris which can interfere with other unit processes or cause damage to mechanical equipment. The method includes screening and grit removal; the screening section helps remove floating substances such as papers, plastics, and rags via screens of varying sizes. The removal of a small grain of sand, stone and gravels is achieved at the grit removals section via settling in the grit channels (Templeton and Butler 2011).

Primary treatment: At this stage, various suspended particles which are in flowing water are removed via conventional sedimentation process or dissolved air floatation or membrane bioreactors (Qasim 2017). This treatment aims at a separation of solids from water and concentration of the separated solids.

Secondary treatment: This treatment aims to remove dissolved organic contaminants from wastewater; it is mainly a biological process followed by subsequent sedimentation process. The aerobic process is usually employed because of its release of relatively inoffensive

products (such as H_2O and CO_2) and its speed in achieving the aim (Templeton and Butler 2011). Equation 2.1 shows an overview of the aerobic process.

$$Organic\ matter + bacteria + O_2 \rightarrow new\ cell\ biomass\ (sludge) + CO_2, H_2O, NH_3 \ldots\ldots\ldots\ldots\ldots (2.1)$$

One of the aerobic processes in which bacteria are suspended in wastewater is the "activated sludge process" (Templeton and Butler 2011). It consists of microorganisms in high concentration (> 2000 mg/L) used as flocs. Adsorption, carbonaceous oxidation, and nitrification techniques are involved in the removal of organic matter with carbon dioxide produced and oxygen consumed during the microbial growth cycle (Templeton and Butler 2011). This treatment stage along with the primary treatment produces vast amounts of sludge (Qasim 2017), often known as sewage sludge which is further treated to avoid secondary pollution because of its characteristics (Wang *et al*. 2015).

Tertiary treatment: This process is usually for disinfection, chlorination, or ultraviolet disinfection or ozonation. These methods are employed before discharging the treated water (Templeton and Butler 2011, Qasim 2017).

2.1.2 Municipal sewage sludge

Municipal sewage sludge (MSS), often known as waste-activated sludge is the by-product of wastewater treatment. MSS is usually a combination of primary sludge, secondary sludge due to aerobic activated sludge treatment and tertiary sludge (Evans 2016, Fytili and Zabaniotou 2008). MSS contains particles removed from wastewater which are rich in nutrients and organic matter (Bachmann *et al*. 2015).

MSS is liquid or semi-solid liquid with solid content ranging from 0.25 -12% based on treatment while the rest is moisture (Fytili and Zabaniotou 2008). The characteristics of sewage sludge vary in pH, organic acid content, inorganic content, heavy metals, alkalinity, nutrient, biological component (viruses, protozoa and bacteria) and energy content based on the method used (Fytili and Zabaniotou 2008). The constituents of MSS can also include organic pollutants such as adsorbable organohalogens (AOX), polychlorinated biphenyls (PCBs), surfactants, polycyclic aromatic hydrocarbons (PAHs), pharmaceuticals and others (Siebielska 2014). Kacprzak et al. (2017), indicated that dry MSS contain 50 - 70% organic matter, 30 - 50%

mineral matter, 3.4 - 4% nitrogen, 0.5 - 2.5% phosphorus and a significant amount of macro and micro-nutrients. Table 2.3 indicates the typical characteristics of MSS. Based on the above characteristics and presence of pathogenic organisms and parasitic helminths, its disposal to the environment is of concern to both humans, animals, and plants.

Table 2.3: Characteristics of municipal sewage sludge (Kacprzak *et al*. 2017)

Parameter	Untreated primary sludge	Secondary sludge
pH	5 – 8	6.5 - 8
TS (%)	2 - 8	0.8 – 1.2
VS (% TS)	60 -80	59 - 88
Cellulose (% TS)	8 -15	7 – 9.7
Protein (% TS)	20 -30	32 - 41
Grease and fats (% TS)	7 – 35	5 - 12
Nitrogen (% TS)	1.5 – 4	2.4 - 5
Phosphorus (% TS)	0.8 – 2.8	2.8 - 11
Potassium (% TS)	0 – 1	0.5 – 0.7

Disposal into the sea, agricultural re-use, landfilling, and incineration are the earliest forms of sewage sludge management with the latter three still in practice (Kacprzak *et al*. 2017). Treatment of MSS is undertaken to eliminate the pathogenic organisms and organic pollutants in it. One management strategy that is more favourable in terms of low energy input and high energy output is the AD process. This process helps in minimising the organic and pathogenic content while generating renewable energy (Cieślik *et al*. 2015, Kacprzak *et al*. 2017, Siebielska 2014).

2.1.3 Dairy wastewater

The dairy industry is one of the food industry's that utilises a large volume of water to produce its products (Liu and Haynes 2011, Trevor *et al*. 2005). Its products include condensed milk, powdered and skimmed milk, yoghurt, butter, cheese, and whey. Water consumption in dairy industries ranges from 1 - 5 times the volume of the raw milk being processed depending on the production (Liu and Haynes 2011, Slavov 2017). Wastewater from dairy industries varies in composition, amount and flow rates based on the product produced at a time. Dairy wastewater has high organic content typically measured as COD or BOD in addition to lactose, organic acids, protein, fats, minerals, and salts. Cheese whey, a by-product of the dairy industry has higher COD than any other dairy effluent (Gelegenis *et al*. 2007, Prazeres *et al*. 2012). This

variation in effluents organic load makes treatment of dairy wastewater challenging. The nitrogen and phosphorous contents of dairy wastewater are lower when compared to other industrial sources. It also has a high concentration of solids with almost 50% in volatile form. The pH of dairy wastewater is usually neutral except for slightly acidic ice-cream wastewater and 4.46 in whey wastewater (Karadag *et al.* 2015).

Direct recycling and re-use of waste content of dairy wastewater for animal feed is one way for reducing wastewater pollution from the industry. Various treatment processes are also being employed, such as physicochemical means, aerobic and anaerobic biological means (Kasmi 2018).

2.1.4 Sugar wastewater

Sugar cane industry is one of the largest industries in South Africa (The South African Sugar Industry 2014). Most of the world's sucrose are produced from sugar cane as either liquid sugar, invert sugar, granulated or brown sugar. The sugar production process involves washing, extraction, clarification/purification, concentration, and separation steps (Kolhe, Sarode and Ingale 2009). Most production stages for sugar involve the use of water and likewise generation of water, which is present in the cane itself leading to the use of about 200m^3 water per tonne of produced sugar (Herve Macarie 2006). Herve Macarie's report has it that even with water management process being implemented in sugar factories, about 1.5 - 2m^3 water per tonne of processed cane is needed and this generates about 1 m^3 of wastewater (Herve Macarie 2006).

Sugar wastewater is contaminated with high organic matter and suspended solids (Fito *et al*. 2019). The physicochemical properties of sugar factories effluent from the combined wastewater is quite different from that of each stage involved in the sugar production and the effluent quality varies based on the chemicals used during the production stages (Fito *et al*. 2019). The COD content of sugar factory wastewater is said to be within the range of 1360-2000 mg/L (Kolhe *et al*. 2009), 1100.3-2148.9 mg/L (Fito *et al*. 2019), 2000-8000 mg/L (Herve Macarie 2006) and can sometimes be lower to about 317 mg/L (Saurabh and Shailja 2014). Other physicochemical properties of treated and untreated sugar wastewater include TS (870 – 21498 mg/L), BOD (970 – 2740 mg/L), pH (4.4 – 8.8), TDS (400 – 1650 mg/L), Chloride (26 – 455 mg/L), sulphate (11.2 – 650 mg/L), Total Kjeldahl Nitrogen (4 – 70 mg/L) and oil and grease (7 -12 mg/L) (Sahu and Chaudhari 2015).

According to Sahu and Chaudhari (2015), sugar wastewater just like other industrial wastewaters, has adverse effects on both the environment, plant and aquatic biota if not properly managed because of the presence of nitrogen, phosphorus and high COD content as can also be seen in a study by Saurabh and Shailja (2014).

2.1.5 Brewery wastewater

The brewery industry uses a large amount of water for its blending and fermentation processes in the production of beer (Enitan et al., 2015). It generates 3 – 10L of wastewater for every litre of beer and its wastewater constitutes soluble starch, sugars, volatile fatty acids, ethanol with suspended solids (Chen et al., 2016). The primary pollutant of water from brewery industries is its by-products, mainly, spent grains generated from the mashing and yeast surplus or the fermentation process mixed with water (Simate et al., 2011). The wastewater contains high levels of biodegradable organic matter characterised by high BOD, COD and TS with a wide range of other organic pollutants (Simate *et al*. 2011).

A review on brewery wastewater Arantes *et al*. (2017) indicates the range of COD to be between 2000 and 32 500 mg/L, TSS content between 500 and 3000 mg/L and its pH between 3 and 11. This is in agreement with the study conducted by Enitan *et al*. (2015) which indicates a high solid content in the brewery wastewater in KwaZulu- Natal. Brewery wastewater has been used for the generation of biogas, and results have been reported in the literature (Chen *et al*. 2016). The typical range of biogas yield from the anaerobic process is said to be 0.75 - 1.12 m^3/kg solids processed and its methane content between 55 and 70% (Arantes *et al*. 2017). Chen *et al*. (2016), also indicated a biogas yield of 530 mL/g COD for brewery wastewater using an anaerobic membrane reactor operating at 35ºC.

2.2 Anaerobic digestion

Anaerobic digestion (AD) is the microbial degradation of organic matter in the absence of oxygen to produce mainly biohydrogen and biogas (Lohani and Havukainen, 2018). It is a series of biochemical reactions where microorganisms anaerobically convert organic materials into products to be finally converted into biogas (Khan *et al*. 2017). Microorganisms breakdown higher molecular mass such as polysaccharides, proteins, fats, cellulose and hemicellulose into a smaller molecular mass which is later converted into biogas (Khan *et al*. 2016), thereby, making the efficiency of anaerobic digestion dependent on the substrate's

component and the activity of microorganisms. Appels *et al*. (2011), stated that AD is a biochemical conversion process that is robust and has well-proven applications.

The AD technique is very attractive because it treats wastewater, generates renewable energy, and produces by-product that can be used as fertilisers on farms, thereby, making it an environmentally friendly process (Ruiz and Flotats 2014). The AD process has the following advantages when compared to the aerobic process of wastewater treatment: fewer nutrients required and generation of less biological sludge which need only drying as further treatment (Buitrón *et al.* 2014). It also requires a small reactor volume and no oxygen, therefore saving power needed for the supply of oxygen in the aerobic method and the organic loading on the system is not limited to an oxygen supply. Thus, in AD increased loading rate can be applied which allows for rapid response to substrate addition after long periods without feeding and semi-feed strategies for some months, this does favours to the system making AD a suitable option for seasonal industrial wastewater treatment and elimination of off-gas that causes air pollution.

AD has been widely applied in the treatment of organic waste streams, and its development for biogas is from as far back as the 16th century (Grando *et al*. 2017). The general equation to produce methane from organic matter is given in Equation 2.2:

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 \quad (2.2)$$

The conversion of organic matter into biogas is represented in Figure 2.4.

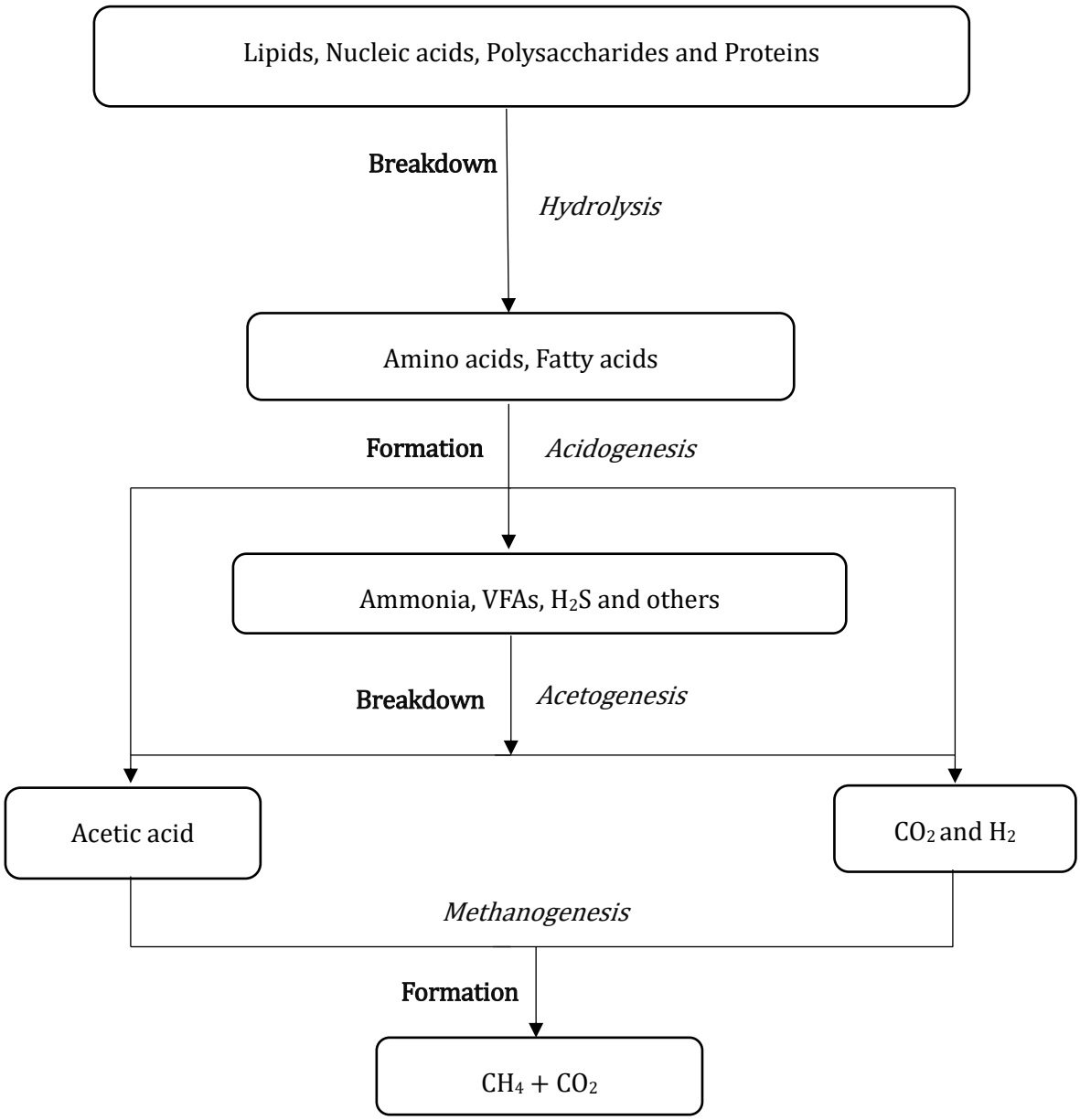

Figure 2.4: Anaerobic digestion degradation process

2.2.1 Anaerobic digestion process

The AD process consists of various processes which occur almost simultaneously, different microorganisms with the varying ability or mechanism to act on the substrate, the product or by-product from a process (Ali Shah *et al.* 2014). Generally, there are four steps in the accomplishment of the overall process to produce biogas. Hydrolysis, which is the first step, involves the degradation of higher molecular-mass compounds into smaller ones appropriate for usage as a supplier of energy and carbon (Mehta and Sirari 2018). The second stage is

acidogenesis, it includes the bacterial transformation of compounds from the hydrolysis stage into distinguishable lower-molecular-mass transitional compounds. Lower chain VFAs formed during the second step are utilised by a consortium of bacteria to generate acetate; this process is the acetogenesis stage (Meegoda *et al*. 2018). The last step, which entails the conversion of the transitional compounds into simpler end products such as CH_4 and CO_2, is known as methanogenesis (Mehta and Sirari 2018, Meegoda *et al*. 2018).

2.2.1.1 Hydrolysis

Hydrolytic bacteria hydrolyse the substrate (macromolecule) into short-chain organic acids and other micro-molecules, which can be acted upon and transformed into soluble short-chain organic molecules (Meegoda *et al*. 2018). At this phase, the hydrolytic bacteria catalyse the breakdown of large complex soluble and insoluble organic molecules present in the wastewater or agricultural biomass into smaller soluble monomers which could be transported into cells of non-hydrolytic fermentative bacteria and metabolised. This stage is usually the rate-limiting step of the AD process as the impact on the substrates with high organic content is significant (Mehta and Sirari 2018).

2.2.1.2 Acidogenesis

Acidogenesis is the process during which simpler organic material is metabolized to form CO_2, H_2, acids and alcohols through the action of the genera namely, *Pseudomonas, Bacillus, Clostridium, Micrococcus* or *Flavobacterium* (Ziemiński and Frąc, 2012*)*. The major intermediate compounds which result from the transformation of the substrate during acid fermentation are acetate (CH_3COOH), propionate (CH_3CH_2COOH), butyrate ($CH_3CH_2CH_2COOH$), hydrogen gas (H_2), carbon dioxide (CO_2), lactate ($CH_3CHOHCOOH$), formate ($HCOOH$), ethanol (CH_3CH_2OH), valeric acid ($CH_3CH_2CH_2CH_2COOH$), isovaleric acid ($(CH_3)_2CHCH_2COOH$), and caproic acid ($CH_3CH_2CH_2CH_2CH_2COOH$). The distribution of the final product varies based on the acidogenic bacteria species and environmental factors such as pH and temperature (Rabii *et al*. 2019).

2.2.1.3 Acetogenesis

The acetogens are said to be obligate hydrogen-producing bacteria that can only survive at deficient H_2 concentrations (Yang 2018). Acidogenesis products are further oxidized to acetate, H_2, and CO_2 by the activity of acetogenic bacteria. For example;

Propionate: $CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$ ……(2.3)

Butyrate: $CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$ ……(2.4)

Therefore, for acetogenic bacteria to maintain a low partial pressure of H_2 less than 10^{-5} atmospheres, they live in symbiosis with the H_2-utilizing methanogens (Meegoda *et al*. 2018). Hydrogen exploiting methanogens accept hydrogen as a substrate from hydrogen producing acetogenic bacteria. The interrelationship amongst these two groups of bacteria is called interspecies hydrogen exchange, which also occurs among acidogenic and methanogenic bacteria (Meegoda *et al*. 2018).

2.2.1.4 Methanogenesis

This process by methanogens produces methane and CO_2. Methanogens are slow-growing bacteria with a generation time between 2 to 10 days at 35˚C and 55˚C, but may take as long as 50 days at 10˚C (Enitan, 2015). They are divided into two, namely the lithotrophs and acetotrophs.

Hydrogen users (lithotrophs): $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ ……..(2.5)

Acetic acid users (acetotrophs): $CH_3COOH \rightarrow CH_4 + CO_2$ ……(2.6)

Most methanogens found in anaerobic wastewater treatment and natural anaerobic environments consume H_2 and single carbon compounds as substrates for generation of CH_4. Additionally, two main types of methanogens which can utilize the two-carbon compound, acetic acid, comprise species of *Methanosarcina* and *Methanothrix (Methanosaeta*). The *Methanothrix species* are incapable to utilizing H_2 in combination with CO_2 as these are non-hydrogen-oxidizing acetotrophs (Enzmann *et al.* 2018).

Conversely, *Methanosarcina* utilise H_2 and CO_2 as well as acetate, carbon monoxide, methanol, and methylamines as growing substrates. Due to their capability to utilise H_2, CO_2 and CH_3COOH, they are categorised as Hydrogen Oxidizing Acetotrophs (HOA). Hydrogen Oxidizing Methanogens (HOM) do not cleave acetate but utilise H_2, formate and CO_2 as substrates (Hunger *et al* 2011). The HOA can make use of multiple (one and/or two carbon) substrates. This ability provides a higher possibility for survival when competing with sulphur reducing bacteria (SRB) and nitrate-reducing bacteria (NRB) during consumption H_2 and CH_3COOH. At low hydrogen partial pressure, HOA uses H_2 and CO_2 rather than acetate, though acetate cleavage by non-hydrogen-oxidising acetotrophs is unaffected by hydrogen (Meegoda *et al*. 2018, Mehta and Sirari, 2018).

2.2.2 Factors influencing anaerobic digestion

Optimum production of methane-containing biogas is affected by factors that affect the methanogenesis stage (methane-producing stage) of the AD process. The factors are; temperature; pH; retention time; nutrients; mixing; inhibition; characteristics of sewage sludge.

2.2.2.1 Temperature

The temperature at which the reactor operates is a crucial parameter always considered in AD and this is due to its effect on the microbial community and their operation, (Kim *et al*. 2017) and consequently the whole process. The variations of temperature during AD are psychrophilic (< 20ºC), mesophilic (30 - 45ºC) and thermophilic (45 - 65ºC). Kim *et al*. (2017) results indicate that a higher organic loading rate (OLR) can be achieved at a mesophilic temperature as compared to a thermophilic temperature, 230mL CH_4/g COD was achieved at 6.7g COD/L day. A methane yield of 220mL/L day was observed at 55ºC which was twofold that at 37ºC (Watanabe *et al*. 2017). In the same study by Watanabe *et al*. (2017), it was observed that the microbial community differed in percentage for the two temperatures. This was in agreement with the study of Jingquan (2006) which indicated that at 70ºC or 75ºC methanogenic activity was inhibited while acidogenesis was favoured.

In another study by Lin *et al*. (2017), a biogas yield of 555.62mL/g VS was observed at 50ºC which was attributed to the interaction between the microbes at elevated temperature as compared to 301.72 and 128.97mL/g VS for 35ºC and 25ºC, respectively. Therefore, though higher biogas yield could be achieved at higher temperature but lower OLRs will be required and likewise, the higher the temperature the higher the likelihood of the inhibition of methanogenic activity.

2.2.2.2 pH

The pH of the substrates and reactor is another crucial factor that affects the stability of the AD process, this is because the growth of the microbial community in an AD process is greatly affected by the operating pH (Khan *et al*. 2016). The pH of AD ranges from approximately 6 to 8.5 for biogas production, and it is noted that a decrease or increase from this range may lead to low methane yield (Kougias and Angelidaki 2018, Mehta and Sirari 2018, Mao *et al*. 2015). The pH of the reactor has been linked to concentrations of other parameters such as ammonia, CO_2 and fatty acids (Fang and Liu, 2002). Wang *et al*. (2014) while working on aerobic and

anaerobic waste activated sludge stated that regardless of the inoculum used higher volatile fatty acids (FAs) were produced at pH of 6 as compared to pH of 5.5 by Kuruti *et al*. (2017).

According to Mao *et al*. (2015), methanogenesis process is more efficient at a pH range of 6.5 – 8.2 and the optimal pH value for methanogens efficiency and active growth is 7. The above statement agreed with the result obtained by Zhai *et al*. (2015) whose study achieved a maximum methane yield of 8579 mL and VS reduction of 179.8 mL/g VS at pH of 7.5 as compared to pH of 6.0. Uncontrolled pH causes the process to fail while co-digesting kitchen waste and cow manure. In another study on pig manure, when the pH value was set to 7, the biogas and methane percentage was 16,607 mL and 51.81%, respectively, as compared to pH of 6 and 8 with 6916 mL, 42.9% and 9739 mL, 35.6% respectively (Zhou *et al*. 2016). In contrast to the above statements, Dai *et al*. (2016) observed that at pH value of 12, the maximum methane yield of 340 mL/g VS was obtained for co-digesting waste activated sludge and perennial ryegrass. Therefore, pH range of 6 – 8 is preferable for the AD process and optimum pH value of 7 to 7.5 is likely to have higher biogas production with more methane content.

2.2.2.3 Retention time

According to Shi *et al*. (2017), longer hydraulic retention time (HRT) is needed for AD of lignocellulosic waste as it gives time for access to the cellulosic part of the substrate. This was revealed in his work on wheat straw which had about 91% increase in biogas yield from 55.2 to 105.2 mL /g VS when operated for 20 and 60 days, respectively. Shorter HRT usually have advantages such as improve process efficiency and reduction in capital cost. However, short HRT could accommodate high organic loading rate but have potential for accumulation of volatile fatty acids which could inhibit the whole process. Higher operating temperature, however, tends to reduce the reaction time (Meegoda *et al*. 2018). Generally, the operating temperature of the system and the substrate constituent are factors that may determine the HRT of the AD.

2.2.2.4 Nutrients

According to Vidal *et al*. (2000), the amounts of carbohydrates, fats, and proteins in dairy wastewaters cause problems during anaerobic treatments. A study on carbohydrate and fat-rich dairy wastewater reveal that anaerobic biodegradation of carbohydrate-rich dairy wastewater was faster compared to fat-rich wastewater, but produced free ammonia (FA) at a level close to the inhibitory level which is caused by the accumulation of volatile fatty acids (VFA). On

the other hand, the fat-rich wastewater overall process was favoured because VFA accumulation was prevented and this supports the claim that fat produces more biogas than protein and carbohydrates. A report for biogas produced by fat is said to be 1.5 to 1.7 times higher than that produced by carbohydrates and protein (Long *et al*. 2012).

Therefore, the nutrient components of the substrate may have an overall effect on the volume of biogas produced during the AD process and subsequently increase or reduce the lag phase.

2.2.2.5 Mixing

The AD process is a process which requires constant contact between the substrate and the microbes for efficient biogas generation. Mixing provide the avenue for consistent contact/ interaction between the substrate and the microbes. Research on the influence of mixing on the methane production during AD process have shown that mixing does have a profound effect on the volume of biomethane generated as compared to the same experiment without mixing (Kozłowski *et al*. 2018).

Ghanimeh *et al*. (2012) reveals that slow mixing at 100 revolutions per minute (rpm) improve the methane generation by 4% over non-mixed assay from 314 mL CH_4/g VS to 327 mL CH_4/g VS. The study also reveals that accumulation of VFAs, propionate and VFA to alkalinity ratio fantastically reduced during mixing giving allowance for higher organic loading rates as compared to non-mixed assay. Though a study by Wang *et al*. (2019) reveals mixing does not affect the overall biogas produced rather it improves the quality of methane produced by 4% and 5% when continuously and intermittently mixed.

Overall, the use of mixing has shown to improve either the microbial community, the contact between the microbes and substrates, the methane yield and reduce inhibition caused by other parameters. However, vigorous/ continuous mixing could cause decrease in methane yield and microbial community (Lindmark *et al*. 2014).

2.2.2.6 Inhibition

Several substances are said to be responsible for the inhibition of biogas, methane-producing bacteria, and archaea community during the AD process. These substances are either intermediate products or toxic in nature, and once in excess can cause the AD process to fail

(Kougias and Angelidaki 2018). They are high ammonia, volatile fatty acids, and long chain fatty acid concentration.

Ammonia inhibition can cause 50% reduction in methane production if between the range of 1.7 to 14g/L and pH values of 8.5 – 8.8 (Kougias and Angelidaki 2018, Zhang *et al*. 2011). In a study by Angelidaki and Ahring (1993), it was observed that aceticlastic methanogens are more sensitive to ammonia concentration at 3.5g/L than hydrogenotrophic methanogens at 7.0g/L which was seen by the growth rate of aceticlastic methanogens being halved at this concentration, and therefore affected the overall yield. Dai *et al*. (2017) study indicated that acclimation of the microbes to high ammonia-ammonium- pH environment help in maintaining the methane content of the biogas while reducing the H_2S content as compared to the control. In this same study, it was observed that due to high ammonia concentration, the methanogens were inhibited but the AD process was stable. Also, once the concentration of free ammonia-nitrogen exceeds 600mg/L the activity of methanogens is affected thereby reducing the methane yield (Dai *et al*. 2013).

Another inhibiting substance is VFA if present in high concentration. VFAs are intermediate products which are transformed to acetic acid prior to conversion to methane, with the most common ones being butyric and propionic acids. It is reported that the concentration of VFAs at a value of 10g/L inhibits the activity of methanogens and may cause imbalance to the system (Kougias and Angelidaki 2018).

Other inhibitory substances in the AD process are long chain fatty acids (LCFA), which are said to be formed when a lipid-rich co-substrate is used (Xie *et al*. 2016). It has been noted that LFCA affect bacteria at every stage of AD even at low concentrations. Appels *et al*. (2008) reported that even at a concentration of 1g/L for stearic and oleic acid, there will be inhibition of the microbial growth by adhesion to the cell membrane. LFCAs are said to affect both hydrogenotrophic and acetoclastic methanogens more at thermophilic than mesophilic temperatures.

2.2.2.7 Characteristics of sewage sludge

The substrate concentration at the beginning have an influence on the process and the yield of methane to be produced (Mehta and Sirari 2018). This sometimes occurs due to low organic content in terms of VS/TS ratio which inhibit the process. Low organic content sludge has

lower organic degradation rate of about 25 – 35% as compared to sludge with higher organic content whose degradation rate is around 56 – 65% for VS/TS ratio of 60% -80% (Kim *et al*. 2011, Liu *et al*. 2016). Therefore, high organic content substrate can be used to co-digest low organic content sludge for improve biogas yield as shown in the study by Liu *et al*. (2016).

2.3 Anaerobic co-digestion

The digestion of two or more substrates of distinct characteristics is called anaerobic co-digestion (AcoD). AcoD is a practical approach to manage organic matters simultaneously (Xie *et al*. 2016) while improving biogas yield. It is an important topic of discussion in the last few decades as far as the AD process is concerned. It is a feasible option because it helps overcome the drawbacks encountered during digestion of a single substrate (Mata-Alvarez *et al*. 2014) and more effective than other current waste management processes (Edwards *et al*. 2017). The following are the advantages of AcoD as stated by (Hagos *et al*. 2017):

- Improved process stability
- Balanced micro and macro-nutrients
- Inhibitory substances are well diluted
- Synergetic effects of microorganism achieved through diversification
- Increase in biodegradable organic matter load
- Improved moisture content
- Reduction of greenhouse gases to the atmosphere
- Economic saving in energy in terms of cost of operation

Studies indicate that the sensitivity of the AD process may be improved by combining several waste streams. According to Hidalgo *et al*. (2018), the addition of process water from a pig farm slaughterhouse to sewage sludge aids the start of the digestion process with little or no lag phase which is attributed to the presence of a readily accessible substrate in solution while improving the yield of biogas. Zhang *et al*. (2011), indicated that AcoD of food wastes with piggery wastewater helps provide necessary trace elements needed to improve anaerobic digestion of food wastes. In the same study, it was observed that there was no accumulation of VFAs which was attributed to the effect of the trace elements supplied by the piggery wastewater, and this has been confirmed by other studies (Zhang *et al*. 2014).

Likewise, anaerobic co-digestion has also been reported to aid C:N ratio by improving substrates deficient of the necessary ratio to the optimum ratio of 20:1 – 30:1 (Velásquez Piñas

et al. 2018). A methane yield of 269mL/g VS was obtained for co-digesting cow manure with paper sludge as compared to 14.7mL/g VS for mono-digestion of paper sludge and this was attributed to an optimum C:N when co-digestion was applied (Priadi *et al*. 2014). AcoD is said to accommodate high organic loading rate as compared to single substrate digestion, and it is important to note that an increase in acclimation of microbial communities has been linked to gradual increase in the organic loading rates which helps in mitigating the effect of inhibition caused by VFAs (Owamah and Izinyon, 2015, Xie *et al*. 2016). This was also indicated in a result obtained by Di Maria *et al*. (2016) were the methane yield was increased from 140 NL/m^3 day to 900 NL/m^3 day for 1.46 kg VS/m^3 day and 2.8 kg VS/m^3 day, respectively. A similar result was obtained for OLR of 6.1g VS/L day producing 0.2 m^3 CH_4/kg VS_{added} (Kim *et al*. 2011). Di Maria *et al*. (2016) results based on co-digestion also indicate reduced HRT from 14 to 10 days.

Also, AcoD of waste activated sludge with combined cheese whey and fruit waste was found to improve the enzymatic activity by 22% compared to mono-digestion. The COD and VS removal efficiency was found to be 9 and 7% more compared to digestion of the waste activated sludge only, while improving the overall cumulative methane - by 31% (Hallaji *et al*. 2019). This result agrees with the study of Zahan *et al*. (2016) on co-digestion of municipal wastewater sludge with food waste whose result indicate 25% to 50% increase in the specific biogas production when 1% to 5% food waste was used as co-substrate. The results also indicate improved removal of tCOD and VS over mono-digestion of the sludge.

However, some studies have also indicated that the addition of some substrates have no momentous effect on the yield of biogas while some reported lower yield as contrasted to mono-digestion of the digestates. The results indicate inhibition caused by furfurals which were produced during acidic pre-treatment (Alatriste-Mondragón *et al*. 2006, Neumann *et al*. 2015).

It is noteworthy to know that the AcoD techniques for sewage sludge, over recent decade, have been geared towards the use of agricultural biomass while the limitless possibilities of utilising wastewater from industries as co-substrates have been literally unexplored though wastewater contains organic matter in dissolved form which can aid the digestion process and also increase the yield of biogas.

2.4 Kinetic models

Kinetic models are used to understand and evaluate the anaerobic digestion process, as it describes the connection between the substrate utilised, microbial growth and the overall output. Kinetic models for AD which depend on growth-limiting substrate concentration are based on the substrate consumption rates and the microbial growth (Xie *et al*. 2016). The models can be utilised for prediction, to control digester functionality and for optimisation of reactor design (Ondari 2015). According to Xie *et al*. (2016), kinetic models that have been applied to both AD/AcoD are First-order kinetic, Monod, Contois, Haldane, Chen and Hashimoto, modified Gompertz and dual pooled first order kinetic model. These models help in simulating the AcoD process and can be modified for the inclusion of inhibition parameters.

2.4.1 First order kinetic model

This model is used to estimate the hydrolysis rate constant and the theoretical maximum methane yield. The model is based on the rate of cumulative methane yield measured during the batch experiment (Dennehy *et al*. 2016). To determine the rate and extent of biodegradation, a linear regression model is applied using the empirical relationship (Membere and Sallis 2018). Sometimes, this model may not be accurate for determining the cumulative methane production because it accounts for the entire process using the exponential form. First order kinetics is represented in Equation 2.7.

$$\frac{dS}{dt} = -KS \qquad (2.7)$$

Where
K= first order disintegration rate constant (1/d)
t = digestion time (day)
S = biodegradable substrate concentration (VS or COD)

Re-arranging equation 2.7 and integrating for time = 0 to t (days) gives Equation 2.8

$$\left[\frac{S_t}{S_o}\right] = e^{-Kt} \qquad (2.8)$$

It is easier to derive the model using gas measurement instead than measuring substrate which may be difficult. The relationship between VS, COD and methane production can be described by Equation 2.9

$$\frac{S_t}{S_o} = \frac{M_m - M(t)}{M_m} \quad \ldots\ldots (2.9)$$

Therefore:

$$M(t) = Mm\ (1 - e^{-K_H t}) \quad \ldots\ldots (2.10)$$

Where
Mm = maximum methane yield (mL) or methane potential of the substrate
M(t) = Cumulative methane yield (mL)
t = time (day)
K_H = rate constant for hydrolysis

2.4.2 Contois model

Contois (1959) indicated an inverse relationship between microbial concentration and their specific growth rate observed. The growth kinetics for a microbial colony is described (Wang and Li 2014) starting from Equation 2.11 as follows:

$$\frac{dX}{dt} = kX_a \quad \ldots\ldots (2.11)$$

Where:
X = microbial mass per unit medium volume
X_a = amount of microbial mass utilising substrate per unit medium volume
t = growth time
k = constant

This takes into consideration the amount of microbial mass in the substrate per unit medium volume, denoted as X_s. This is represented by Equation 2.12

$$\mu = \frac{dX}{(X_a + X_S)dt} \quad (2.12)$$

Substituting Equation 2.12 into Equation 2.11 gives Equation 2.13

$$\mu = \frac{KX_a}{X_a + X_s} \quad (2.13)$$

If $X_a \gg X_s$

$$\mu_{max} = k \quad (2.14)$$

Subtract equation 2.13 from equation 2.14 to get equation 2.15

$$\mu_{max} - \mu = k - \frac{kX_a}{X_a + X_s} \quad (2.15)$$

Re-arranging equation 2.15 gives equation 2.16

$$\mu_{max} - \mu = \frac{kX_s}{X_a + X_s} \quad (2.16)$$

Considering the relationship between the amounts of microbial mass utilising the substrate per unit medium volume to the substrate (S) is expressed in Equation 2.17

$$\frac{X_a}{S} = \theta \quad or \quad X_a = S\theta \quad (2.17)$$

Substituting Equation 2.17 into Equation 2.16 gives Equation 2.18

$$\mu_{max} - \mu = \frac{kX_s}{S\theta + X_s} \quad (2.18)$$

Replacing k with μ_{max} and rearranging gives Equation 2.19

$$\mu = \frac{\mu_{max} S\theta}{S\theta + X_s} \qquad (2.19)$$

Further rearrangement gives, $\mu = \frac{\mu_{max} S}{S + \frac{X_S}{\theta}}$

Therefore, $\mu = \frac{\mu_{max} S}{S + k_c X_S} \qquad (2.20)$

Where:

$k_c = 1/\theta$ = growth coefficient

Equation 2.20 is the Contois kinetic model.

2.4.3 Chen and Hashimoto model

The Chen and Hashimoto model described in the Equation 2.21 was built on fundamental biochemical principles and the Contois model (Contois 1959), and it has been recognised as a reliable tool for predictions of substrates digestion with substantial TS content (Kafle and Chen 2016). The model has been applied successfully in both batch and continuous AD processes

$$\frac{S_t}{S_o} = \frac{K_{CH}}{HRT \times \mu_m + K_{CH} - 1} \qquad (2.21)$$

Where:

HRT = digestion time or hydraulic retention time (days)

K_{CH} = Chen and Hashimoto kinetic constant

μ_m = maximum specific growth rate of microorganisms (1/day)

Substituting in Equation 2.5 in Equation 2.21 and making M (t) subject of the formula, gave the Chen and Hashimoto kinetic model for methane production as in Equation 2.22

$$M(t) = M_m \left(1 - \frac{K_{CH}}{HRT \times \mu_m + K_{CH} - 1}\right) \qquad (2.22)$$

2.4.4 Dual pooled first order

This model is fit for simulating the batch digestion of substrates whose digestion has two distinct generation periods.

$$M(t) = Mm\ (1 - \alpha \times e^{-K_f t} - (1-\alpha) \times e^{-K_L t}) \ldots \ldots \ldots (2.23)$$

Where:

K_f = rate constant for rapidly degradable substrate

K_L= rate constant for slowly degradable substrate

α = ratio of rapidly degradable substrate to total degradable substrate

2.4.5 Modified Gompertz model

This is based on the actual Gompertz model which was used to indicate the exponential relationship between population density and specific growth. The modification describes the variation in cell density during the growth of bacteria exponentially in terms of growth rates and the lag phase period as shown in Equation 2.24. It is commonly used in the simulation of methane accumulation (Kafle and Chen 2016).

$$M(t) = Mo\ \times \exp\left\{-\exp\left[\frac{Rmax\ \times e}{Mo}\ (\lambda - t) + 1\right]\right\} \ldots \ldots \ldots (2.24)$$

Where:

R_{max} = maximum methane production rate (mL/g VS.day)

λ = lag phase (day)

t = time (day)

e = exp (1) = 2.7183

2.4.6 Monod kinetics model

This model has been extensively applied in the expression of anaerobic digestion kinetics process. Monod kinetics model is used to describe the transfer of substrates into a cell. It is also used to express the rate of substrate removal as given in Equation 2.25.

$$\frac{ds}{dt} = \frac{-\mu_m SX}{Y\ (k_s + S)} \ldots \ldots \ldots (2.25)$$

Where:

S = substrate concentration (g/L)
k_x = Monod's constant (g/L)
X = microorganism concentration (g/L)
Y = growth yield coefficient (dimensionless)
μ_m = maximum specific growth rate (h^{-1}).

2.4.7 Haldane kinetics model

Haldane model was a development based on the Monod kinetic model, as it takes into consideration inhibition of the methanogenesis stage caused by high acetate concentrations. According to Lokshina *et al*. (2001), the Haldane model was the best fit for a wide range of initial acetate concentrations when applied to an Upflow anaerobic sludge blanket (UASB) biomass at 11ºC and 22ºC. It is represented as shown in Equation 2.26.

$$\frac{dS}{dt} = -\frac{\mu_m S X}{Y\ (K_s + S + S(S/k_I)^n} \quad (2.26)$$

Where:
S = substrate concentration (g/L)
k_s = half saturation coefficient (g/L)
X = microorganism concentration (g/L)
Y = growth yield coefficient (dimensionless)
μ_m = maximum specific growth rate (h^{-1})
k_I = inhibition constant;
n = Haldane index (n = 1 or 2)

2.5 Computer-aided experimental design

In designing experiments, computer-aided software built with basic integral design development can be easily employed in the generation of both simple and complex experimental design. Software such as Design Expert® from Statease, Minitab® and JMP® are common software used by experimenters for design and analysis of the whole process after the experiments. Designs such as factorial, response surface method (RSM) and mixture design are most frequently carried out to either determine the effect of certain variables or check the interaction of each variable for optimisation and prediction purposes.

Process optimisation is an essential part of engineering design since it allows for easy simulation of a process without wasting time and resources. Therefore, when the main objective is to optimise the response, RSM: a collection of mathematical and statistical methods used for modelling and evaluation of problems is an essential tool when the response of interest is subjective to several factors. The experimental result is better appreciated when it can be understood, interpreted and implemented easily. One of the ways by which quantitative analysis and interpretation are derived is via regression analysis which helps generate empirical models, which aid in the optimisation of the process, prediction and control. It is stated that regression analysis has a strong interplay with experimental design, indicating analysis of data generated from both planned and unplanned experiments could be handled via regression methods (Montgomery 2017).

In determining the optimum operating conditions, RSM utilises first or second-order polynomial models as shown in equation 2.27 and 2.28. Response surface designs are used for fitting response areas. Design such as Central Composite Design usually known as CCD is a good design for second-order model fitting and it varies each factor over 5- levels (Design Expert 2005). The Box-Behnken design is another response surface design which is used for analysis when each factor must be varied over 3 levels and it is a spherical design (Montgomery 2017). The designs do not give space for some adjustment; therefore, experimenters make use of optimal design (OD). This design is usually used when a conventional standard design could not accommodate the choice of the experimenter, as it allows for easy adjustment of the level of the independent variables.

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k + \epsilon \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.27)$$

$$y = \beta o + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j}\sum \beta_{ij} x_i x_j + \epsilon \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.28)$$

Where:

y = dependent variable

x = independent variable

β0 = intercept

$\beta_{1,2,k,I}$ = regression coefficients

Ɛ = error

The first order polynomial model as shown in Equation 2.27 is usually used until the system is found to have a curvature, at this point the second-order polynomial model is employed. Generally, the probability value (p-value) is one of the major values investigated to determine the significance of an empirical model. A p-value less than 0.05 makes the model significant and the p-value for the lack-of-fit must be greater than 0.05 to make the model lack-of-fit non-significant (Design Expert 2005). The least squares method is employed in determining the p-value of the model and the factors involved (Montgomery 2017).

CHAPTER 3

METHODOLOGY

3.0 Introduction

The experimental approach undertaken to achieve the objectives of this study are presented in this chapter which outlines the steps taken for sample collection, preparation and characterisation, preliminary and main analysis. It also details the design of experiment and the kinetic studies undertaken to analyse the data obtained.

3.1 Wastewater and sludge collection

The wastewaters used in this study were collected from dairy, sugar, and brewery industries. The sewage sludge and inoculum were collected from municipal WWTPs in Durban, KwaZulu-Natal (KZN). Each industrial wastewater and the sewage sludge were collected for characterisation and for running batch laboratory tests which were used in evaluating BMP as well as organic pollutant removal. Samples were analysed for TSS, TS, VS TDS and COD according to the standard methods (APHA 2005).

The sewage sludge used was obtained from Amanzimtoti wastewater treatment plant and it was collected immediately after the primary settling tank into two 5 L containers, the temperature and pH of the samples were measured immediately after collection. The inoculum used was taken from one of the anaerobic digesters at the same wastewater treatment plant, whereby the digester is operated at 25^{o}C and the pH of the inoculum was 7.19 as at the time of sampling. The samples were also analysed for TS, VS, TSS and VSS on the same day before storing in a cold room at 4^{o}C.

Brewery wastewater used in this study was sampled before the effluent from the factory was fed into the digester and it was collected in a 25 L container, the temperature and pH of the sample were measured immediately after collection. The sample was also analysed for TS, TSS and VSS on the same day before storing in a cold room at 4^{o}C.

Two samples of dairy wastewater were sampled; the first sampling point was before treatment and the second after the treatment (separation of fat). While sampling, the pH, temperature, conductivity, and the dissolved oxygen were determined for the two samples. The TSS and

VSS were also determined immediately before storing in a cold room. The COD, TS and VS content were determined the second day following the standard method.

The sugar wastewater used in this study was collected from the treatment effluent section of a sugar processing mill in KZN. The sample was kept in a cold room at 4ºC and analysed within 48 hours from the time of sampling using standard procedures.

3.2 Sludge and wastewater characterisation

3.2.1 Analytical methods

The pH, TS, VS and COD of the raw samples and the biodegraded samples was carried out to for comparison and to determine the efficiency of the process. Analysis was done according to the standard procedure as outlined by APHA (2015). Composition of gases in the biogas was analysed using gas chromatography (Yang, Zhang and Wang 2015). Outlined below are the physicochemical parameters analysed before and after the co-digestion experiment. This section contains the summary of the analysis procedure, and the detail procedure can be found in Appendix A1 for some of the equipment used in the study.

3.2.1.1 pH

pH measurements were carried out to determine the degree of acidity or alkalinity of the inoculum, sewage sludge and the wastewaters. This was done to ascertain the pH range is at the required standard for both discharge and co-digestion process to be carried out or corrected if not. The pH range for discharge according to SA and DWA is 5 – 9.5 while pH range of 5.5 – 8.5 has been reported to favour anaerobic digestion process.

3.2.1.2 Chemical oxygen demand

COD was determined according to APHA (2005) standard section 5220D. COD determination is a measure of the oxygen equivalent of that fraction of the organic matter in a sample that is prone to oxidation by a strong chemical oxidant. The oxygen requirement value helps in stating toxic situation and presence of biologically resistant substances. COD test was used to measure a load of organic pollutants in industrial wastewater. The limit of COD concentration for wastewater as specified by BIS and SA discharge limits is 250 and 75mg/L, respectively.

The COD for each wastewater and the sewage sludge were carried out by diluting 1mL of the substrate to 9 mL of distilled water. The mixture has been shaken to homogenise and then 2 mL of the mixture was added to the already prepared COD reagents. The blank sample is the COD vial with 2 mL of distilled water. The COD vials are then shaken before been placed in the thermoreactor block for 2 hours heating at 120ºC. Thereafter, the vials are removed and made to cool under room temperature. Using the 432 HR program on the HACH 3900 Spectrophometer (Figure A.1.1), The blank sample is measured, and the actual samples under study are measured. The result obtained was multiplied by the dilution factor.

3.2.1.3 Total solids

This is the residue left after evaporation of a sample and subsequent drying till constant weight is achieved. The defined temperature once evaporation of sample is complete is 103º – 105ºC according to APHA (2005) section 2540B.

Total solid for each sample was determined by measuring 5mL of the samples in preheated crucibles of known weight. The crucible and the wastewater samples were then placed in the oven for 90 minutes at 105ºC. The crucible along with the residue was then weighed. The value of the total solid was calculated using the Equation 3.1.

$$Total\ solids\ \left(\frac{mg}{L}\right) = \frac{(A - B) \times 1000}{C} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.1)$$

Where:

A = weight of crucible + residue (mg)

B = weight of crucible (mg)

C = sample volume in mL

3.2.1.4 Total suspended solids (TSS)

TSS for each sample was determined by filtering 5mL of the samples through a pre-weighed 47mm glass fibre paper with 1.2µm pore size from Munktell Ahlstrom using a suction kit. The fibre papers were then placed in crucibles of known weight before heating the oven for 1 hour at 105ºC. The crucible along with the residue on the fibre paper was then weighed. The value

of the total dissolved solids was obtained by subtracting TSS from TS. The value of the total suspended solid was calculated using the Equation 3.2.

$$TSS\left(\frac{mg}{L}\right) = \frac{(A_1 - B_1) \times 1000}{C} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.2)$$

Where:

A_1 = weight of filter and crucible + residue in mg

B_1 = weight of filter and crucible in mg

C = volume of sample filtered in mL

3.2.1.5 Total dissolved solids

TDS is an indication of salinity in water (Asadollahfardi, Taklify and Ghanbari 2012). It is the measure of total inorganic salts and other substances that are dissolved in water. Many salts are found dissolved in natural waters, the common ones are carbonates, bicarbonates, chlorides, sulphates, phosphates and nitrates of calcium, magnesium, sodium, potassium, iron, manganese, etc (Sillanpää *et al.* 2018). The high content of dissolved solid elements affects water density, influences osmo-regulation of freshwater in organisms reduces gaseous solubility (such as oxygen) and water use for drinking, irrigation, and industrial purposes. Classification of water based on the concentration of TDS as desirable for drinking (up to 500 mg/L), permissible for drinking (up to 1,000 mg/L), useful for irrigation (up to 2,000 mg/L), not useful for drinking and irrigation (above 3,000 mg/L) (Ravikumar, Somashekar and Prakash 2015).

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3.2.1.6 Volatile solids

This is the term used to ascertain the weight loss on ignition in a furnace at 550°C for 1 hour for solids (thickened sludge) and 30 minutes for liquids (slurry or wastewaters) (APHA 2005). Determination of VS is needed prior and after AcoD process since some calculation of the biogas produced are based on the VS as well as organic loading rate determination and %VS reduction.

VS for each sample was determined by igniting the crucible and residue obtained after TS in a muffle furnace for 30 mins at 550°C. The value of the volatile suspended solid was calculated using the Equation 3.3.

$$VS\left(\frac{mg}{L}\right) = \frac{(B - D) \times 1000}{C} \quad \ldots\ldots (3.3)$$

Where:

B = weight of residue and crucible in mg from Total solids test

D = weight of residue and crucible in mg after ignition

3.2.1.7 Volatile suspended solids (VSS)

VSS for each sample was determined by igniting the crucible, fibre paper and residue gotten after TSS in a muffle furnace for 30mins at 550°C. The value of the volatile suspended solid was calculated using the Equation 3.4.

$$VSS\left(\frac{mg}{L}\right) = \frac{(B_1 - D_1) \times 1000}{C} \quad \ldots\ldots (3.4)$$

Where:

B_1 = weight of residue + filter and crucible in mg from total suspended solids test

D_1 = weight of residue + filter and crucible in mg after ignition

3.2.2 Sewage sludge characterisation

Analysis was done according to standard procedures for wastewater (APHA 2005), the samples were analysed within 48 hours of sampling for TS, TSS, VSS in duplicate.

3.2.3 Wastewater pre-treatment procedure

The industrial wastewater used for the study was collected from effluents stream of industries in Durban KwaZulu-Natal, and these streams have undergone primary pre-treatment. However, the wastewater was filtered to eliminate suspended solids less than 1 mm in diameter for increased contact with microorganism.

3.3 Experimental set-up

3.3.1 Biochemical methane potential test

The BMP test was a preliminary test process which was carried out on three wastewaters, namely brewery, dairy and sugar industries. This was done to carry out selective measure to choose the best industrial wastewater from the three based on their performance for the duration of the digestion process.

Preliminary AD runs were done in batches of 1L schott bottles which serves as the bioreactors, the bioreactors were tightly fastened using screw caps with ports. The schott bottle used as the bioreactor in this study were graduated and it had a total capacity of 1000 mL with clearly labelled graduation up to 950 mL. These opening screw caps were fitted with silicone tubes to prevent leakage, while the remaining openings were closed with small screw caps. The purging process was conducted prior to total closure of the openings, N_2 gas was utilised for the purging of the constituent of the bioreactors to achieve an anaerobic environment for the anaerobes to effectively work (Wu *et al.*, 2016). Each bioreactor was purged for a minimum of 4 - 5 minutes (Banu J, Yukesh Kannah and Kavitha 2017). Silicone tubing was used for biogas collection into a cylinder using the downward water displacement technique. The digesters were mechanically mixed via magnetic stirrer for two hours before measurement of the volume of biomethane produced. The BMP test was carried out in duplicate, and a blank control sample was also duplicated. The schematic diagram of the batch anaerobic bio-digesters used for the BMP test is shown below in Figure 3.1.

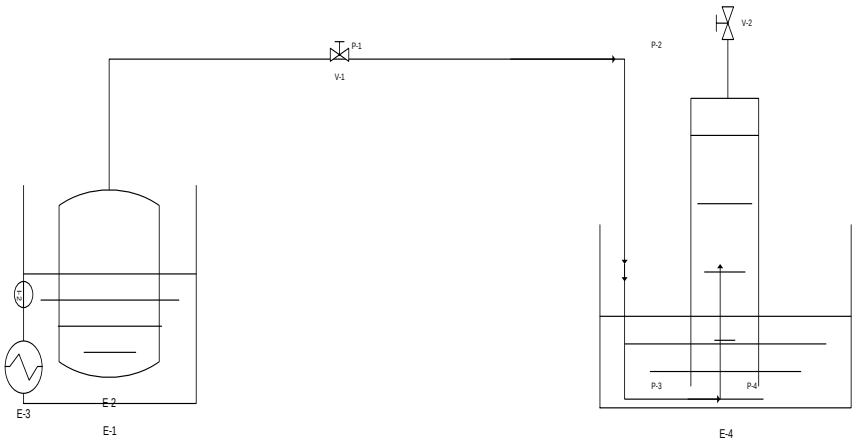


Figure 3. 1: Schematic diagram of the BMP Test

3.3.2 Preliminary screening test

3.3.2.1 Effect of industrial wastewater

The 1L anaerobic bioreactors were used to evaluate the effect of each industrial wastewater as co-substrates for SS as shown in Figure 3.2. Equal volume of each wastewater was co-digested with SS in different bioreactors and the biodegradability of the wastes under temperature was evaluated. The following parameters were investigated, pH change and methane yield. The inoculum- substrate ratio (ISR) used was 1:1; all bioreactors contain a specified amount of inoculum, as shown in Table 3.1. SS mixed with industrial wastewater was used to load each reactor while the blank contains the SS as the only substrate.

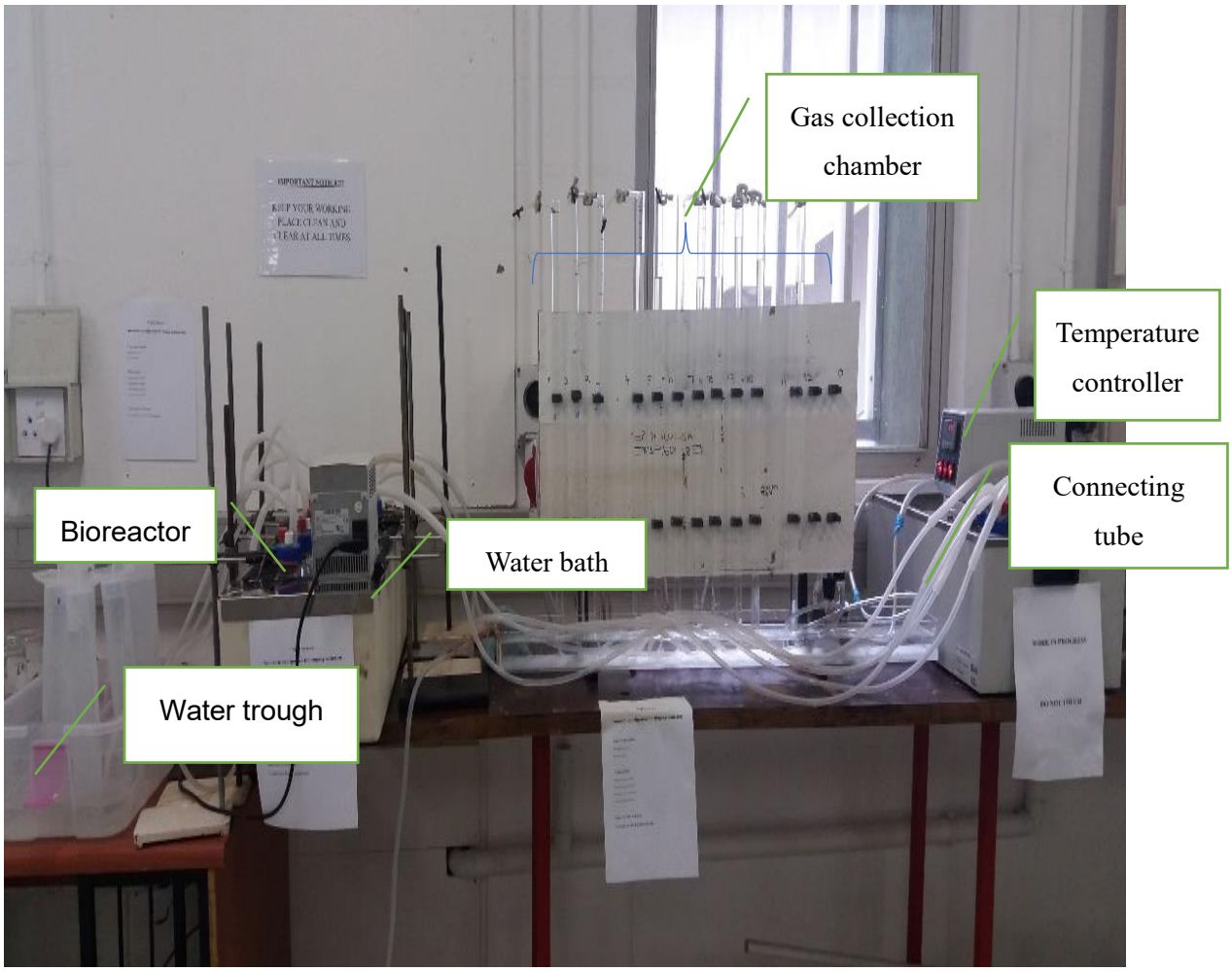


Figure 3.2: Experimental set-up

Table 3. 1: Composition of each mixture

Mixture	I (mL)	SS (mL)	SW (mL)	BW (mL)	DW (mL)
A	400	400	--	--	--
B	400	200	200	--	--
C	400	200	--	200	--
D	400	200	--	--	200

3.3.2.2 Effect of temperature

To evaluate the impact of temperature, a duplicate number of runs were done at ambient (25^{o}C) and mesophilic conditions (35^{o}C).

3.3.3 Experimental design

3.3.3.1 Effect of mix-ratio

The experimental design was carried out after the initial preliminary test and the selection of one or two of the industrial wastewater were to be considered in varying mixtures with the SS. Design Expert 11.0 software from Stat-Ease was used to design the experimental runs, the optimal (combined) option was used because it can accommodate varying of the mixture while taking into consideration other numerical factors. The optimal combined design (OCD) was used when the mixture design and response surface was to be considered at the same time.
Four different mixture ratios were obtained from the design generated by the OCD which is 1:0, 3:1, 1:3 and 1:1 for SS to wastewater respectively, and the three temperatures considered were 25^{o}C, 35^{o}C and 55^{o}C as represented by -1, 0 and 1 respectively in the experimental design generated as shown in Table 3.2.

Table 3. 2: Experimental design for mixture ratio in coded form

Run	SS	WW	TEMP
S1	0.25	0.75	-1
S2	0	1	-1
S3	0.5	0.5	-1
S4	0.25	0.75	1
S5	0.5	0.5	0
S6	0.75	0.25	1
S7	0.5	0.5	1
S8	1	0	-1
S9	0.5	0.5	0
S10	0	1	1
S11	1	0	0
S12	1	0	1
S13	0	1	0
S14	0	1	0
S15	0.75	0.25	0

3.3.3.2 Effect of inoculum to substrate ratio

Another factor considered in this study is the varying of the inoculum to substrates ratio (ISR). Three ISRs was considered in this study which are: 1:2, 1:1 and 2:1. The calculations for the ISR were based on the VS content of the inoculum and the substrates. The value of the VS for the inoculum, sludge and the wastewater used are shown in Table 3.3, and sample calculations for the ISR are shown in Appendix A3 section A3.1 using Equation 3. 5.

$$ISR\ \left(\frac{mgVS}{mgVS}\right) = \frac{VS\ of\ Inoculum}{(VS\ of\ Sludge) + (VS\ of\ wastewater)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.5)$$

Table 3.3: Volume of inoculum and substrate for ISR experiment

Inoculum		Substrate		ISR
Value (mg)	Volume (mL)	Value (mg)	Volume (mL)	
2232	300	4464	482	1:2
4464	400	4464	321	1:1
4464	546	2232	220	2:1

3.3.4 Experimental start-up

Each bioreactor for the mix-ratio was filled with the 400mL of the inoculum and the required volume of the two substrates (SS and WW) as specified in the experimental design for mix-ratio in Section 3.3.3.1. The total volume of the mix-solution in each bioreactor was 800mL leaving a headspace of 200mL. Each bioreactor was purged with N_2 for at least 2 minutes to remove O_2 that may be present in the solution before being closed. Each bioreactor was then kept in the water bath with their corresponding temperature of 25^{o}C, 35^{o}C and 55^{o}C, respectively. The connecting tube on each bioreactor was then connected to the water displacement /gas collection compartment as shown Figure 3.2.

Similarly, the above procedure was carried out for the ISRs experimental start-up, the volume of each substrate and inoculum added to each bioreactor is shown in Section 3.3.3.2. The ISRs experiment was operated at two temperatures (25^{o}C and 35^{o}C), this was done because the result from the mix-ratio experiment indicated that assays operated at 25^{o}C and 35^{o}C had the highest methane production and COD reduction efficiency as compared to those operated at 55^{o}C. and

3.4 Gas collection and characterisation

The technique of downward water displacement (Tetteh *et al.* 2017) was used for accounting the volume of gas generated throughout this study. The gas production from the preliminary and mix-ratio experiment were passed through sodium hydroxide (NaOH) solution rather than water only to absorb any CO_2 present in the biogas produced to account for the volume of biomethane produced. Therefore, the results in Chapter Four were reported as biomethane because the CO_2 was absorbed in the NaOH solution.

The biogas produced from the ISRs experiment was passed through water, therefore, to ascertain the content of the gas, characterisation using Shimadzu GC-2014 equipped with a thermal conductivity detector and a Poropak column. The left and right column flow was 20 mL/min while the temperature of the column was 40ºC and pressure of 78 – 106 and 0.5 – 1.6 KPa were maintained for the right and left-hand side of the column. The temperature at the injection port was 120ºC and that of the detector was 250ºC. The carrier gas used was N_2.

The GC was calibrated with pure samples of CH_4, H_2 and CO_2 to determine the retention time of each pure component. A triplicate of each sample was analysed at varying percentage as indicated in Table 3.4. Figure 3.3 shows the calibration curves and the coefficient of determination indicating its precision to determine the retention time for each pure component. Gas collection for analysis on the GC was done using a 100µm syringe.

Table 3.4: Calibration data for hydrogen, methane, and carbon dioxide standard

Gas retention time	Composition/ Area	0%	20%	40%	60%	80%	100%
H_2 0.753 mins	1	0	58140.9	117799.4	167717.7	225339.7	272688.6
	2	0	57827.6	117843.2	166917.5	226327.3	273403.5
	3	0	57734.3	117861.8	167298.6	225873.5	273056.1
	AVERAGE	**0**	**57900.93**	**117834.8**	**167311.3**	**225846.8**	**273049.4**
CH_4 1.441 mins	**1**	0	45636.3	78773.2	108617.5	122631.7	165126.1
	2	0	46102.3	78427.2	109063.2	122237.1	164454
	3	0	45424.9	79003.7	108487.3	122627.4	165778.2
	AVERAGE	**0**	**45721.17**	**78734.7**	**108722.7**	**122498.7**	**165119.4**
CO_2 2.229 mins	**1**	0	31919.2	54812.5	69776.3	86617.2	97349.7
	2	0	30019.2	53982.3	70103.4	87076.8	96248.2
	3	0	32034.6	55097.9	69227.5	86293.6	96494.7
	AVERAGE	**0**	**31324.33**	**54630.9**	**69702.4**	**86662.53**	**96697.53**

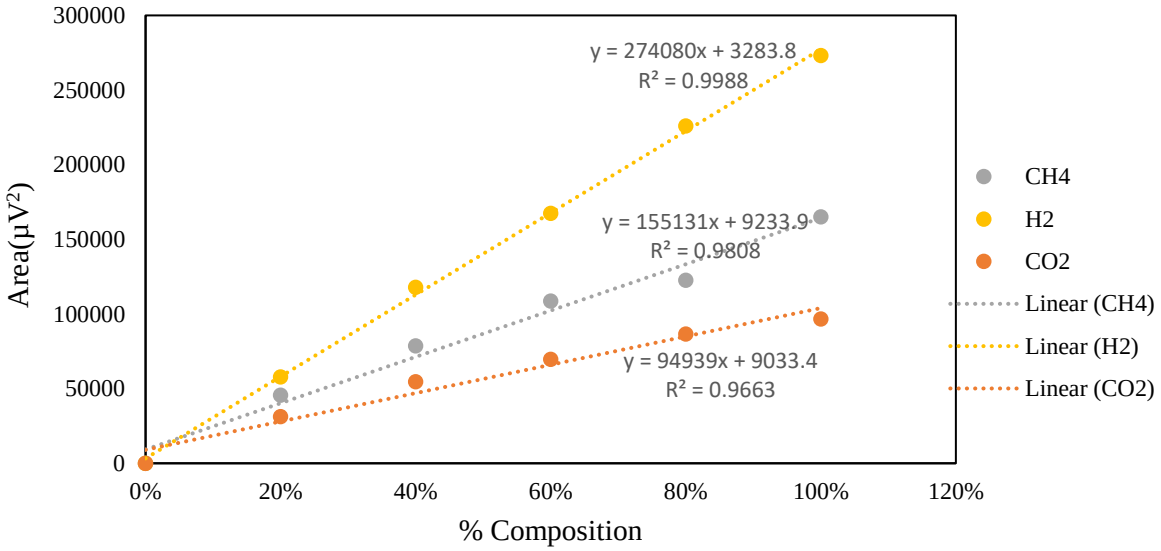


Figure 3.3: Calibration curves for H_2, CH_4, and CO_2

3.5 Kinetics analysis

3.5.1 Experimental procedure

The optimum run from the ISR was used for the kinetic model analysis. An equal volume of substrate and the corresponding volume of inoculum for ISR of 1:2 as shown in Section 3.2.3.2 was taken for the kinetic analysis. The experiment was run for 24 hours at 25°C and was made to run continuously by mixing using magnetic stirrer for the duration of the experiment. Downward water displacement method was used for gas measurement which was taken at an interval of 2 hours.

3.5.2 Statistical analysis

All tests were carried out in duplicate and cumulative data were analysed using the solver add-on of Excel by Microsoft® while the total yield of biomethane and COD reduction with their correlation with each component/factor were analysed using Design expert.

For the curve fitting of the kinetics models that were analysed, the sum of squares error method was utilised to calculate the deviation between the measured and the predicted value by the models (Bechmann and Lomborg 2013). The sum of squares error was set as the objective function, which was then minimised using Solver add-in while changing the other parameters. Iteration was carried out until the predicted values fitted closely to the measured values using the generalised reduced gradient non-linear solver for the optimisation and curve fitting process. A constraint precision of $1E-6$ was used, likewise, the use of regression technique

in analysis toolpak was used to get the regression values such as the R-squared value, and the graph of predicted versus the measured values among others.

Curve fitting application for non-linear in OriginPro Software by OriginLab® was also used to ascertain the value of the constants and the regression coefficients.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.0 Introduction

In this chapter, the results of the characterisation, the preliminary screening analysis, the main experiment, and the process by which the selection of the wastewater for the final experimental work are discussed.

4.1 Characterisation of wastewater and SS

The solids, pH, and COD value of the first set of wastewaters and sludge collected are shown in Table 4.1.

Table 4. 1: Inoculum and Sewage Sludge Characteristics

	I	SS	SW	BW	DW
pH	7.19	5.72	6.30	5.00 ± 0.12	9.13
TS (mg/L)	12100	42210	4515	7778.8	4602.5
TSS (mg/L)	9720	40220		623.3	767.5
TDS (mg/L)	2380	1990		7155.5	3835
VS (mg/L)	6460	30980	3413.7	5050	3976.3
VSS (mg/L)	5380	29880		503.3	467.5
VS/TS (%)	53.4	73.4	75.6	64.9	86.4
COD (mg/L)			7130	6462.5	3012.5

4.1.1 Results of inoculum and SS

As shown in Table 4.1, the result of the SS indicates that it has high TS content which could be an issue when it comes to the management of the solids. Results also indicate that the sludge is highly biodegradable because the VS/TS ratio is above 50% which is an indication of biodegradability as stated by Kayhanian (1995). The results of the analysis also indicate that the TDS of the sludge is low whereas the suspended solids are high. The pH of the sludge used in this study was slightly acidic whereas that of the inoculum was close to neutral, making it fit for anaerobic process (Mao *et al*. 2015).

4.1.2 Results of industrial wastewaters

Physio-chemical analyses of the three wastewaters used for the preliminary studies indicated that these wastewaters effluents were not in conformity with the discharge standards as required by Department of Water Affairs (DWA 2010) and the wastewater discharge limits according to European Union (EU) (Enitan *et al*. 2015, University of Pretoria 2013) as the COD and TS values were above the limits. As stated by Enitan (2015), industrial wastewaters with COD higher than 800 mg/L are reported to be very suitable for the anaerobic process. All the wastewater in this study were suitable for anaerobic digestion process because they all have a high COD content of 7130, 6462.5 and 3012.5 mg/L for SW, BW and DW, respectively.
The results of the sugar wastewater were consistent with results in the literature as reported by Kushwaha (2015) and Fito *et al*. (2018), likewise, the result of the analysis carried out on dairy wastewater was also consistent with the result obtained by Karadag *et al*. (2015). The solids content of the wastewater was considerably lower than that of the SS. This aids the balancing of the moisture content that was required for the efficient digestion process. Likewise, the ratio of VS to TS of the wastewaters indicated that all the industrial wastewaters are biodegradable. The pH of the wastewater was also within the ranges reported for various wastewater worked before from these industries (Enitan *et al*. 2015, Kushwaha 2015 and Karadag *et al*. 2015).

4.2 Biochemical methane potential results

4.2.1 Effect of temperature

Güngör-Demirci and Demirer (2004) stated that temperature is the main factor that plays a vital role in the yield of biogas, degradability of the substrates and stability of the process. Two temperatures were considered in the preliminary analysis which were 25ºC and 35ºC. These two temperatures were chosen since most anaerobic process reported in the literature were said to be carried out within 35 – 37ºC, while consideration for ambient temperature was determined to reduce the cost of heating.

The effect of temperature on the yield of biogas is shown in Figure 4.1. As a general trend usually stated in literature (Lin *et al* 2017), increases in temperature aids increase in biogas, so it was observed for the control and SW mix (samples with sugar wastewater as co-substrate), while the reverse was the case with the DW mix and BW mix whose yield was higher at ambient temperature than at 35ºC. This could be because the inoculum used was taken from a digester being operated at ambient temperature.

The results indicated that temperature is a significant factor in the AD process because the variation between the yield for SW mix was a bit high while that of DW mix suggested that the temperature may not play a significant role for the high yield obtained at 25°C, but rather some other factor. It was noteworthy that it was observed during the process that when the process temperature dropped from 35°C to a lower temperature due to the drop in the water level. During the process of increasing the temperature, the production rate of biogas was higher as compared to the time when there were no alterations in the temperature.

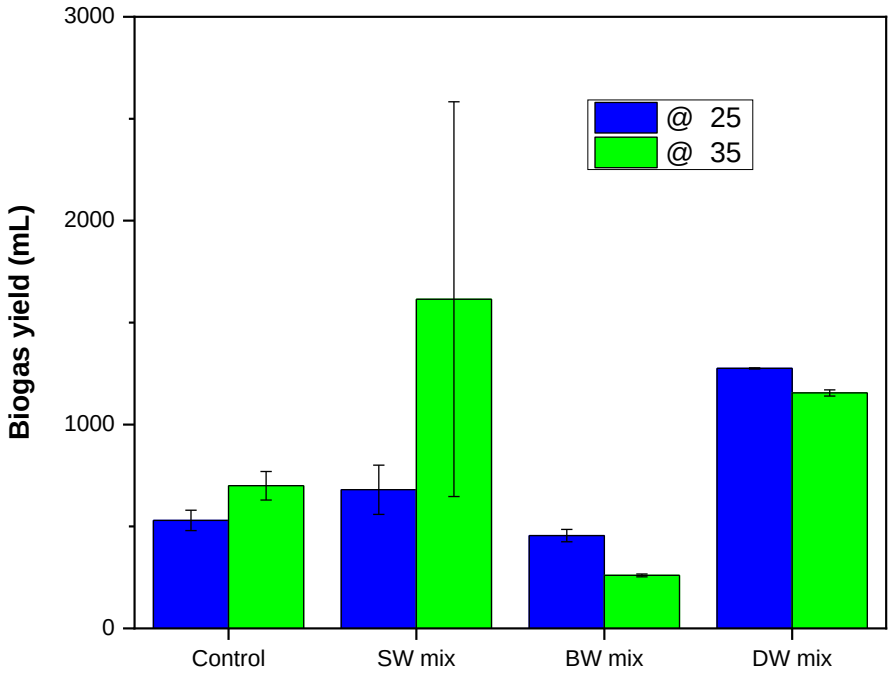


Figure 4. 1: Overall biomethane production

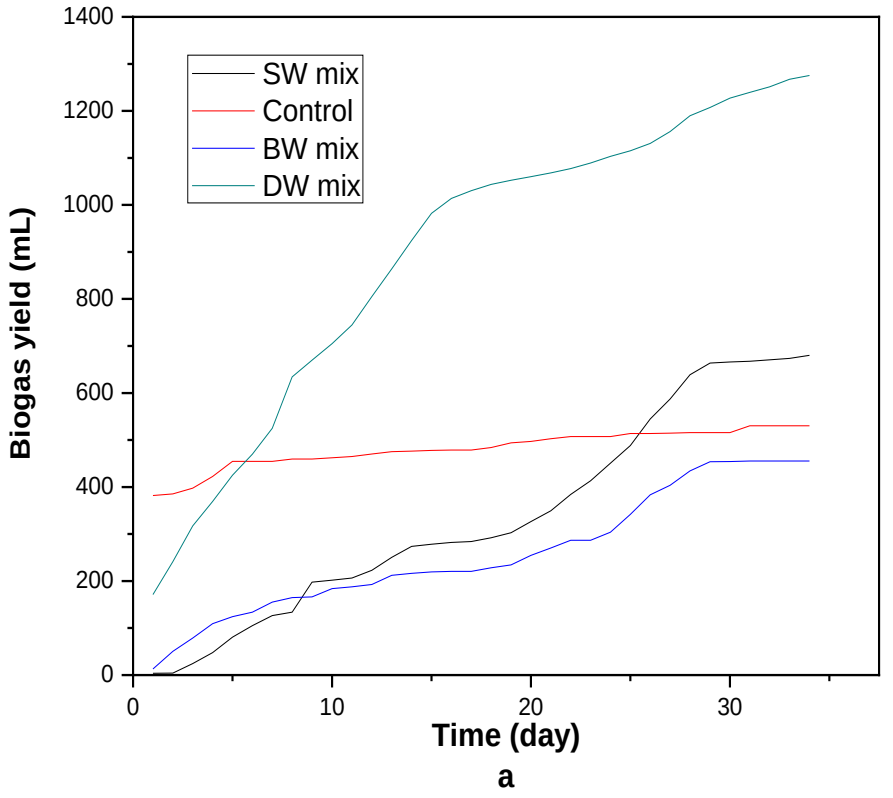
4.2.2 Comparison of biogas yield from each industrial wastewater

As shown in Figure 4.2(a) and (b), the addition of wastewater helps reduce the lag phase duration which is usually encountered when using agricultural biomass as co-substrates for MSS as observed by Hidalgo *et al*. (2018). Figure 4.2(a) shows the yield of biogas for the BMP assay at 25°C where it indicated that the BMP assay with wastewater produced more biogas than the control except for the BW mix. The result shows a steady increase in the production of biogas for the assay with wastewater as compared to the control which had a maximum increase within the first five days, but later had a declined production rate after the 5th day. The reason for the low biogas production for BW mix may be due to inhibition by volatile fatty

acids (Kougias and Angelidaki 2018), because literature has reported favourably high yield for digestion of BW (Arantes *et al*. 2017).

However, it was observed that at 25ºC the production of biogas for the control had a sharp increase from the second day of the experiment to the 5th day after which there was a decline. The reason for the decline could be a drop in the pH of the assay because lower pH has been reported to affect the yield of biogas during the AD process. Likewise, as observed from Figure 4.2(a) the production of DW mix assay was high as compared to SW and BW mix. The yield of biogas from the DW mix in Figure 4.2(a) was 56%, 50% and 42%, higher than the yield for SW mix, control, and BW mix, respectively.

The volume of biogas generated in the decreasing order of SW mix (1615 mL) >DW mix (1155 mL) > control (690 mL) > BW mix (260 mL) and respectively as shown in Figure 4.2(b). From Figure 4.2(b), it was observed that between day 15 and 19, there was a sharp increase in the yield of biogas from all the assays, but minimal increment was observed after this day for all the wastewater mix assay. This is an indication that the AcoD gas production peak for this study was on the 20th day which agrees with Rabii *et al*. (2019).



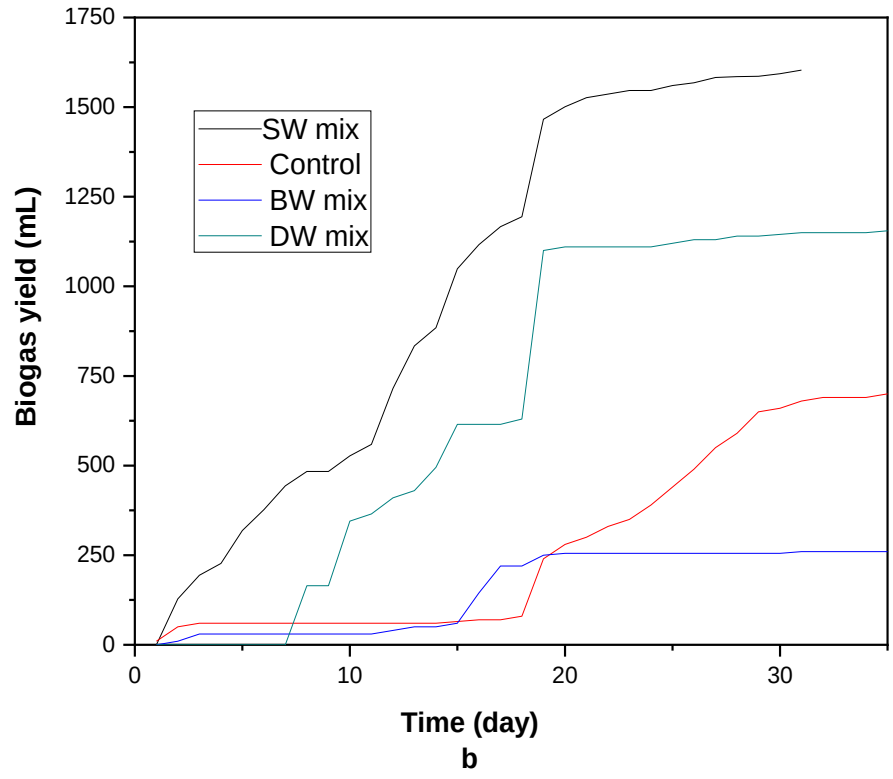


Figure 4. 2: BMP assays cumulative biomethane production at (a) 25°C and (b) 35°C

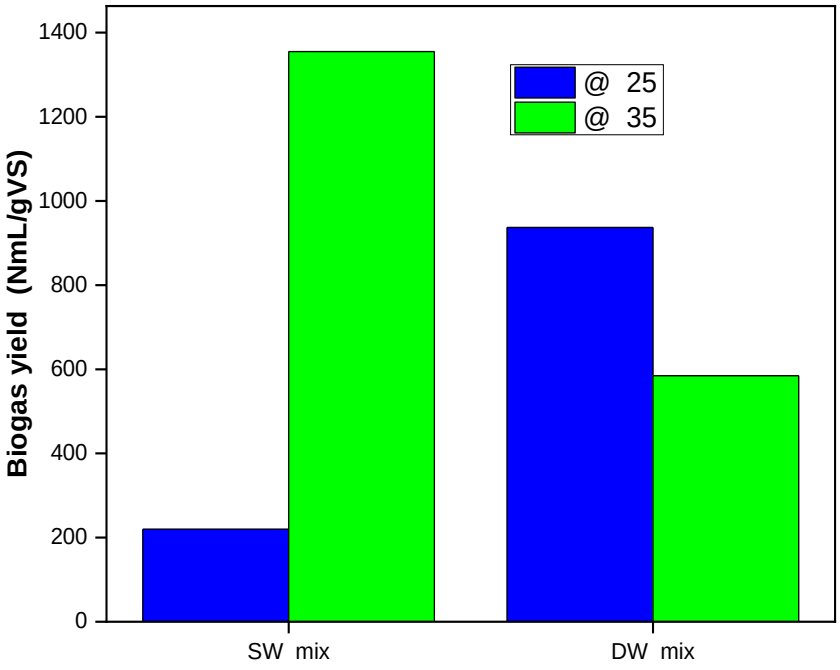


Figure 4. 3: Overall Biomethane Yield

4.3 Anaerobic batch reactors

4.3.1 Effect of SW mix ratio

4.3.1.1 Effect of pH on the AD process

The pH of the substrates at the time of the digestion is one of the major factors that determine the stability of the AD process. The result of the analysis of the pH of the assays before and after the hydraulic retention time indicate the effect of pH on the system. Most of the reactors had both the initial and final pH within the specified range for optimum operation of the AD process as shown in Table 4.2 and Figure 4.4. Five of the reactors had pH above the specified range, the reactors were S1, S7, S9, S12 and S14 with final pH value of 9.68, 10.46, 11.08, 12.41 and 12.83, respectively. The result of the methane production indicates that three (3) of these reactors namely S1, S7 and S12 had the lowest methane production which could be an indication of the pH changes. Likewise, the COD reduction efficiencies of four (4) of these reactors were also the lowest recorded in this study, probable cause may be because of pH change in these reactors (Kougias and Angelidaki 2018, Mao *et al*. 2015).

Table 4. 2: Initial and final pH value of SW mix

Sample	Mix-ratio (SS: SW)	Operating Temperature (°C)	Initial pH	Final pH
S1	1:3	25	6.9	9.68
S2	0:1	25	7.06	7.22
S3	1:1	25	6.67	7.99
S4	1:3	55	6.92	8.75
S5	1:1	35	6.68	8.19
S6	3:1	55	6.76	8.73
S7	1:1	55	6.77	10.46
S8	1:0	25	6.63	7.79
S9	1:1	35	6.66	11.08
S10	0:1	55	7.15	8.82
S11	1:0	35	6.53	8.24
S12	1:0	55	6.51	12.41
S13	0:1	35	7.07	8.45
S14	0:1	35	7.06	12.83
S15	3:1	35	6.62	8.42

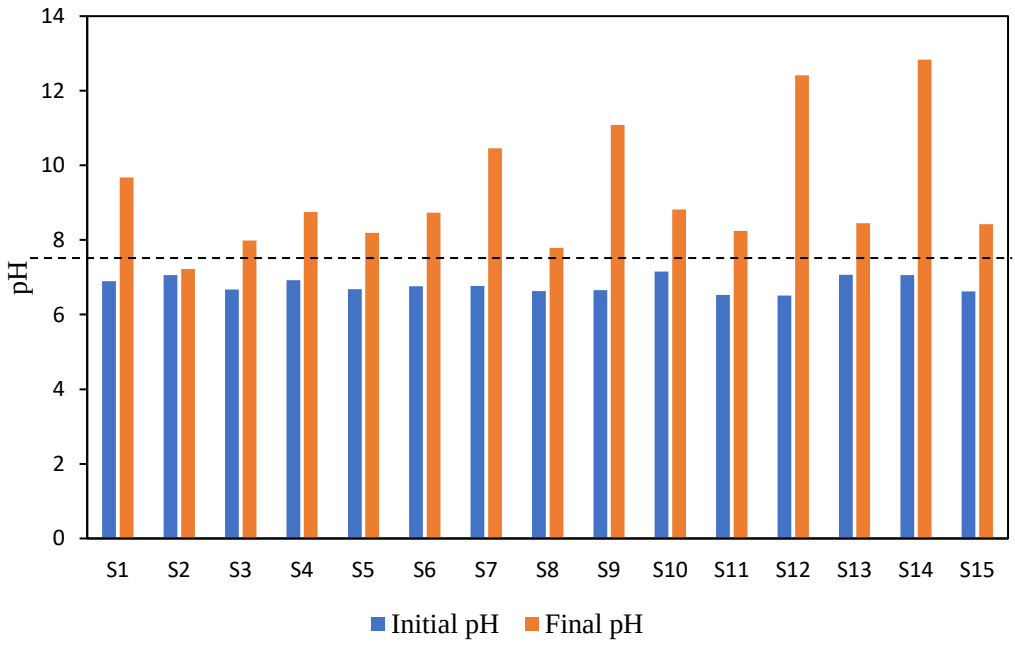


Figure 4. 4: Initial and final pH for SW mix

4.3.1.2 Biomethane production

The first set of the experiments carried out was for screening purposes for the selection of the industrial wastewater with the best biogas yield. The two industrial wastewaters with the best output (i.e. SW and DW) were used in the next phase of experimentation by varying the volume ratio to that of the municipal sludge, to determine the optimum best mix-ratio for the combination of both substrates.

Table 4.3 shows the varying mix-ratio considered in this study, where the result of the varying mix-ratio for SW shows that the assays operated at 35°C had the highest biomethane production overall after 30 days. The highest production of 6857 mL was observed with S5 assay of 1:1 mix operated at 35°C and 875.7 mL/g COD_{added} as shown in Table 4.3. Comparing the result of S5 with that of the assay with sludge only (S11 at 35°C) which had a production of 6811 mL and 869.9 mL/g COD_{added} and wastewater only (S13/S14 at 35°C) with production of 1545mL and 695mL respectively as shown in Table 4.3, it is clearly seen that AcoD had a significant effect over the digestion of individual substrate. The overall effect would be clearly seen in the cost reduction in relation to facilities needed if each substrate were digested separately.

Table 4. 3: Biomethane production for sugar wastewater mix-ratio

Run	Mix-ratio (SS: SW)	Operating Temperature (°C)	Overall Biomethane Production (mL)	Biomethane Production (mL/g COD_a)	Biomethane Production (mL/g CODr)
S1	1:3	25	235.00	30.90	35.98
S2	0:1	25	575.00	74.30	78.4
S3	1:1	25	3662.70	417.50	442.36
S4	1:3	55	1918.19	235.20	262.31
S5	1:1	35	6857.00	875.70	938.21
S6	3:1	55	603.90	78.00	92.27
S7	1:1	55	215.10	27.60	84.29
S8	1:0	25	2636.80	336.80	353.93
S9	1:1	35	2830.00	361.40	1400.99
S10	0:1	55	2409.60	297.70	333.48
S11	1:0	35	6811.00	869.90	960.65
S12	1:0	55	135.70	17.30	-2088.22
S13	0:1	35	1545.00	197.30	215.48
S14	0:1	35	695.00	88.80	367.73
S15	3:1	35	408.00	52.10	62.39

[a] indicate added while [r] indicates removed

For assays operated at 25ºC, a similar trend was shown for the biomethane production as those operated at 35ºC. The assay with equal mix-ratio (S3) had the highest production of 417.5 mL/g COD_a followed by assay with sludge only (S8), then SW only (S2) with 336.8 and 74.3 mL/g COD_a respectively. In contrast to the result observed at 35ºC, biomethane data for assays operated at 55ºC revealed that digestion of wastewater only (S10) had more production of 297.7mL/g COD_a as compared to that of sludge only (S12) and when co-digested (S6 and S7) of 17.3, 78 and 27.6 mL/g COD_a respectively. Overall, assays for the SW mix had lower production at 55ºC as compared to other temperatures considered in this study.

In conclusion, the effect of varying mix-ratio can be seen on the overall biomethane production as observed in the assays at 25ºC and 55ºC, as the result of co-digestion mix-ratio can be predicted from the mono-digestion of each substrate. Higher biomethane production from a specific substrate was reflected in the mix-ratio production also, as seen with S8, S2, S10 and S12 which were mono-digestion of either SS or SW as compared to S1, S4, S7 and S3 when co-digested. For instance, S2 which had a mix-ratio of 0:1 (SS: SW) with a production of 575 mL CH_4 had a resulting effect on S1 (mix- ratio of 1:3) with total production of 235 mL as compared to S3 (equal mix-ratio) of 3662 mL which was due to higher production when sludge only was digested at 25ºC. This same trend was observed for assays at 55ºC. However, the deviation was observed for the assays at 35ºC which had higher production for sludge only but could not be seen when the ratio of sludge was more in the mix-assay as observed for S15.

4.3.1.3 COD reduction efficiency

The resulting effect of varying mix-ratio on COD reduction efficiency is shown in Table 4.4. The results indicated that the highest reduction efficiency was observed at S8 and S2 when each substrate was mono digested with the percentage reduction of 95.15 and 94.76% respectively. Based on the mix-ratio, COD highest removal of 92.08% was achieved for the S5 (1:1) assay at 35ºC. The results indicated that varying mix-ratio does not have much effect on the COD reduction, likewise, there was no correlation between COD reduction and the volume of biogas since COD reduction can sometimes not be a measure for the amount of biogas produced (Arici and Koçar 2015). The S12 assay's final COD value increased thereby having a negative reduction, and this may be due to accumulation of CO_2 and pH below or above the optimum range required for the AD process. However, it was observed that temperature had significant effect on the removal of COD, and assays operated at 25ºC was more effective in COD removal as compared to those at other temperature considered in this study.

Table 4. 4: Percentage COD reduction for SW mix

Sample	Mix-ratio	Temp	Initial	Final	%Reduction
S1	1:3	25	7830	1110	85.82
S2	0:1	25	7830	410	94.76
S3	1:1	25	7830	440	94.38
S4	1:3	55	7830	810	89.66
S5	1:1	35	7830	620	92.08
S6	3:1	55	7830	1210	84.55
S7	1:1	55	7830	5270	32.69
S8	1:0	25	7830	380	95.15
S9	1:1	35	7830	5810	25.80
S10	0:1	55	7830	840	89.27
S11	1:0	35	7830	740	90.55
S12	1:0	55	7830	7895	-0.83
S13	0:1	35	7830	660	91.57
S14	0:1	35	7830	5940	24.14
S15	3:1	35	7830	1290	83.52

4.3.1.4 VS reduction efficiency

The effect of the SW mix-ratio on the VS reduction indicated that the mix ratio does improve the overall VS removal of the co-substrates. There was notable % VS removal increase of 62.5% in the S6 and S15 assay whose mix-ratio is 3:1, but a slight increase in the volume of wastewater does decrease the % VS removal efficiency to 25% when compared to mono-digestion of the sewage sludge only of 50%. Likewise, there was no direct inference of the effect of temperature on the % VS reduction because reduction efficiency varies across temperature ranges as shown in Table 4.5.

Table 4. 5: Percentage VS reduction for SW mix

Sample	Mix-ratio	Temp	VS after(mg/L)	VS before(mg/L)	% Reduction
S1	1:3	25	4000	6000	33.33
S2	0:1	25	3000	6000	50.00
S3	1:1	25	3000	4000	25.00
S4	1:3	55	3000	6000	50.00
S5	1:1	35	3000	4000	25.00
S6	3:1	55	3000	8000	62.50
S7	1:1	55	4000	4000	0.00
S8	1:0	25	4000	8000	50.00
S9	1:1	35	2000	4000	50.00
S10	0:1	55	2000	6000	66.67
S11	1:0	35	3000	8000	62.50
S12	1:0	55	3000	8000	62.50
S13	0:1	35	2000	6000	66.67
S14	0:1	35	3000	6000	50.00
S15	3:1	35	3000	8000	62.50

4.3.2 Effect of DW mix ratio

4.3.2.1 Effect of pH on the AD process

The initial and final pH of the DW mix-ratio assays were also determined to ascertain if pH was a determining factor for its biomethane production and COD reduction efficiency. The results obtained indicate that all the reactors had pH within the specified range for the AD process as shown in Table 4.6. Though it was observed that reactors with high final pH had lower methane production as compared to the other reactors. Therefore, it can be clearly stated that pH is one of the determining factors for methane production and COD reduction in the AD process.

Table 4. 6: Initial and final pH value of DW mix

Sample	Mix-ratio (SS: DW)	Temp (ºC)	Initial pH	Final pH
D1	1:3	25	7.23	7.9
D2	0:1	25	7.05	8.1
D3	1:1	25	6.99	7.88
D4	1:3	55	7.26	8.1
D5	1:1	35	7.03	7.99
D6	3:1	55	7.06	8.23
D7	1:1	55	7.02	8.02
D8	1:0	25	7.04	8
D9	1:1	35	7.14	7.79
D10	0:1	55	7.07	7.89
D11	1:0	35	7.03	8.4
D12	1:0	55	7.03	7.68
D13	0:1	35	7.12	8.1
D14	0:1	35	7.14	8.23
D15	3:1	35	7.1	8.3

4.3.2.2 Biomethane production

Biomethane production data obtained during the DW mix-ratio experiment is depicted in Figure 4.5. The five topmost productions were observed in descending order in D4>D10>D12>D6>D5 with production volume of 1589.3 >1205.5>749.5>633.2>385 mL respectively for 30 days as shown in Table 4.7. The mix-ratio of 1:3 (SS: DW) had the overall highest production indicating that mix-ratio does have effect on the generation of biomethane when varying sludge with dairy wastewater. The above result is followed by D10 (DW only) indicating a higher production for DW at higher temperature and D12 (Sludge only) with 1205.5 and 749.5 mL respectively at 55ºC.

A similar trend was observed for the assays at 35ºC, the highest production of 385 mL biomethane was obtained from equal mix-ratio (D5) followed by the assay with DW only (D13) and sludge only (D11) with 280.3 and 225 mL, respectively. On the other hand, the assays operated at 25ºC were observed to have the highest production for assay with sludge only (D8) followed by the mix assay of 1:3. The order of production in descending order for assay at 25ºC is as follows: D8>D1>D3>D2 with 225, 215,177.5 and 175 mL, respectively. Therefore, addition of more dairy wastewater to sludge does improve the biomethane production rate.

Table 4. 7: Biomethane production for dairy wastewater mix-ratio

Run	Mix-ratio (SS: DW)	Temp (°C)	Overall Biomethane production (mL)	Biomethane Production (mL/g COD_{added})
D1	1:3	25	215	89.6
D2	0:1	25	175	63.9
D3	1:1	25	178	74.9
D4	1:3	55	1589	691
D5	1:1	35	385	162.4
D6	3:1	55	633	236.3
D7	1:1	55	170	71.7
D8	1:0	25	225	102.3
D9	1:1	35	218	91.8
D10	0:1	55	1206	440
D11	1:0	35	225	102.3
D12	1:0	55	749	340.7
D13	0:1	35	280	102.3
D14	0:1	35	123	44.7
D15	3:1	35	118	43.8

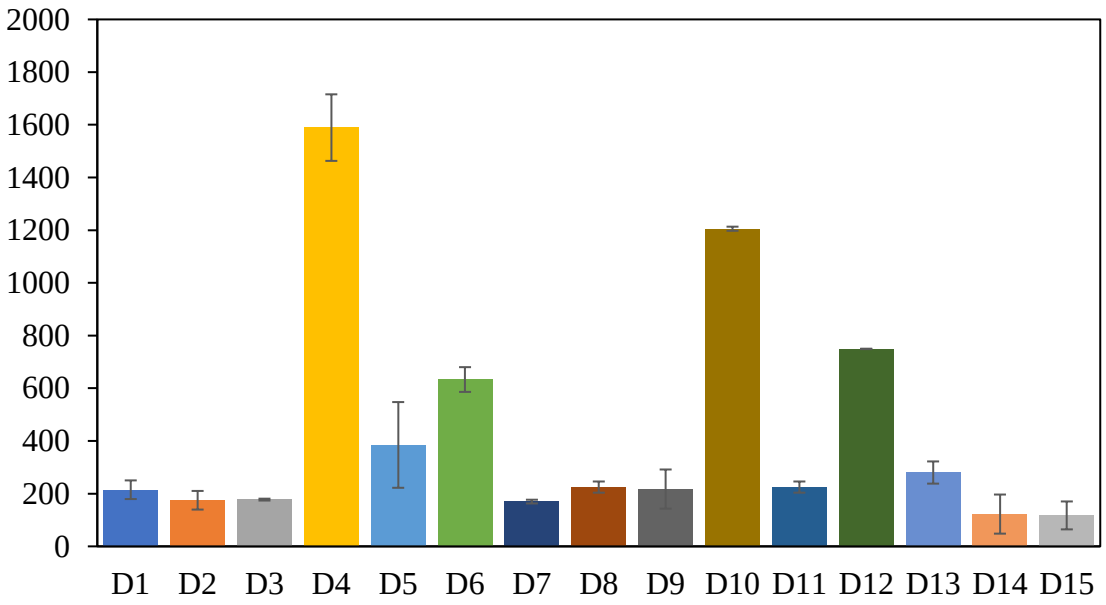


Figure 4. 5: Biomethane production for DW mix

4.3.2.2 COD reduction efficiency

Table 4.8 show the effect of DW mix-ratio in reducing the COD present in each assay. The highest reduction was observed in the assays at 25ºC, this was also observed in the SW mix-ratio and like the SW mix, the assay with sugar only (D2) had the highest reduction efficiency of 88.76%.

Results for 25ºC indicated that varying mix ratio had effect on the reduction efficiency, and an increase in the amount of DW in the mix caused a reduction in the overall COD. The increasing order of COD reduction efficiency is as follows for 25ºC: D8<D3<D1<D2 with efficiencies of 70.41, 83.50, 86.67 and 88.76% respectively. The trend as observed for 25ºC was shown for assays at 35ºC, and assays with DW only had the highest reduction efficiency followed by the assay with equal mix of both substrates, the assay with 3:1 (SS: DW) and then the assay with sludge only. COD reduction efficiencies in increasing order was as follows: D11< D9<D15<D5<D13<D14 with 3.82, 52.32, 56.87, 61.81,76.97 and 83.69%, respectively.

There was a deviation for assays operated at 55ºC because the highest reduction efficiency was observed for the assay with sludge only (D12) with 71.32%, while the lowest was observed for the assay with DW only (-0.44%). COD reduction efficiency for assays operated at 55ºC was differing to those at 25ºC and 35ºC. In conclusion, as observed for SW mix-ratio experiment, the effectiveness of COD reduction does not correlate well with the amount of biomethane generation for the DW mix-ratio as well.

Table 4. 8: Percentage COD reduction for DW mix

Sample	Mix-ratio (SS: DW)	Temp (°C)	Initial	Final	%Reduction
D1	1:3	25	2400	320	86.67
D2	0:1	25	2740	308	88.76
D3	1:1	25	2370	391	83.50
D4	1:3	55	2300	2192	4.70
D5	1:1	35	2370	905	61.81
D6	3:1	55	2680	2239	16.46
D7	1:1	55	2370	2137	9.83
D8	1:0	25	2200	651	70.41
D9	1:1	35	2370	1130	52.32
D10	0:1	55	2740	2752	-0.44
D11	1:0	35	2200	2116	3.82
D12	1:0	55	2200	631	71.32
D13	0:1	35	2740	631	76.97
D14	0:1	35	2740	447	83.69
D15	3:1	35	2680	1156	56.87

4.3.3 Analysis of the SW mix-experiment using Design Expert

This section provides a detailed explanation of the design expert analysis results which include the ANOVA, model generated, response surface plots for factors interaction and the effect of individual on the responses. The design and result obtained from the experiment are shown in Table 4.9, it shows the fractional volume of the substrates used and temperature as explained in Section 3.3.3.1. The methane yield is the volume of methane produced divided by the CODr and the COD reduction as explained in Section 4.3.1.3.

4.3.3.1 Methane yield analysis

Table 4.10 shows the statistical model summary for the generation of the most optimum response surface model for the SW mix-ratio under study. The model with the best R^2 and adjusted R^2 value was chosen, a Cubic * Quadratic model was selected for the yield as indicated in Table 4.11. The model had a standard deviation of 270.99 and adjusted R^2 of 0.8715, this model was the only model with high R^2 value and non-significant lack of fit.

Table 4. 9: Result for biomethane yield and % COD reduction of SW mix

Run	SS	WW	TEMP	Yield (mL/g CODr)	% COD Reduction
S1	0.25	0.75	-1	35.98	85.82
S2	0	1	-1	78.4	94.76
S3	0.5	0.5	-1	442.36	94.38
S4	0.25	0.75	1	262.31	89.66
S5	0.5	0.5	0	938.21	92.08
S6	0.75	0.25	1	92.27	84.55
S7	0.5	0.5	1	84.29	32.69
S8	1	0	-1	353.93	95.15
S9	0.5	0.5	0	1400.99	25.8
S10	0	1	1	333.48	89.27
S11	1	0	0	960.65	90.55
S12	1	0	1	-2088.22	-0.83
S13	0	1	0	215.48	91.57
S14	0	1	0	367.73	24.14
S15	0.75	0.25	0	62.39	83.52

Table 4. 10: Combined model fit summary for methane yield

Mixture Order	Process Order	Mixture p-value	Process p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
M	M						
M	L		0.2922	0.0860	0.0145	-0.2343	
M	Q		0.0881	0.1001	0.1706	-0.0933	
M	C	*	*	0.1001	0.1706	-0.0933	Aliased
L	M	0.4717		0.0821	-0.0332	-0.4189	
L	L	0.1480	0.1142	0.0992	0.1770	-0.8164	
L	Q	0.1009	0.0789	0.1342	0.4280	-0.1563	
L	C	0.1009*	*	0.1342	0.4280	-0.1563	Aliased
Q	M	0.2257		0.0848	0.0146	-0.6204	
Q	L	0.2309	0.1325	0.1069	0.2738	-1.7678	
Q	Q	0.2726	0.1430	0.1423	0.5315	-2.1573	
Q	C	0.2726*	*	0.1423	0.5315	-2.1573	Aliased
C	M	0.8273		0.0770	-0.0701	-0.7535	
C	**L**	**0.8380**	**0.2835**	**0.0815**	**0.1123**	**-2.8556**	**Suggested**
C	**Q**	**0.0826**	**0.0372**	**0.3208**	**0.8715**		**Suggested**
C	C	0.0826*	*	0.3208	0.8715		Aliased

* The combined model is aliased.

Table 4. 11: Fit summary for model order abbreviation

Mean	Linear	Quadratic	Cubic
M	L	Q	C

Mixture	Process
Cubic	**Linear**
Cubic	**Quadratic**

The model generated for the yield was a second order regression model which was later used to develop the response surface. Equation 4.1 shows the reduced cubic and quadratic model for the pseudo components and the coded factor while Equation 4.2 shows the model for the actual parameters (real components/actual factors). The model is useful for identifying the relative impact of each factor/component and their corresponding combination by comparing the coefficients. The equation shows both individual effects of the components, the antagonistic and the synergetic effect of each combination on the response as depicted by the negative and positive signs before each model terms. The term with high impact on the yield as shown in

Equation 4.1 is the A^2BC^2 and shown in Table 4.12 is the standard error of each coefficients and the variance inflation factors (VIFs). All the VIFs are greater than 1 which indicate multi-colinearity, but they are also less than 10 which indicates that the correlation of the factors is tolerable and that the model is reliable.

$$Methane\ yield = 960.65\ A + 291.61\ B + 2173.89\ AB - 1202.02\ AC + 146.59\ BC + 1851.98\ ABC - 1808.74AC^2 - 66.613\ BC^2 - 12145.1\ AB * (A - B) + 582.87\ ABC^2 + 1277.1\ ABC(A - B) + 16418.99\ ABC^2(A - B) \ldots \ldots\ldots (4.1)$$

$$Methane\ yield = 960.65\ Sludge + 291.605\ SW + 2173.89\ Sludge * SW - 1202.023\ Sludge * Temp + 146.593\ SW * Temp + 1851.976\ Sludge * SW * Temp - 1808.743\ Sludge * Temp^2 - 66.613\ SW * Temp^2 - 12145.1\ Sludge * SW * (Sludge - SW) + 582.866\ Sludge * SW * Temp^2 + 1277.1\ Sludge * SW * Temperature * (Sludge - SW) + 16418.987\ Sludge * SW * Temp^2 * (Sludge - SW) \ldots \ldots\ldots (4.2)$$

Table 4. 12: Coefficients in terms of coded factors for methane yield

Component	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
A-Sludge	960.65	1	270.99	98.23	1823.07	5.25
B-WW	291.61	1	191.62	-318.22	901.43	3.13
AB	2173.89	1	1013.96	-1052.98	5400.76	5.47
AC	-1202.02	1	190.93	-1809.66	-594.38	1.58
BC	146.59	1	190.93	-461.05	754.23	1.83
ABC	1851.98	1	881.10	-952.06	4656.02	2.44
AC^2	-1808.74	1	331.50	-2863.73	-753.76	4.77
BC^2	-66.61	1	270.51	-927.49	794.26	3.67
AB(A-B)	-12145.10	1	3456.25	-23144.44	-1145.76	5.72
ABC^2	582.87	1	1343.30	-3692.10	4857.83	5.66
ABC(A-B)	1277.10	1	2212.64	-5764.51	8318.71	1.76
ABC^2(A-B)	16418.99	1	4103.83	3358.75	29479.22	6.05

The Analysis of variance for the methane model is shown in Table 4.13. A p-value of 0.0439 which is less than 0.05 indicates the significance of the model to predict the experiments accurately (Design Expert 2005). The decomposition of the sum of squares of the model terms that make up the model indicate the individual significance base on the p-value. AC, AC^2, AB(A-B) and ABC^2(A-B) had p-values less than 0.05 has shown in Table 4.13. Likewise, the actual R^2 value of 0.9725 indicate a good correlation exists between the experimental values and the predicted values.

Table 4. 13: Methane yield response ANOVA for cubic x quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	Comments
Model	7,780E+06	11	7.073E+05	9.63	0.0439	significant
(1)Linear Mixture	3.245E+05	1	3.245E+05	4.42	0.1263	
AB	3.376E+05	1	3.376E+05	4.60	0.1214	
AC	2.911E+06	1	2.911E+06	39.63	0.0081	
BC	43287.79	1	43287.79	0.5895	0.4985	
ABC	3.244E+05	1	3.244E+05	4.42	0.1263	
AC^2	2.186E+06	1	2.186E+06	29.77	0.0121	
BC^2	4453.16	1	4453.16	0.0606	0.8214	
AB(A-B)	9.068E+05	1	9.068E+05	12.35	0.0391	
ABC^2	13826.32	1	13826.32	0.1883	0.6937	
ABC(A-B)	24464.77	1	24464.77	0.3331	0.6043	
ABC^2(A-B)	1.176E+06	1	1.176E+06	16.01	0.0280	
Residual	2.203E+05	3	73436.59			
Lack of Fit	1.016E+05	1	1.016E+05	1.71	0.3208	not significant
Pure Error	1.187E+05	2	59336.35			
Cor Total	8.001E+06	14				

The predicted versus actual value is shown in Figure 4.6(a) which shows the degree of alignment of the data points while the normal probability plot indicate equal distribution of the data point as shown in Figure 4.6 (b) which is make a good validity of the model.

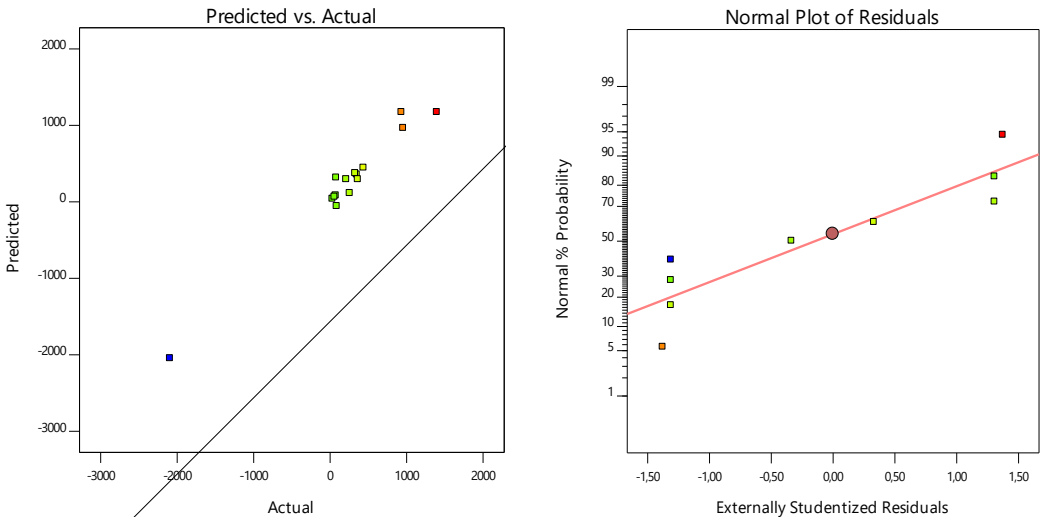


Figure 4. 6: Predicted vs Actual plot (left) and The Normal Probability plot (right) of Methane Yield for SW mix

4.3.3.2 COD reduction analysis

The result obtained for the COD reduction is discussed below in detail. Table 4.14 shows the combined fit summary models and the suggested one for the generation of the COD reduction response of the SW mix. Two models were suggested as the best namely Mean*Linear model whose adjusted R^2 value was 0.0946 and p-value greater than 0.05. The second combined model suggested was Quadratic*Quadratic model with adjusted R^2 value of -0.1924 and p-values of 0.9382 and 0.5829 for mixture and process, respectively. The p-values of both models were greater than 0.05 which indicate that both models would not be significant (Design Expert 2005).

Table 4. 14: Combined model fit summary for COD reduction

Mixture Order	Process Order	Mixture p-value	Process p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
M	M						
M	**L**		**0.1406**	**0.9090**	**0.0946**	**-0.0970**	**Suggested**
M	Q		0.6524	0.8923	0.0363	-0.2461	
M	C	*	*	0.8923	0.0363	-0.2461	Aliased
L	M	0.6286		0.8623	-0.0570	-0.3858	
L	L	0.3821	0.1632	0.9144	0.1016	-0.3285	
L	Q	0.2676	0.3109	0.9367	0.1531	-0.3198	
L	C	0.2676*	*	0.9367	0.1531	-0.3198	Aliased
Q	M	0.9405		0.8353	-0.1445	-0.7075	
Q	L	0.9101	0.3458	0.8600	-0.0752	-1.4419	
Q	**Q**	**0.9382**	**0.5829**	**0.8247**	**-0.1924**	**-2.0474**	**Suggested**
Q	C	0.9382*	*	0.8247	-0.1924	-2.0474	Aliased
C	M	0.9473		0.8029	-0.2480	-0.8360	
C	L	0.8894	0.5529	0.7699	-0.3369	-2.9594	
C	Q	0.9456	0.8356	0.4067	-1.1392		
C	C	0.9456*	*	0.4067	-1.1392		Aliased

* The combined model is aliased.

ANOVA for COD reduction

The ANOVA summary for COD reduction is shown in Table 4.15, which indicate the interaction and individual sum of squares and p-value of each term and their corresponding effect on the model and lack of fit value. The lack of fit obtained was non-significant though the model was not significant, this gives an indication that the model cannot predict effectively the COD reduction efficiency of the SW mix and that the design space is not well managed with all terms in the model having a p-value greater than 0.05. The model's F-value of 0.47 implies the model is not significant relative to noise and that there is an 84.67% chance due to noise for the large F-value obtained. The model shown in Table 4.15 is a reduced Cubic * Cubic model based on adjustment of some terms in the model tab in Design Expert.

The fit statistics as shown in Table 4.16 reveal a lower R^2 value of 0.5415 and adjusted R^2 value of -0.6047 for the reduced Cubic *Cubic model. There is a big difference between the actual R^2, the adjusted R^2 and the predicted R^2 value (-11.0114), which indicate why the model could not be able to navigate the design space appropriately. Likewise, the adequacy precision which measure the signal to noise ratio with value of 2.53 indicates an inadequate signal.

Table 4. 15: ANOVA for COD reduction

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	8148.44	10	814.84	0.4724	0.8467	not significant
[(1)]Linear Mixture	278.85	1	278.85	0.1617	0.7082	
AB	280.29	1	280.29	0.1625	0.7075	
AC	4086.56	1	4086.56	2.37	0.1986	
BC	0.1030	1	0.1030	0.0001	0.9942	
ABC	135.58	1	135.58	0.0786	0.7931	
AC^2	1156.14	1	1156.14	0.6703	0.4589	
BC^2	1401.40	1	1401.40	0.8125	0.4184	
AB(A-B)	457.87	1	457.87	0.2655	0.6336	
ABC^2	375.11	1	375.11	0.2175	0.6652	
AC^3	0.0000	0				
BC^3	0.0000	0				
ABC(A-B)	63.69	1	63.69	0.0369	0.8570	
Residual	6899.33	4	1724.83			
Lack of Fit	2429.41	2	1214.70	0.5435	0.6479	not significant
Pure Error	4469.92	2	2234.96			
Cor Total	15047.77	14				

[(1)] Inference for linear mixtures uses Type I sums of squares.

Table 4. 16: Statistics fit summary for COD reduction

Std. Dev.	41.53	**R^2**	0.5415	**C.V. %**	58.05	**Predicted R^2**	-11.0114
Mean	71.54	**Adjusted R^2**	-0.6047			**Adeq Precision**	2.5316

Table 4.17 shows the coefficient of the terms in coded factors and the standard error and the terms that were not included in the final model but aliased since they have no effect on the model. The VIF were all above 1 which indicate multi-colinearity occurrence though it is still tolerable since none of the VIFs value was greater than 10.

The coded factors equation is shown in Equation 4.3 while the actual components/ factors equation could not be generated because it is aliased. The equation indicates a positive effect of most terms on the model apart from AB, AC and AC^2 whose coefficient indicate a negative effect on the response. The final equation is also a second order regression model.

$$COD\ reduction = 90.23A + 57.91B - 61.79AB - 45.02AC + 0.226\,BC + 37.51\,ABC - 40.05\,AC^2 + 36.92\,BC^2 + 147.14\,AB(A-B) + 95.94\,ABC^2 + 63.06\,ABC(A-B) \quad \ldots\ldots (4.3)$$

Table 4. 17: Coefficients in terms of coded factors for COD reduction

Component	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
A-Sludge	90.23	1	40.06	-20.98	201.45	4.88
B-SW	57.91	1	29.31	-23.47	139.29	3.11
AB	-61.79	1	153.27	-487.34	363.77	5.32
AC	-45.02	1	29.25	-126.22	36.19	1.58
BC	0.2260	1	29.25	-80.98	81.43	1.83
ABC	37.51	1	133.79	-333.95	408.97	2.39
AC^2	-40.05	1	48.92	-175.87	95.77	4.42
BC^2	36.92	1	40.96	-76.80	150.64	3.59
AB(A-B)	147.14	1	285.59	-645.78	940.07	1.66
ABC^2	95.94	1	205.74	-475.28	667.16	5.66
AC^3	ALIASED					
BC^3	ALIASED					
ABC(A-B)	63.06	1	328.19	-848.14	974.27	1.65

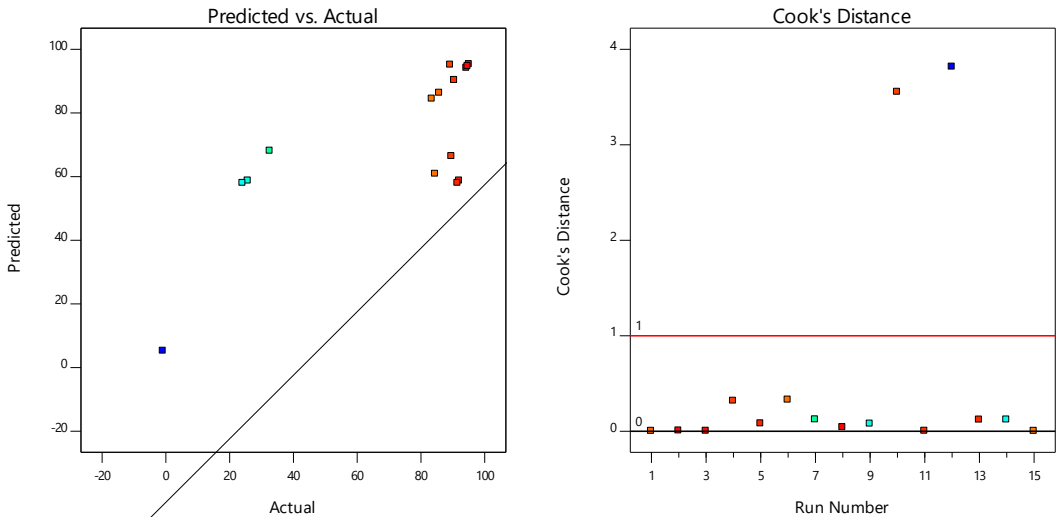


Figure 4. 7: Predicted vs Actual plot (left) and The Cook's distance plot (right) for COD reduction of SW mix

Figure 4.7 (a) and (b) show the predicted versus actual value plot and the cook distance, the data points on Figure 4.7 (a) were scattered which shows why the R^2 value was low. The cook distance figure indicated that two data points from run 10 and 12 exceeded the limit which make it difficult for the model to accurately fit the experiment under study.

4.3.3.3 Optimisation of SW mix assay

Optimisation of the responses were carried using the Numerical optimisation section of Design Expert. The optimisation objective was set to maximise the methane yield while accommodating the lower limit of -2088.22 mL/g COD and upper limit of 1400.99 mL/g COD while other factors were set in range.

The optimisation result that were selected was three (3) optimal solution with the following desirability of 0.965, 0.738 and 0.665 as shown in Figure 4.8, 4.9 and 4.10 respectively. The result with the highest methane yield and COD reduction was the one with desirability of 0.965 though it does not accommodate the SW in its solution. It has a methane yield of 1160.35 mL/g COD with optimal conditions of 100% sludge and 33.25ºC as the operating temperature. The second solution with desirability of 0.738 had a methane yield of 698.64 mL/g COD, COD reduction of 100% with optimal conditions of 68% sludge, 32% SW and 25ºC as the operating temperature. The second solution had accommodation of about 30% SW for its solution. The third had both the lowest yield of 81.37 mL/g COD and COD reduction of 64.17% as shown

in Figure 4.10. From Figure 4.10, it can be deduced that temperature of 55°C had minimal effect based on this study on both the methane yield and COD reduction when sludge and SW are co-digested.

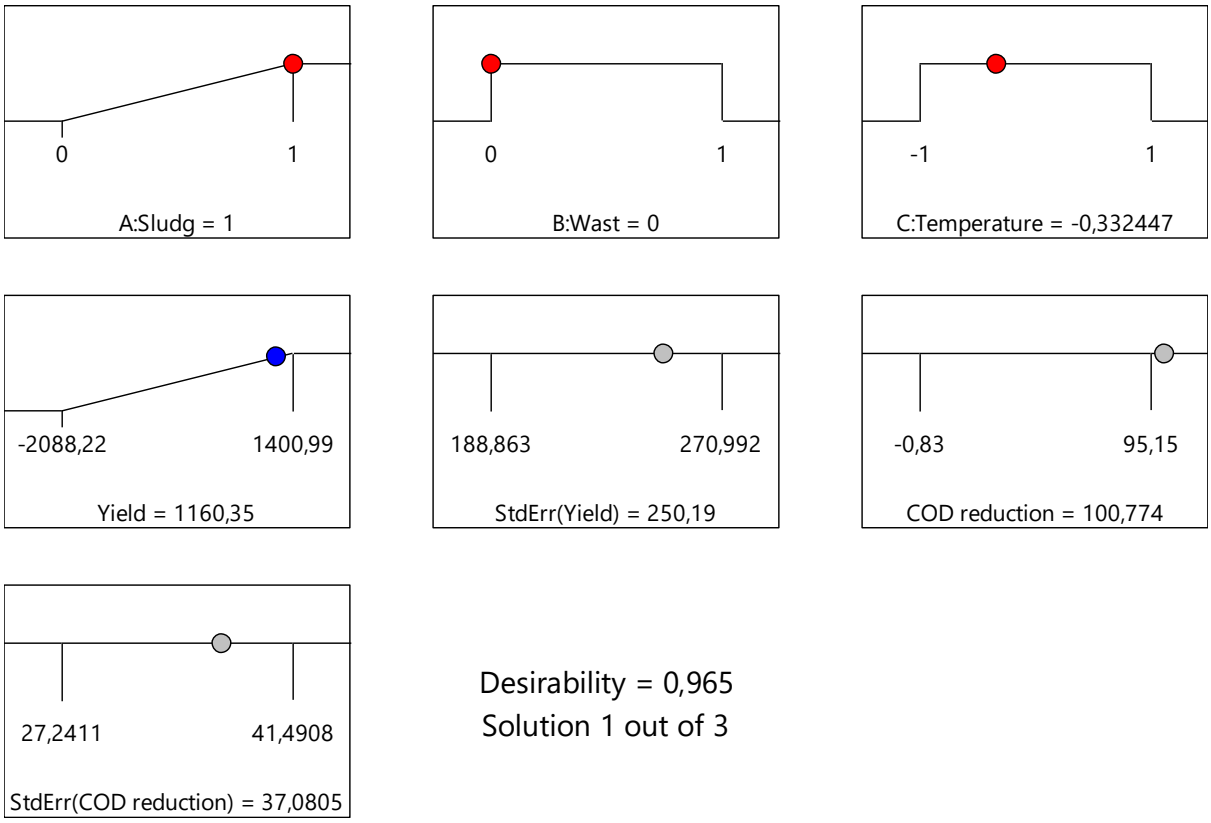


Figure 4. 8: Ramp plot for optimum solution with 96.5% desirability

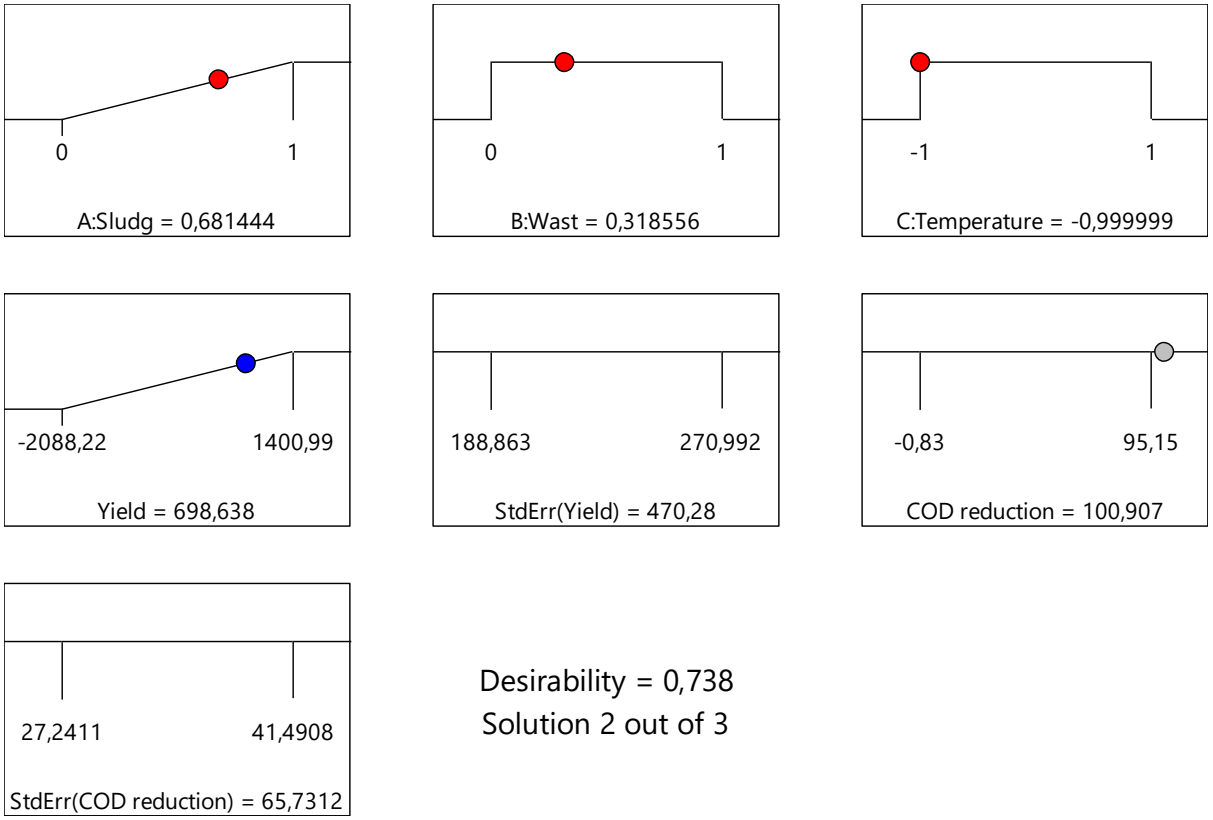


Figure 4. 9: Ramp plot for optimum solution with 73.8% desirability

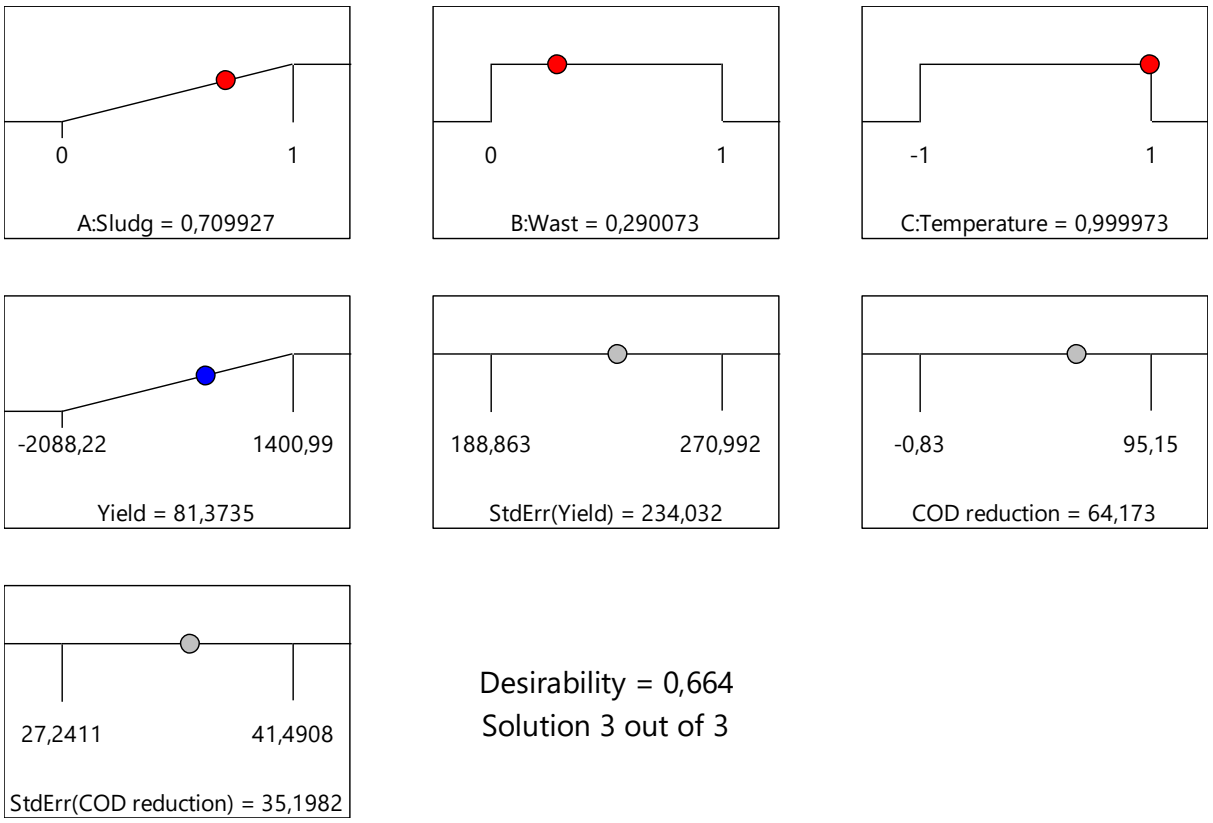


Figure 4. 10: Ramp plot for optimum solution with 66.4% desirability

To accommodate the use of SW as co-substrate in this study, the optimisation parameter was later adjusted to maximisation of both the methane yield and the SW. The result obtained is shown in Figure 4.11, all four (4) solutions had a desirability of 0.628, with the best result selected to give a methane yield of 1400.99 mL/g COD and COD reduction of 54.33% with optimum conditions of 35ºC temperature, 46% Sludge and 54% SW. This solution was chosen and was incorporated in the ISR ratio experiment by using almost equal volume of the substrates for any given ISRs.

The response surface showing the interaction effect of the component/factors is shown in Figure 4.12, 4.13, 4.14 and 4.15 for desirability of 0.965, 0.738, 0.664 and 0.628, respectively. The rest of the response surface plots are shown in Appendix D.

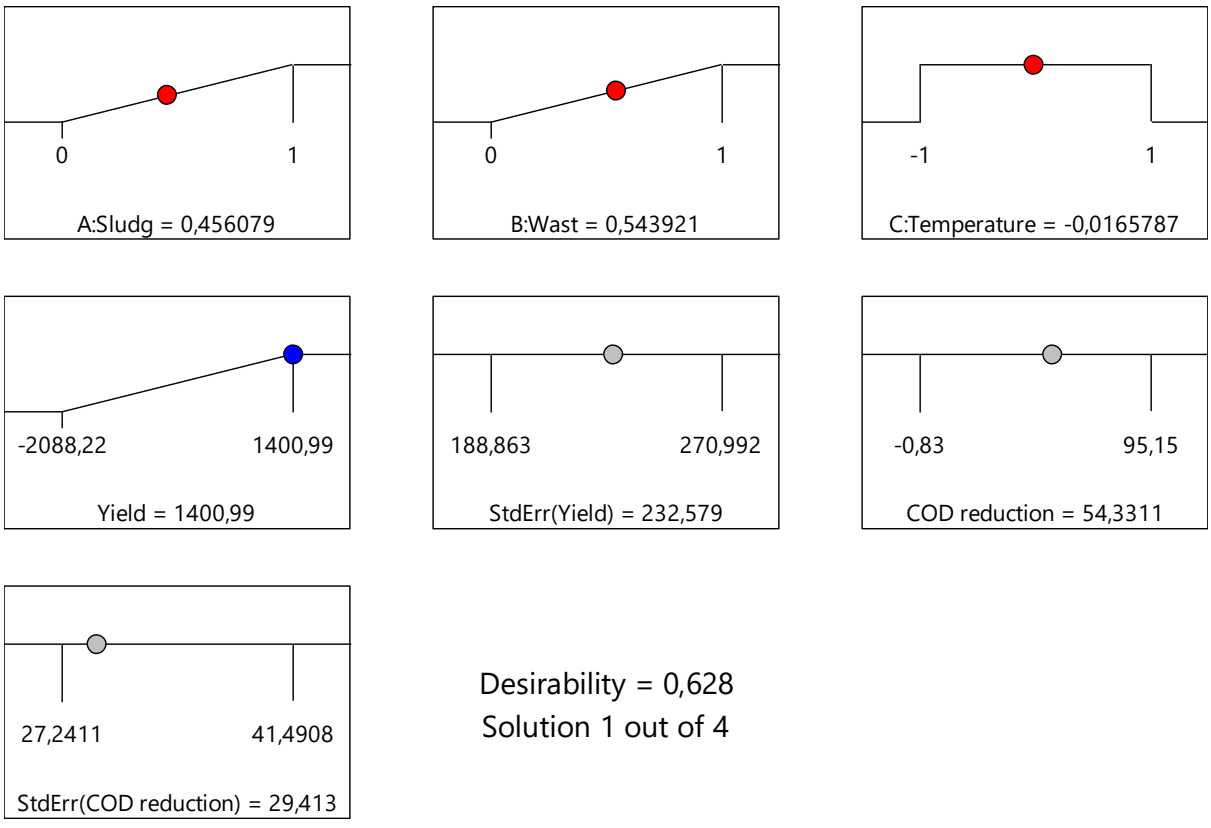


Figure 4. 11: Ramp plot for optimum solution for maximization of methane yield and SW

Design-Expert® Software
Component Coding: Actual
Factor Coding: Actual

All Responses
Design Points
0 1

X1 = A: Sludg
X2 = B: Wast
X3 = C: Temperature

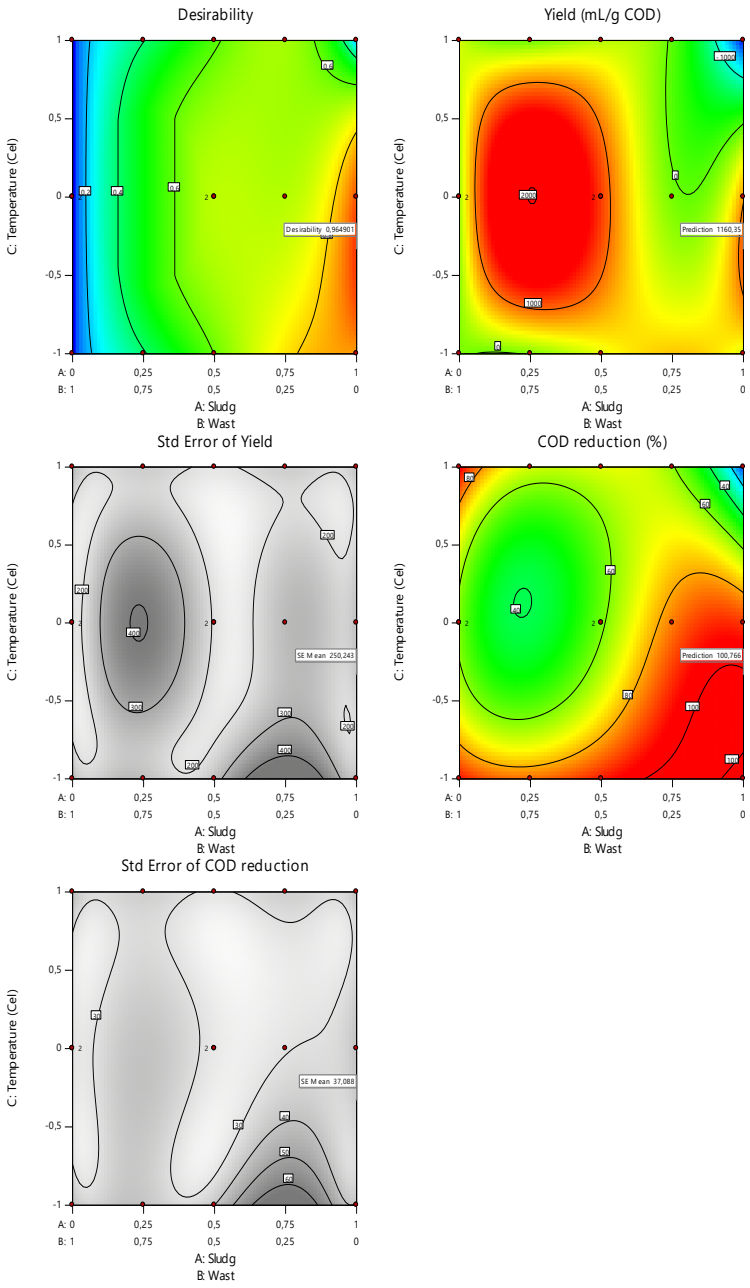


Figure 4. 12: Contour plot for optimum solution with 96.5% desirability

Design-Expert® Software
Component Coding: Actual
Factor Coding: Actual

All Responses
Design Points
0 1

X1 = A: Sludg
X2 = B: Wast
X3 = C: Temperature

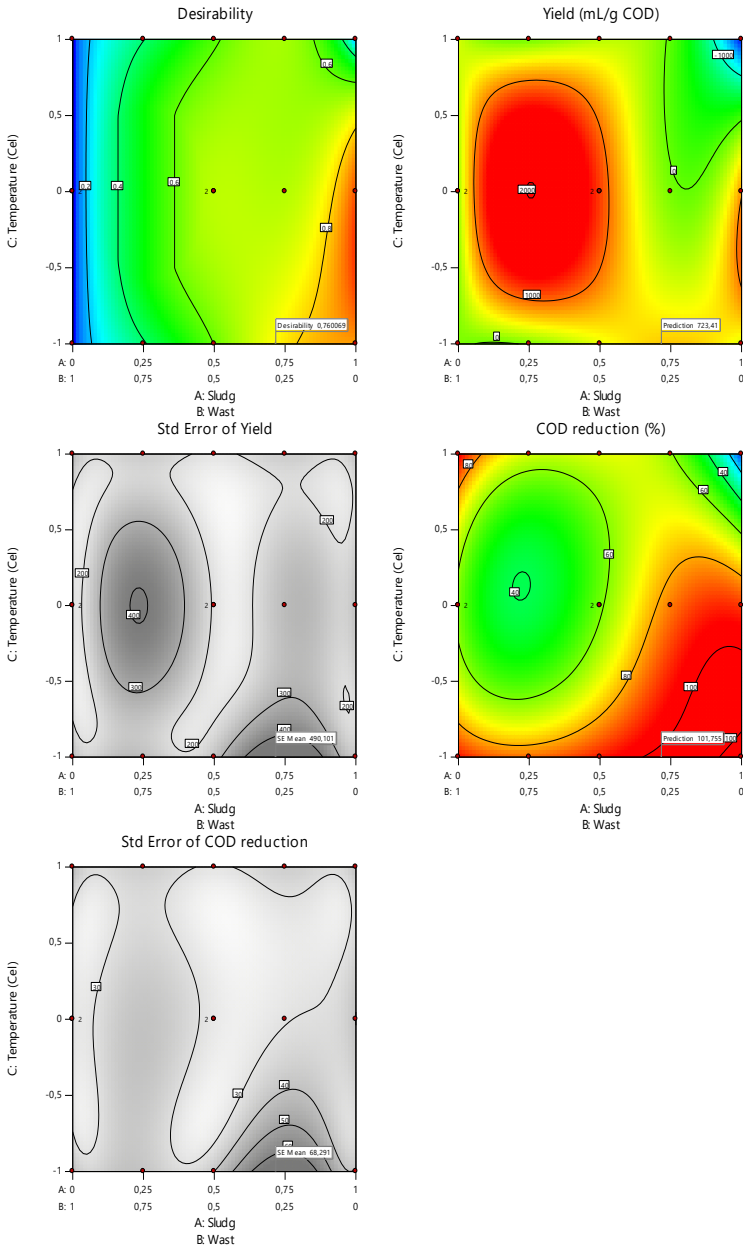


Figure 4. 13: Contour plot for optimum solution with 76.3% desirability

Design-Expert® Software
Component Coding: Actual
Factor Coding: Actual

All Responses
Design Points
0 1

X1 = A: Sludg
X2 = B: Wast
X3 = C: Temperature

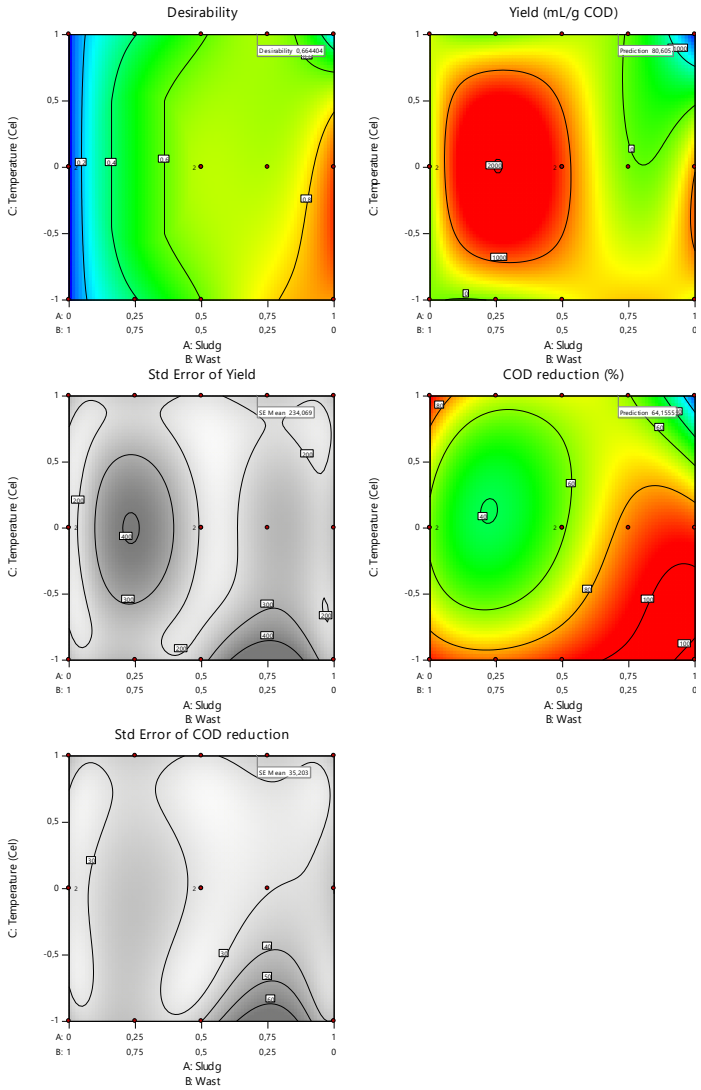


Figure 4. 14: Contour plot for optimum solution with 66.4% desirability

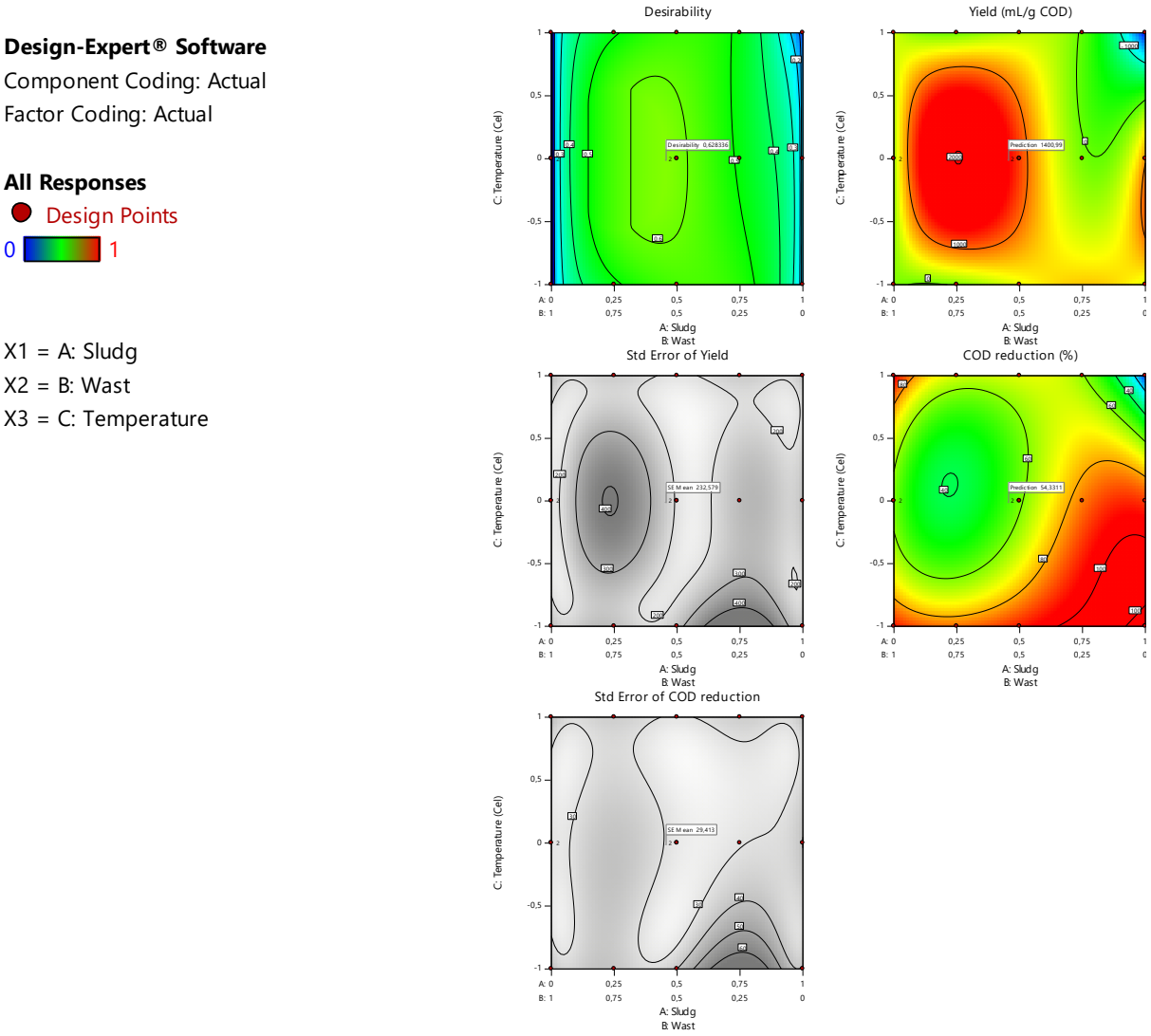


Figure 4. 15: Contour plot for optimum solution for maximization of methane yield and SW

4.3.4 Effect of inoculum-substrate ratio (ISR)

4.3.4.1 Biomethane production

In order to ascertain if various ISRs will affect the yield of biomethane, three ISRs were considered which were 1:2, 1:1 and 2:1. Table 4.18 shows the result of the daily biomethane production for each reactor, where the subscript 25 and 35 indicate the temperatures at which each assay was operated at. The number 12, 11 and 21 indicate the ISR contained by each assay, while D and S indicate the assay with dairy and sugar wastewater, respectively.

Table 4.18 shows that the assays with dairy wastewater had their maximum production mainly within the first seven (7) days of the setup with exception of some assays operated at 35ºC,

while little production was observed in the remaining days. The above trend was not observed in assays with sugar wastewater as there was a steady daily production for most of the assay operated at both temperatures. The highest daily production of biomethane for all the assays was observed at 25^{o}C for DW mix with ISR of 2:1 as show in Table 4.18. This agrees with Pellera and Gidarakos (2016) and Nazaitulshila et al. (2015) studies on winery and juice industry and fat, oil and grease waste streams respectively, whose optimum methane production was observed at substrate to inoculum ratio (SIR) of 0.5 or ISR of 2 (VS basis).

The result reveals that ISR does affect the overall biomethane production as seen in Figure 4.14. The overall result indicated that both wastewaters performed best at 25^{o}C as opposed to the result obtained during the preliminary runs where SW mix had a maximum biomethane production at 35^{o}C. SW mix with ISR of 1:2 had the highest production of 1088.7mL biomethane while the DWmix with ISR of 2:1 had the second highest overall production of 969mL. Generally, the SW mix assay performed better as compared to the DWmix as seen in Figure 4.16. The progression from highest to lowest is $S12_{25}$>$D21_{25}$>$S11_{25}$>$S11_{35}$>$D12_{35}$>$S12_{35}$>$S21_{35}$>$S21_{25}$>$D21_{35}$>$D12_{25}$>$D11_{25}$ with production rate of 40.32, 35.88, 34.48, 30.05, 28.86, 26.33, 21.18, 14.09, 12.52, 2.96 and 1.22 mL/day, respectively.

The result obtained was in agreement with Yoon et al. (2014) and Ma et al. (2019), whose study indicated that SIR of 1.5 and 2 which is commensurate to ISR of 0.75 and 0.5 had the highest methane generation when they worked with piggery slaughterhouse wastes and rape straw co-digested with dairy manure respectively. Though this was in contrast with Nazaitulshila et al. (2015) whose work on fat, oil and grease revealed less than 60% methane production at SIR of 2 - 4.

4.3.4.2 Gas characterisation

The biogas produced from the ISR experiment was passed through the water as opposed to the initial experiments which were passed through NaOH solution to absorb the CO_2 produced during the AD process. To determine the biogas content, gas analysis was carried out using gas chromatography (Shimadzu GC-2014). The first characterisation was done in the first week of the experiment and results indicated that most of the assay had mainly biomethane only after passing through water. Table 4.19 shows the result of the characterisation for the gas collected at tip of the reactor for the first and third week of the experiment while Figure 4.17 - 4.19 shows the chromatograph of $S12_{25}$, $S11_{35}$ and $D12_{35}$ for first and third week respectively.

Four assays had 100% methane production during the first week namely $D11_{25}$, $D21_{25}$, $S21_{25}$ and $S21_{35}$, while $S11_{35}$, $D12_{35}$, $S12_{35}$, $S11_{25}$, $D11_{35}$, $D21_{35}$ and $D12_{25}$ had methane content of 94.12%, 88.08%, 73.61%, 46.87%, 46.63%, 44.74% and 44.27% respectively as shown in Table 4.19. The lowest methane content was observed with $S12_{25}$ (42.29%) while the rest is CO_2 though it had the highest overall methane production.

On the other hand, during the third week of the experiment, the assay with the highest methane content was $S12_{25}$ (45.45%) while $D21_{35}$ had no methane generation again with 100% CO_2. Overall, the assays with ISR of 1:2 for both sugar and dairy had more methane content as compared to other ISRs for both temperatures at the third week as shown in Table 4.19. This similar trend can also be seen in the overall production of methane as discussed in Section 4.3.4.1.

Likewise, the best co-substrate was sugar wastewater as seen in this study with both the highest methane content and volume generated.

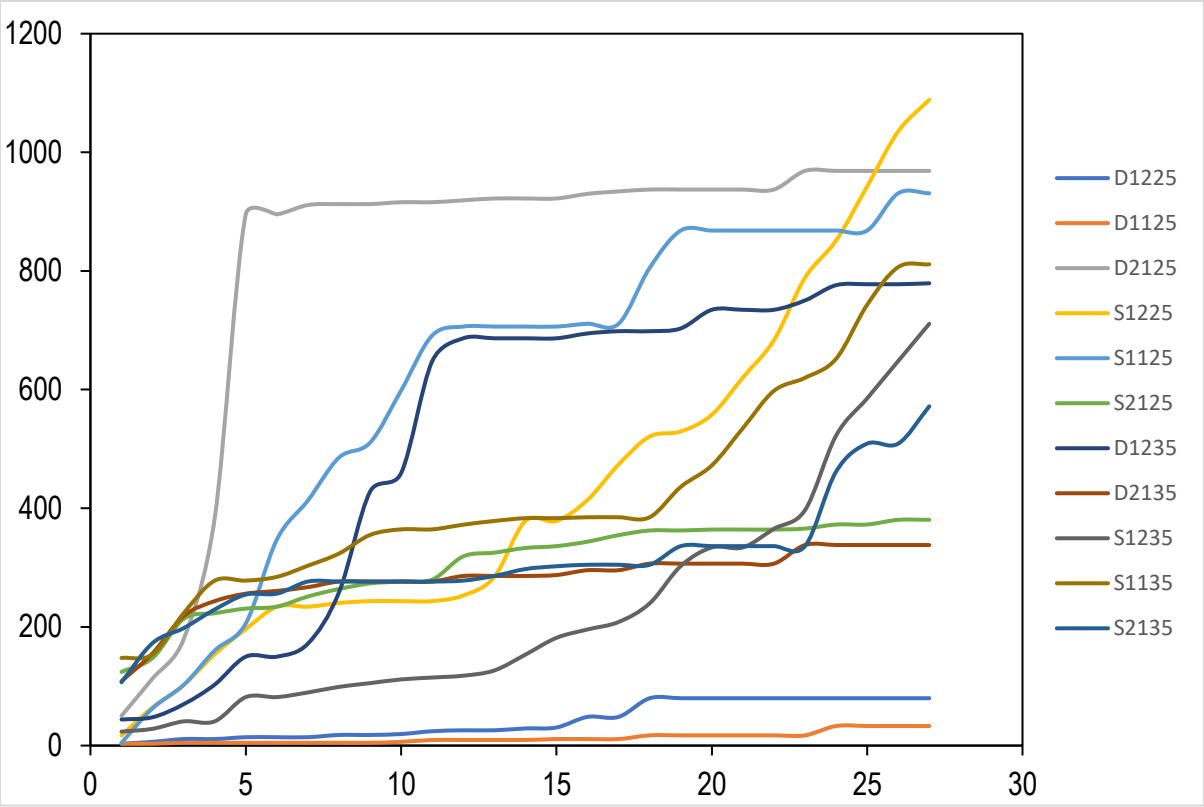


Figure 4. 16: Cumulative biomethane production for varying ISR assay

Table 4. 18: Daily production of biomethane using various ISR

Sample	$D12_{25}$	$D11_{25}$	$D21_{25}$	$S12_{25}$	$S11_{25}$	$S21_{25}$	$D12_{35}$	$D21_{35}$	$S12_{35}$	$S11_{35}$	$S21_{35}$
pH	7.23	7.05	6.99	7.26	7.03	7.06	7.02	7.04	7.14	7.07	7.03
Day1	3	3	50	17	5	124	44	108	24	148	107
Day2	3	0	63	47	58	24	4	47	5	6	66
Day3	5	2	66	38	39	66	22	63	13	69	25
Day4	0	0	204	52	58	9	33	25	0	55	31
Day5	3	0	512	42	46	8	47	13	41	0	25
Day6	0	0	0	38	141	3	0	5	0	6	2
Day7	0	0	16	0	66	17	22	6	8	19	20
Day8	4	0	2	6	72	13	86	9	9	20	0
Day9	0	0	0	3	25	9	170	0	6	31	0
Day10	2	2	3	0	88	3	31	0	6	9	0
Day11	5	3	0	0	93	3	189	0	3	0	0
Day12	2	0	3	9	15	39	39	9	3	8	2
Day13	0	0	3	31	0	6	0	0	9	6	8
Day14	3	0	0	94	0	8	0	0	27	5	12
Day15	2	2	0	0	0	3	0	2	28	0	5
Day16	18	0	8	36	5	8	8	8	14	2	3
Day17	0	0	4	60	0	11	4	0	13	0	0
Day18	31	6	3	47	94	8	0	11	31	0	0
Day19	0	0	0	8	63	0	5	0	63	51	31
Day20	0	0	0	28	0	2	31	0	31	36	0
Day21	0	0	0	63	0	0	0	0	0	63	0
Day22	0	0	0	63	0	0	0	0	31	63	0
Day23	0	0	31	105	0	2	16	31	31	22	0
Day24	0	16	0	63	0	7	26	0	126	32	126
Day25	0	0	0	91	0	0	2	0	63	91	47
Day26	0	0	0	93	63	8	0	0	63	63	0
Day27	0	0	0	53	0	0	2	0	63	5	63

Table 4. 19: Gas Characterisation for ISR Experiment

Sample	First Week		Third Week	
	@ 25ºC			
	% CH_4	%CO_2	% CH_4	% CO_2
D12	44.272	55.728	15.740	84.260
D11	100	0	6.029	93.971
D21	100	0	11.217	88.783
S12	42.292	57.708	45.447	54.553
S11	46.871	53.129	16.782	83.218
S21	100	0	7.813	92.187
	@ 35ºC			
D12	88.077	11.923	39.328	60.672
D11	46.626	53.374	**	**
D21	44.744	55.256	0	100
S12	73.606	26.394	35.429	64.571
S11	94.12	5.88	39.630	60.370
S21	100	0	7.945	92.055

** indicate missing due to technical error

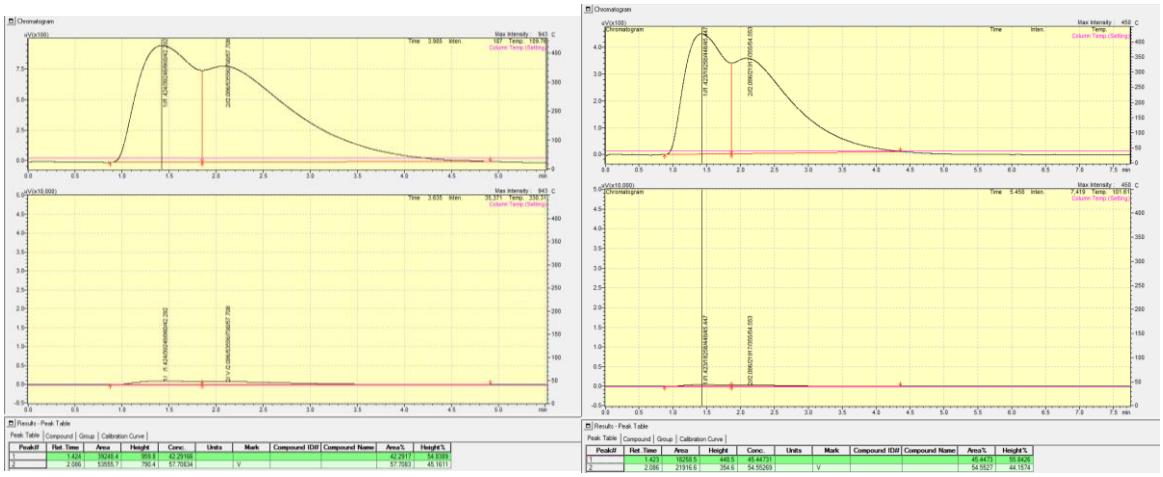


Figure 4.17: Chromatograph for $S12_{25}$ first week(left) and third week (right)

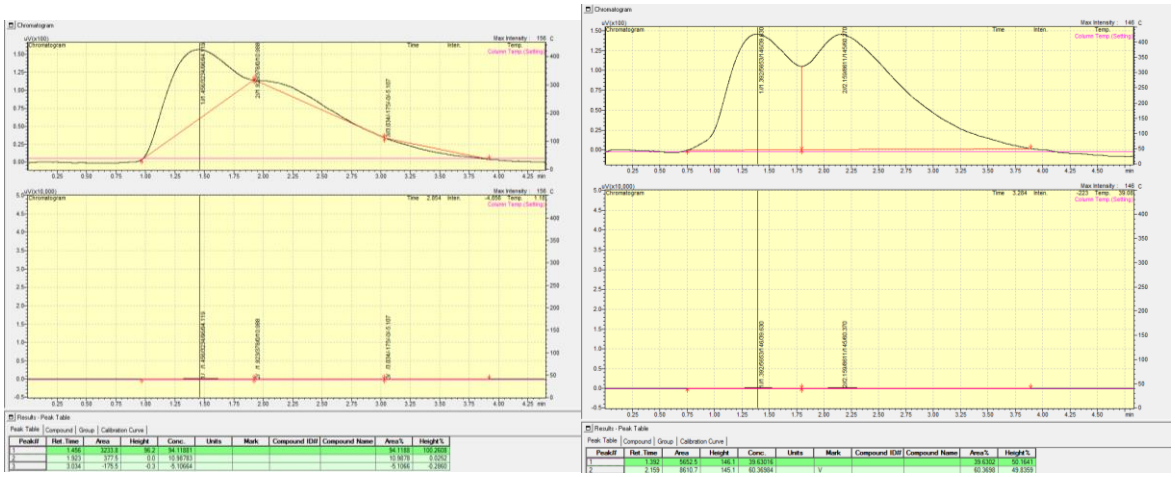


Figure 4.18: Chromatograph for $S11_{35}$ first week(left) and third week (right)

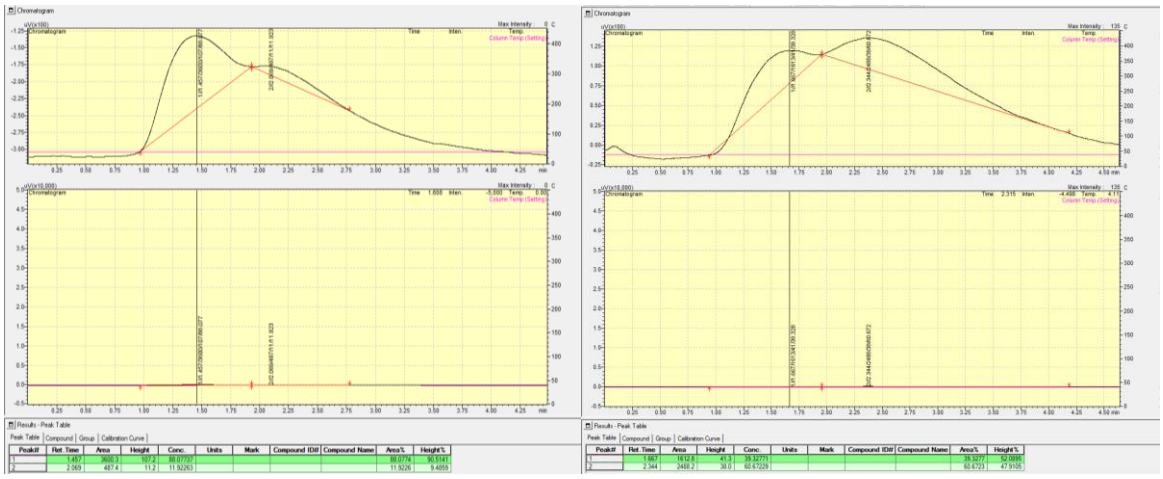


Figure 4.19: Chromatograph for $D12_{35}$ first week(left) and third week (right)

4.3.3.3 COD reduction for ISR

The assay with the highest methane production i.e. ISR of 1:2 with sugar wastewater operated at 25^{o}C was further analysed for COD reduction potential. The sample was run in duplicate and the average result as shown in Table 4.20 was plotted in Figure 4.20. The result obtained indicate decrease in the COD concentration for the first 8 hours, then an increase after 10 h before further decrease was observed. This is an indication that steady decrease in the concentration of COD occur during the AcoD of SW with sewage sludge, therefore, the potential of COD reduction is feasible during co-digestion of sewage sludge with sugar wastewater. The overall COD reduction within the 12 hours of experimentation was 60% which was obtained from the initial and final COD values.

Table 4.20: COD reduction for ISR for SW

Sample / Time(hours)	0	2	4	6	8	10	12
S1	2550	1020	820	1020	970	1220	890
S2	1850	1070	840	970	880	960	870
Ave	2200	1045	830	995	925	1090	880

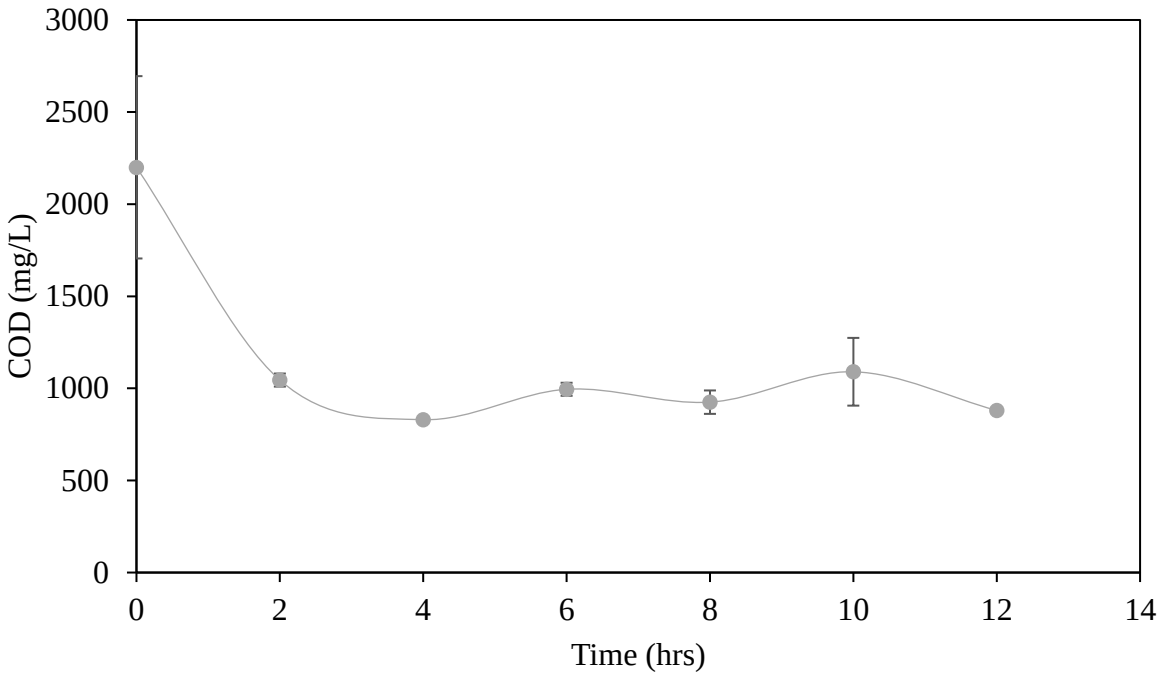


Figure 4. 17: COD reduction for ISR for SW mix

4. 4 Kinetics analysis

The data shown in Table 4.21 is the predicted values for each model considered whereas Figure 4.21 shows the plots of the measured values during the experiments for the kinetics analysis evaluations and the four kinetics models that were fitted to the measured values. The first-order kinetic model was the best fit for this experiment with an R-squared value of 0.9962697 followed by dual-pooled model (R^2 = 0.9962660) whereas Chen and Hashimoto model and Gompertz model had an R^2 value of 0.995222 and 0.9878497 respectively. The dual-pooled model result was close to that of the first-order model since dual-pooled was a derivation from first order with fractional consideration of two different substrates. The results validate the

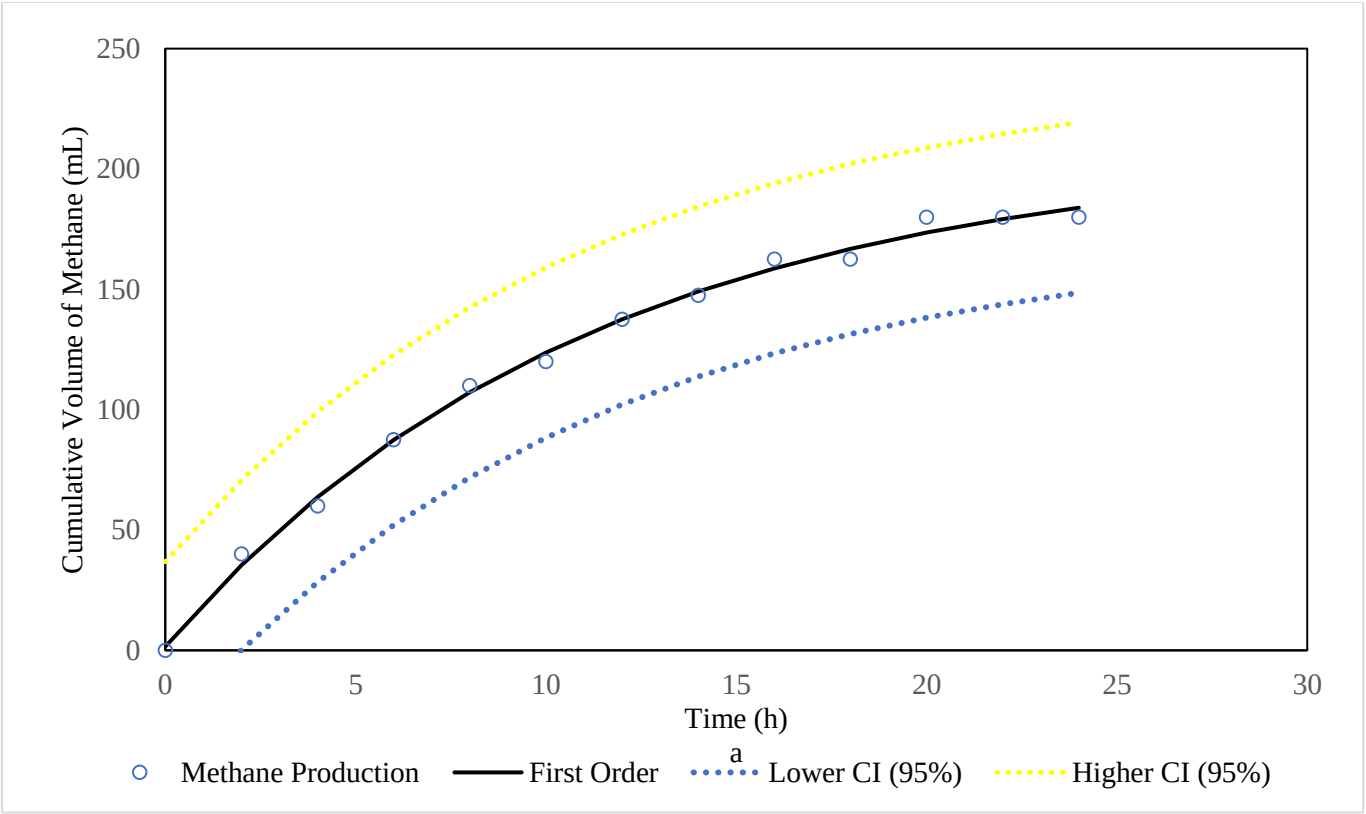
assertion of Xie et al. (2016) whose work state that first order, dual-pooled first order and Gompertz model have been used to simulate the production of methane for co-digestion system. Figure 4.21(a) shows the first order model prediction and the experimental values with high and low confidence interval of 95%. The data for the plot of the confidence interval is shown in Table C.3 in Appendix C.

The rate of methane production as modelled by Excel and OriginPro software (results are shown in Figures C.2 to C.4) had similar values for all parameters in the modified Gompertz model when approximated. The maximum methane (Mm) production for the Gompertz model (184.27mL) was quite close to that of the experimental value (180mL). Although it had a lower coefficient of determination as compared to other models considered in this study, it is still a good fit. The maximum methane production obtained for the first order model was 206.58 mL and 0.092 as the hydrolysis constant. The coefficient of determination and other parameters of the models are as presented in Table 4.21.

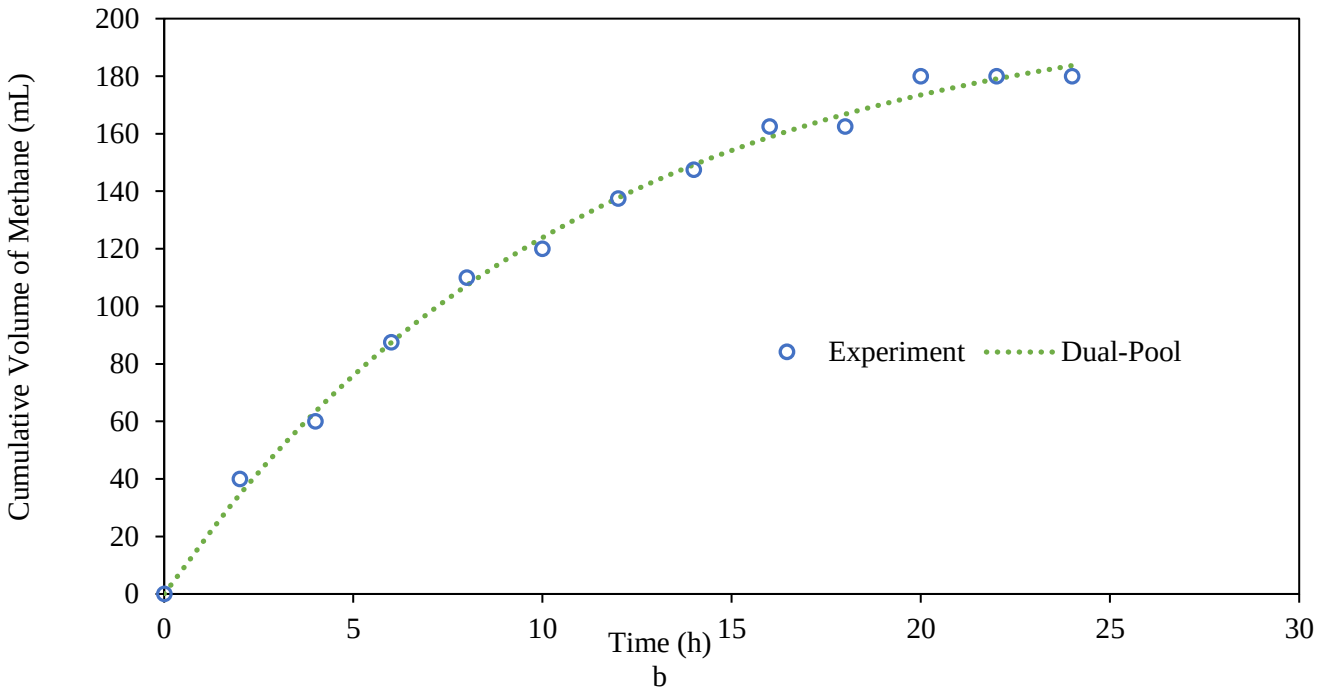
In conclusion, the use of kinetic models can be used as preliminary assessment for the AcoD of a given substrate for sewage sludge as seen in Table 4.20, the models closely predict the methane production rate and overall methane production of the experimental process (Xie et al., 2016).

Table 4.21: Measured and predicted values for each kinetic model

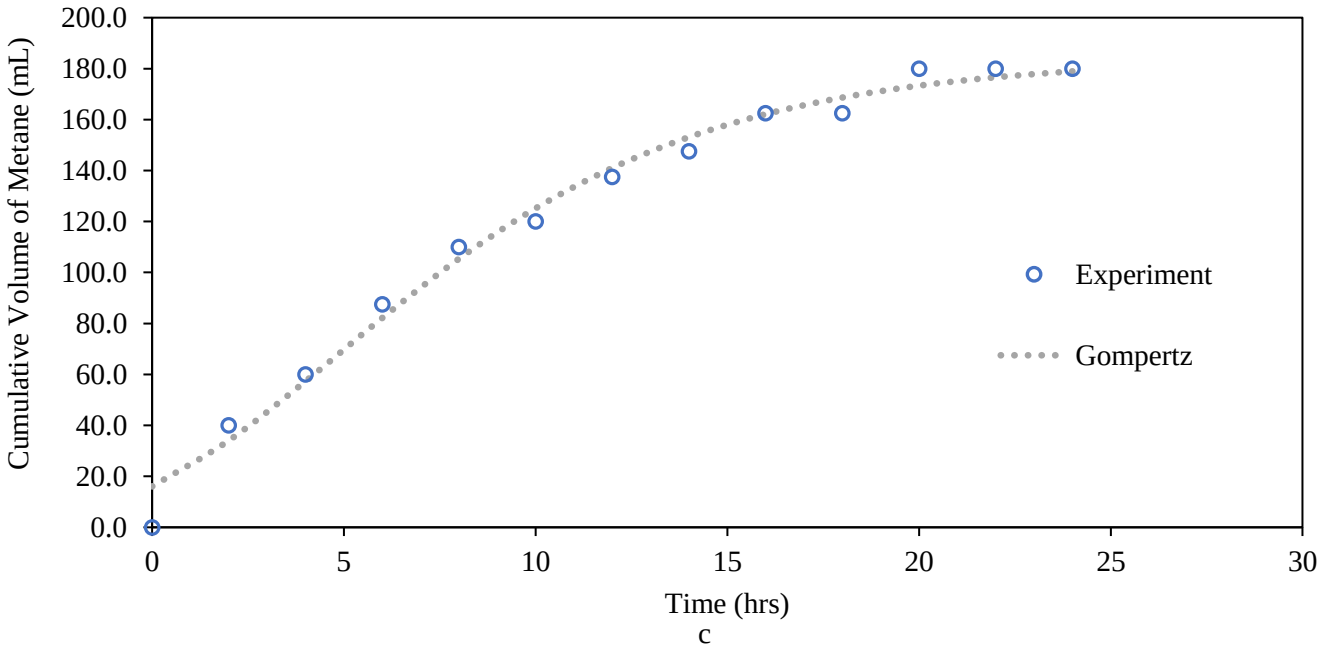
Time (Hours)	Methane Production	First Order	Gompertz	Dual-Pool	Chen and Hashimoto
0	0.0	0	16.05207	0	-0.01043
2	40.0	34.57028	34.05493	34.62818	38.61674
4	60.0	63.35529	57.3008	63.41747	67.86717
6	87.5	87.32319	82.12921	87.36315	90.78562
8	110.0	107.2801	105.3547	107.289	109.2273
10	120.0	123.8973	125.1636	123.877	124.3873
12	137.5	137.7336	141.0076	137.6923	137.0699
14	147.5	149.2544	153.1278	149.2031	147.8366
16	162.5	158.8473	162.1172	158.798	157.091
18	162.5	166.8348	168.6431	166.799	165.1308
20	180.0	173.4856	173.311	173.4736	172.1805
22	180.0	179.0234	176.6157	179.0438	178.4122
24	180.0	183.6345	178.9388	183.6943	183.9606


Cumulative Volume of Methane (mL)
250
200
150
100
50
0
0
5
10
15
20
25
30
Time (h)
Methane Production
First Order
Lower CI (95%)
Higher CI (95%)

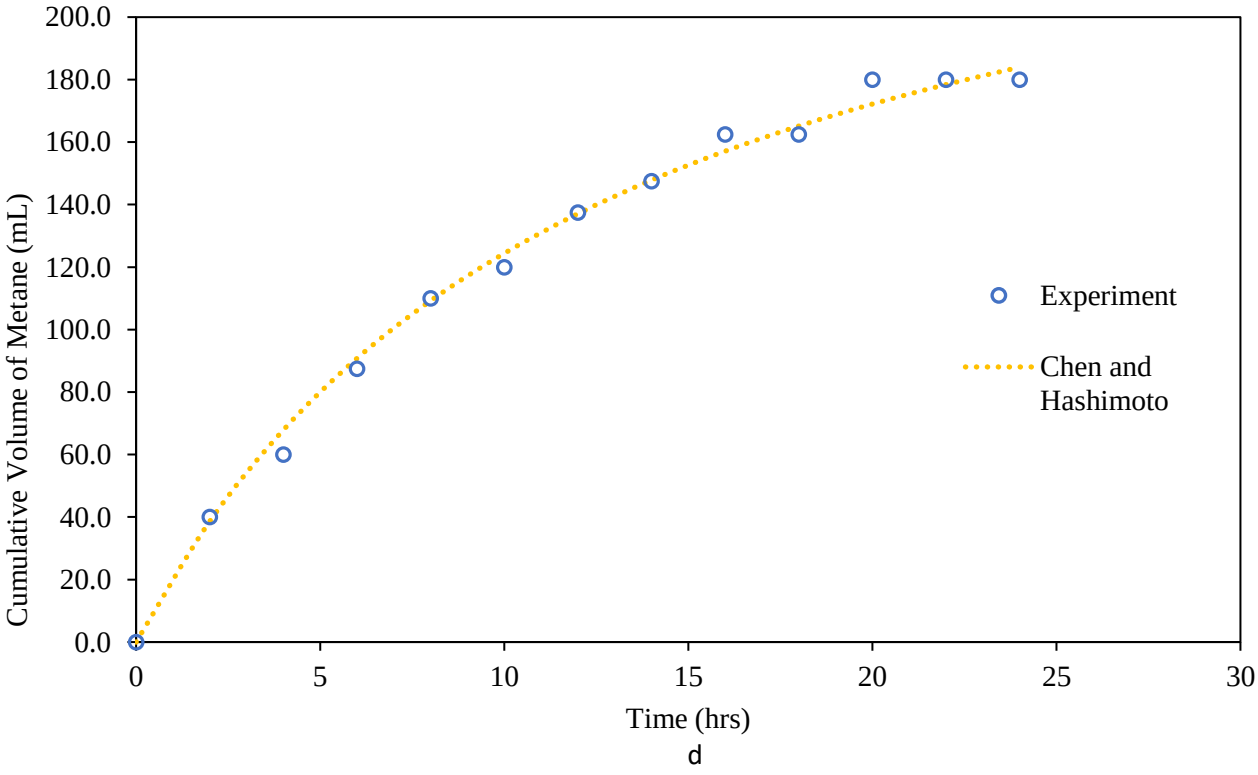
a


Cumulative Volume of Methane (mL)
200
180
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (h)
Experiment
Dual-Pool

b



c



d

Figure 4. 218: Kinetics Models Fitting for Cumulative Biomethane Production (a) First Order Kinetic Model (b) Dual-pooled First Order Model (c) Simplified Gompertz Model (d) Chen and Hashimoto Kinetic Model

Table 4. 21: Computed value for the parameters of the kinetic models

	MS Excel				OriginPro		
Model	First Order	Dual-pooled First Order	Chen and Hashimoto	Modified Gompertz	First Order	Dual-pooled First Order	Modified Gompertz
R^2	0.996270	0.996266	0.99522	0.98785	0.9963	0.9963	0.98489
Mm (mL)	206.58	207.50	279.61	184.27	207.88± 6.214	207.89 ±34.724	184.27
k or k_f (day^{-1})	0.0920	0.1067	**	0.1842	0.090	0.0896	0.1842
k_L(day^{-1})	**	0.0845	**	**	**	0.0896	**
α	**	0.312	**	**	**	2.0158	**
λ (day)	**	**	**	4.843	**	**	4.843
K_{CH}	**	**	26815.63	**	**	**	**
μ_m(h^{-1})	**	**	2148.97	**	**	**	**

** denote "parameters not applicable to the model"

In conclusion, from the study it has been established that wastewater from agro- based industries can enhance the yield of biomethane and help in COD reduction as well. Table 4.22 show the summary of the study as compared to some other studies where sewage sludge was co-digested with other waste streams.

Table 4.22: Summary of findings

	This Study	**Wickham et al. (2016)**	**(Zahan et al., 2016)**
Substrates	SS and SW	SS and Liquid waste	SS and Food Waste
Yield (mL CH4/g VS)	1354.8	94	284 - 327
% methane increases over mono-digestion	134.0	NSP	221
COD reduction (%)	93.1	102	59

NSP – not specified

CHAPTER 5

5.1 Conclusion

This study considered the use of three (3) industrial wastewaters namely; brewery, dairy and sugar wastewater as co-substrates for anaerobic digestion of sewage sludge. The characteristics of these agro-industrial base wastewaters indicate their suitability for the AD process in that they supply the necessary nutrients needed for methanogens to work on. Preliminary analysis was used for selection of the best wastewater, which was further used for analysing the effect of mix-ratio of the substrates on the biomethane yield and percentage COD reduction. Lastly, inoculum to substrate ratio analysis was carried out to ascertain its effects on biomethane production and then, the results were fitted to existing anaerobic kinetics models.
The following are the conclusions that can be drawn from this study:

1. Preliminary results indicated that addition of wastewaters as co-substrate to sewage sludge increases the volume of biomethane produced as compared to digestion of sewage sludge only. The results obtained show an increase of 134% and 67% increase when sugar and dairy wastewater were added as co-substrates over the control (sludge only).
2. The pH of the system was quite stable throughout the duration of the experiment. The deviations observed in some of the bioreactors with increase in pH clearly indicate that pH increase above optimum range of 6.0 – 8.5 could hinder the AD process and ultimately reduce biomethane production.
3. The effect of temperature was clearly observed to have a direct proportionality to the overall yield of methane production during the mix-ratio experiment, but inverse proportionality to the reduction of the COD content as observed for both SW mix and DW mix experiment.
4. Sugar wastewater was the most suitable co-substrate for sewage sludge because the yield of methane and COD reduction percentage was higher when compared to that of dairy wastewater for both the mix-ratio and ISRs experiment. Additionally, an equal mix of sewage sludge and wastewater gave a higher yield and COD reduction at mesophilic temperature as generated in the design expert optimum result.
5. The methane yield model generated for the SW mix, shown below, closely predicted the experimental yield.

$$Methane\ yield = 960.65\ Sludge + 291.605\ SW + 2173.89\ Sludge * SW - 1202.023\ Sludge * Temp + 146.593\ SW * Temp + 1851.976\ Sludge * SW * Temp - 1808.743\ Sludge * Temp^2\ - 66.613\ SW * Temp^2 - 12145.1\ Sludge * SW * (Sludge - SW) + 582.866\ Sludge * SW * Temp^2 + 1277.1\ Sludge * SW * Temperature * (Sludge - SW) + 16418.987\ Sludge * SW * Temp^2 * (Sludge - SW) \ldots \ldots (4.2)$$

6. The DW ISR experimental data indicate that inoculum to substrate ratio of 2 :1 operated at 25ºC performed better than other ISRs considered in this study.
7. The ISR of 1:2 was performed better than other ISRs for SW as co-substrate also operated at 25ºC. Kinetic study indicates that three (3) of the four (4) models were easily fitted for the SW, the first order and dual-pooled first order were the models that correctly fitted the AcoD process with R^2 value of close to unity (1).
8. In conclusion, agro-industrial based industrial wastewater with high COD shows promise as efficient co-substrates for improved biomethane yield as well as COD reduction efficiency.

5.2 Recommendations for future work

The following are the recommendations observed in this study for further research;

❖ Continuous system of the study is recommended for further studies: In the present study, a batch process experiment was conducted. This case study may not be applicable on an industrial scale level. Therefore, the use of a continuous system is recommended for industrial application purposes.

❖ Intermittent operation at two different temperatures should be considered to ascertain it overall biomethane production: During this present study, it was noted that fluctuation between two set of temperature (i.e. 25ºC and 35ºC) did increases the amount of biogas production. This was an observation; it is necessary to confirm if it does improve the overall biomethane production for an AcoD process.

❖ Economic evaluation of the whole process is required for better understanding of the cost implication for generating heat, energy efficiency of the methane produced and the estimation of the return on investment.

REFERENCES

Alatriste-Mondragón, F., Samar, P., Cox, H. H., Ahring, B. K. and Iranpour, R. 2006. Anaerobic codigestion of municipal, farm, and industrial organic wastes: a survey of recent literature. *Water Environment Research,* 78**:** 607-636.

Ali Shah, F., Mahmood, Q., Maroof Shah, M., Pervez, A. and Ahmad Asad, S. 2014. Microbial ecology of anaerobic digesters: the key players of anaerobiosis. *The Scientific World Journal*, *2014*.

Angelidaki, I. and Ahring, B. 1993. Thermophilic anaerobic digestion of livestock waste: the effect of ammonia. *Applied Microbiology and biotechnology,* 38**:** 560-564.

APHA, A. P. H. A. 2005. Water Environment Federation (APHA-AWWA-WEF). 2005. *Standard Methods for the Examination of Water and Wastewater, 21st ed. Alexandria, Virginia: Water Environment Federation*.

Appels, L., Baeyens, J., Degrève, J. And Dewil, R. 2008. Principles andand potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science,* 34**:** 755-781.

Appels, L., Lauwers, J., Degrève, J., Helsen, L., Lievens, B., Willems, K., Van Impe, J. And Dewil, R. 2011. Anaerobic digestion in global bio-energy production: potential and research challenges. *Renewable and Sustainable Energy Reviews,* 15**:** 4295-4301.

Arantes, M. K., Alves, H. J., Sequinel, R. And Da Silva, E. A. 2017. Treatment of brewery wastewater and its use for biological production of methane and hydrogen. *International Journal of Hydrogen Energy,* 42**:** 26243-26256.

Arici, Ş. And Koçar, G. 2015. The Effect of Adding Maize Silage as a Co-Substrate for Anaerobic Animal Manure Digestion. *International Journal of Green Energy,* 12**:** 453-460.

Asadollahfardi, G., Taklify, A. and Ghanbari, A. 2012. Application of artificial neural network to predict TDS in Talkheh Rud River. *Journal of Irrigation and Drainage Engineering*, *138*(4):363-370.

Bachmann, N., la Cour Jansen, J., Bochmann, G. and Montpart, N. 2015. *Sustainable biogas production in municipal wastewater treatment plants*. Massongex, Switzerland: IEA Bioenergy.

Banu J, R., Yukesh Kannah, R., Kavitha, S. 2017. BMP A Tool for Assessing Energy Efficient Biogas Production. *Advances in Biotechnology and Microbiology* 5(3): 1-5.

Bechmann, A., Lomborg, S. 2013. Model Diagnostics for Regression. *New Media and Society*.

Buitrón, G., Kumar, G., Martinez-Arce, A. and Moreno, G. 2014. Hydrogen and methane production via a two-stage processes (H2-SBR+ CH4-UASB) using tequila vinasses. *international journal of hydrogen energy*, *39*(33):19249-19255.

Chen, H., Chang, S., Guo, Q., Hong, Y. And Wu, P. 2016. Brewery wastewater treatment using an anaerobic membrane bioreactor. *Biochemical Engineering Journal,* 105**:** 321-331.

Cieślik, B. M., Namieśnik, J. And Konieczka, P. 2015. Review of sewage sludge management: standards, regulations and analytical methods. *Journal of Cleaner Production,* 90**:** 1-15.

Contois, D. 1959. Kinetics of bacterial growth: relationship between population density and specific growth rate of continuous cultures. *Microbiology,* 21**:** 40-50.

Dai, X., Duan, N., Dong, B. And Dai, L. 2013. High-solids anaerobic co-digestion of sewage sludge and food waste in comparison with mono digestions: Stability and performance. *Waste Management,* 33: 308-316.

Dai, X., Hu, C., Zhang, D., Dai, L. And Duan, N. 2017. Impact Of A High Ammonia-ammonium-pH system on methane-producing archaea and sulfate-reducing bacteria in mesophilic anaerobic digestion. *Bioresource Technology,* 245**:** 598-605.

Dai, X., Li, X., Zhang, D., Chen, Y. And Dai, L. 2016. Simultaneous enhancement of methane production and methane content in biogas from waste activated sludge and perennial ryegrass anaerobic co-digestion: The effects of pH and C/N ratio. *Bioresource Technology,* 216**:** 323-330.

Dennehy, C., Lawlor, P. G., Croize, T., Jiang, Y., Morrison, L., Gardiner, G. E. And Zhan, X. 2016. Synergism and effect of high initial volatile fatty acid concentrations during food waste and pig manure anaerobic co-digestion. *Waste Management,* 56**:** 173-180.

Design Expert. 2005. Multifactor RSM Tutorial (Part 1 – The Basics) Response Surface Design and Analysis. *Design Expert 9 User's Guide*.

Deublein, D. And Steinhauser, A. 2011. *Biogas from waste and renewable resources: an introduction,* Wiley VCH. John Wiley and Sons.

Di Maria, F., Micale, C. And Contini, S. 2016. Energetic and environmental sustainability of the co-digestion of sludge with bio-waste in a life cycle perspective. *Applied Energy,* 171**:** 67-76.

Edwards, J., Othman, M., Crossin, E. And Burn, S. 2017. Anaerobic co-digestion of municipal food waste and sewage sludge: A comparative life cycle assessment in the context of a waste service provision. *Bioresource Technology,* 223**:** 237-249.

Enitan, A.M. 2015. *Microbial community analysis of a UASB reactor and application of an evolutionary algorithm to enhance wastewater treatment and biogas production* (Doctoral dissertation).

Enitan, A. M., Adeyemo, J., Kumari, S., Swalaha, F. M. And Bux, F. 2015. Characterization of brewery wastewater composition. *World Academy of Science, Engineering and Technology, International Journal of Environmental, Chemical, Ecological, Geological and Geophysical Engineering,* 9**:** 1015-1018.

Enzmann, F., Mayer, F., Rother, M. and Holtmann, D. 2018. Methanogens: biochemical background and biotechnological applications. *AMB Express*, *8*(1):1-22.

Ersahin, M.E., Ozgun, H., Dereli, R.K. and Ozturk, I. 2011. Anaerobic treatment of industrial effluents: an overview of applications. *Waste water-treatment and reutilization*, 9-13.

Evans, T. D. 2016. Sewage sludge: Operational and environmental issues. *Review of Current Knowledge.* 4th ed. Marlow, UK: Foundation for Water Research.

Fang, H. H. And Liu, H. 2002. Effect of pH on hydrogen production from glucose by a mixed culture. *Bioresource Technology,* 82**:** 87-93.

Fito, J., Tefera, N. And Van Hulle, S. W. H. 2019. Sugarcane biorefineries wastewater: bioremediation technologies for environmental sustainability. *Chemical and Biological Technologies in Agriculture,* 6**:** 6-12.

Fytili, D. And Zabaniotou, A. 2008. Utilization Of Sewage Sludge In Eu Application Of Old And New Methods—A Review. *Renewable And Sustainable Energy Reviews,* 12**:** 116-140.

Gelegenis, J., Georgakakis, D., Angelidaki, I. And Mavris, V. 2007. Optimization of biogas production by co-digesting whey with diluted poultry manure. *Renewable Energy,* 32**:** 2147-2160.

Ghanimeh, S., El Fadel, M. And Saikaly, P. 2012. Mixing effect on thermophilic anaerobic digestion of source-sorted organic fraction of municipal solid waste. *Bioresource Technology,* 117**:** 63-71.

Grando, R. L., De Souza Antune, A. M., Da Fonseca, F. V., Sánchez, A., Barrena, R. And Font, X. 2017. Technology overview of biogas production in anaerobic digestion plants: A European evaluation of research and development. *Renewable and Sustainable Energy Reviews,* 80**:** 44-53.

Güngör-Demirci, G. And Demirer, G. N. 2004. Effect of initial COD concentration, nutrient addition, temperature and microbial acclimation on anaerobic treatability of broiler and cattle manure. *Bioresource Technology,* 93**:** 109-117.

Hagos, K., Zong, J., Li, D., Liu, C. And Lu, X. 2017. Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives. *Renewable and Sustainable Energy Reviews,* 76**:** 1485-1496.

Hallaji, S. M., Kuroshkarim, M. And Moussavi, S. P. 2019. Enhancing methane production using anaerobic co-digestion of waste activated sludge with combined fruit waste and cheese whey. *BMC biotechnology,* 19(1)**:** 19-28.

Herve Macarie, J. L. M. 2006. Overview of the biological processes available for the treatment of sugarcane mill wastewater. *International Sugar Journal,* 108**:** 431-439.

Hidalgo, D., Martín-Marroquín, J. M. And Corona, F. 2018. The effect of feed composition on anaerobic co-digestion of animal-processing by-products. *Journal of Environmental Management,* 216**:** 105-110.

Horváth, I.S., Tabatabaei, M., Karimi, K. and Kumar, R. 2016. Recent updates on biogas production-a review. *Biofuel Research Journal*, 10:394-402.

Hunger, S., Schmidt, O., Hilgarth, M., Horn, M.A., Kolb, S., Conrad, R. and Drake, H.L. 2011. Competing formate-and carbon dioxide-utilizing prokaryotes in an anoxic methane-emitting fen soil. *Applied and Environmental Microbiology*, *77*(11):3773-3785.

Jingquan, L. 2006. Optimization Of Anaerobic Digestion Of Sewage Sludge Using Thermophilic anaerobic pre-treatment. *BioScience and Technology, BioCentrum-DTU, Technical University of Denmark DK-2800: Lyngby Denmark,* (Doctoral Dissertation).

Kacprzak, M., Neczaj, E., Fijałkowski, K., Grobelak, A., Grosser, A., Worwag, M., Rorat, A., Brattebo, H., Almås, Å. And Singh, B. R. 2017. Sewage sludge disposal strategies for sustainable development. *Environmental Research,* 156**:** 39-46.

Kafle, G. K. And Chen, L. 2016. Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models. *Waste Management,* 48**:** 492-502.

Karadag, D., Köroğlu, O. E., Ozkaya, B. And Cakmakci, M. 2015. A review on anaerobic biofilm reactors for the treatment of dairy industry wastewater. *Process Biochemistry,* 50**:** 262-271.

Karim, K., Klasson, K. T., Drescher, S. R., Ridenour, W., Borole, A. P. And Al-DAHHAN, M. H. 2007. Mesophilic digestion kinetics of manure slurry. *Applied Biochemistry and Biotechnology,* 142**:** 231-242.

Karray, R., Karray, F., Loukil, S., Mhiri, N. And Sayadi, S. 2017. Anaerobic co-digestion of Tunisian green macroalgae Ulva rigida with sugar industry wastewater for biogas and methane production enhancement. *Waste Management,* 61**:** 171-178.

Kasmi, M. 2018. Biological Processes as Promoting Way for Both Treatment and Valorisation of Dairy Industry Effluents. *Waste Biomass Valorisation* **9:** 195–209.

Kayhanian, M. 1995. Biodegradability of the organic fraction of municipal solid waste in a high-solids anaerobic digester. *Waste Management andand Research,* 13**:** 123-136.

Khan, M., Ngo, H. H., Guo, W., Liu, Y., Nghiem, L. D., Hai, F. I., Deng, L., Wang, J. And Wu, Y. 2016. Optimization of process parameters for production of volatile fatty acid, biohydrogen and methane from anaerobic digestion. *Bioresource Technology,* 219**:**738-748.

Khan, M. A., Ngo, H. H., Guo, W., Liu, Y., Zhang, X., Guo, J., Chang, S. W., Nguyen, D. D. And Wang, J. 2017. Biohydrogen production from anaerobic digestion and its potential as renewable energy. *Renewable Energy,* 124:754-768.

Kim, H.-W., Nam, J.-Y. And Shin, H.-S. 2011. A comparison study on the high-rate co-digestion of sewage sludge and food waste using a temperature-phased anaerobic sequencing batch reactor system. *Bioresource Technology,* 102**:** 7272-7279.

Kim, M.-S., Kim, D.-H. And Yun, Y.-M. 2017. Effect of operation temperature on anaerobic digestion of food waste: Performance and microbial analysis. *Fuel,* 209**:** 598-605.

Kolhe, A., Sarode, A. And Ingale, S. 2009. Study of effluent from sugar cane industry. *Sodh Samiksha Mulyankan,* 2**:** 303-306.

Kougias, P. G. And Angelidaki, I. 2018. Biogas and its opportunities—A review. *Frontiers of Environmental Science and Engineering,* 12**:** 1-12.

Kozłowski, K., Mazurkiewicz, J., Chełkowski, D., Jeżowska, A., Cieślik, M., Brzoski, M., Smurzyńska, A., Dongmin, Y. And Wei, Q. 2018. The Effect of Mixing During Laboratory Fermentation of Maize Straw with Thermofilic Technology. *Journal of Ecological Engineering,* 19**:** 93-98.

Kuruti, K., Nakkasunchi, S., Begum, S., Juntupally, S., Arelli, V. And Anupoju, G. R. 2017. Rapid generation of volatile fatty acids (VFA) through anaerobic acidification of livestock organic waste at low hydraulic residence time (HRT). *Bioresource Technology,* 238**:** 188-193.

Kushwaha, J. P. 2015. A review on sugar industry wastewater: sources, treatment technologies, and reuse. *Desalination and Water Treatment,* 53: 309-318.

Lin, Q., De Vrieze, J., Li, C., Li, J., Li, J., Yao, M., Hedenec, P., Li, H., Li, T., Rui, J., Frouz, J. And Li, X. 2017. Temperature regulates deterministic processes and the succession of microbial interactions in anaerobic digestion process. *Water Research,* 123**:** 134-143.

Lindmark, J., Thorin, E., Bel Fdhila, R. And Dahlquist, E. 2014. Effects of mixing on the result of anaerobic digestion: Review. *Renewable and Sustainable Energy Reviews,* 40**:** 1030-1047.

Liu, C., Li, H., Zhang, Y. And Liu, C. 2016. Improve biogas production from low-organic-content sludge through high-solids anaerobic co-digestion with food waste. *Bioresource Technology,* 219**:** 252-260.

Liu, Y. Y. And Haynes, R. J. 2011. Origin, Nature, and Treatment of Effluents From Dairy and Meat Processing Factories and the Effects of Their Irrigation on the Quality of Agricultural Soils. *Critical Reviews in Environmental Science and Technology,* 41: 1531-1599.

Lohani, S. P. And Havukainen, J. 2018. Anaerobic Digestion: Factors affecting anaerobic digestion process. *In:* S, V., E, G., B, G., D, P. and Z, Z. (eds.) *Waste Bioremediation.* Singapore: Springer.

Lokshina, L. Y., Vavilin, V. A., Kettunen, R. H., Rintala, J. A., Holliger, C. And Nozhevnikova, A. N. 2001. Evaluation of kinetic coefficients using integrated monod and haldane models for low-temperature acetoclastic methanogenesis. *Water Research,* 35**:** 2913-2922.

Ma, X., Jiang, T., Chang, J., Tang, Q., Luo, T. And Cui, Z. 2019. Effect of Substrate to Inoculum Ratio on Biogas Production and Microbial Community During Hemi-Solid-State Batch Anaerobic Co-digestion of Rape Straw and Dairy Manure. *Applied Biochemistry and Biotechnology,* 189**:** 884-902.

Mao, C., Feng, Y., Wang, X. And Ren, G. 2015. Review on research achievements of biogas from anaerobic digestion. *Renewable and Sustainable Energy Reviews,* 45**:** 540-555.

Mata-Alvarez, J., Dosta, J., Romero-Güiza, M. S., Fonoll, X., Peces, M. And Astals, S. 2014. A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renewable and Sustainable Energy Reviews,* 36**:** 412-427.

Meegoda, J. N., Li, B., Patel, K. And Wang, L. B. 2018. A Review of the Processes, Parameters, and Optimization of Anaerobic Digestion. *International Journal of Environmental Research and Public Health,* 15: 2224.

Mehta, C. And Sirari, K. 2018. Comparative study of aerobic and anaerobic composting for better understanding of organic waste management: A mini review. *Plant Archives,*18(1):44-50.

Membere, E. And Sallis, P. 2018. Effect of temperature on kinetics of biogas production from macroalgae. *Bioresource Technology,* 263**,** 410-417.

Mhlanga, F. T. And Brouckaert, C. J. 2012. Influent characterisation of wastewater. *Water and Sanitation Africa* [Online], 7(4): 30-35.

Montgomery, D. C. 2017. *Design and analysis of experiments,* John Wiley and Sons.

Nazaitulshila, R., Idris, A., Harun, R. And Wan Azlina, W. A. K. G. 2015. The Influence of Inoculum to Substrate Ratio on the Biochemical Methane Potential of Fat, Oil, and Grease in Batch Anaerobic Assays. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects,* 37**,** 590-597.

Neumann, P., Torres, A., Fermoso, F. G., Borja, R. And Jeison, D. 2015. Anaerobic co-digestion of lipid-spent microalgae with waste activated sludge and glycerol in batch mode. *International Biodeterioration and Biodegradation,* 100**,** 85-88.

Oberholster, P. J. And Ashton, P. J. 2008. State of the nation report: An overview of the current status of water quality and eutrophication in South African rivers and reservoirs. *Parliamentary Grant Deliverable. Pretoria: Council for Scientific and Industrial Research (CSIR).*

Ondari, J. M. 2015. *Anaerobic co-digestion of abattoir and textile industry wastewater in a UASB reactor* (Doctoral Dissertation).

Owamah, H. I. And Izinyon, O. C. 2015. The effect of organic loading rates (OLRs) on the performances of food wastes and maize husks anaerobic co-digestion in continuous mode. *Sustainable Energy Technologies and Assessments,* 11**,** 71-76.

Pellera, F.-M. And Gidarakos, E. 2016. Effect of substrate to inoculum ratio and inoculum type on the biochemical methane potential of solid agroindustrial waste. *Journal of Environmental Chemical Engineering,* 4**,** 3217-3229.

Prazeres, A. R., Carvalho, F. And Rivas, J. 2012. Cheese whey management: A review. *Journal of Environmental Management,* 110**,** 48-68.

Priadi, C., Wulandari, D., Rahmatika, I. And Moersidik, S. S. 2014. Biogas production in the anaerobic digestion of paper sludge. *APCBEE procedia,* 9**,** 65-69.

Qasim, S. R. 2017. *Wastewater treatment plants: planning, design, and operation*, Routledge.

Rabii, A., Aldin, S., Dahman, Y. And Elbeshbishy, E. 2019. A review on anaerobic co-digestion with a focus on the microbial populations and the effect of multi-stage digester configuration. *Energies,* 12**,** 1106.

Radwan, E.H., Fahmy, G.H., Saber, M.A.K. and Saber, M.E.D.K. 2017. The impact of some organic and inorganic pollutants on fresh water (Rashid branch, River Nile). *Egypt. Journal. of Advances in Biology*, *10*(2):2133-2145.

Ravikumar, P., Somashekar, R.K. and Prakash, K.L. 2015. Suitability assessment of deep groundwater for drinking and irrigation use in the parts of Hoskote and Malur Taluks, Karnataka (India). *Environmental Research, Engineering and Management*, *71*(1):15-26.

Ruiz, B. and Flotats, X., 2014. Citrus essential oils and their influence on the anaerobic digestion process: An overview. *Waste Management*, *34*(11):2063-2079.

Sahu, O. P. And Chaudhari, P. K. 2015. The characteristics, effects, and treatment of wastewater in sugarcane industry. *Water Quality, Exposure and Health,* 7**,** 435-444.

Saurabh, S. And Shailja, P. 2014. Physico-chemical analysis of sugar mill effluent and their impact on changes of growth of wheat (Triticum aestivum) and maize (Zea mays L.). *Journal of Environmental Science, Toxicology and Food Technology,* 8**,** 57-61.

Shi, X.-S., Dong, J.-J., Yu, J.-H., Yin, H., Hu, S.-M., Huang, S.-X. And Yuan, X.-Z. 2017. Effect of hydraulic retention time on anaerobic digestion of wheat straw in the semicontinuous continuous stirred-tank reactors. *BioMedical Research International,* 2017:1-6.

Siebielska, I. 2014. Comparison of changes in selected polycyclic aromatic hydrocarbons concentrations during the composting and anaerobic digestion processes of municipal waste and sewage sludge mixtures. *Water Science and Technology,* 70**,** 1617-1624.

Sillanpää, M., Ncibi, M.C., Matilainen, A. and Vepsäläinen, M., 2018. Removal of natural organic matter in drinking water treatment by coagulation: A comprehensive review. *Chemosphere*, *190*:54-71.

Simate, G. S., Cluett, J., Iyuke, S. E., Musapatika, E. T., Ndlovu, S., Walubita, L. F. And Alvarez, A. E. 2011. The treatment of brewery wastewater for reuse: State of the art. *Desalination,* 273**,** 235-247.

Slavov, A. K. 2017. General Characteristics and Treatment Possibilities of Dairy Wastewater - A Review. *Food Technology and Biotechnology*, *55*(1): 14–28.

Tariq, M., Ali, M. And Shah, Z. 2006. Characteristics of industrial effluents and their possible impacts on quality of underground water. *Soil and Environmental,*25(1): 64-69.

Templeton, M. R. And Butler, D. 2011. *Introduction to wastewater treatment,* UK, Bookboon.

Tetteh, E.K., Amano, K.A., Asante-Sackey, D. and Armah, E.K. 2017. Biochemical Methane Potential (BMP) of *Miscanthus Fuscus* for Anaerobic Digestion. *International Journal of Scientific and Research Publications*, *7*(12):434-439.

The South African Sugar Industry, 2013/2014 URL http://www.smri.org/include/sugarfacts/sasugarindustry.htm (accessed 9.1.20).

Trevor, J. B., Corne, V. S. And Yung-Tse, H. 2005. Treatment of dairy processing wastewaters. *Waste treatment in the food processing industry.* 1-28. CRC Press.

University Of Pretoria, S. A. L. I. I. 2013. *National Water Act 36 0f 1998* [Online]. Available: https://cer.org.za/wp-content/uploads/2014/02/Revision-of-General-Authorisations-2013 [Accessed 1-11-2018 2019].

Velásquez Piñas, J. A., Venturini, O. J., Silva Lora, E. E. And Calle Roalcaba, O. D. 2018. Technical assessment of mono-digestion and co-digestion systems for the production of biogas from anaerobic digestion in Brazil. *Renewable Energy,* 117**,** 447-458.

Vidal, G., Carvalho, A., Méndez, R. And Lema, J. M. 2000. Influence of the content in fats and proteins on the anaerobic biodegradability of dairy wastewaters. *Bioresource Technology*, 74**,** 231-239.

Von Sperling, M. 2007. *Wastewater Characteristics, Treatment and Disposal,* London, IWA Publishing.

Wang, D., Zhao, J., Zeng, G., Chen, Y., Bond, P. L. And Li, X. 2015. How Does Poly(hydroxyalkanoate) Affect Methane Production from the Anaerobic Digestion of Waste-Activated Sludge? *Environmental Science and Technology,* 49**,** 12253-12262.

Wang, H., Larson, R. A., Borchardt, M. And Spencer, S. 2019. Effect of mixing duration on biogas production and methanogen distribution in an anaerobic digester. *Environmental Technology***,** 1:1-7.

Wang, K., Yin, J., Shen, D. And Li, N. 2014. Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: effect of pH. *Bioresource Technology,* 161**,** 395-401.

Wang, Z.-W. And Li, Y. 2014. A theoretical derivation of the Contois equation for kinetic modeling of the microbial degradation of insoluble substrates. *Biochemical Engineering Journal,* 82**,** 134-138.

Watanabe, K., Koyama, M., Ueda, J., Ban, S., Kurosawa, N. And Toda, T. 2017. Effect of operating temperature on anaerobic digestion of the Brazilian waterweed Egeria densa and its microbial community. *Anaerobe,* 47**,** 8-17.

Wickham, R., Galway, B., Bustamante, H. And Nghiem, L. D. 2016. Biomethane potential evaluation of co-digestion of sewage sludge and organic wastes. *International Biodeterioration and Biodegradation,* 113**,** 3-8.

Wu, P., Wang, G., Wang, G., Børresen, B.T., Liu, H. and Zhang, J. 2016. Butanol production under microaerobic conditions with a symbiotic system of Clostridium acetobutylicum and Bacillus cereus. *Microbial cell factories*, *15*(1):1-11.

WWF-SA, W. W. F. F. N.-S. A. 2017. Scenarios for the future of water in South Africa. *In:* Christine, C. and Dean, M. (eds.). South Africa.

Xie, S., Faisal, I. H., Xinmin, Z., Wenshan, G., Hao, H. N., William, E. P. and Long, D. N. 2016. Anaerobic co-digestion: A critical review of mathematical modelling for performance optimization. *Bioresource Technology*, 222**,** 498-512.

Xu, R., Zhang, K., Liu, P., Khan, A., Xiong, J., Tian, F. and Li, X. 2017. A critical review on the interaction of substrate nutrient balance and microbial community structure and function in anaerobic co-digestion. *Bioresource Technology*. 247:1119-1127.

Yang, C. 2018. Acetogen communities in the gut of herbivores and their potential role in syngas fermentation. *Fermentation*, *4*(2):40-57.

Yang, G., Zhang, G. and Wang, H. 2015. Current state of sludge production, management, treatment and disposal in China. *Water Research,* 78**,** 60-73.

Yongabi, K. 2010. Biocoagulants for water and waste water purification: a review. *International Review of Chemical Engineering,* 2**,** 444-458.

Yoon, Y.-M., Kim, S.-H., Shin, K.-S. and Kim, C.-H. 2014. Effects of substrate to inoculum ratio on the biochemical methane potential of piggery slaughterhouse wastes. *Asian-Australasian Journal of Animal Sciences,* 27**,** 600-607.

Zahan, Z., Othman, M. Z. and Rajendram, W. 2016. Anaerobic codigestion of municipal wastewater treatment plant sludge with food waste: a case study. *BioMedical Research International,* 2016:1-13.

Zhai, N., Zhang, T., Yin, D., Yang, G., Wang, X., Ren, G. and Feng, Y. 2015. Effect of initial pH on anaerobic co-digestion of kitchen waste and cow manure. *Waste Management,* 38**,** 126-131.

Zhang, C., Su, H., Baeyens, J. and Tan, T. 2014. Reviewing the anaerobic digestion of food waste for biogas production. *Renewable and Sustainable Energy Reviews,* 38**,** 383-392.

Zhang, L., Lee, Y.-W. and Jahng, D. 2011. Anaerobic co-digestion of food waste and piggery wastewater: Focusing on the role of trace elements. *Bioresource Technology,* 102**,** 5048-5059.

Zhou, J., Zhang, R., Liu, F., Yong, X., Wu, X., Zheng, T., Jiang, M. and Jia, H. 2016. Biogas production and microbial community shift through neutral pH control during the anaerobic digestion of pig manure. *Bioresource Technology,* 217**,** 44-49.

Ziemiński, K. and Frąc, M. 2012. Methane fermentation process as anaerobic digestion of biomass: Transformations, stages and microorganisms. *African Journal of Biotechnology,* 11**,** 4127-4139.

APPENDIX A1

Wastewater Characterization Procedure

A.1.1 TOTAL SOLIDS

Apparatus:

i. Porcelain Crucibles

ii. Oven/ heating mantle

iii. Analytical Balance

iv. Desiccator

v. Muffle furnace

Procedure

i. Heat clean porcelain crucibles in the oven at 105°C for 1 hour or in muffle at 550°C for 15 minutes (if determination of volatile solid). Then cool in desiccator until needed.
ii. Weigh crucibles immediately before use.
iii. Pipette 5 or 10 mL the well-mixed sample to the pre-weighed crucible.
iv. Place crucible + sample solution in an oven at 105°C for 24 hours or heat in a water bath until solution is dry, then place in an oven for 1 hour.
v. Remove crucible from oven and place in desiccator to cool.
vi. Weigh the crucible + residue.

Calculate Total solid as follows:

Total Solid (mg/L) = $\frac{(A-B) \times 1000}{sample\ volume,\ mL}$ .....

A= weight of dried residue + crucible (mg)

B= weight of crucible (mg)

Calculation in %:

$$\%TS = \frac{(A-B) \times 100}{C-B}$$

C = weight of wet sample + crucible (mg)

A.1.2 VOLATILE SOLIDS

Apparatus:

i. Muffle furnace

ii. Crucible + residue from TS

iii. Furnace tongs

iv. Insulated gloves

Procedure

i. Place the crucible + residue from TS in the muffle furnace.
ii. Ignite at 550 °C ± 50 °C for 30 minutes.
iii. Partially air cool, desiccate and weigh.

Calculate Volatile Solid as follow;

Volatile Solid (mg/L) = $\frac{(A-B) \times 1000}{Sample\ volume,\ mL}$ ----------

Where: A = weight of crucible + residue from TS test (mg)

B = weight of crucible + residue after ignition (mg)

Calculation in %

$$\%VS = \frac{(A-B) \times 100}{A - A_1}$$

A_1 = weight of crucible (mg)

A.1.3 TOTAL SUSPENDED SOLID

Apparatus:

i. Micropipette

ii. Analytical balance

iii. Glass fibre filters

iv. Desiccator

v. Oven

vi. Vacuum filtration apparatus

Procedure

i. Dry glass fibre filters for 10minutes at 105 °C in an oven.
ii. Remove from oven, cool in a desiccator for 5 minutes and weigh.
iii. Place the filter in the filtration unit.
iv. Measure 5 or 10 mL of the well-mixed sample and add slowly to the filtration unit while applying suction through a vacuum pump. Continue adding sample until the whole of the liquid has passed through the filter.

v. Rinse remains of sample in measuring cylinder with distilled water and pour into the filtration unit.

vi. Allow solution to continue filtering until the excess water to remove from filter.

vii. Remove filter, place in pre-weighed crucible as TS and heat at 105°C for 1 hour in an oven.

viii. Thereafter, cool in desiccator for 5 minute and weigh.

Calculation for total suspended solid

Total Suspended Solid (mg/L) = $\frac{(A-B) \times 1000}{Sample\ volume,\ mL}$.....

Where: A = weight of filter, crucible + residue (mg)

B = weight of filter + crucible (mg)

Calculation in %:

$$\%TSS = \frac{(A-B) \times 100}{C-B}$$

C = weight of wet residue on filter + crucible (mg)

A.1.4 TOTAL DISSOLVED SOLIDS

Apparatus Required

i. Porcelain Crucibles

ii. Drying Oven

iii. Analytic Balance

iv. Desiccator

v. Vacuum filtration apparatus

Procedure

i. Heat clean crucible at 180°C for 1 hour.

ii. Remove and cool in desiccator until needed. Weigh immediately before use.

iii. Pour filtrate from TSS into the crucible.

iv. Place in oven at 180°C for 24 hours, cool in a desiccator, and weigh.

Calculate Total dissolved solid as follows:

Total dissolved solid (mg/L) = $\frac{(A-B) \times 1000}{Sample\ volume,\ mL}$.....

A= weight of crucible + dried residue (mg)

B= weight of crucible (mg)

A.1.5 VOLATILE SUSPENDED SOLIDS

Apparatus:

i. Muffle furnace

ii. Filter, crucible + residue from TSS

iii. Furnace tongs

iv. Insulated gloves

Procedure

i. Place the filter, crucible + residue from TSS in the muffle furnace.

ii. Ignite at 550 °C ± 50 °C for 30 minutes.

iii. Partially air cool, desiccate and weigh.

Calculate Volatile Suspended Solid as follow;

Volatile suspended solid (mg/L) = $\frac{(A-B) \times 1000}{Sample\ volume,\ mL}$ ----------

Where: A = weight of filter, crucible + residue from TSS test (mg)

B = weight of filter, crucible + residue after ignition (mg)

Calculation in %

$$\%VSS = \frac{(A-B) \times 100}{A - A_1}$$

A_1 = weight of crucible + filter (mg)

A.1.6 CHEMICAL OXYGEN DEMAND (COD)

Apparatus:

i. Thermoreactor

ii. Erlenmeyer flasks

iii. Micropipettes

iv. 0.45µm filter

v. Spectrophotometer

Reagents:

i. Standard Potassium Dichromate (K_2CrO_7) Digestion Solutions: 0.0167M

ii. Sulphuric Acid H_2SO_4 and Silver Sulphate reagent Ag_2SO_4 (COD reagent)

Standard Preparation

Prepare a standard K_2CrO_7 solution by dissolving 12.26g in distil water to make 1000mL. In addition, dissolve 5.5g Ag_2SO_4 in 1 kg concentrated H_2SO_4 and add distil water to make 1000mL.

Procedure

i. Pipette 2 mL sample to each digestion tube.
ii. Add 2.5mL distilled water to another digestion tube (blank).
iii. Add 1.5mL potassium dichromate digestion solution into each tube.
iv. Add 3.5mL COD reagent in each tube.
v. The acid should be poured down the wall of the flask while flask is tilted. If sample is too concentrated, it will turn green, and higher dilution of sample should be used.
vi. Prepare a blank with each set of samples consisting of 2 mL distilled water in place of sample together with all reagents and digest together with samples.
vii. Digest for 2 hours at 150 °C using the thermoreactor.
viii. Analyse using the HACH 3900 spectrophotometer."

Figure A1.1: HACH® (Model 200) COD reactor and HACH® (Model 3900) spectrometer (right).

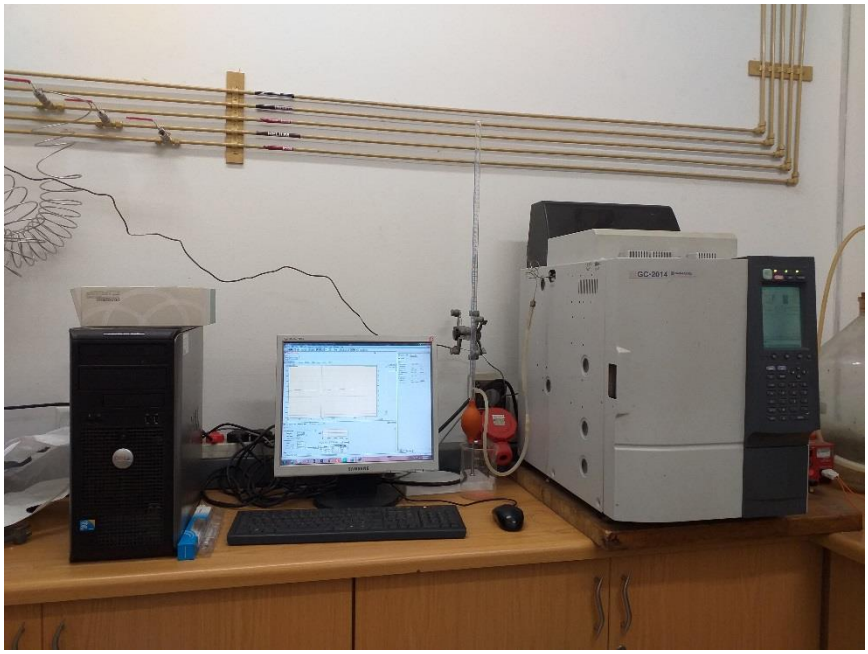


Figure A1.2: Shimadzu 2014 Gas Chromatography

APPENDIX A2

Preliminary Run Characterisation Raw Data

This section contains the raw data for the characteristics of each wastewater, municipal sludge and the inoculum utilized in this study.

Table A2. 1: Total Solids Raw Data before Digestion (Preliminary Run)

Sample	B	a1	a2	A	Volume	Total solid(mg/L)
Brewery W_1	43.3279	43.4806	43.4809	43.4808	20	7645
Brewery W_2	42.6342	42.7863	42.7878	42.7871	20	7642.5
Brewery W_1	44.3262	44.4804	44.4819	44.4812	20	7747.5
Brewery W_2	40.2918	40.4466	40.4494	40.448	20	7810
Dairy W_1	42.6812	42.7634	42.7698	42.7666	20	4270
Dairy W_2	43.3242	43.411	43.4165	43.4138	20	4477.5
Dairy W_1	44.9011	44.9924	44.9889	44.9907	20	4477.5
Dairy W_2	42.0732	42.1686	42.1669	42.1678	20	4727.5
Sugar W_1	44.8999	44.9873	44.9892	44.9883	20	4417.5
Sugar W_2	42.6803	42.7715	42.7736	42.7726	20	4612.5
SS_1	43.3276	43.5349	43.5351	43.535	5	41480
SS_2	44.9024	45.1171	45.1171	45.1171	5	42940
$Inoculum_1$	44.3287	44.3898	44.3896	44.3897	5	12200
$Inoculum_2$	42.0754	42.1353	42.1355	42.1354	5	12000

Sample Calculation for all TS

Total Solid

$$Total\ solids\ \left(\frac{mg}{L}\right) = \frac{(A-B)\times 1000}{C}$$

$$A = \frac{(a1+a2)}{2}$$

Brewery W_1(mg/L) $= \frac{(43.4808-43.3279)g * 1000mL * 1000mg}{20mL * 1g * 1L}$

$= \frac{0.1529 * 1000000mg}{20L}$

$= \frac{152900mg}{20L}$

= 7645mg/L

Table A2. 2: Volatile Solids Raw Data Before Digestion (Preliminary Run)

Sample	A	b1	b2	B	Volume	Volatile solid(mg/L)
Brewery W_1	44.4812	44.3788	44.3812	44.38	20	5060
Brewery W_2	40.448	40.3467	40.3476	40.3472	20	5040
Dairy W_1	44.9907	44.914	44.9132	44.9136	20	3855
Dairy W_2	42.1678	42.0864	42.0851	42.0858	20	4100
Sugar W_1	44.9883	44.9198	44.9217	44.9208	20	3375
Sugar W_2	42.7726	42.7038	42.7032	42.7035	20	3452.5
SS_1	43.535	43.3828	43.383	43.3829	5	30420
SS_2	45.1171	44.9595	44.9593	44.9594	5	31540
$Inoculum_1$	44.3898	44.3575	44.3575	44.3575	5	6460
$Inoculum_2$	42.1353	42.103	42.1028	42.1029	5	6480

Sample calculation of all VS

Volatile Solids, mg/L = $\frac{(A-B)*1000}{Sample\ volume,\ mL}$

Brewery W_1(mg/L) = $\frac{(44.4812-44.3800)g*1000mL*1000mg}{20mL*1g*1L}$

$= \frac{0.1012*1000000mg}{20L}$

$= \frac{101200mg}{20L}$

= 5060 mg/L

Table A2. 3: Total Suspended Solids Raw Data Before Digestion (Preliminary Run)

Sample	B1	b1	a1	a2	A	Volume	TSS (mg/L)
Brewery W_1	44.2459	0.0997	44.3689	44.3703	44.3696	40	600.0
Brewery W_2	43.3266	0.099	43.4504	43.4515	43.451	40	633.8
Dairy W_1	42.6336	0.0965	42.748	42.7457	42.7469	20	837.5
Dairy W_2	43.9806	0.0979	44.0929	44.092	44.0925	20	697.5
SS	40.2949	0.0969	40.5927		40.5927	5	40220
Inoculum	42.6856	0.0967	42.8311		42.8311	5	9720

Sample Calculation for all TSS

Total Suspended Solid

$$Total\ suspended\ solids\ \left(\frac{mg}{L}\right) = \frac{(A-B)\times 1000}{C}$$

$$A = \frac{(a1+a2)}{2}$$

$$B = B1 + b1$$

Where b1 is the weight of filter paper and B1 weight of the crucible

Brewery W_1(mg/L) $= \frac{(44.3696-44.3456)g * 1000mL * 1000mg}{40mL * 1g * 1L}$

$= \frac{0.024*1000000mg}{20L}$

$= \frac{24000mg}{40L}$

= 600 mg/L

Table A2. 4: Volatile Suspended Solids Raw Data Before Digestion (Preliminary Run)

Sample	A	b1	b2	B	Volume	VSS (mg/L)
Brewery W_1	44.3696	44.3492	44.3498	44.3495	40	502.5
Brewery W_2	43.451	43.4304	43.4308	43.4306	40	508.8
Dairy W_1	42.7469	42.7284	42.7457	42.7371	20	490.0
Dairy W_2	44.0925	44.0751	44.092	44.0836	20	445.0
SS_1	40.5927	40.4433	40.4435	40.4434	5	29860
SS_2	44.9024	44.7171	44.7174	44.7173	5	37030
$Inoculum_1$	42.8311	42.8042		42.8042	5	5380
$Inoculum_2$	42.0754	42.1353		42.1353	5	11980

Sample calculation of all VSS

Volatile suspended solids, mg/L $= \frac{(A-B)*1000}{Sample\ volume,\ \ mL}$

Brewery W_1(mg/L) $= \frac{(44.3696-44.3495)g * 1000mL * 1000mg}{40mL * 1g * 1L}$

$= \frac{0.0201 * 1000000 mg}{40L}$

$= \frac{20100 mg}{40L}$

= 502.5 mg/L

Table A2. 5: COD Raw Data Before Digestion (Preliminary Run)

Sample	COD Read(mg/L)	COD Actual(mg/L)	Abs read	Trans read (%)
Brewery W_1	1414	7070	0.628	23.6
Brewery W_2	1171	5855	0.52	30.2
Dairy W_1	586	2930	0.26	54.9
Dairy W_2	619	3095	0.275	53.1

Dilution Factor = 5

Sample Calculation

$$COD\ Actual\ \left(\frac{mg}{L}\right) = \ COD\ read\left(\frac{mg}{L}\right) * 5$$

Brewery W_1 COD actual (mg/L) = 1414*5 = 7070 mg/L

Table A2. 6: Total Solids Raw Data after Digestion (Preliminary Run) for 25°C

Sample	B	a1	a2	B	C (volume)	TS (mg/L)
I + SS	40.2894	40.4212	40.4214	40.4213	5	26380
I + SS + SW	42.0720	42.1292	42.1288	42.1290	5	11400
I + SS + BW	44.3245	44.4063	44.4060	44.40615	5	16330
I + SS + SW	43.3230	43.3860	43.3863	43.38615	5	12630
I + SS + DW	44.8997	44.9862	44.9864	44.9863	5	17320
I + SS + DW	44.3256	44.4005	44.4010	44.40075	5	15030

Table A2. 7: Total Solids Raw Data after Digestion (Preliminary Run) for 35°C

Sample	B	a1	a2	A	Volume	TS (mg/L)
I + SS	42.6822	42.8129	42.8133	42.8131	5	26180
I + SS + SW	42.0737	42.1497	42.1493	42.1495	5	15160
I + SS + BW	40.2914	40.4234	40.4230	40.4232	5	26360
I + SS + DW	44.3273	44.3967	44.3969	44.3968	5	13900
I + SS + SW	43.3259	43.4078	43.4080	43.4079	5	16400

Volatile Solids after Digestion for 25°C

Table A2. 8: Volatile Solids Raw Data after Digestion (Preliminary Run) for 25°C

Sample	A	b1	b2	B	Volume	VS (mg/L)
I + SS	40.4212	40.3636	40.3634	40.3635	5	11540
I + SS + SW	42.1292	42.1034	42.103	42.1032	5	5200
I + SS + BW	44.4063	44.3601	44.3605	44.3603	5	9200
I + SS + SW	43.386	43.3554	43.3552	43.3553	5	6140
I + SS + DW	44.9862	44.9558	44.9561	44.95595	5	6050
I + SS + DW	44.4005	44.366	44.3662	44.3661	5	6880

Volatile Solids after Digestion for 35°C

Table A2. 9: Volatile Solids Raw Data after Digestion (Preliminary Run) for 35°C

Sample	A	b1	b2	B	Volume	VS (mg/L)
I + SS	42.8129	42.7498	42.7495	42.74965	5	12650
I + SS + SW	42.1497	42.1191	42.1193	42.1192	5	6100
I + SS + BW	40.4234	40.3572	40.357	40.3571	5	13260
I + SS + DW	44.3967	44.3641	44.364	44.36405	5	6530
I + SS + SW	43.4078	43.3715	43.372	43.37175	5	7210

Table A2. 10: Preliminary run showing daily biogas production for the three industrial wastewaters used at 25°C

Experimental	I + SS	I + SS + SWW	I + SS + BWW	I + SS + SWW*	I + SS + DWW	I + SS + DWW*
pH	6.78	6.74	6.51	6.81	6.69	6.8
Day1	382.1	6.9	12.9	0.6	314.5	28.3
Day2	3.1	0.0	37.7	0.9	131.0	8.8
Day3	12.3	15.7	28.3	24.5	92.7	59.7
Day4	24.8	30.8	30.5	15.7	50.3	53.4
Day5	32.4	33.3	15.1	33.0	31.7	79.8
Day6	0.0	19.5	9.4	29.2	37.7	51.8
Day7	0.0	25.1	21.4	17.9	41.5	68.5
Day8	5.0	5.0	9.4	9.4	8.2	210.5
Day9	0.0	0.0	1.6	127.3	32.4	38.6
Day10	2.5	1.6	17.6	7.2	37.7	32.4
Day11	2.5	8.8	4.1	0.0	64.4	15.7
Day12	5.7	17.3	4.7	15.7	87.7	33.0
Day13	4.7	34.2	19.5	21.4	102.1	15.4
Day14	1.3	19.5	4.1	27.0	90.5	30.8
Day15	1.6	4.7	3.1	4.4	82.9	32.7
Day16	0.6	1.9	1.3	5.7	34.6	28.3
Day17	0.0	0.0	0.0	3.8	3.1	29.8
Day18	5.3	3.1	7.5	12.6	11.0	15.7
Day19	10.1	0.0	6.3	22.0	3.1	14.1
Day20	3.1	19.5	20.1	27.6	0.0	15.7
Day21	5.7	20.7	15.7	25.1	0.0	15.7
Day22	4.7	20.7	16.7	49.0	0.0	18.9
Day23	0.0	26.7	0.0	31.4	0.0	23.6
Day24	0.0	34.6	17.3	40.2	7.9	20.4
Day25	6.3	37.1	37.7	37.7	0.0	23.6
Day26	0.0	40.8	41.5	72.3	0.6	30.8
Day27	0.9	37.7	20.4	47.1	1.6	48.7
Day28	1.3	82.6	30.5	20.7	0.0	67.6
Day29	0.0	26.4	19.5	23.6	0.0	35.5
Day30	0.0	2.2	0.6	1.9	1.6	37.7
Day31	14.1	0.0	0.9	3.1	3.1	21.4
Day32	0.0	1.6	0.0	5.0	5.7	18.2
Day33	0.0	6.3	0.0	0.0	0.0	32.4
Day34	0.0	9.4	0.0	3.1	0.0	15.7
Day35						
Total	530.1	593.8	455.3	766.3	1277.5	1273.1

Table A2. 11: Preliminary run showing daily biogas production for the three industrial wastewaters used at 35^{o}C

Experimental	I + SS	I + SS + SWW	I + SS + BWW	I + SS + DWW	I + SS + SWW
pH	6.77	6.73	6.53	6.69	6.82
Day1	10	1385	0	0	15
Day2	40	110	10	0	46.5
Day3	10	85	20	0	15
Day4	0	55	0	0	3.4
Day5	0	160	0	0	7.5
Day6	0	100	0	0	5.2
Day7	0	70	0	0	20.2
Day8	0	55	0	165	8
Day9	0	0	0	0	0
Day10	0	65	0	180	6.8
Day11	0	15	0	20	15.8
Day12	0	140	10	45	54.8
Day13	0	80	10	20	50
Day14	0	80	0	65	6.8
Day15	5	200	10	120	41
Day16	5	115	85	0	6.2
Day17	0	100	75	0	0.2
Day18	10	45	0	15	3.4
Day19	160	540	30	470	1.2
Day20	40	70	5	10	0
Day21	20	50	0	0	0
Day22	30	20	0	0	0
Day23	20	20	0	0	0
Day24	40	0	0	0	0
Day25	50	20	0	10	2.5
Day26	50	15	0	10	0
Day27	60	30	0	0	0
Day28	40	5	0	10	0
Day29	60	0	0	0	0.5
Day30	10	15	0	5	0
Day31	20	20	5	5	0
Day32	10	5	0	0	1
Day33	0	10	0	0	0
Day34	0	5	0	0	0
Day35	10	0	0	5	0
Total	690	3685	260	1150	311

APPENDIX A3

Characterisation Raw Data for the Mix-ratio

Total Solids

Table A3. 1: Total Solids Raw Data for Mix-ratio

Sample	B	a1	a2	A	Volume	Total solid(mg/L)
SS_1	42.1	42.36		42.36	5	52000
SS_2	40.31	40.66		40.66	5	70000
Dairy W_1	43.34	43.36		43.36	5	4000
Dairy W_2	44.88	44.89		44.89	5	2000
Sugar W_1	44.8999	44.9873	44.9892	44.9883	20	4417.5
Sugar W_2	42.6803	42.7715	42.7736	42.7726	20	4612.5
$Inoculum_1$	44.37	44.42		44.42	5	10000
$Inoculum_2$	42.71	42.77		42.77	5	12000

Volatile Solids

Table A3. 2: Volatile Solids Raw Data for Mix-ratio

Sample	A	b1	b2	B	Volume	VS (mg/L)
$Sludge_1$	42.36	42.2		42.2	5	32000.0
$Sludge_2$	40.66	40.41		40.41	5	50000.0
Dairy W_1	43.36	43.34		43.34	5	4000.0
Dairy W_2	44.89	44.88		44.88	5	2000.0
Sugar W_1	44.9883	44.9198	44.9217	44.9208	20	3375.0
Sugar W_2	42.7726	42.7038	42.7032	42.7035	20	3452.5
$Inoculum_1$	44.42	44.39		44.39	5	6000.0
$Inoculum_2$	42.77	42.74		42.74	5	6000.0

Table A3. 3: Daily Biomethane Production for SW mix

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Day1	0	150	900	31.4	3000	0	0	1700	2780	0	1400	0	1500	300	0
Day2	30	180	0	31.4	3300	111.5	34.5	0	0	113.1	3050	14.1	0	200	20
Day3	40	0	10	86.4	0	0	1.5	0	10	6.3	1700	0	0	0	20
Day4	30	10	0	86.4	0	0	8	0	0	17.3	0	0	5	10	0
Day5	10	0	10	4.7	0	5	4	0	0	7.9	0	0	0	0	0
Day6	15	5	2.5	72.9	0	3.1	9	0	0	76.4	0	2.5	0	0	0
Day7	0	0	0	81.7	20	0	11.5	0	0	75.4	0	20.4	0	0	0
Day8	10	0	0	47.1	40	0	16	0	0	34.6	100	0	0	0	40
Day9	0	0	0.2	1.6	40.5	25.1	10.3	12.3	0	0	0	0	0	0	0
Day10	15	0	0	6.3	0	13.2	1.7	0	0	0	0	0	0	0	5
Day11	5	0	0	7.9	0	0	3.7	0	0	79.2	0	0	0	0	0
Day12	40	0	0	39.3	0	5.3	6	0	0	25.1	40	6.9	10	0	10
Day13	30	0	0	10.1	0	0	0	0	0	0	0	0	0	0	0
Day14	0	0	0	0	20	5.3	6	0	0	100.5	20	6.3	0	0	5
Day15	0	0	10	62.8	0	10.1	0	0	20	25.1	80	0.9	0	0	0
Day16	0	0	0	43	0	33.9	0	0	0	32	15	0.9	0	0	0
Day17	0	0	0	0	1.2	0.6	1	9.5	0	0	0	0	0	0	0
Day18	0	0	0	103.7	0	61.9	8.5	0	0	94.3	0	0	0	0	0
Day19	0	0	5	17.3	0	9.4	3.7	0	0	11	0	29.2	0	0	0
Day20	0	0	0	89.2	0	0	8	0	0	106.8	0	0	0	0	0
Day21	0	0	70	9.4	0	15.7	1.4	720	0	11	0	1.6	0	0	5
Day22	0	0	15	106.8	20	105.3	7.3	0	0	108.4	20	0	15	0	20
Day23	0	0	70	58.8	1	8.5	1.5	15	0	58.1	25	5.7	0	5	10
Day24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day25	0	0	0	30.8	60	0	0	0	0	25.1	140	0	0	0	20
Day26	0	0	10	144.5	30	0	12	150	0	146.1	0	4.7	10	0	40
Day27	0	0	40	40.8	80	40.8	1.5	10	0	51.8	20	6.3	0	5	30
Day28	0	0	1320	67.6	60	1.6	0	0	0	77	100	0	5	5	25
Day29	0	0	0	89.5	40	0	7	0	0	65.4	100	0	0	0	70
Day30	10	70	800	72.3	20	3.1	9.5	10	0	89.2	0	0	0	0	10
Day31	0	70	0	136.7	0	47.1	13	0	0	628.4	1	0	0	170	44
Day32	0	0	0	75.4	0	94.3	10.5	10	20	62.8	0	31.4	0	0	24
Day33	0	80	0	86.4	15	1.6	6.5	0	0	105.3	0	0	0	0	0
Day34	0	10	0	92.7	10	1.3	5.5	0	0	97.4	0	4.7	0	0	10
Day35	0	0	400	83.3	0	0	6	0	0	78.6	0	0	0	0	0

Sugar Mix Experimental

Volatile Solids before and after Digestion

Table A3. 4: Volatile Solids before and after Digestion for SW mix

Runs	A	B	Volume	VS after(mg/L)	VS before(mg/L)	% Reduction
S1	39.08	39.04	10	4000	6000	33.33
S2	41.40	41.37	10	3000	6000	50.00
S3	41.39	41.36	10	3000	4000	25.00
S4	40.76	40.73	10	3000	6000	50.00
S5	40.09	40.06	10	3000	4000	25.00
S6	40.08	40.05	10	3000	8000	62.50
S7	41.48	41.44	10	4000	4000	0.00
S8	44.46	44.42	10	4000	8000	50.00
S9	43.42	43.40	10	2000	4000	50.00
S10	42.78	42.76	10	2000	6000	66.67
S11	44.93	44.90	10	3000	8000	62.50
S12	44.46	44.43	10	3000	8000	62.50
S13	41.42	41.40	10	2000	6000	66.67
S14	43.46	43.43	10	3000	6000	50.00
S15	42.78	42.75	10	3000	8000	62.50

Sample calculation of all VS after

$$\text{Volatile Solids, mg/L} = \frac{(A-B)*1000}{Sample\ volume,\ mL}$$

$$\text{S1(mg/L)} = \frac{(39.08-39.04)g*1000mL*1000mg}{10mL*1g*1L}$$

$$= \frac{0.04*1000000mg}{10L}$$

$$= \frac{40000mg}{10L}$$

$$= 4000 \text{ mg/L}$$

Sample calculation of all % VS reduction

Volatile Solids % reduction, $= \frac{VS\ before - VS\ after}{VS\ before} * 100$

S1(mg/L) $= \frac{(6000-4000)}{6000} * 100$

$= \frac{2000}{6000} * 100$

= 0.3333*100

= 33.33%

COD Values

Table A3. 5: Initial and Final COD reading for SW mix

Sample	Initial	Final	%Reduction	Abs read	Transmittance (%)
S1	7830	1110	85.82	0.049	89.3
S2	7830	410	94.76	0.018	95.9
S3	7830	440	94.38	0.020	95.6
S4	7830	810	89.66	0.036	92.1
S5	7830	620	92.08	0.028	93.8
S6	7830	1210	84.55	0.054	88.4
S7	7830	5270	32.69	0.234	58.4
S8	7830	380	95.15	0.017	96.1
S9	7830	5810	25.80	0.258	55.2
S10	7830	840	89.27	0.037	91.8
S11	7830	740	90.55	0.033	92.7
S12	7830	10580	-35.12	0.470	33.9
S13	7830	660	91.57	0.029	93.5
S14	7830	5940	24.14	0.264	54.5
S15	7830	1290	83.52	0.057	87.7

Inoculum to Substrate Experiment

Sample Calculation for ISR

For every 200mL of sample, we have $VS\frac{mg}{L} * \frac{1L}{1000mL} * 200mL$

$$For\ sewage\ sludge, \quad \frac{24420 * 200}{1000}\ mg = 4884\ mg$$

$$For\ wastewater, \quad \frac{3414 * 200}{1000}\ mg = 682.7\ mg$$

$$For\ Inoculum, \quad \frac{11160 * 200}{1000}\ mg = 2232\ mg$$

The ratio of inoculum to substrate based on 800 mL, $\frac{2 * 2232mg}{(4884+682.7)mg} = 0.802$

Then, for every 400 mL of inoculum, 400*0.802 = 321 mL of substrate is needed.

Therefore, 400 mL of Inoculum to 321 mL of substrate gave an ISR of 1:1 (base on VS). Equal volume of sludge and wastewater was used as gotten from the mix-ratio experiment. Likewise, 200 mL of Inoculum to 321 mL of substrate gave an ISR of 1:2 and 400mL of Inoculum to 161 mL of substrate gave an ISR of 2:1.

The overall working volume of the reactor was 800 mL; therefore, volumes of substrate and inoculum were adjusted respectively to fill up the remaining volume.

Table A3. 6: Cumulative Biomethane production for various ISR

Sample	$D12_{25}$	$D11_{25}$	$D21_{25}$	$S12_{25}$	$S11_{25}$	$S21_{25}$	$D11_{35}$	$D21_{35}$	$S12_{35}$	$S11_{35}$	$S21_{35}$
Day1	3	3	50	17	5	124	44	108	24	148	107
Day2	6	3	113	64	63	148	48	156	28	154	173
Day3	11	5	179	102	102	214	70	218	41	223	198
Day4	11	5	383	154	160	223	103	244	41	278	229
Day5	14	5	895	196	206	231	150	256	82	278	255
Day6	14	5	895	234	347	234	150	261	82	284	256
Day7	14	5	911	234	413	251	172	267	90	303	276
Day8	18	5	913	240	485	264	258	276	99	324	276
Day9	18	5	913	244	511	273	428	276	105	355	276
Day10	19	6	916	244	599	276	459	276	112	364	276
Day11	24	9	916	244	691	280	648	276	115	364	276
Day12	26	9	919	253	706	319	687	286	118	372	278
Day13	26	9	922	284	706	325	687	286	127	379	286
Day14	29	9	922	379	706	333	687	286	153	383	298
Day15	30	11	922	379	706	336	687	287	182	383	302
Day16	48	11	930	414	711	344	695	296	196	385	305
Day17	48	11	934	474	711	355	698	296	208	385	305
Day18	80	17	937	521	805	363	698	307	240	385	305
Day19	80	17	937	529	868	363	703	307	303	436	336
Day20	80	17	937	558	868	364	735	307	334	472	336
Day21	80	17	937	621	868	364	735	307	334	535	336
Day22	80	17	937	683	868	364	735	307	365	598	336
Day23	80	17	969	789	868	366	750	338	397	620	336
Day24	80	33	969	851	868	373	776	338	523	652	462
Day25	80	33	969	943	868	373	778	338	585	743	509
Day26	80	33	969	1035	931	380	778	338	648	807	509
Day27	80	33	969	1089	931	380	779	338	711	811	572

Note: S and D represent sample containing sugar and dairy wastewater respectively.

While the subscripts 25 and 35 indicate the temperature of operation.

APPENDIX B

Chromatographs for Biochemical Methane Potential

Standards

CH_4

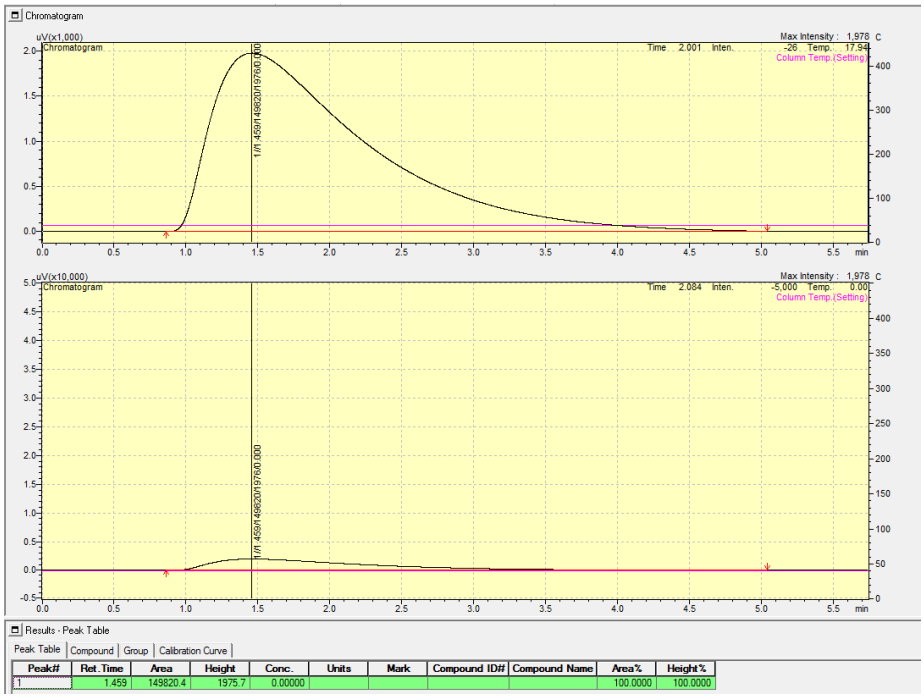


Figure B. 2: Chromatograph for Standard CH_4

CO_2

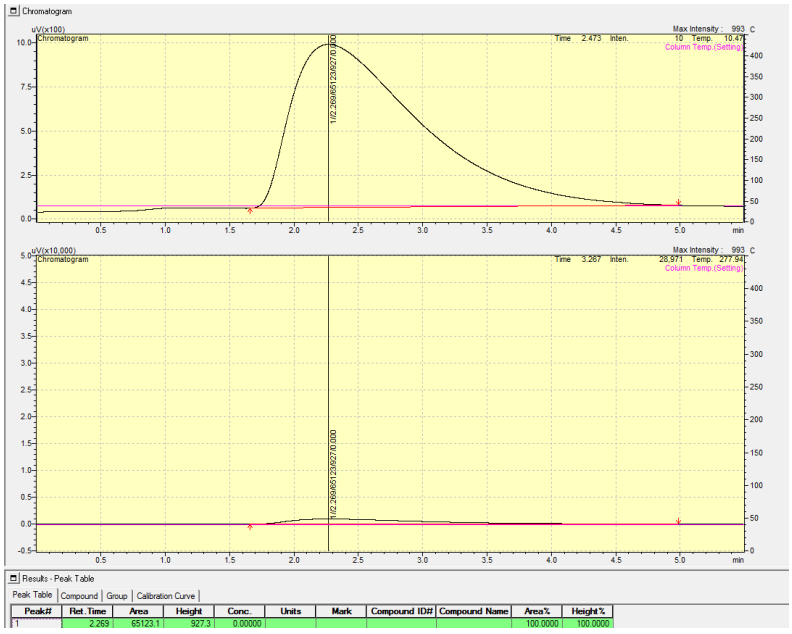


Figure B. 3: Chromatograph for Standard CO_2

H_2

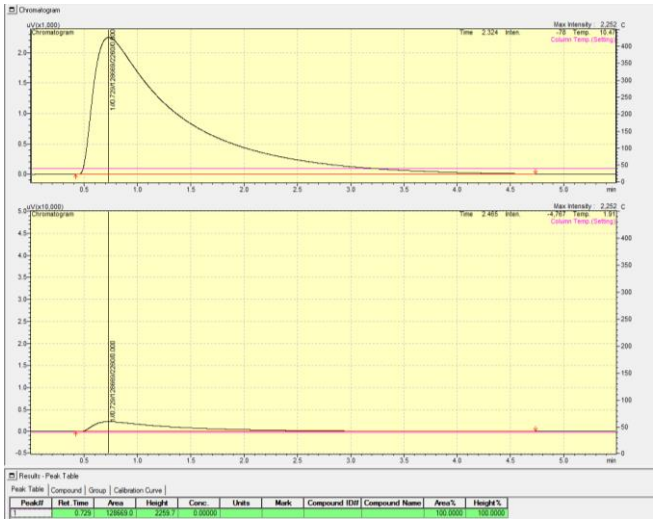


Figure B. 4: Chromatograph for Standard H_2

Last week of Experimental Start-up

D1

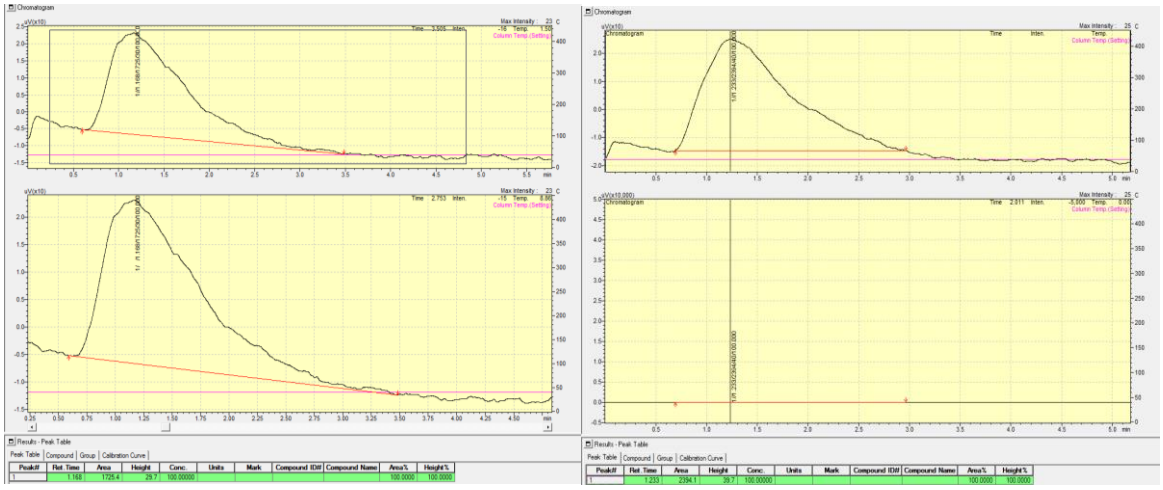


Figure B. 5: Chromatograph for DW mix (SS: DW - 1:3)

D2

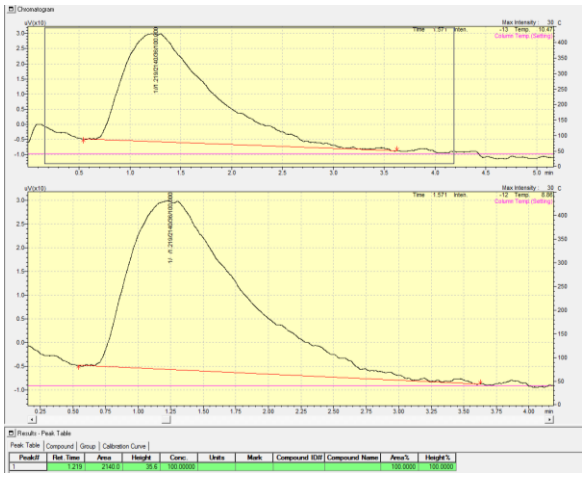


Figure B. 6: Chromatograph for DW mix (SS: DW - 0:1)

D3

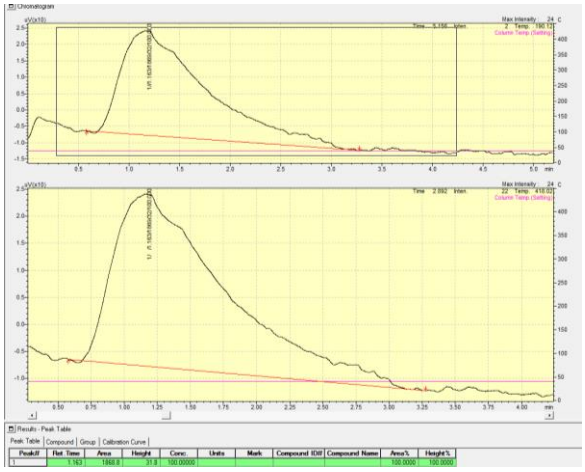


Figure B. 7: Chromatograph for DW mix (SS: DW - 1:1)

D4

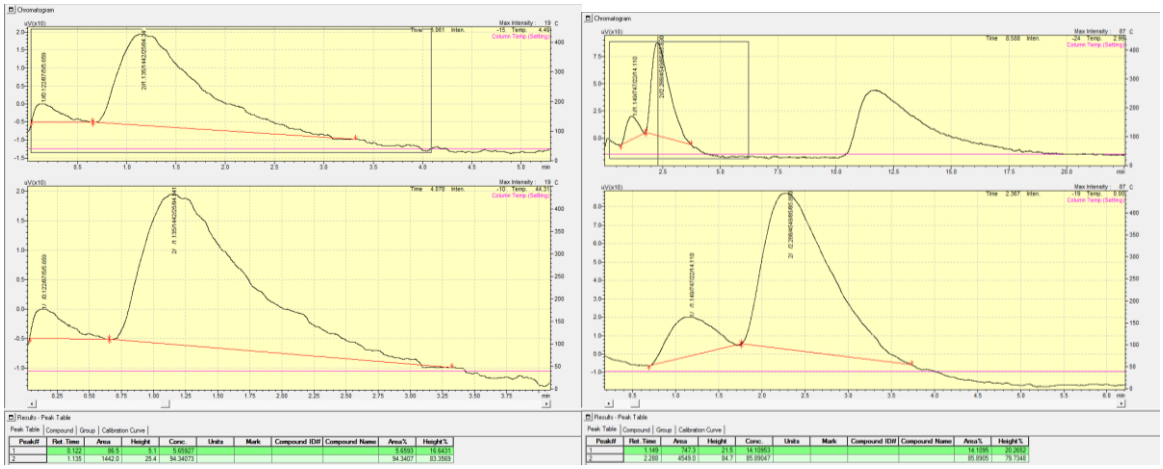


Figure B. 8: Chromatograph for DW mix (SS: DW - 1:3) @ 55ºC

D5

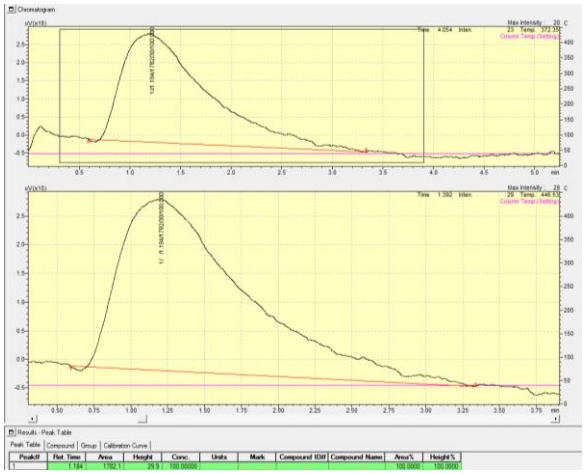


Figure B. 9: Chromatograph for DW mix (SS: DW - 1:1) @ 35ºC

D6

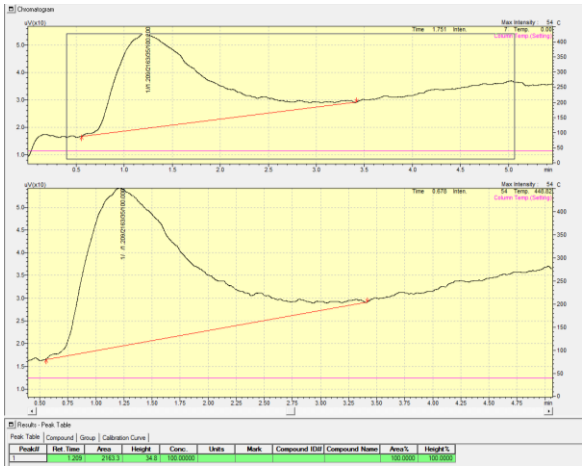


Figure B. 10: Chromatograph for DW mix (SS: DW - 3:1) @ 55ºC

D7

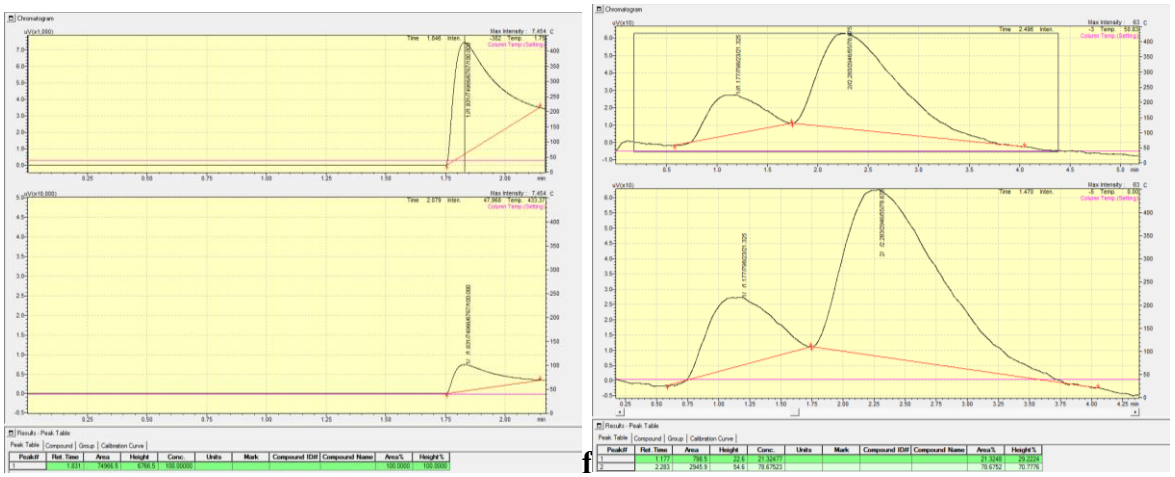


Figure B. 11: Chromatograph for DW mix (SS: DW - 1:1) @ 55ºC

D8

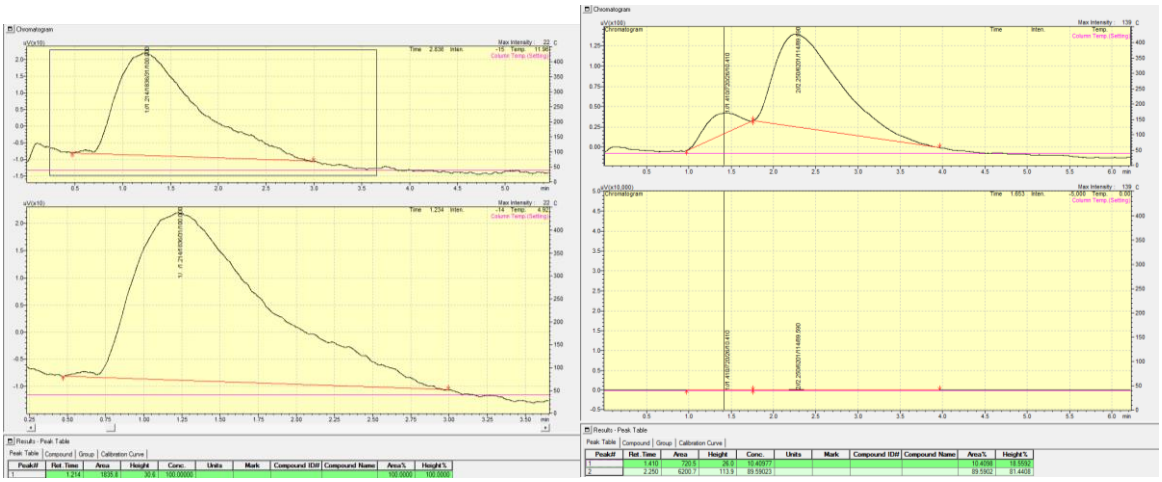


Figure B. 12: Chromatograph for DW mix (SS: DW - 1:0) @ 25ºC

D9

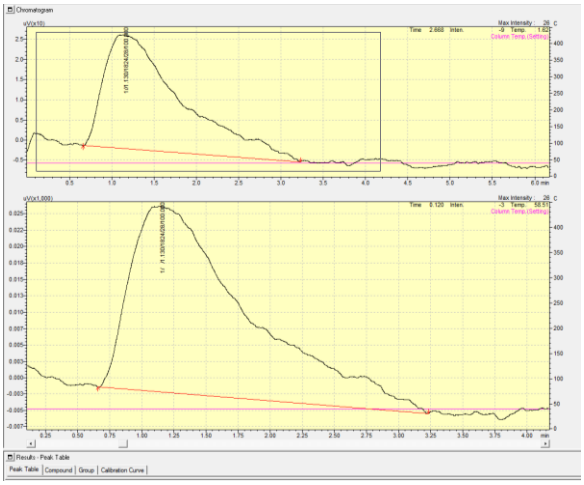


Figure B. 13: Chromatograph for DW mix (SS: DW - 1:1) @ 35ºC

D10

Figure B. 14: Chromatograph for DW mix (SS: DW - 0:1) @ 55ºC

D11

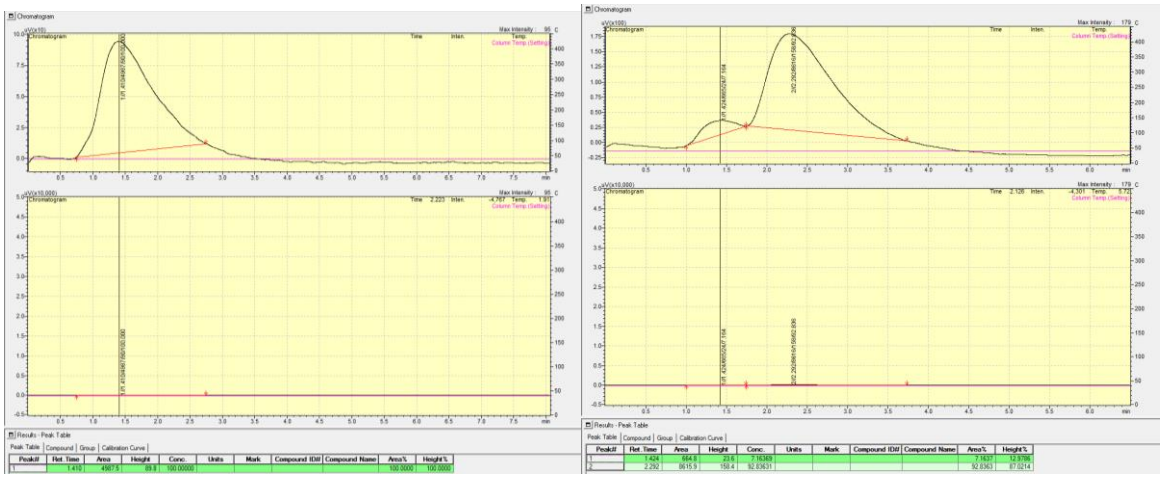


Figure B. 15: Chromatograph for DW mix (SS: DW - 1:0) @ 35ºC

D12

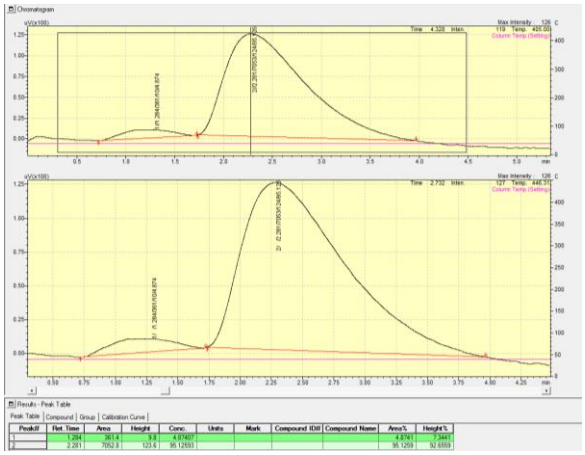


Figure B. 16: Chromatograph for DW mix (SS: DW - 1:0) @ 55ºC

D13

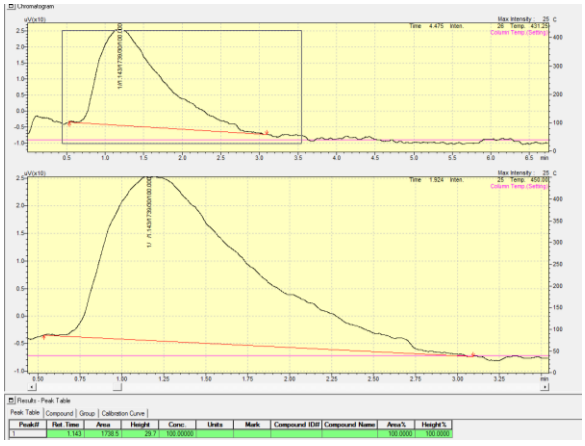


Figure B. 17: Chromatograph for DW mix (SS: DW - 0:1) @ 35ºC

D14

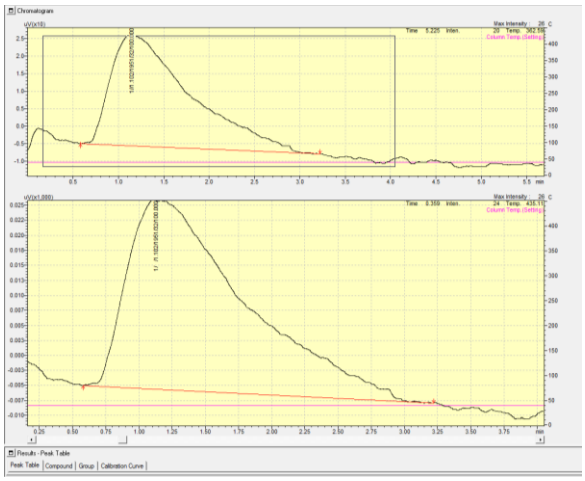


Figure B. 18: Chromatograph for DW mix (SS: DW - 0:1) @ 35°C

D15

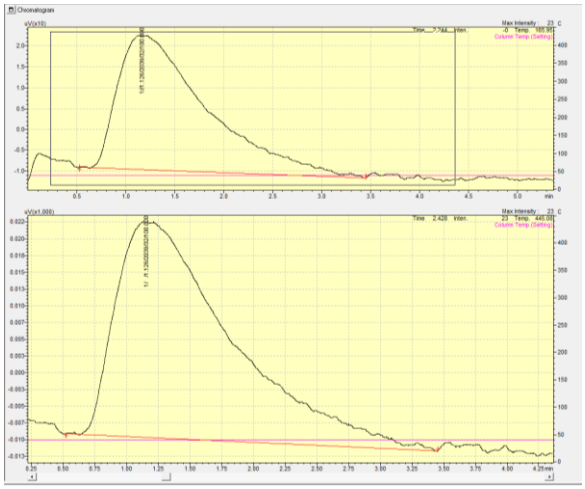


Figure B. 19: Chromatograph for DW mix (SS: DW - 3:1) @ 35°C

Chromatograph for ISR Experiment

Week 1

D12

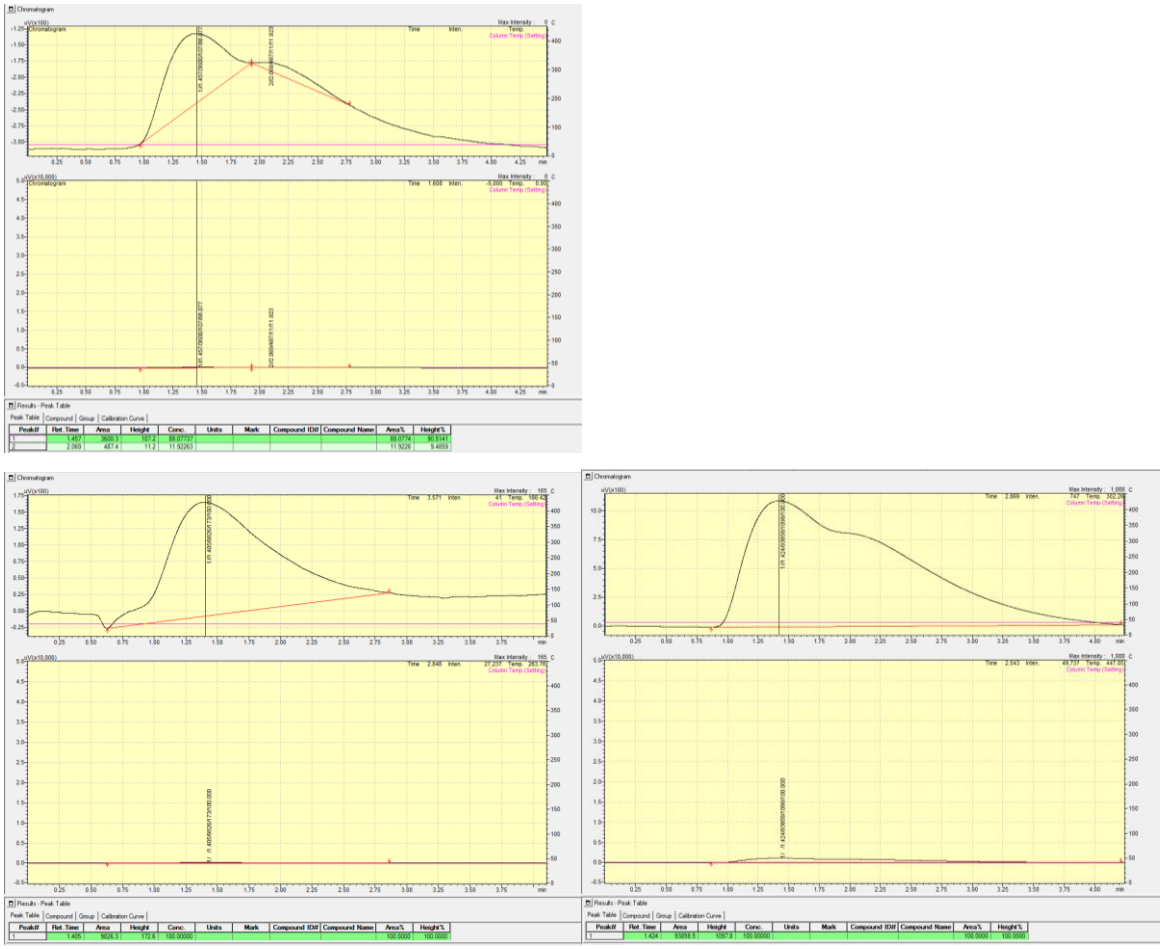


Figure B. 20: Chromatograph for DW (ISR - 1:2) @ 35°C; at the gas collection section(left) and at the tip of the bioreactor (right)

D22

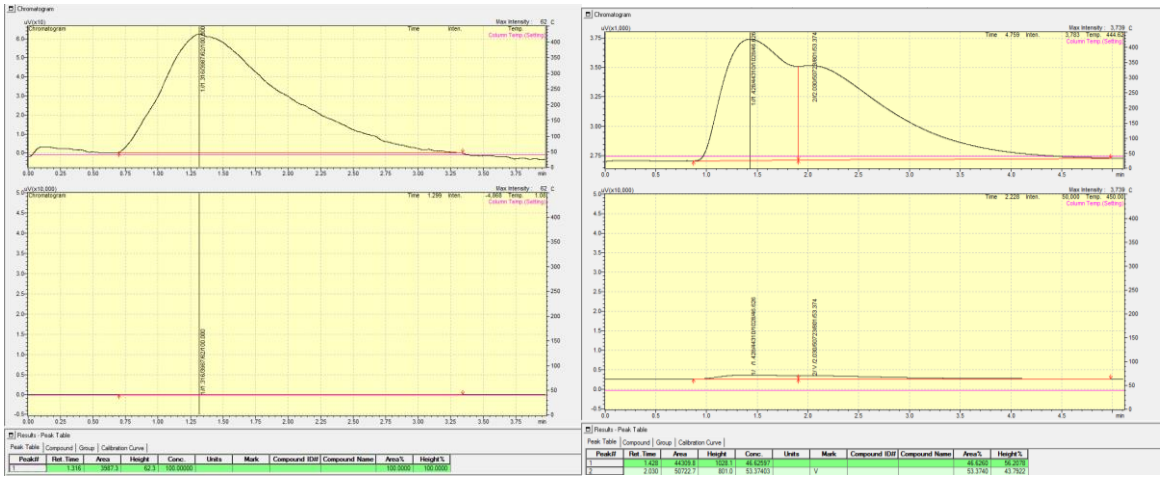


Figure B. 21: Chromatograph for DW (ISR - 1:1) @ 35°C; at the gas collection section(left) and at the tip of the bioreactor (right)

D32

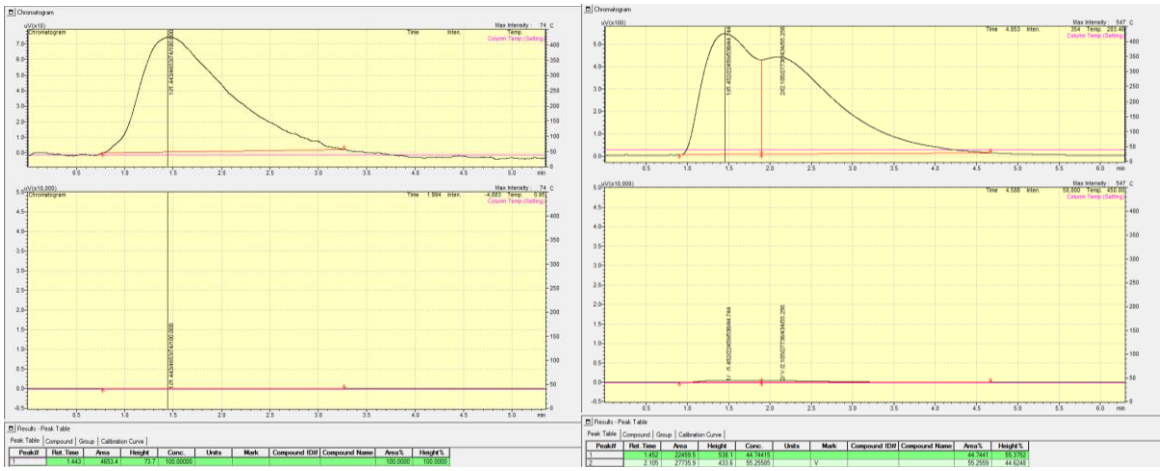


Figure B. 22: Chromatograph for DW (ISR - 2:1) @ 35°C; at the gas collection section(left) and at the tip of the bioreactor (right)

S12

Figure B. 23: Chromatograph for SW (ISR - 1:2) @ 35°C at the tip of the bioreactor

S22

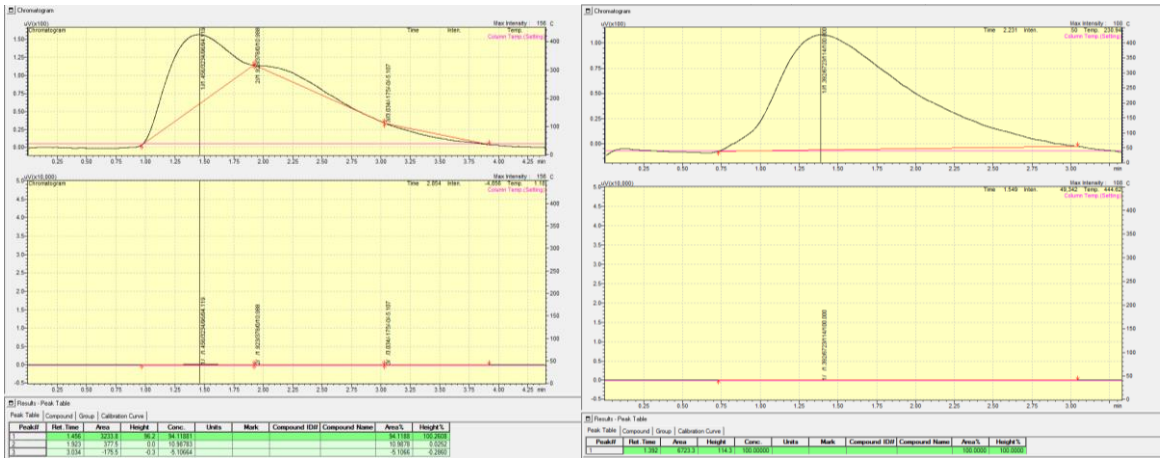
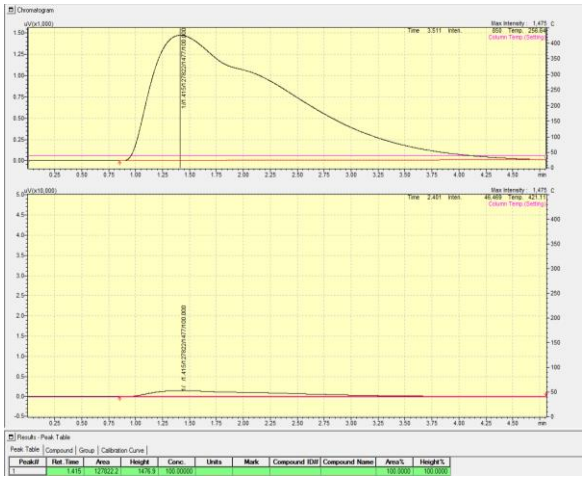


Figure B. 24: Chromatograph for SW (ISR - 1:1) @ 35°C; at the gas collection section(right) and at the tip of the bioreactor (left)



S1

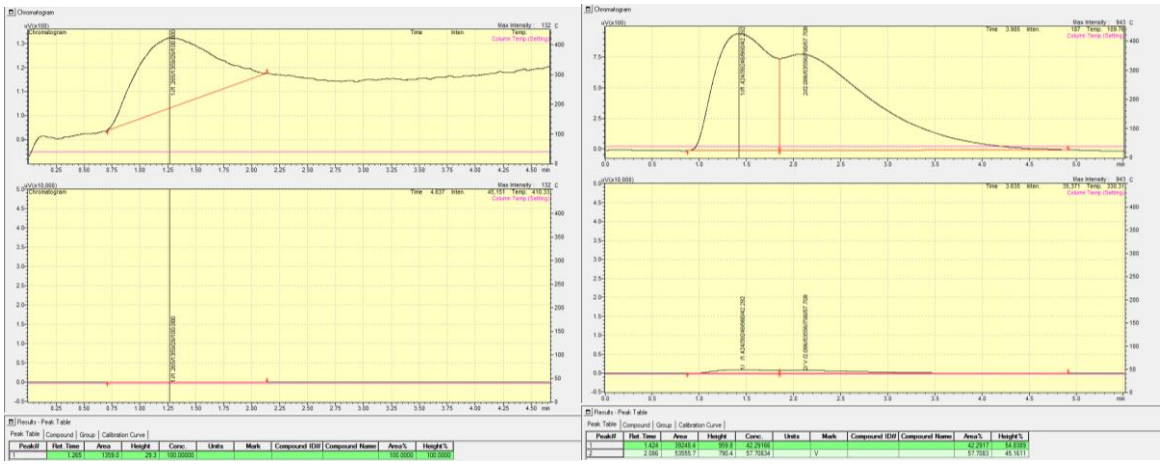


Figure B. 25: Chromatograph for SW (ISR - 1:2) @ 25ºC; at the gas collection section(left) and at the tip of the bioreactor (right)

S2

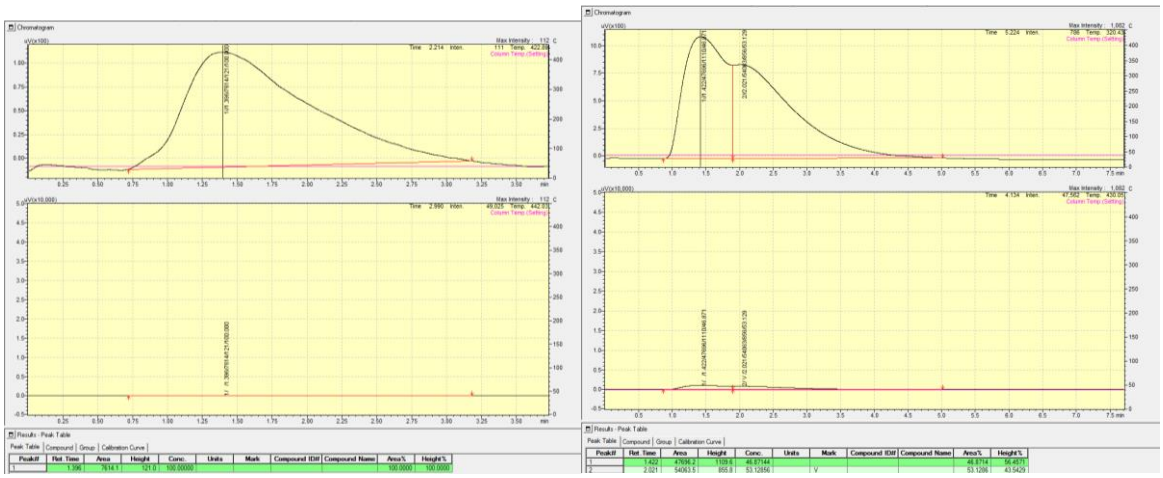


Figure B. 26: Chromatograph for SW (ISR - 1:1) @ 25ºC; at the gas collection section(left) and at the tip of the bioreactor (right)

S3

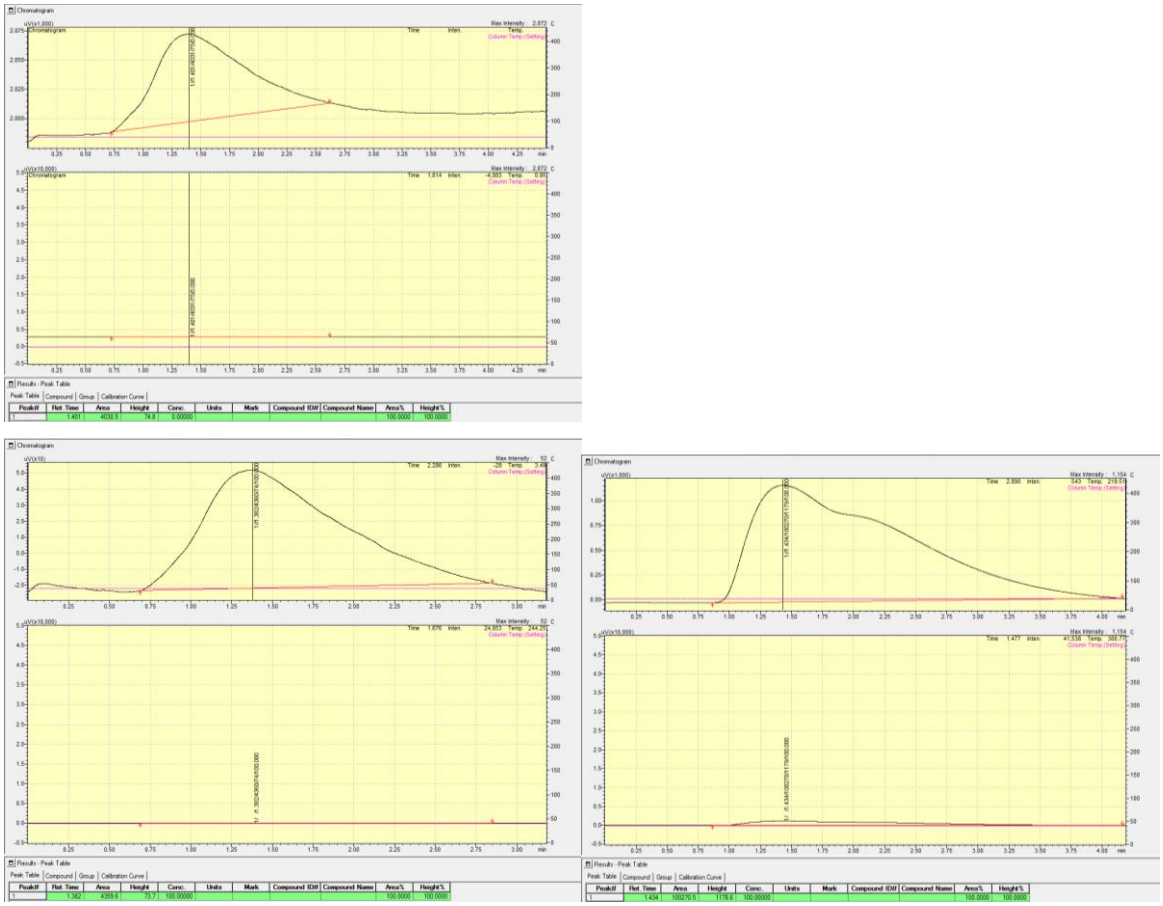


Figure B. 27: Chromatograph for SW (ISR - 2:1) @ 25°C; at the gas collection section(left) and at the tip of the bioreactor (right)

D1

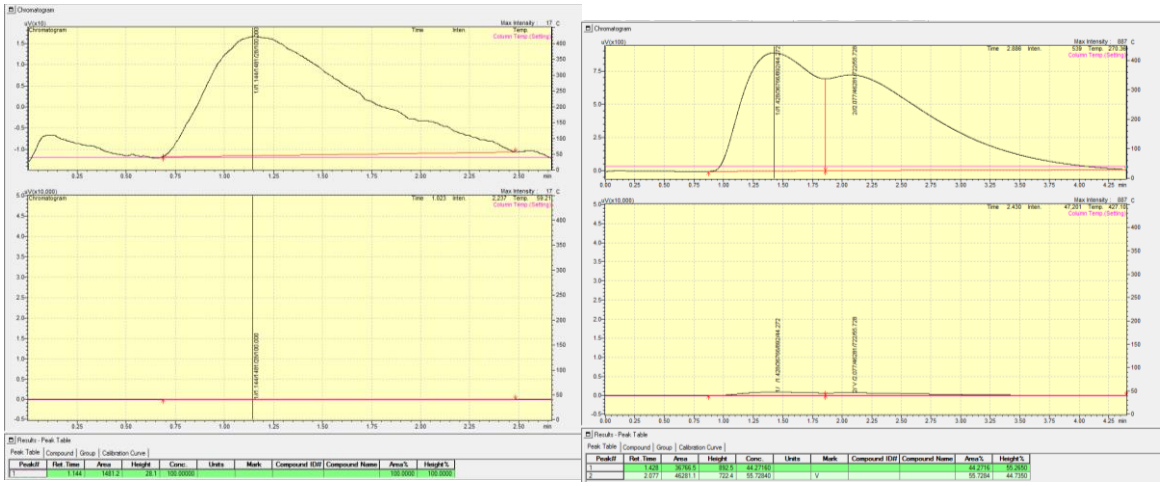


Figure B. 28: Chromatograph for DW (ISR - 1:2) @ 25°C; at the gas collection section(left) and at the tip of the bioreactor (right)

D2

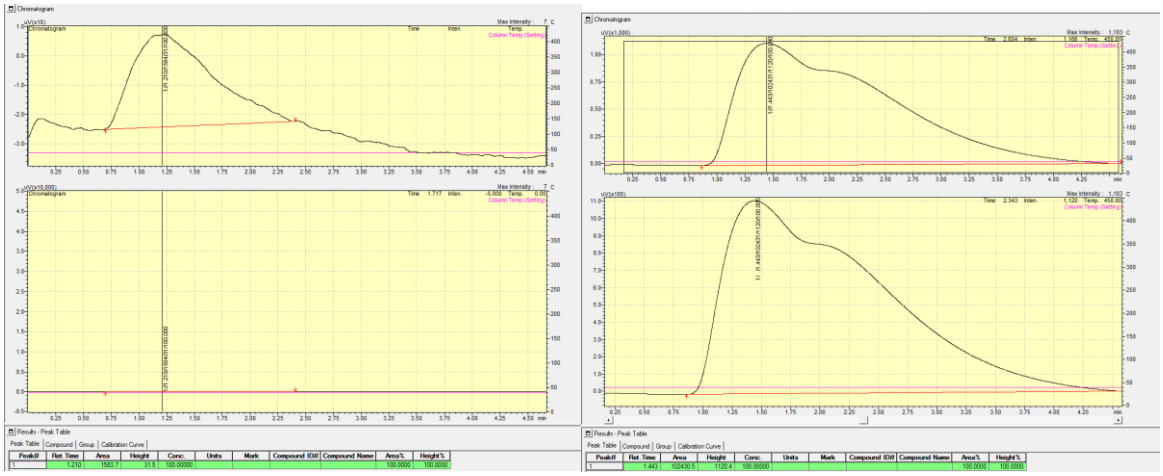


Figure B. 29: Chromatograph for DW (ISR - 1:1) @ 25ºC; at the gas collection section(left) and at the tip of the bioreactor (right)

D3

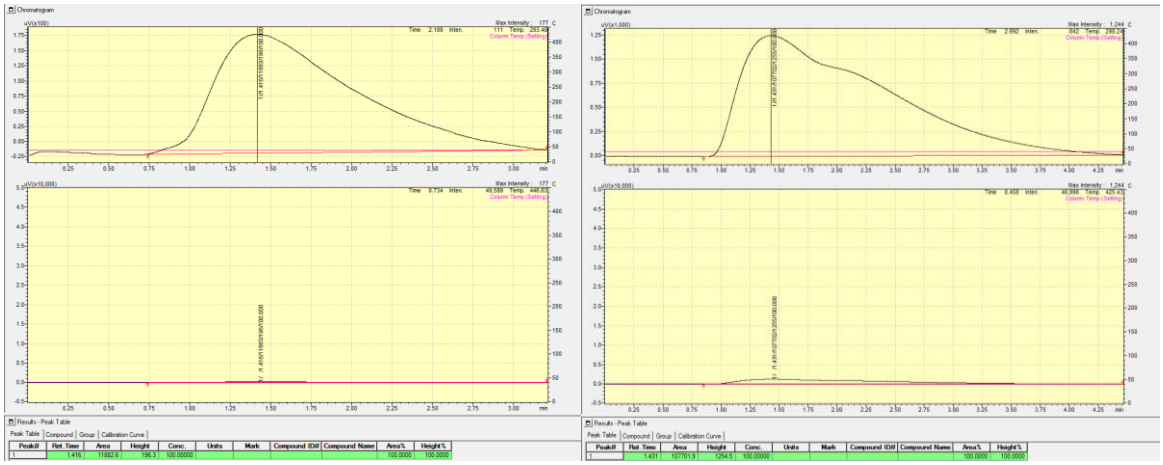


Figure B. 30: Chromatograph for DW (ISR - 2:1) @ 25ºC; at the gas collection section(left) and at the tip of the bioreactor (right)

Chromatograph for ISR Experiment

Week 3

D1

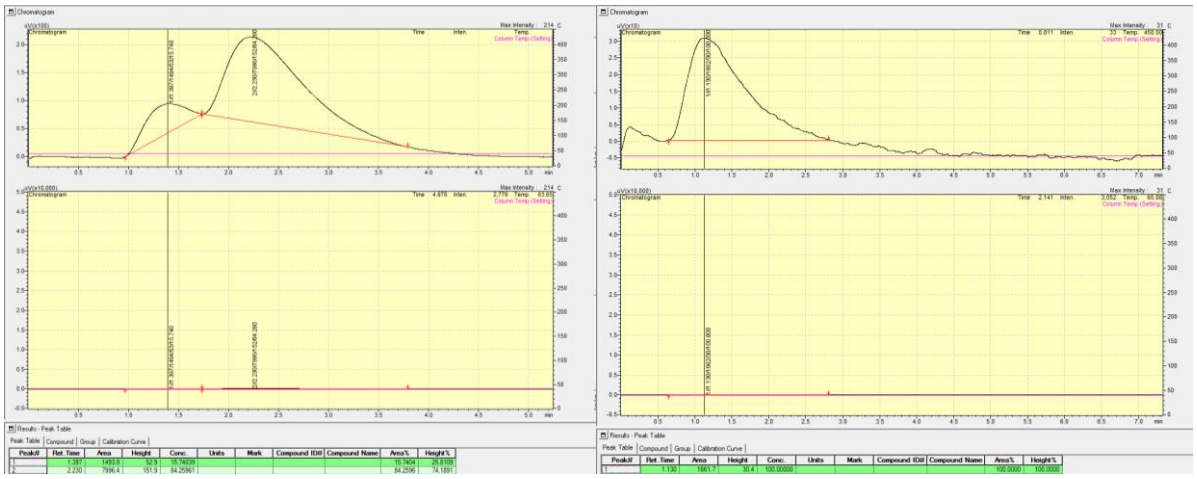


Figure B. 31: Third Week Chromatograph for DW (ISR - 1:2) @ 25ºC; at the gas collection section(right) and at the tip of the bioreactor (left)

D2

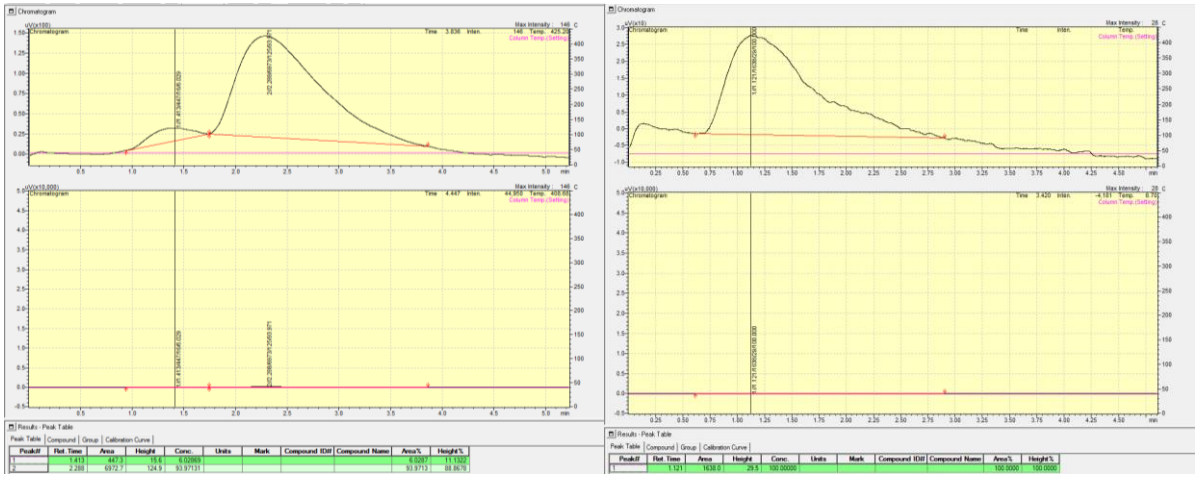


Figure B. 32: Third Week Chromatograph for DW (ISR - 1:1) @ 25ºC; at the gas collection section(right) and at the tip of the bioreactor (left)

S3

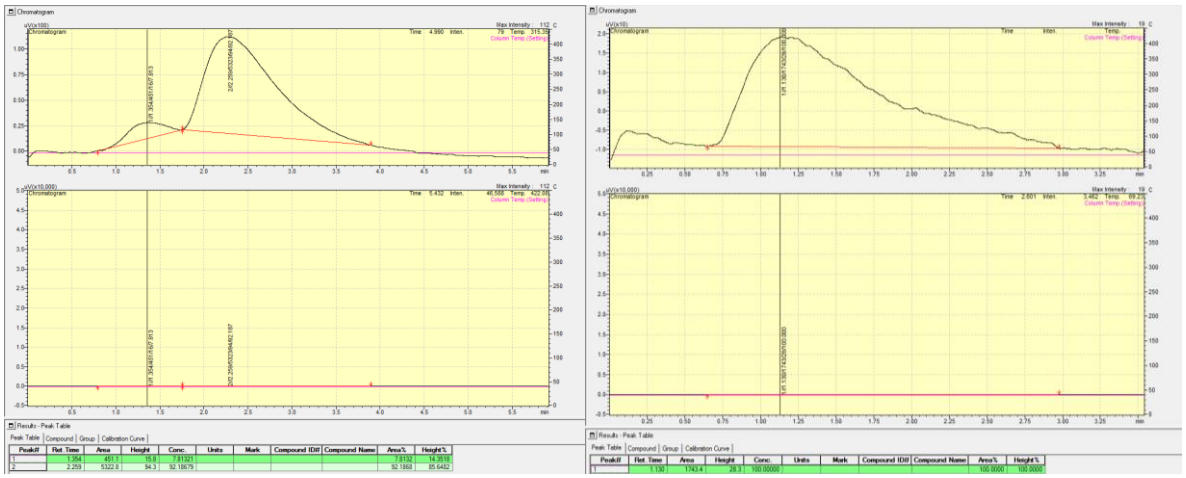


Figure B. 33: Third Week Chromatograph for SW (ISR - 2:1) @ 25°C; at the gas collection section(right) and at the tip of the bioreactor (left)

S2

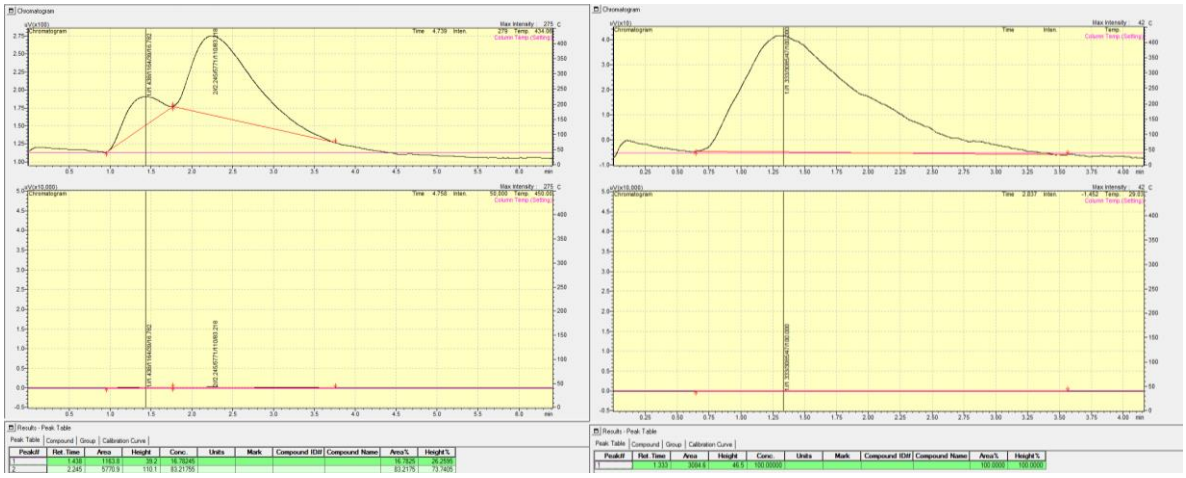


Figure B. 34: Third Week Chromatograph for SW (ISR - 1:1) @ 25°C; at the gas collection section(right) and at the tip of the bioreactor (left)

D3

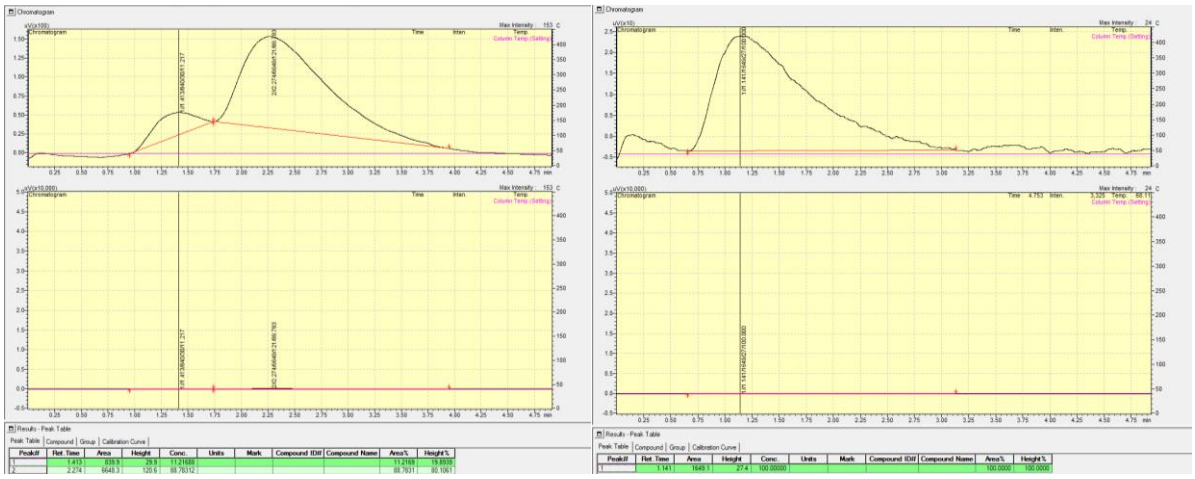


Figure B. 35: Third Week Chromatograph for DW (ISR - 2:1) @ 25°C; at the gas collection section(right) and at the tip of the bioreactor (left)

S1

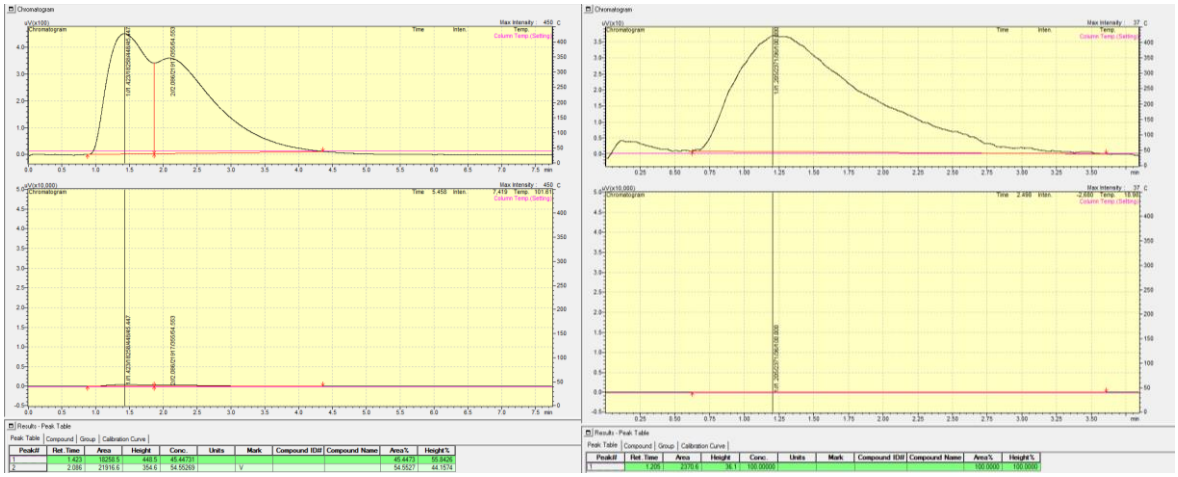


Figure B. 36: Third Week Chromatograph for SW (ISR - 1:2) @ 25°C; at the gas collection section(right) and at the tip of the bioreactor (left)

S12

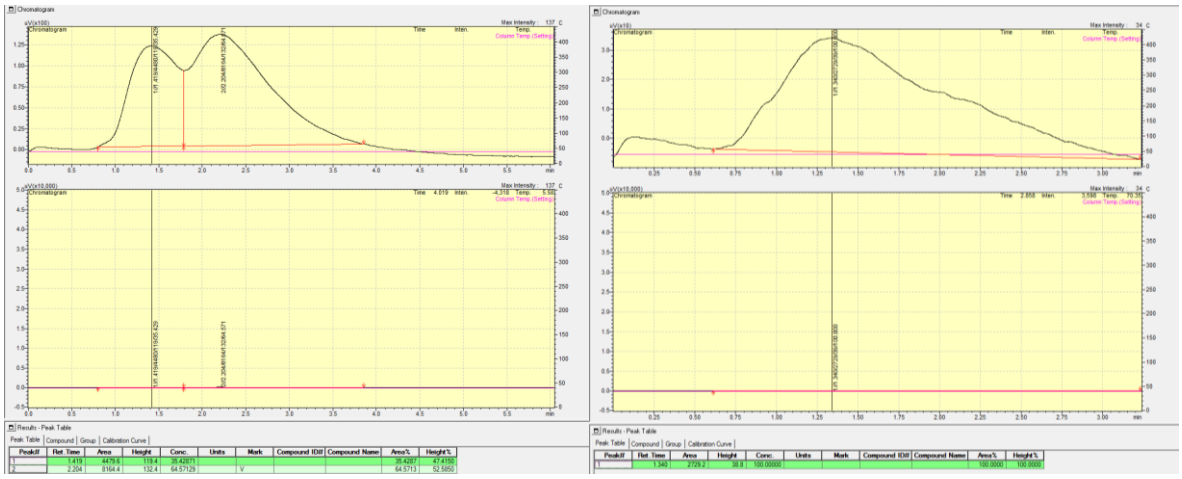


Figure B. 37: Third Week Chromatograph for SW (ISR - 1:2) @ 35°C; at the gas collection section(right) and at the tip of the bioreactor (left)

S22

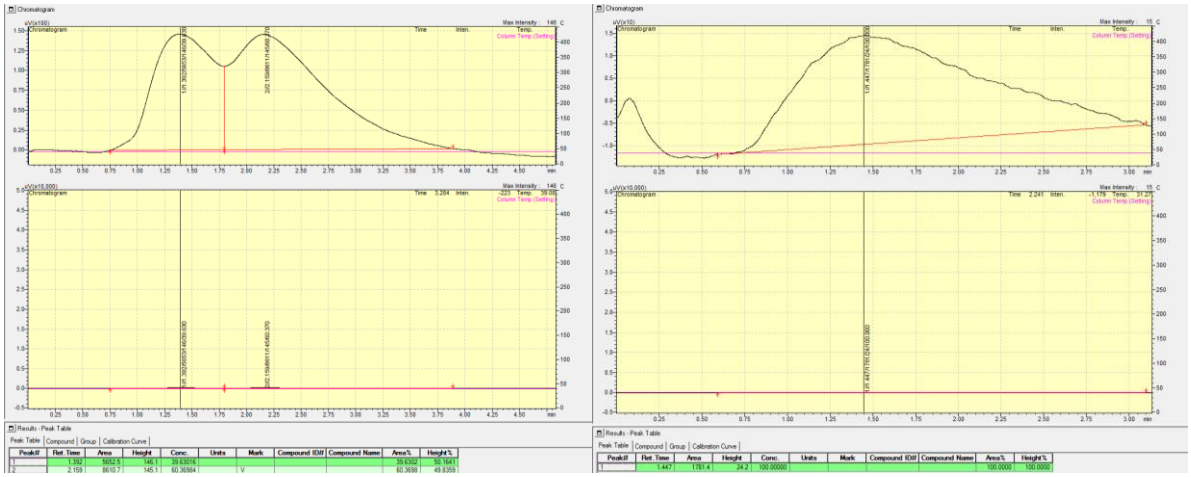


Figure B. 38: Third Week Chromatograph for SW (ISR - 1:1) @ 35°C; at the gas collection section(right) and at the tip of the bioreactor (left)

S32

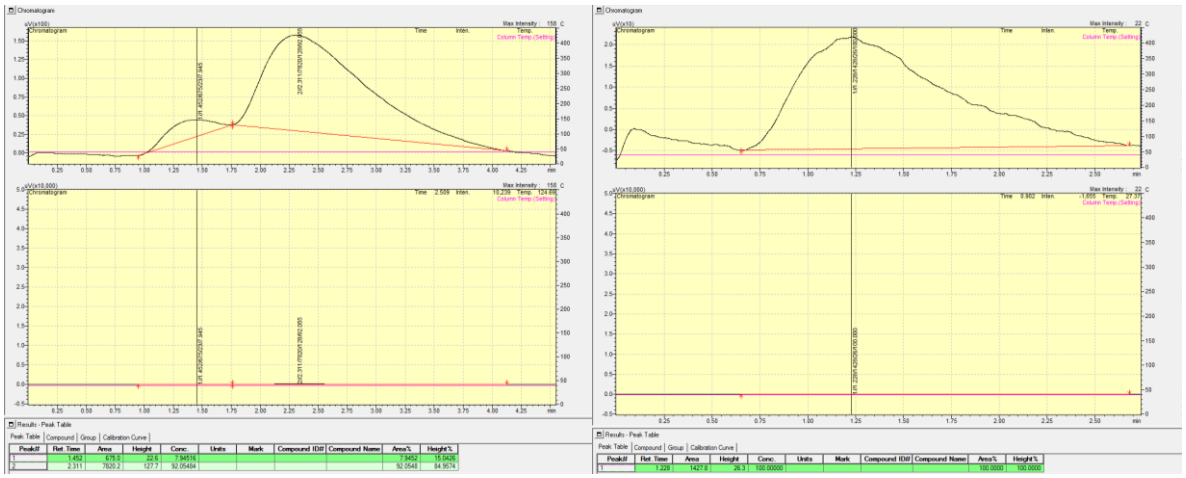


Figure B. 39: Third Week Chromatograph for SW (ISR - 2:1) @ 35°C; at the gas collection section(right) and at the tip of the bioreactor (left)

D12 D32

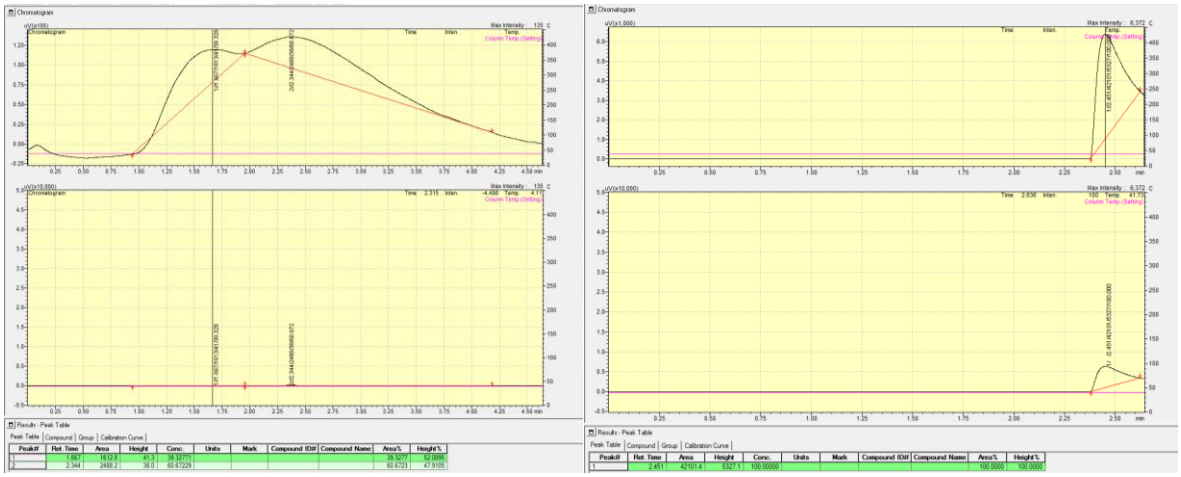


Figure B. 40: Third Week Chromatograph for DW @ 35°C at the tip of the bioreactor; (ISR - 1:2) (left) and (ISR - 2:1) (right)

APPENDIX C

Kinetics Analysis Data and Graph

This section contains the data used for fitting the kinetic models and other graphs that were not shown in the main chapter of the write-up.

Table C. 1: Raw Data for Curve Fitting in Excel and the Values for each Model

Time (Hours)	Methane Production	First Order	Gompertz	Dual-Pool	Chen
0	0.0	0	16.05207	0	-0.01043
2	40.0	34.57028	34.05493	34.62818	38.61674
4	60.0	63.35529	57.3008	63.41747	67.86717
6	87.5	87.32319	82.12921	87.36315	90.78562
8	110.0	107.2801	105.3547	107.289	109.2273
10	120.0	123.8973	125.1636	123.877	124.3873
12	137.5	137.7336	141.0076	137.6923	137.0699
14	147.5	149.2544	153.1278	149.2031	147.8366
16	162.5	158.8473	162.1172	158.798	157.091
18	162.5	166.8348	168.6431	166.799	165.1308
20	180.0	173.4856	173.311	173.4736	172.1805
22	180.0	179.0234	176.6157	179.0438	178.4122
24	180.0	183.6345	178.9388	183.6943	183.9606

Table C. 2: Models Parameters from Non-linear Regression (Excel) with their Total Sum of Squares Error

First Order		Simplified Gompertz		Dual-Pooled First Order		Chen and Hashimoto	
Parameter	Value	Parameter	Value	Parameter	Value	Parameter	Value
Mm	206,5772	Mm	184,2655	Mm	207,5022	Mm	279,6094
k	0,09157	**k**	4,843275	$\mathbf{k_f}$	0,106689	μ_m	2148,973
SSR	155,2236	**c**	0,184218	$\mathbf{k_l}$	0,084456	**k**	26815,63
		SSR	516,568	**a**	0,312085	**HRT**	7
				SSR	155,2149	**SSR**	210,2761

Table C.3: Descriptive statistics for kinetic analysis methane production

Methane Value	
Mean	120.5769231
Standard Error	16.2170619
Median	137.5
Mode	180
Standard Deviation	58.47144822
Sample Variance	3418.910256
Kurtosis	-0.242339033
Skewness	-0.854574727
Range	180
Minimum	0
Maximum	180
Sum	1567.5
Count	13
Confidence Level (95.0%)	35.33394253

Table C.4: Methane Data with confidence interval values.

Time (Hours)	Methane Production	First Order	Lower CI (95%)	Higher CI (95%)
0	0.0	1.54847	-33.7855	36.88241
2	40.0	35.41101	0.077067	70.74495
4	60.0	63.71625	28.38231	99.05019
6	87.5	87.37621	52.04227	122.7102
8	110.0	107.1533	71.81932	142.4872
10	120.0	123.6846	88.35069	159.0186
12	137.5	137.503	102.169	172.8369
14	147.5	149.0536	113.7196	184.3875
16	162.5	158.7085	123.3746	194.0425
18	162.5	166.779	131.445	202.1129
20	180.0	173.525	138.191	208.8589
22	180.0	179.1639	143.8299	214.4978
24	180.0	183.8773	148.5434	219.2113

Table C.5: ANOVA for First order model (OriginPro 9)

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Regression	2	40875.20297	20437.60149	1347.06	6.92E-13
residual	10	151.72011	15.17201		
Uncorrected Total	13	230031.25			
Corrected Total	12	41026.92308			

Table C.6: ANOVA for Simplified Gompertz model (OriginPro 9)

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Regression	3	229514.682	76504.89398	1481.023	1.54E-13
Residual	10	516.56805	51.6568		
Uncorrected Total	13	230031.25			
Corrected Total	12	41026.92308			

Table C.7: ANOVA for Dual -pooled first order model (OriginPro 9)

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Regression	4	40875.20297	10218.80074	538.8238	9.32E-10
Residual	8	151.72011	18.96501		
Uncorrected Total	13	230031.25			
Corrected Total	12	41026.92308			

Table C.8: Fit Summary for the kinetic models (OriginPro 9)

	First Order	Simplified Gompertz	Dual-pooled first order
Number of Points	13	13	13
Degrees of Freedom	10	10	8
Reduced Chi-Sqr	15.17201	51.6568	18.96501
Residual Sum of Squares	151.72011	516.56805	151.72011
R-Square (COD)	0.9963	0.98741	0.9963
Adj. R-Square	0.99556	0.98489	0.99445
Fit Status	Succeeded (100)	Succeeded (100)	Succeeded (100)

Table C.9: Predicted value for methane production for each kinetic models

First Order	Gompertz	Dual pooled
1.54847	16.05212	1.54847
35.41101	34.05494	35.41101
63.71625	57.30076	63.71625
87.37621	82.12913	87.37621
107.1533	105.35462	107.15326
123.6846	125.16357	123.68463
137.503	141.00765	137.50298
149.0536	153.12791	149.05355
158.7085	162.11733	158.70852
166.779	168.64337	166.77898
173.525	173.3113	173.52497
179.1639	176.61605	179.16385
183.8773	178.93914	183.87731

OriginPro Graph for the Kinetic Models Fitting

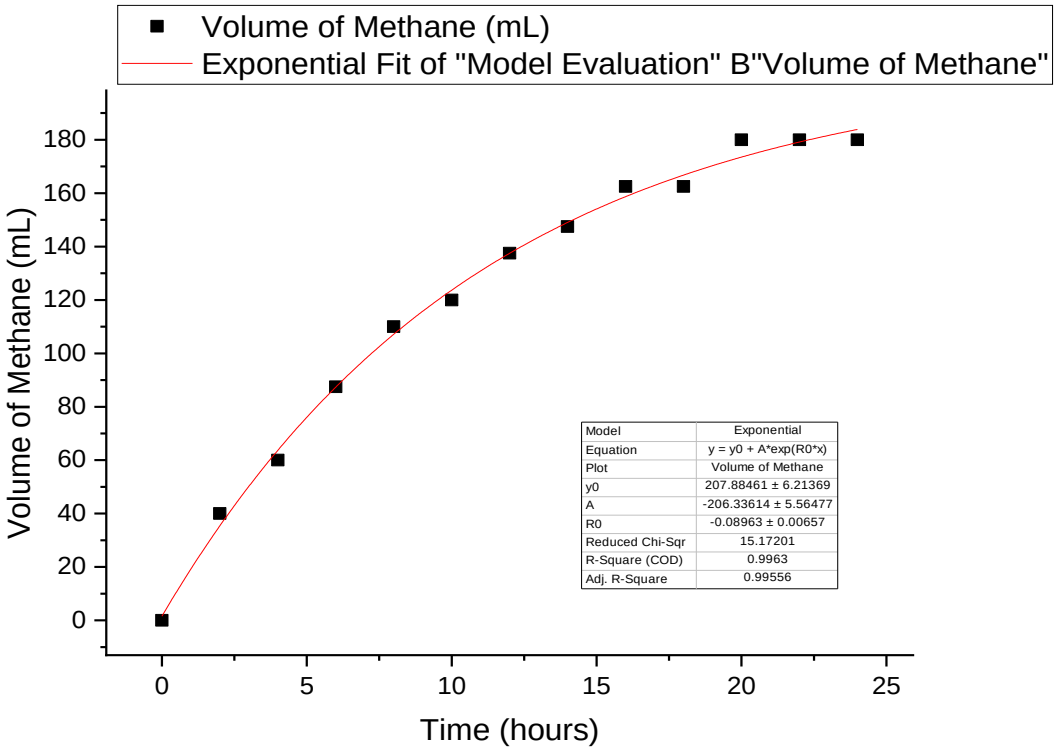


Figure C. 1: Curve fitting plot for First Order Kinetic Model (OriginPro)

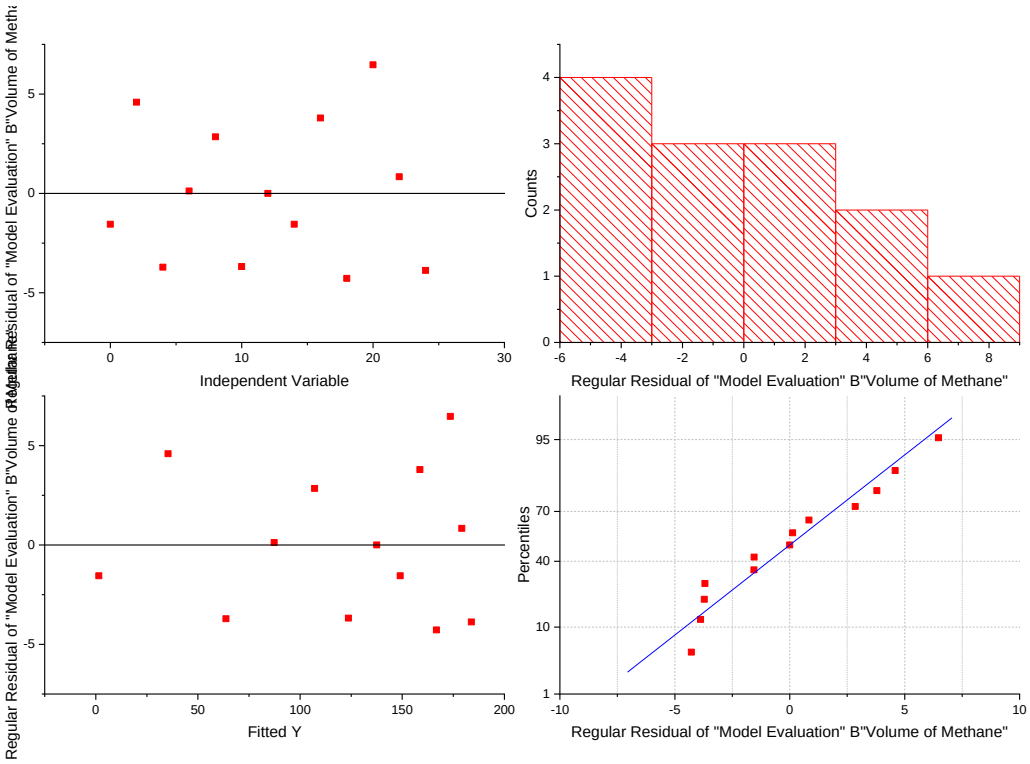


Figure C. 2: Residual Plot for the First Order Model (OriginPro)

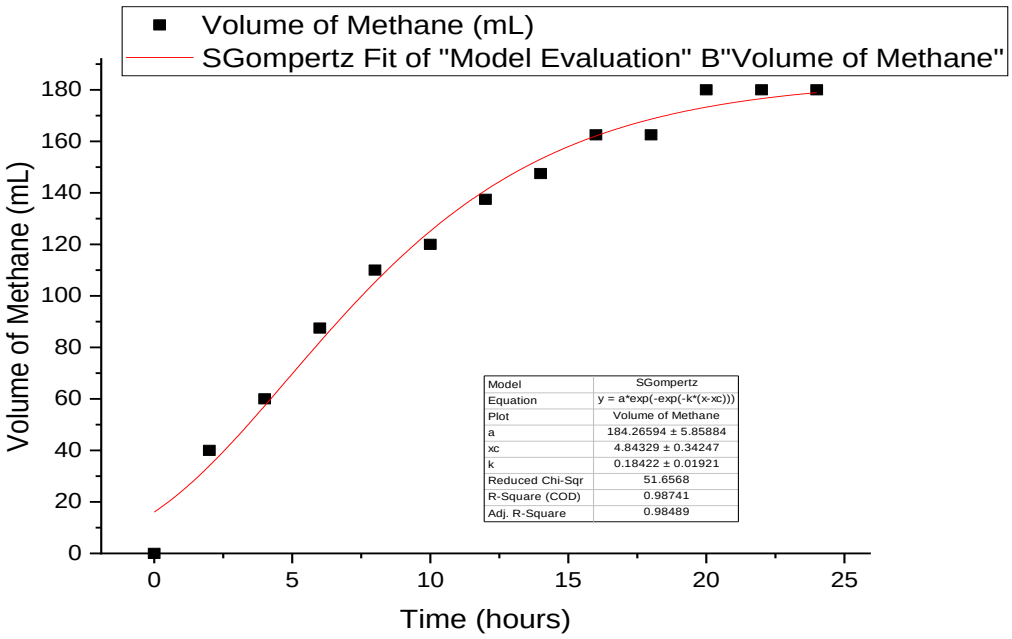


Figure C. 3: Curve fitting plot for Simplified Gompertz Kinetic Model (OriginPro)

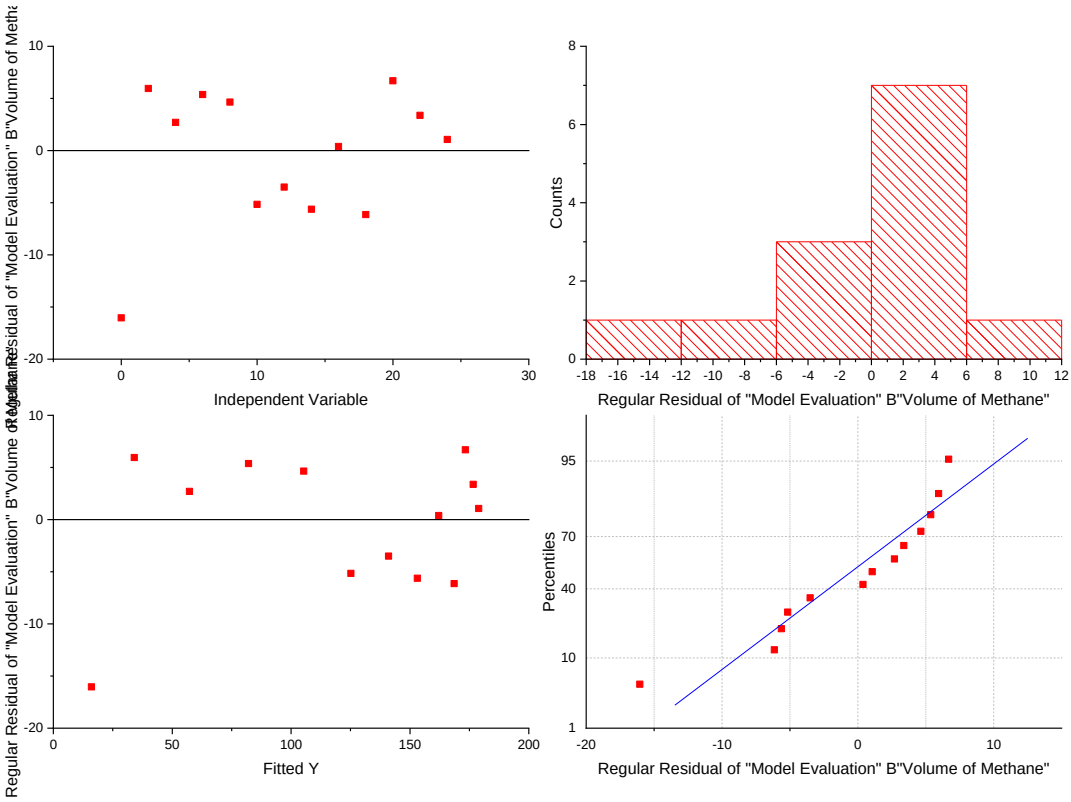


Figure C. 4: Residual Plot for the Simplified Gompertz Model (OriginPro)

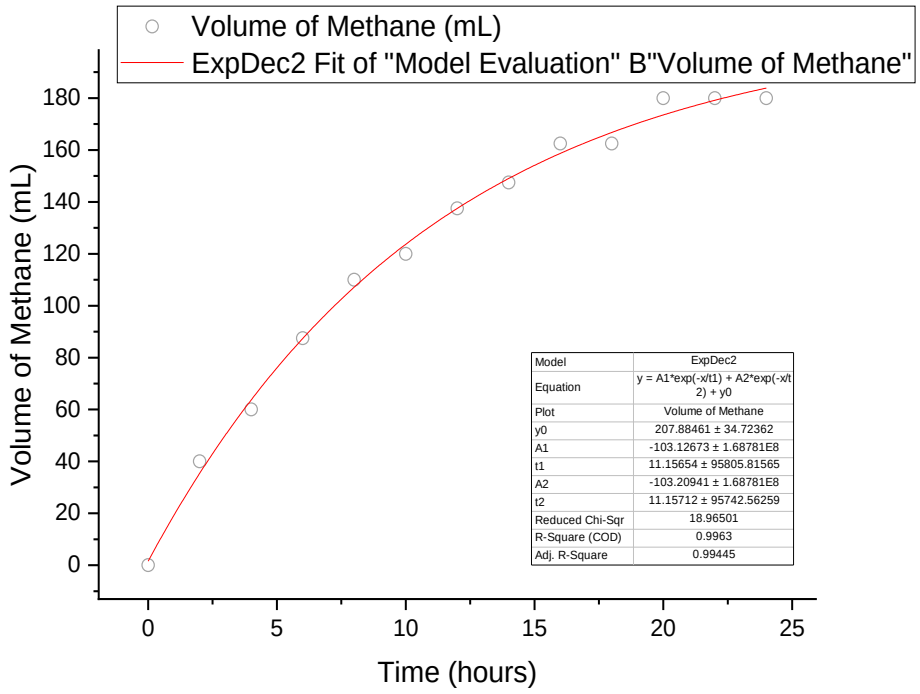


Figure C. 5: Curve fitting plot for Dual-Pooled First Order Kinetic Model (OriginPro)

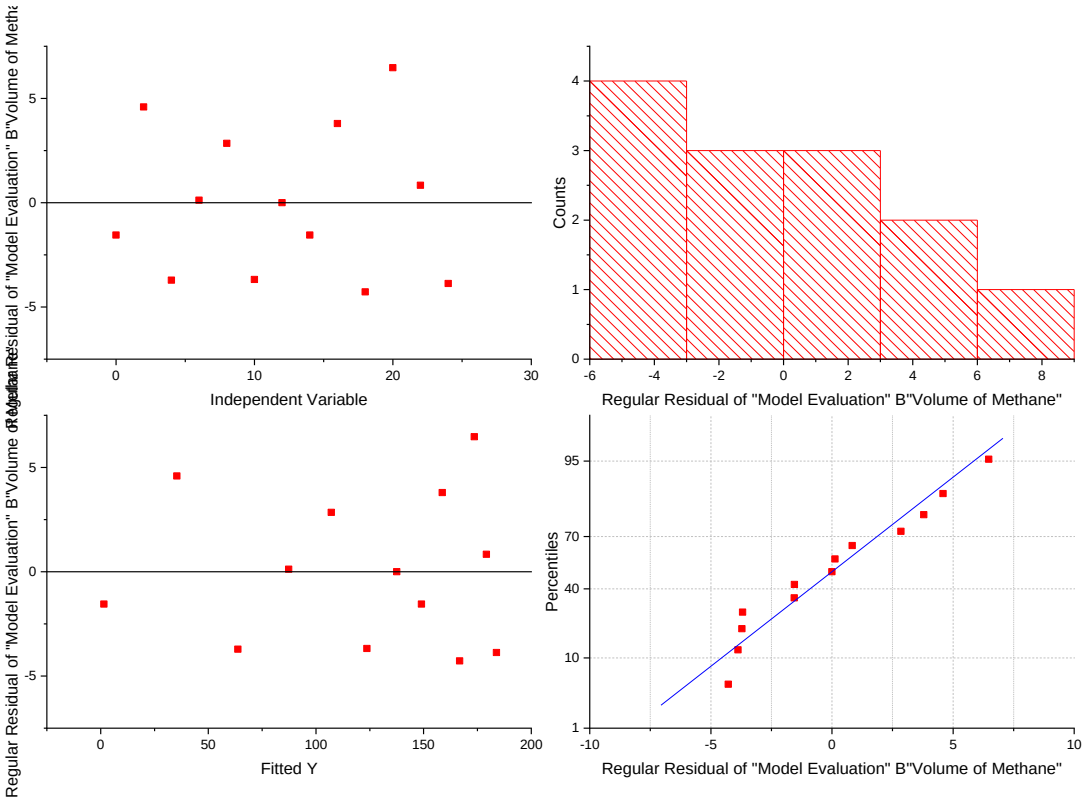


Figure C. 6: Residual Plot for the Dual-Pooled First Order Model (OriginPro)

APPENDIX D

The section contains the design of experiment for the Sugar and Dairy wastewater mix-ratio in coded form. The result, model graph and report are also shown in this section.

Table D. 1: Experimental Design from Design Expert

Run	SLUDGE	DAIRY WW	TEMP (ºC)	Biogas Yield ($mL/gCOD_{added}$)	% COD Reduction
1	1	0	0	35.98	85.82
2	0	1	1	78.4	94.76
3	0.5	0.5	0	442.36	94.38
4	0.25	0.75	0	262.31	89.66
5	1	0	1	938.21	92.08
6	0.5	0.5	1	92.27	84.55
7	1	0	0	84.29	32.69
8	0.25	0.75	1	353.93	95.15
9	0.5	0.5	1	1400.99	25.8
10	0	1	0	333.48	89.27
11	0.75	0.25	1	960.65	90.55
12	0.5	0.5	0	-2088.22	-0.83
13	0	1	0	215.48	91.57
14	0	1	0	367.73	24.14
15	0.75	0.25	0	62.39	83.52

Note: Sludge and dairy wastewater are in fraction since they are mixture component and 0 and 1 under temperature stand for 25ºC and 35ºC respectively.