UNIVERSITI TEKNOLOGI MARA

ENHANCEMENT OF METHANE PRODUCTION BY ANAEROBIC CO-DIGESTION OF IWK SLUDGE AND FOOD WASTES

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NUR ANISAH BINTI ABDUL LATIF

Thesis submitted in fulfilment
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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 5 Mac 2026 to conduct the final examination of Nur Anisah Binti Abdul Latif on her Masters of Science thesis entitled "Enhancement Of Methane Production By Anaerobic Co-Digestion Of IWK Sludge And Food Wastes" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The Panel of Examiners was as follows:

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ABSTRACT

Indah Water Konsortium (IWK) sludge, a wastewater treatment by-product in Malaysia, contains substantial organic matter and presents promising potential for bioenergy recovery through anaerobic digestion. However, mono-digestion of sewage sludge often yields low methane production due to limited biodegradability, low solids content, and microbial imbalance. This study investigates the enhancement of methane production via anaerobic co-digestion (AcoD) of IWK sludge with food wastes, specifically non-dairy creamer waste (NDCW) and instant coffee waste (CW), using IWK thickened sludge as inoculum. The study objectives were to optimize the inoculum-to-substrate (I:S) ratio and hydraulic retention time (HRT), evaluate the effect of chemical pre-treatment on methane yield, and assess system performance at pilot scale. Physicochemical characterization of substrates and inoculum included pH, total solids (TS), volatile solids (VS), chemical oxygen demand (COD), volatile fatty acids (VFA), alkalinity, total suspended solids (TSS), and volatile suspended solids (VSS). Batch experiments were conducted using biochemical methane potential (BMP) tests in 125 mL serum bottles, followed by scale-up in a 40 L continuous stirred tank reactor (CSTR) operated under mesophilic conditions (35–37 °C) and pH 6.8–7.0. The optimal I:S ratio of 2:1 (IWK sludge: CW) for the batch experiments produced the highest methane yield of 17.31 mL CH_4/gVS. Acid pre-treatment using 2 M H_2SO_4 further enhanced methane production to 18.49 mL CH_4/gVS, representing a 45% improvement. In contrast, the control treatments—Control-CW (0.013 ± 0.0007 mL CH_4/g VS) and Control-NDCW (0.005 ± 0.0003 mL CH_4/g VS)—produced negligible methane, emphasizing the necessity of an active inoculum and balanced substrate interactions. At pilot scale, the pre-treated 2:1 mixture achieved a methane yield of 40,936.7 mL CH_4/gVS and COD removal efficiency of 61.6%. Methane composition remained stable at 50–70%, confirming effective digestion performance. Overall, the findings demonstrate that optimizing operational parameters and applying chemical pre-treatment significantly improve anaerobic digestion efficiency. The successful scale-up in 40L continuous stirred tank reactor confirms the feasibility of co-digesting sewage sludge with food waste for sustainable biogas production.

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LIST OF SYMBOLS

Symbols

Cp(s)	Concentration Of The Component (P) In The Sample
Cp(std)	Concentration Of The Standard In The Reference
ap(s)	Area Of The Peak Of The Component (P) In The Sample Chromatogram

LIST OF ABBREVIATIONS

Abbreviations	
AcoD	Anaerobic Co-Digestion
AD	Anaerobic Digestion
AL	Alkalinity
BMP	Biochemical Methane Potential
CH_4	Methane
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
CW	Coffee Waste
GC-FID	Gas Chromatography- Flame Ionization Detector
GHG	Greenhouse Gases
H_2S	Hydrogen Sulphide
H_2SO_4	Sulphuric Acid
HRT	Hydraulic Retention Time
IWK	Indah Water Konsortium
M	Molarity
MC	Moisture Content
mg/L	Miligram/Litre
NDCW	Non-Dairy Creamer Waste
O_2	Oxygen
OLR	Organic Loading Rate
S	Thickened Sludge
STP	Sewage Treatment Plant
TS	Total Solid
TSS	Total Suspended Solid
VFA	Volatile Fatty Acid

VS	Volatile Solid
VSS	Volatile Suspended Solid
WWTP	Wastewater Treatment Plant

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Malaysia's national sewerage system (mostly operated by IWK) produces large and increasing sludge volumes by about 5.3 to 7.4 million m³ of sewage sludge annually. This is expected to increase to around 10 million m³ per year by 2035 due to urban growth and expanding wastewater treatment coverage. Sewage sludge is the by-product of the wastewater treatment process that is a rich source of organic matter with cellulose, hemicellulose, and lignin content similar to plant tissues. Consequently, sewage sludge can be considered as biomass (Romdhana et al., 2009 & Goyal et al., 2008). Interest in energy recovery from sewage sludge has recently grown because this idea simultaneously and positively addresses both the energy issue and the environmental issues associated with conventional sludge treatment, such as landfilling and land application (Figure 1.1). The extraction of useable energy from sewage sludge has been accomplished using a variety of processes, such as pyrolysis and anaerobic digestion. One of the most advanced and a cost-effective method for converting sludge into methane-rich bioenergy (biogas/electricity) is anaerobic digestion (AD).

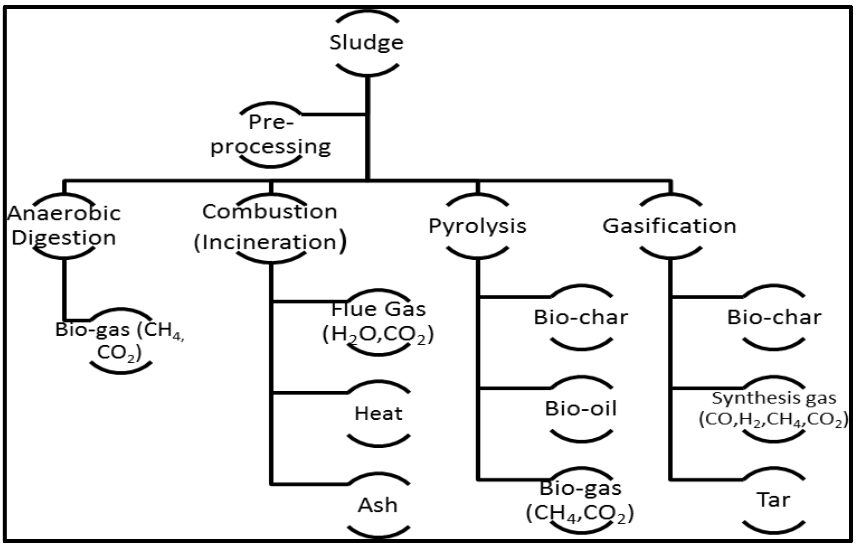


Figure 1.1 The Potential Sludge -To-Energy Recovery Routes (Oladejo et al., 2019)

In 2011, Malaysia's national wastewater management business, Indah Water Konsortium (IWK), began converting sludge into biogas by anaerobic digestion (AD). Using the energy potential of biogas generated during the treatment process, this project was a component of IWK's endeavors to enhance sustainability in wastewater treatment. By producing electricity from biogas, which is mostly made up of methane, wastewater treatment facilities are able to operate more sustainably and with less dependency on outside energy sources. However, the biogas produced did not meet the expected capacity designed for the biogas plant with only 2500 m^3/day produced instead of recommended 8000 m^3/day (Pantai 2, STP), which could be due to mono-digestion of sewage sludge and therefore on September 2019, IWK has made an initiative by adding food wastes without any treatments to enhance biogas production. The coffee and non-dairy creamer (NDC) waste are by-products from the food and beverage sector. These wastes were used as substrates for this anaerobic co-digestion (AcoD). Surprisingly, the biogas generated was doubled reaching 5000 m^3/day compared to mono-digestion, but still did not achieved the total biogas volume of the digester. The NDC, however, is lactose-free. It is a liquid or powder substitute for milk or cream that creates a high water-dissolved fat emulsion from vegetable oil (30% hydrogenated coconut or palm oil) to reduce the amount of milk or swap out milk powder in foods like oatmeal, baked goods, coffee, and others (Dedin et al., 2016). According to Huang et al., (2019), NDC is composed of 60-65% glucose or corn syrup, 2-5% sodium caseinate, stabilizers, and emulsifier (Ramesh et al., 2008) and contains the least amount of minerals at 1.2%, the most amount of protein at 8.5%, the least amount of fat at 32.5%, and the highest amount of carbohydrates at 58%. The by-products of the NDC sector are liquid waste and sewage sludge. The inadequate treatment of NDC wastewater will impact the environment.

NDC sludge and wastewater contain substantial amounts of organic matter, particularly proteins, lipids, and carbohydrates (Dewi et al., 2016a, 2016b; Triyaswati et al., 2020. Wastewater from coffee processing plants is highly acidic and has a high chemical oxygen demand (COD), containing abundant organic matter and suspended solids (Navitha, 2018; Sahana, 2018). According to Cardenas et al., (2009), most ligands are found mainly in coffee pulp, coffee husk, coffee wastewater and spent coffee grounds are not biodegradable. Therefore, anaerobic co-digestion (AcoD) is considered a viable treatment for both NDC and coffee waste by improving its biodegradability, reduces inhibitory effects and increases methane production (Karki et al., 2025).

Anaerobic digestion (AD) is a process in which organic waste is broken down by biological means without oxygen, producing biogas and other organic compounds as byproducts. An efficient method of wastewater treatment for industrial waste is anaerobic co- digestion (AcoD). This involves the simultaneous anaerobic digestion of two or more substrates, overcoming the disadvantages of mono-digestion and improving economics through enhanced methane production. It offers enormous potential for energy production and environmental management. Natural gas and carbon dioxide (CO_2) (30%–50%), methane (CH_4) (50%–70%), and varying amounts of water vapour and hydrogen sulphide (H_2S) are the main constituents of biogas (Andre et al., 2016). Hydrolysis, acidogenesis (or fermentation), acetogenesis, and methanogenesis are the four phases involved. Biogas produced from AD is the renewable resource that can be used to generate electricity, as well as energy for cooking and heating. It will contribute to reducing greenhouse gas emissions and odor issues (Neils et al., 2015; Agyeman and Tao, 2014).

This study is intended to serve as a source of information for methane production by anaerobic co-digestion (AcoD) of IWK sludge and food wastes. By applying the best pre-treatment techniques from experimental scale to up- scale, the results of this study will help to improve biogas production by anaerobic co-digestion (AcoD) of sewage sludge. Waste types, pH, temperature, hydraulic retention time (HRT), volatile fatty acids (VFA), chemical oxygen demand (COD), and alkalinity among the variables that affect the AcoD process. In addition, the composition of food waste strongly influences how well microbes can break it down in anaerobic digestion, which affects how efficient the whole process is (Jaiganesh et al., 2015).

1.2 Problem Statements

Anaerobic co-digestion (AcoD) is a process that can provide a clean and renewable energy source while helping to reduce landfill issues. However, the success of this process hinges on several critical factors. The efficiency of anaerobic co- digestion largely depends on the inoculum-to-substrate (I:S) ratio and the hydraulic retention time (HRT). An unsuitable I:S ratio can cause problems such as volatile fatty acid accumulation, pH imbalance, and reduced methane production. Likewise, an inappropriate HRT may lead to incomplete substrate degradation if too short, or process instability and lower efficiency if too long. Despite their importance, limited studies have examined the optimization of I:S ratio and HRT for the co-digestion of IWK sludge with food waste. This gap in knowledge makes it challenging to achieve stable performance and maximize methane yield.

The growing amounts of sewage sludge and food waste present serious environmental and disposal challenges. Conventional anaerobic digestion often produces low methane yields due to the complex composition and slow biodegradability of these wastes. Chemical pre-treatment can enhance the breakdown of organic matter, increase substrate availability and boost methane production. However, the optimal pre-treatment conditions for maximizing methane yield during the anaerobic co-digestion of IWK sludge and food waste are not yet well understood. Therefore, studying the effects of chemical pre-treatments on methane production is essential for developing a more efficient and sustainable waste-to-energy process.

Anaerobic co-digestion of IWK sludge and food waste offers a promising route for renewable energy production, yet accurately measuring methane output at the pilot scale remains challenging. Conventional measurement methods may lack precision or practicality for on-site monitoring. Assessing methane production using GC-FID and portable gas analyzers is therefore crucial to optimize the performance of a 40 L pilot digester and improve understanding of co-digestion efficiency.

1.3 Research Questions

i) What is the optimized anaerobic co-digestion parameter of IWK sludge and food wastes in lab scale by BMP test?

ii) What is the effect of pre-treatments to anaerobic co-digestion of IWK sludge and food wastes for the production of methane in lab scale by BMP test?

iii) How much methane produced from anaerobic co-digestion of IWK sludge and food wastes of 40L pilot scale digester?

1.4 Objectives of Study

The objectives of this study are as follows**:**

i) To optimize the inoculum to substrate (I:S) ratio and hydraulic retention time (HRT) of anaerobic co-digestion parameter of IWK sludge and food wastes in lab scale by BMP test.

ii) To investigate the chemical pre-treatment of IWK sludge and food wastes in the production of methane by anaerobic co-digestion in lab scale by BMP test.

ii) To quantitatively evaluate methane production from the co-digestion of IWK sludge and food waste in a 40 L pilot-scale anaerobic digester, employing GC-FID and a portable gas analyzer for biogas composition analysis.

1.5 Significance of Study

The findings of this study provide useful insights for enhancing methane production through anaerobic co-digestion (AcoD) of IWK sludge, coffee waste, and non-dairy creamer waste. IWK sludge was selected because it is readily available and supplies a stable microbial population, although its biogas potential is relatively low on its own. Coffee waste was included for its high content of easily degradable organic matter, which boosts microbial activity, while non-dairy creamer waste contributes additional organic and lipid content to increase methane production, despite its acidity that can impact pH if used excessively. By combining these substrates, the system benefits from complementary properties: the sludge buffers the digester, and the food wastes improve carbon balance and energy availability. Therefore, anaerobic digestion (AD) is recognized as an environmentally friendly method for processing various organic wastes and serves as an effective approach to managing the growing volume of organic waste in Malaysia.

This study was conducted to optimize the parameters of AcoD that influence the production of biogas especially methane such as the temperature, pH, volatile solid and total solid, and hydraulic retention time. At mesophilic temperatures between 35-37^{o}C, gas production rises. However, temperatures below 35^{o}C reduce biogas output and may take the process less cost-effective. In addition to temperature, factors such as pH, HRT, TS, VS and mixing conditions also play important roles in AD by affecting microbial activity, substrate degradation, and methane generation. Next, the pretreatment techniques for sewage sludge and food wastes by AD would help to enhance the biogas production. Sludge and food wastes possess unique characteristics, including lignocellulosic content and cellulose structure, among others. Pre-treatment improves digestibility by disrupting and breaking down the cellulose and lignin structure, thereby enhancing the substrate's biodegradability. This facilitates the acid's easy access, enabling it to hydrolyze into monomers. This study applied both thermal and chemical pretreatments to enhance the breakdown of sludge and coffee waste prior to anaerobic digestion. These pretreatments help disrupt complex organic structures and solubilize organic matter, making it more accessible to microbes. By increasing the biodegradability of the feedstock, these methods improve microbial activity, accelerate digestion, and enhance methane production.

Lastly, the main reasons to evaluate the biogas by AcoD are to know the potential of food wastes co-digest together with the sludge in a production of methane, CH_4. The biogas production from the AD can be converted into electricity. As we know, methane is a potent greenhouse gas (GHG) that can persist in the atmosphere. The reduction of CH_4 emissions which can reduces the GHG effects and also the global warming.

1.6 Scope and Limitations of Study

The main aim objective of this study is to enhance methane production through the anaerobic co-digestion of IWK sludge and food wastes. Laboratory-scale biochemical methane potential (BMP) tests were carried out in the Environmental

Technology Laboratory of the Faculty of Applied Sciences (FSG), UiTM Shah Alam, using 125 ml serum bottles over a 30-day hydraulic retention time (HRT). Thickened sludge from IWK STP Titiwangsa 2, Kuala Lumpur, was used as inoculum, while coffee waste and non-dairy creamer wastewater from Nestlé Manufacturing (Malaysia) Sdn Bhd, Shah Alam, served as substrates. The primary parameters investigated were the inoculum-to-substrate (I:S) ratio and hydraulic retention time (HRT), alongside the application of various pre-treatment methods, including chemical treatment with 2 M H_2SO_4, thermal treatment (90°C for 180 min), combined thermal–acid treatment, and alkali treatment. The optimized batch experiments conditions were then scaled up to a pilot study using a 40-liter digester at the Indah Water Research Center (IWRC), Kuala Lumpur. Biogas production was measured using a portable gas analyzer and gas chromatography-flame ionization detector (GC-FID) to evaluate overall process performance. However, the study is limited by the laboratory and pilot-scale setups, which may not fully reflect full-scale operations, and by the specific types of food wastes used, whose composition may vary and affect the applicability of the results.

CHAPTER 2

LITERATURE REVIEW

2.1 Sludge

2.1.1 Overview

Sewage sludge (SS) is the solid residue, semi-solid substance, or sludge residue produced as a byproduct of the treatment of domestic or industrial wastewater. Sludge types for this residue include primary and secondary sludge. Primary sludge is produced by processes such as sedimentation, chemical precipitation, and others, while secondary sludge is an activated sludge resulting from biological treatment processes. In terms of production, previous studies have reported that aerobic treatment systems such as activated sludge processes can convert approximately 60–80% of removed organic matter into sludge, whereas anaerobic systems typically generate significantly lower amounts, around 20–30% (Tchobanoglous et al., 2014; Metcalf & Eddy, 2014). Additionally, primary sludge generally accounts for about 50% of the total sludge generated in wastewater treatment plants (Kintep, 2008). According to Abbas et al. (2011), sewage sludge can produce fuels. Sewage sludge can function as an inoculum in anaerobic digestion as it contains active microbial communities, including methanogens that support the initiation and continuation of methane production. However, its application may pose concerns due to the presence of potential toxic substances originating from wastewater. Nevertheless, its high organic matter and nutrient content make it a viable option for biogas generation.

Table 2.1 presents the physicochemical characteristics of sewage sludge from wastewater treatment plants in Malaysia. In general, the sludge has high moisture content up to (80-90%) and contains a large amount of organic matter, especially volatile solids (50-70%), which are important for anaerobic digestion as they act as a substrate for biogas production. The pH of the sludge is typically near neutral to slightly alkaline (6.4-8), providing suitable conditions for microbial activity, particularly for methane-producing bacteria. Typical sewage sludge COD values may range from 20,000-80,000 mg/L. For example, one study reported sewage sludge with

COD around 82,000 mg/L and total solids of 8.52%, indicating high biogas potential (Usevičiūtė et al.,2025). Total solids (TS) represent the total amount of solid material present in sewage sludge, including organic and inorganic solids. Typical raw sewage sludge contains about 2-8%, meanwhile for thickened sludge may contain at range 6-12% of TS.

Table 2.1
Characteristics of Sewage Sludge in Malaysia

Parameter	Typical Range / Value	Unit	Notes / Source Highlights
pH	6.4 –8.5	–	Generally near neutral to slightly alkaline; favorable for biological processes (Ganji et al., 2024; Tanor et al., 2016; Singh and Agrawal 2007)
Moisture Content	80 –89	%	High moisture content typical in sludge (Tanor et al., 2016)
Total Solids (TS)	4 –20	%	Inverse of moisture content (Tanor et al., 2016)
Volatile Solids (VS)	50 –70 (of TS)	%	Organic fraction that is biodegradable (Tanor et al., 2016)
Chemical Oxygen Demand (COD)	20,000-80,000	mg/L	Indicates organic pollutant load ing sludge (Ganji et al., 2024)
Nickel(Ni)	5.5 –30	mg/k g	
Total Suspended Solids (TSS)	33 –83	mg/L	Particulate matter concentration

Due to the significant volume of sewage sludge produced during wastewater treatment, Jin et al., (2015) highlighted sewage sludge concentrate as a viable substrate for bioenergy generation, contributing to sustainable energy solutions. The effectiveness of biogas production from sewage sludge largely depends on the treatment method applied. Generally, sewage sludge exhibits a pH between 6.8 and

7.0 and contains 70–85% moisture. Studies have demonstrated that treating sewage sludge concentrate through coagulation and adsorption processes can result in chemical oxygen demand (COD) levels appropriate for bioenergy production (Gong et al., 2015). Furthermore, Jin et al. (2016) reported that using an advanced coagulation–microfiltration reactor can produce a concentrate with a COD value of approximately 15,000 mg/L, which is suitable for energy recovery.

2.1.2 IWK Sludge (Thickened Sludge)

A private business called Indah Water Konsortium (IWK) Sdn Bhd is in charge of Peninsular Malaysia's majority of states' wastewater management. IWK has traditionally been responsible for the maintenance and operation of 5997 public sewerage treatment facilities, 926 network pump stations, and approximately 16, 328 km of sewer pipelines. Additionally, Bunus Sewage Treatment Plant (STP) in Kuala Lumpur produces biogas at a rate of about 2500 m^3/day (David et al., 2014). This quantity of biogas generated about 15000 kWh/m^3 of electricity. As a result, the power produced serves as a backup source of energy generation and can aid in reducing overall energy use.

Malaysia, a developing country, is characterized by an annual increase in sewage sludge production in line with global trends. Indah Water Konsortium (IWK) Pantai Dalam wastewater treatment plant is one of the IWK branches involved in the biogas production project. According to IWK Sdn Bhd (2010), Malaysia produced about 3 million tonnes of sewage sludge annually, with projections reaching 7 million tonnes by 2020. More recent estimates indicate that sewage sludge generation has continued to rise steadily over the past decade, reaching approximately 6–7 million tonnes annually between 2018 and 2024, driven by population growth, urbanization, and expansion of sewerage infrastructure (WEPA, 2021; IWK Reports, 2022). However, the most common methods of sewage sludge disposal remain landfilling and incineration. Nearly 50% of Malaysia's wastewater treatment plants are automated, while the remaining systems still rely on septic tanks and oxidation ponds. According to Kumaran et al. (2016), several mechanized plants have been upgraded with anaerobic digesters, where sewage sludge is processed anaerobically to utilize methane as a valuable energy source for electricity generation. Figure 2.1 illustrates the increasing trend of sewage sludge production in Malaysia from 2015 to 2024 and the data presented are estimated based on reported trends from available literature.

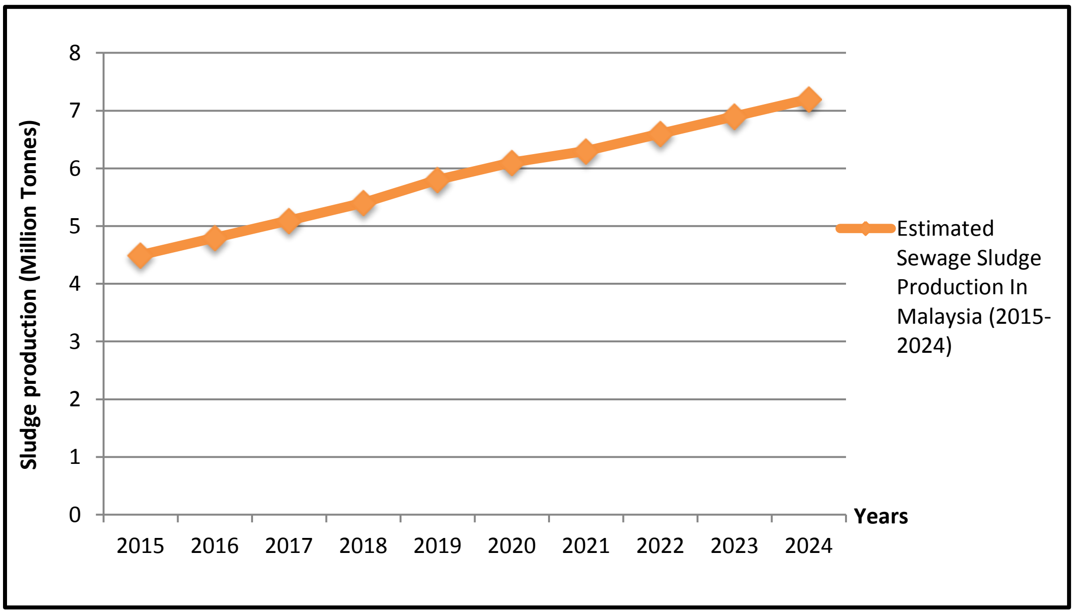


Figure 2.1 Estimated Trend of Sewage Sludge Production in Malaysia from 2015-2024 (WEPA, 2021; IWK, 2022)

Malaysia has recently commissioned three advanced anaerobic digestion wastewater treatment plants, located at Pantai 2 Kuala Lumpur, Jelutong in Penang, and Langat in Selangor. The treatment plants at Pantai 2 and Jelutong are already in operation, but Langat is scheduled for completion in 2018. The Pantai 2 WWTP can produce up to 9,600 m^3 of biogas per day. In 2018, it will generate between 450 and 500 KW of electricity (Hanum et al., 2019). One of the largest plants in Malaysia is the Jelutong wastewater treatment plant. Its goal is to treat 800,000 population equivalents (PE). (Jelutong WWTP-Malaysia). Anaerobic digestion of sewage sludge has not been the subject of much research or study in Malaysia, suggesting that the public is not particularly interested in or aware of these problems. Food waste is a major part of municipal solid waste and can cause environmental problems if sent to landfills, producing methane, a potent greenhouse gas. Anaerobic digestion (AD) offers a way to convert food waste into biogas, mainly methane, and nutrient-rich digestate that can be used as fertilizer.

AD is a sustainable approach that reduces waste, generates renewable energy, and supports a circular economy. However, the high moisture content and variable composition of food waste can limit digestion efficiency and methane yield. Pre-treatment methods or co-digestion with other organic wastes can help improve biogas production.

2.2 Food Waste (FW)

Food waste is considered a suitable substrate for anaerobic co-digestion due to its high organic content and biodegradability. Globally, approximately 1.3 billion tonnes of food waste are generated each year, indicating its strong potential for renewable energy production (Food and Agriculture Organization, 2011). Food waste typically contains 70–90% moisture and is rich in carbohydrates, proteins, and lipids, resulting in high biogas and methane yields (Zhang et al., 2007).

However, the mono-digestion of food waste can lead to process instability due to rapid acidification and the accumulation of volatile fatty acids (VFAs), which may inhibit methanogenic activity (Li et al., 2011). To address these issues, anaerobic co-digestion is commonly applied by combining food waste with other substrates such as sewage sludge, manure, or agricultural residues. In this study, coffee waste (CW) and non-dairy creamer waste (NDCW) was used as food wastes.

2.2.1 Coffee Waste (CW)

Coffee is currently one of the most consumed products in the developed world. There is always an abundant supply of different coffee blends and flavors in supermarkets and cafes. In the past, fresh coffee was provided by percolating, infusing, or boiling the ground and roasted beans for domestic and commercial preparation (Clarke and Macrae, 1987). The Malaysian coffee market is growing and becoming more competitive. Both local cafes and global coffee chains are opening new locations and expanding their business. Coffee is being consumed by more and more people and is becoming an increasingly common beverage, especially among Millennials. Malaysia is ranked among the top 50 countries with the highest coffee consumption globally (Rahim, Jin, and Fong, 2019). Figure 2.2 shows a statistical analysis of coffee consumption in Malaysia from 2013 to 2022 (forecast), which indicates that consumption will increase from 635 thousand coffee bags (60kg/coffee bags) in 2020 to 800 thousand coffee bags (60kg/coffee bags) in 2021 and 2022 (Ramanathan & Ali, 2021). This fact warrants an investigation of current coffee consumption in Malaysia and the variables that influence these changes.

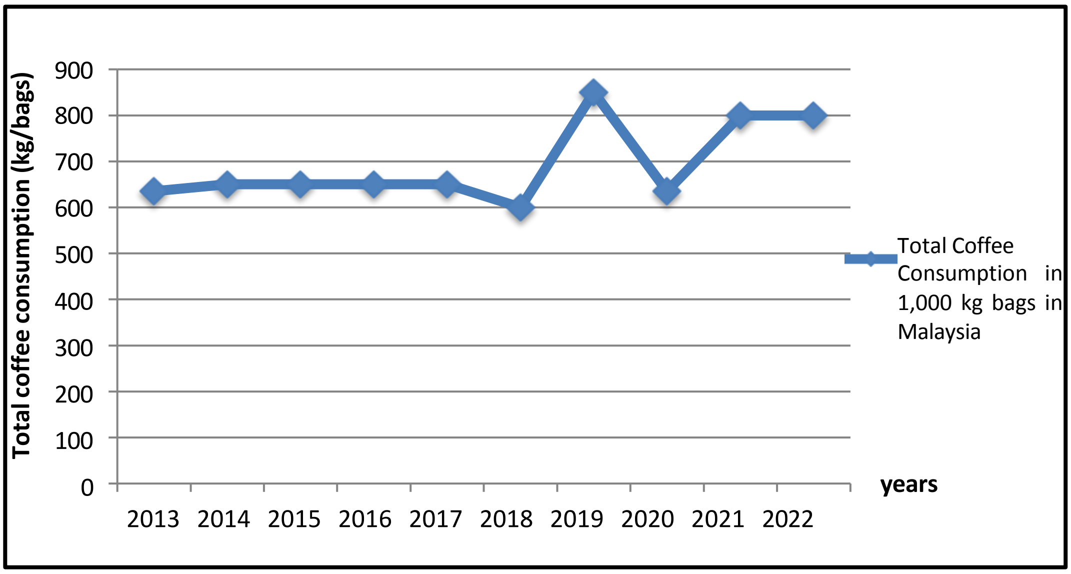


Figure 2.2 Total Coffee Consumption in Malaysia from Year 2013-2022
(Ramanathan & Ali, 2021)

Both dry and wet methods are available for making coffee. Wet processing usually produces large amounts of hazardous wastewater that easily be discharged into a nearby stream or river because it consumes a lot of water. Figure 2.3 illustrates the by-products of coffee processing, which consist of pulp (43%), mucilage (12%), and parchment (6.1%) (Tory Gay, 2018).According to Navitha and Kousar (2018) and Sahana et al. (2018), these effluents usually contain a high concentration of organic waste and suspended solids and are also quite acidic. According to Cardenas et al. (2009), the pollution level of streams and rivers near coffee processing factories is extremely high, so coffee wastewater needs to be treated before being released. In addition, it was found that little to no attention is paid to modern coffee wastewater management practices.

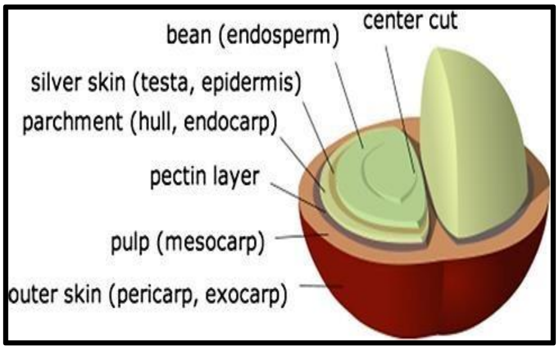


Figure 2.3 The Anatomy of Coffee Berry
(Tory Gay, 2018)

In addition, wastewater from coffee processing plants is colored and contains a variety of macromolecules, including polyphenols (e.g., melanoidin) and caffeine. Therefore, pollution from the discharge of colored wastewater is responsible for turbidity, high chemical oxygen demand up to 50,000 mg/L, and biological oxygen demand (20,000 mg/L) (Table 2.2), eutrophication, and light-blocking and photosynthesis-altering effects (Takashina et al. 2018; Tokumura et al. 2008; Péerez et al. 2007). The strict effluent standards of the Environment Quality Act (EQA) of 1974 must be met by all Malaysian companies discharging effluent. Therefore, AD is one of the alternative methods to address these issues by decomposing organic contaminants in coffee-processing wastewater, leading to substantial reductions in COD, BOD, and turbidity, while also partially breaking down color-causing compounds. Furthermore, it reduces environmental impacts such as eutrophication and produces biogas as a renewable energy source, thereby supporting industries in meeting regulatory discharge requirements.

Table 2.2
Compilation Characteristics of Coffee Wastewater

Parameter	Typical Range / Value	Unit	Notes / Source Highlights
pH	2.5 – 7.7	–	Highly acidic during fermentation (around pH 4), can rise near neutral after treatment (Genanaw et al. (2021b); Takashina et al. (2018)
Biological Oxygen Demand (BOD)	8,000 – 20,000	mg/L	Very high, indicating large amounts of biodegradable organic matter; BOD should be reduced below 200 mg/L before discharge (Murthy and Naidu (2012); Genanaw et al. (2021); and Bisekwa et al. (2021)
Chemical Oxygen Demand (COD)	15,000 – 50,000	mg/L	High COD mainly due to resistant organic compounds like mucilage and polyphenolics (Takashina et al. (2018); Tokumura et al. (2008)
Total Suspended Solids (TSS)	High, exact values vary	mg/L	Includes mucilage solids that precipitate and clog waterways (Tokumura et al. (2008); Péerez et al. (2007)
Total Solids (TS)	Not explicitly specified	%	High organic content from pulp and mucilage. Bisekwa et al. (2021)

Organic Components	Carbohydrates (reducing and non-reducing) 2.0 –12.4% (reducing sugars)	% dry basis	Includes sugars, pectins, proteins, caffeine, tannins, polyphenols

2.2.2 Non-Dairy Creamer Waste (NDCW)

Nowadays, the enormous, expanding industrial sector is under great pressure to contribute to the welfare of the people. However, as industry continues to expand, the waste it produces becomes a problem. A food ingredient company utilizes coconut oil as a raw material in the production of dairy-free creamer. Wastewater from non-dairy creamer (NDC) production becomes polluted due to inadequate management practices. The effluents have also affected the quality of water and caused unpleasant odors due to its characteristics as shown in Table 2.3. The odors are caused by the high content of organic chemicals in the liquid waste. A good management strategy that uses wastes as valuable raw materials is therefore urgently needed.

The food and beverage industry produces commodities such as coffee and non-dairy creamer (NDC). However, NDC is lactose-free. It is a liquid or powdered substitute for milk or cream that forms an emulsion in foods such as oatmeal, baked goods, coffee, and other products with a high percentage of fats dissolved in water from vegetable oil (30% hydrogenated coconut or palm oil) (Dedin et al., 2016). According to Huang et al. (2019), NDC contained minerals (1.2%), the most protein (8.5%), the least amount of fat (32.5%), and the most carbohydrates (58%) of any food. It consists of 60- 65% glucose or corn syrup, 2.5% sodium caseinate, stabilizers, and emulsifiers.

Wastewater from non-dairy creamer production contains high levels of organic compounds derived from carbohydrates, fats, and proteins due to the composition and processing of the creamer product. These organic constituents — coupled with inorganic additives such as phosphates from stabilizers and emulsifiers — contribute to pollutants in the effluent. When discharged without adequate treatment, these pollutants can lead to oxygen depletion, nutrient enrichment, and disruption of aquatic ecosystems, consistent with findings in related food industry wastewater studies (Sulistya Dewi et al., 2016). Inadequate processing of wastewater from the non- dairy creamer industry can harm the environment. According to Dewi et al. (2016)1; 2016b and Triyaswati et al. (2020), the wastewater contains pollutants such as protein-, carbohydrate-, and fat-containing organic compounds, as well as odor-causing inorganic compounds such as suspended or dissolved phosphorus. The ecological balance could be disturbed by the pollutants.

Table 2.3
Compilation of Non-Dairy Creamer Industry Wastewater Characteristics (in Mg/L) Except For pH

Parameters	Typical range/ Value	Unit	References
pH	5.0-7.0	-	Anisah et al., 2022
Chemical Oxygen Demands (COD)	10,000-50,000	mg/L	Nikolić et al., 2025
Biochemical Oxygen Demands (BOD)	5,000-30,000	mg/L	Nikolić et al., 2025
Total Suspended Solid (TSS)	1,000-10,000	mg/L	Nikolić et al., 2025
Color & Odor	Milky/Whitish, may develop odors	-	Nikolić et al., 2025
Nutrients (N & P)	Moderate levels	-	Nikolić et al., 2025

Anaerobic co-digestion using food waste as substrate could therefore be the most practical way to solve this problem. The performance of the AD process is improved by using food waste as substrate and also helps to reduce the high generation of food waste in Malaysia. Other options for food waste disposal include landfill, incineration, and agricultural use. Food waste disposal in landfills creates an important source of methane gas, which traps heat in the atmosphere 20 times more than carbon dioxide. These techniques can be problematic in terms of their environmental and public health impacts. (Fytili and Zabaniotou, 2012).

Basically, for food waste or substrates with high carbohydrate, fat, protein, and fiber content, the characterization process must be completed before the digestion process can begin. The high percentage of biodegradability, moisture content of about 70%, volatile solids of about 95%, and a large number of lipids are other characteristics of food waste (Zhang et al., 2017). In addition, the methane gas produced by the AD process can be used to meet heating and electricity needs.

2.3 Anaerobic Digestion (AD)

The anaerobic digestion (AD) process has been identified worldwide as the most efficient and widely used process for the treatment of municipal solid waste and wastewater sludge. AD also has slowly received people's attention worldwide through promising technologies which help to convert waste into both biogas and odor-free residues. Thus, AD is a natural biochemical process in which organic matter is converted and biologically degraded by microorganisms without oxygen supplies which causes the production of liquid and solid digestate and a mixture of gases called biogas. Thus, the digestate or slurry from the digester is rich in ammonium and another nutrient that can be used as an organic fertilizer.

Avienna et al., (2015) stated that biogas is the end product that is formed from the AD process which mainly consists of methane, CH_4, carbon dioxide, CO_2, and others. Previous studies generally confirmed methane and carbon dioxide are produced as the main composition of the biogas from AD with a percentage of 55-75% and 25- 45% respectively. Besides, a small amount of hydrogen sulfide and ammonia may be produced from the biogas (Mao et al., 2015; Divya et al., 2015). Thus, the composition of the biogas produced depends on the conditions of the process and the substrate characteristics (Stoknes, 2016).

The gas can be used as fuel for heat and/or power generation, processed to be used as fuel for road vehicles, or it can be fed into the natural gas industry (Zhang et al., 2014). Figure 2.4 shows the flowchart of the AD process. AD transforms organic wastewater into biogas and stabilized digestate. After pre-treatment, the feedstock passes through hydrolysis, acidogenesis, acetogenesis, and methanogenesis, breaking down complex organic matter. The process produces renewable biogas and nutrient-rich digestate with lowered COD, BOD, and turbidity, providing effective wastewater treatment and energy recovery. In addition, AD should be commercialized in Malaysia and other countries as it helps to reduce the impact of global warming and reduce methane emissions. While the climate in Malaysia is 30 to 40°C, which is the optimum temperature for the AD process, it is not possible to commercialize the process in other countries.

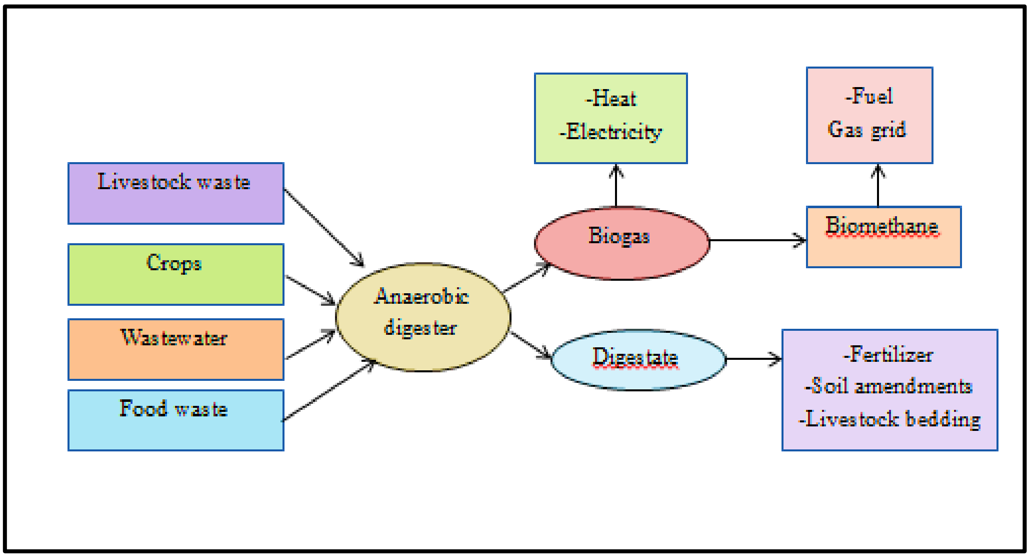


Figure 2.4 Anaerobic Digestion Flow Chart
Source: Environmental And Energy Study Institute, EESI (2017)

2.3.1 Stages of Anaerobic Digestion process

There are four stages of the AD process namely, hydrolysis, acidogenesis (or fermentation), acetogenesis, and end up with methanogenesis (Mao et al., 2015). Each requires a specific group of microorganisms. In the AD process, the interaction between substrate and microorganisms may occur and the end product will be formed. Figure 2.5 shows a simplified diagram of the anaerobic degradation of organic matter.

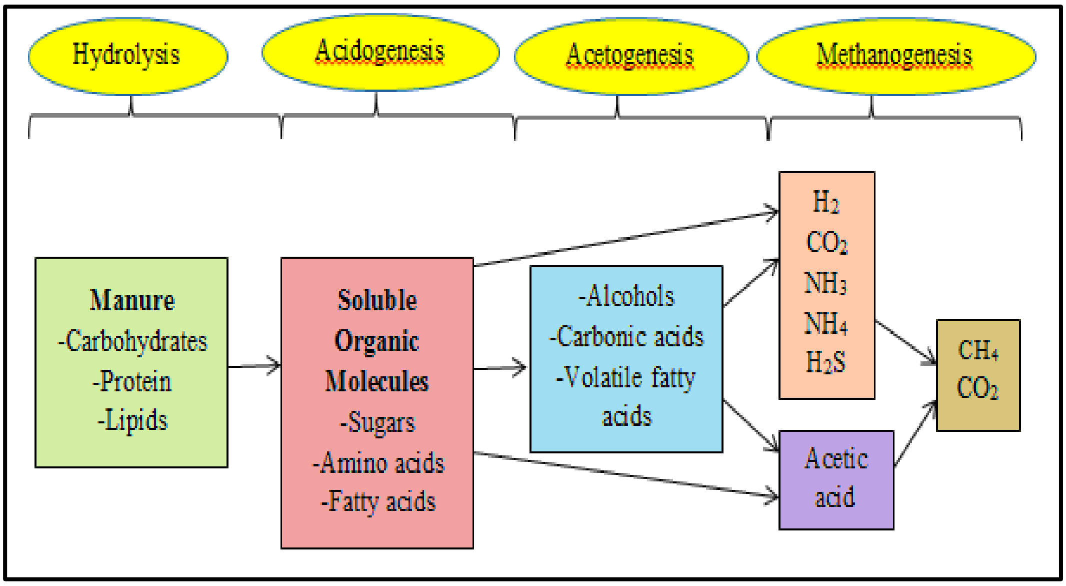


Figure 2.5 Stages of anaerobic digestion process
Source: Girard (2013)

The process of hydrolysis is where insoluble complex organic stuff becomes soluble organic material. (Zhang et al., 2014). Amino acids, fatty acids, and simple sugars will be formed through the breakdown by extracellular enzymes such as cellulose, amylase, and lipase. According to Divya et al. (2015), the connection between the microbes and the enzymes engaged in the process can determine whether hydrolysis acts as the rate-limiting phase in anaerobic digestion.

The results of hydrolysis are converted to acetic acid, volatile acid, volatile fatty acids (VFA), alcohols, hydrogen, and carbon dioxide in the second phase of the AD process, called acidogenesis. (Mata-Alvarez et al., 2014). This phase, also known as fermentation, is usually the one that progresses the fastest. The third phase is called acetogenesis. Acetate, which contains both acids and alcohol, is formed from the VFAs of the second phase of AD (acidogenesis). Acetate is an important component that serves as a substrate for the bacteria that produce methane, according to Darwin (2013). (acetogenic bacteria). At the end of the AD process, these bacteria will produce methane gas. The VFAs are then converted to acetic acid, hydrogen, and carbon dioxide.

Methanogenesis, the process' final stage, is known as a crucial one in the entire anaerobic digesting procedure with the slowest biochemical processes in comparison to other phases. Methanogenic bacteria will act during this phase to create methane and carbon dioxide. Methanogens may be sensitive to changes in temperature, pH, and chemical component concentrations, according to Manser (2015).

2.4 Parameters affecting AD process

Anaerobic digestion (AD) is a complex and sensitive process. Thus, there are several microorganisms responsible for the AD process, and therefore the most essential factors that affect the anaerobic digestion efficiency include temperature, pH, Hydraulic retention time (HRT), C/N ratio, and organic loading rate (OLR).

2.4.1 Effect of Temperature in AD process

Temperature plays an important parameter that determines the AD process, particularly the methanogenesis, and hydrolysis. This is because bacteria required a suitable and optimum temperature to maintain their growth and activity during the AD process. If the temperature is unsuitable, it may disrupt the whole AD process.

The optimum temperature stimulates the microbial activity of microbial communities in the digester. Sibiya et al (2014) revealed that the optimum temperature for bacteria growth is between 30-37^{o}C, which is known as the mesophilic condition.

Three temperature ranges-psychrophilic (20°C), mesophilic (25 – 42°C), and thermophilic (50-65^{o}C) can be used to categorize the AD process. The best environment for AD processes is the mesophilic range (35–38^{o}C). According to Mussoline (2013), the bacteria present were significantly more resilient and less impacted by the changes in the environment, which caused the methane yield to increase to 25% when the temperature changed from 25 to 35^{o}C. In addition, the thermophilic tends to be less stable and requires less energy. Both mesophilic and thermophilic settings are favorable for the microbes to function throughout the AD process, although Li et al. (2017a) noted that at thermophilic circumstances (50–65^{o}C), some microorganisms are particularly sensitive to that temperature shift. As a result, Table 2.4 displays the AD temperature that was employed based on earlier studies.

Table 2.4
Temperature of AD used based on the previous studies.

Author	Temperature	Results
Hansen et al., (2004)	Thermophilic, 55^{o}C.	CH_4 production for the waste sample was on average 495 mL CH_4/g VS.
Leung & Wang (2016)	Mesophilic condition 25^{o}C, 30^{o}C and 35^{o}C	CH_4 yield was 43.8%, 54.8% and 60.4% respectively.
Sibiya et al., (2015)	Mesophilic (30-40^{o}C) Thermophilic (50-60^{o}C)	The CH_4 yield was higher at 45^{o}C with 208.6mL, compared to 35^{o}C which is 178mL
Paritosh et al., (2017)	temperature of 40^{o}C , 45^{o}C , 50^{o}C and 55^{o}C	The result of CH_4 yield produced was 65.6%, 66.2%, 67.4% and 58.9%

2.4.2 Effect of pH in AD process

Most of the studies highlighted that the pH is also one of the important parameters that might affect the whole AD process (Lindner et al., 2015; Obulisamy et al., 2016 Chakraborty et al., 2017). So, it is vital in maintaining the optimal pH to achieve the effectiveness of microorganisms' activities. Thus, the pH of the solution cannot be too high or too low which can disrupt microbial activities. The optimum pH for methane gas production is in the range of 6.8-7.3 which is neutral to slightly alkaline. This range is most favorable for methanogenic bacteria to grow, survive, and reacts.

Agdag and Sponza (2007) stated that pH of 7.0–7.2 is a suitable pH for industrial sludge. The pH range of 6.8–7.2 is the optimal pH in the AD process (Ward et al.,(2008) and Neshat et al.,(2017)). A pH of 6.5– 8.2 was favorable for methanogenesis (Lee et al., (2009b)) meanwhile, the hydrolysis and acidogenesis phase occurs efficiently at pH 5.5 and 6.5 (Kim et al., 2003). Liu et al. (2008) revealed that pH 6.5–7.5 is the ideal range to release a higher biogas production in AD. Sibiya et al (2014) discovered that pH 6.5 produced 195.4mL, followed by pH 6.8 and 7.2 which are 137.2mL and 176.9mL respectively. Thus, Table 2.5 shows the result of methane production based on the pH used from the previous studies.

Table 2.5
pH value used based on the previous studies

Authors	pH	Results
Kheiredin et al., (2014)	At pH 4 and at pH 5.5	Low biogas yield was obtained at pH 4 (1661mL) compared to pH 5.5 (1700mL)
Carotenuto et al., (2016)	at pH 7	The highest result of methane concentration in the biogas (above 65%) was obtained
Sibiya et al., (2014)	The optimal pH in AD process at range 6.5, 6.8 and 7.2	The methane production is 195.4 mL, 137.2mL, and 176.9mL

Deepanraj (2017)	at pH 5,6,7,8 and 9	The maximum result obtained for the cumulative biogas yield by the reactor was 4594, 5021, 5673 and 4889 mL

2.4.3 Effect of Hydraulic retention time (HRT) in AD process

Retention time defines as the time taken or the periods for organic matter completely take place during the degradation process. Retention time can be divided into two conditions which are hydraulic retention time (HRT) and solids retention time (SRT). HRT is known as the time when the fluid remains in the digester, while the SRT is probably where the bacteria remain in the digester (residence time). The HRT is most related to organic loading rate (OLR), for instance, the higher the OLR, the higher the HRT (Liu et al., 2017 Kornaros, 2014). Moreover, findings by Chan et al (2017) mentioned that, HRT also can affect the digestion rate, the C/N ratio of the digestate, and the microbial flux.

Zhang et al., (2015) revealed that the acidogenesis stage needs to stabilize the low pH to increase the group bacteria of hydrolytic acidogenic by having low HRT. However, if the HRT is too low, it may lead to a partial hydrolysis process of organic substances which can affect the efficiency of the AD process. Other than that, HRT also can affect the removal of chemical oxygen demand (COD). The study by Dhar et al., (2016) proved that the anaerobic co-digestion at HRT 25 days, showed the optimum for methane production (maximum of 316 mL CH_4 g^{-1} COD removal) while at HRT 12 and 20 days for the same feed showed the higher accumulation of VFAs, thus, causes inhibition. Another factor that can affect the HRT is the feedstock composition and particle size. Table 2.6 summarized the effect of HRT on AD process from the previous studies.

Table 2.6
Effect of HRT on AD process from the previous studies.

Authors	HRT Condition	Key findings	Impact on AD performances
Liu et al., (2017); Kornaros (2014)	High OLR ↔ Higher HRT needed	HRT strongly linked to organic loading	Maintains system stability

Chan et al. (2017)	Variable HRT	Affects digestion rate, C/N ratio, microbial activity	Influences overall process efficiency
Zhang et al., (2015)	Low HRT	Promotes acidogenesis and growth of hydrolytic bacteria	Improves early-stage digestion but may reduce overall efficiency if too low
Dhar et al. (2016)	25 days (optimum)	Highest methane yield (316 mL CH_4 g^{-1} COD)	Optimal biogas production
	12–20 days	Accumulation of VFAs	Process inhibition, reduced methane yield

2.5 Anaerobic Co-Digestion

Anaerobic co-digestion (AcoD) is the simultaneous digestion of a homogenous mixture of two or more substrates. Thus, the combining of different substrates can reduce the toxic compounds that can slow down digestion such as ammonia and sulfide. Co-digestion is one of the processes where energy-rich organic waste material such as fats, oils, and grease (FOG) or food scraps are added to dairy or wastewater digesters with surplus capacity. These high-energy materials will produce the most methane production rather than the mono-digestion itself. Co-digestion of various biosolid wastes improved the digestion process and the feasibility of biotransformation. Many studies reported the co-digestion of sewage sludge with different types of food wastes enhances methane yields. For instance, the methane yield increased by 60% from the co-digestion of sludge mixed with fat, oil, and grease (FOG) (Luostarinen & Sillanpää, 2009).

Co-digestion also helps to optimize the nutrient balance and enhances the pH buffer capacity. Moreover, the moisture contents of the digester feed can be established. Otherwise, anaerobic co-digestion (AcoD) showed better process efficiency such as better product yield, nutrients availability, bulk density, lower feed volume, substrate variability, toxicity dilution, synergism, divers, and robust microbiome, etc than the mono-digestion process, resulted in low methane yield (Obulisamy et al., 2016; Chakraborty et al., 2017; Li et al., 2017a). Ammonia inhibition reduction has been shown in the co- digestion of chicken manure-cow dung, chicken manure-dairy wastes, and chicken manure-fruits and vegetable wastes (Mata-Alvarez et al., 2015). Therefore, the methane yields from such co-digestions are higher.

2.6 Strategy to improve the Anaerobic Co-digestion (AcoD)

Enhancing anaerobic co-digestion (AcoD) requires optimizing both the types of waste and process conditions. Combining wastes like sewage sludge and food waste balances nutrients and supports microbial growth. Pre-treatments such as heating, grinding, or chemical treatment improve digestibility and increase methane production. Maintaining proper temperature, pH, retention time, and inoculum to substrate ratio ensures process stability, and helps to boost efficiency. These strategies collectively improve methane yield and make the digestion process more reliable.

2.6.1 Pre-Treatment of Sewage Sludge and Food Wastes

A previous study by Ariunbaatar et al. (2015a); Kumar et al. (2014); Menon et al. (2016); Zhang et al. (2016) and Zou et al. (2016) reported that methane production can be optimized by several pre-treatment such as biological, chemical, physical and thermal methods. Thus, Table 2.7 and Table 2.8 show several pre-treatments that applied for FW and sludge before AcoD for the highest production of methane. Thus, the different pre-treatment applied for FW AcoD studies shows different results in methane yield. In addition to that, biological pretreatment (bacterial and fungal) are most beneficial in term of lower operation cost and energy consumption.

The substrate that will be used in this study is food waste samples (coffee, and creamer waste) from the food industry (suggested by IWRC), while; the IWK sludge will be used as inoculum. A previous study by Li et al., (2012) proved that the chemical pre-treatment using strong acids, alkalis, or oxidants would help to destruct the organic compounds. Other than that, Ma et al., (2011) cited that, the methane yield produced was 520 CH_4/g VS at 400^oC, and increased by 60% after hydrochloric acid (HCL) treatment at pH 2 was added. Alkaline pre-treatment with sodium hydroxide (NaOH) breaks down the complex structures of organic wastes, such as lignocellulosic components in vegetables and fruits. NaOH disrupts lignin and hemicellulose in the cell walls, increasing the accessibility of carbohydrates for anaerobic microbes. This improved hydrolysis makes more substrate available for methane-producing microorganisms, enhancing biogas production. For instance, adding 10 g NaOH per 100 g total solids (TS) to vegetable and fruit waste was reported to increase methane yield by 25% (Sambusiti et al., 2013). AD generally requires adjustments of the pH by increasing the alkalinity. The chemical pre-treatments were unsuitable for easily biodegradable substrates which are rich in carbohydrates due to VFAs accumulation which leads to failure of the methanogenesis step. However, the substrates which are rich in lignin and lipids will be no effect.

Other than that, the chemical pre-treatments are considered widely used on wastewater sludge and lignocellulosic substrates (Carrere et al., (2010); Modenbach et al., 2012). This due to acid pre-treatment being highly desirable for lignocellulosic substrates which can help to break down the lignin, and also the hydrolytic microbes are capable to perform in acidic conditions (Mussoline et al., 2012). Lopez et al., (2008) and Neves et al., (2006) cited that the methane yield was increased by 11.5% with alkaline pre-treatment and by using 8.75mL HCL/Kg wet sludge at pH 2, the methane yield was increased up to 14.3% (Devlin et al., 2011).

Table 2.7
Effect of different pre-treatment of FW from the previous study

Author	Pre-treatment	Results
	Biological	
Lim et al., (2013)	(Micro-aeration)	- Batch at 35^{o}C, methane yield produced was 256 CH_4/gVS, methane yield after pre-treatment was increased by 10-21%
Kim et al., (2000)	(Semi-aerobic pre-hydrolysis, anaerobic and methanogenic)	- Continuous at 41^{o}C, methane yield produced was 500 CH_4/gVS, methane yield after pre- treatment was 95% COD removal with enhanced methane yield
Vernier et al., (1987)	(Mesophilic pre-hydrolysis)	- Semi-continuous at 37^{o}C, methane yield produced was 371 CH_4/gVS, methane yield after pre-treatment was 9% and 13% for higher methane yield

Mechanical		
Agyeman et al., (2014)	(Particle size reduction from 8 to 2.5 mm)	- Batch at 36^{o}C, methane yield produced was 530 CH_4/gVS, methane yield after pre-treatment was improved by 9-34%
Izumi et al., (2010)	(Size reduction by beads mill)	- Batch at 36^{o}C, methane yield produced was 330 CH_4/gVS, methane yield after pre- treatment was increased by 28%
Marin et al.., (2010)	(Microwave with intensity of 7.8 ˚C /min)	- Batch at 36^{o}C, methane yield produced was 340 CH_4/gVS, methane yield after pre- treatment was 6% higher biogas production
Chemical and Thermal		
Ma et al., (2011)	(2M H_2SO_4 treatment)	-methane yield produced was 520 CH_4/gVS, methane yield after increased by 60%
Sambusiti et al., (2013)	Alkaline (NaOH): Adding 10g NaOH/100g TS	- the effect of alkaline (NaOH) pre-treatments on vegetables and fruit was increased the methane yield by 25%
Li and Jin., (2015)	(120°C for 50 min)	- Batch at 35 ^{o}C, methane yield produced was 1200 CH_4/gVS, methane yield after pre- treatment was increased by 29-40%
Ariunbaatar et Al., (2015a)	(80^{o}C)	- Batch at 36 ^{o}C, methane yield produced was 647 CH_4/gVS, methane yield after pre- treatment was increased by 52%

Table 2.8

Effect of different pre-treatment on Sludge from the previous study

Author	Pre-treatment	Results
	Ultrasonification	
Braguglia et al., (2011)	Activated Sludge (23g TS/L) 24 kHz, 300 W, 5 000kJ/kg TS	+35% methane yield, 0.86 energy ratio
	Microwave	
Ebenezer et al., (2015)	Activated Sludge 14000 kJ/kg TS (14.2 ± 0.7 g TS/kg)	+570.7% biogas production
Appels et al., (2013)	800 W, 35 min	+50% biogas production, +66.6% DS removal
	Electrokinetic disintegration	
Choi et al., (2006)	Activated Sludge 19kV, 110Hz, 1.5s	+2.5 times higher biogas production
Lee et al., (2011)	$34kWh/m^3$	+33% methane production, +18% TCOD removal, -40% digester size
	Thermal Hydrolysis	
Oosterhuis et al., (2014)	Dewatered activated sludge (16% TS) Pilot-scale CAMBI™, 65^oC,6 Bar,20 min	Increase the biogas production by 30- 40% -improved dewaterability
	Chemical	
Davlin et al., (2011)	Activated sludge 8.75mL HCL/kg wet sludge, pH 2	+14.3% methane yield
Valo et al., (2004)	130^oC, pH 10 (KOH)	+36.4% COD removal, +33%TS removal, +74% biogas production
Li et al., (2012)	Sewage sludge 0.1 mol NaOH/L	+26.4% organic removal, +1.5% biogas yield
Weemaes et al., (2000)	0.1g O_3/g COD	+1.8 times methane yield, +2.2 times production rate, decrease dewaterability

2.6.2 Effect of Inoculum-to-Substrate (I:S) Ratio in AD process

Selecting the appropriate inoculum-to-substrate (I:S) ratio is vital for optimizing anaerobic digestion and maximizing methane production by preventing the buildup of volatile fatty acids (VFAs). If the I:S ratio exceeds the recommended levels, a swift breakdown of carbohydrates can occur, leading to excessive VFA concentrations that can significantly disrupt the digestion process. Conversely, if the I:S ratio is too low, methane (CH_4) production will be adversely impacted (Hinds et al., 2018). Research shows that the ideal I:S ratio for the anaerobic co-digestion of sewage sludge and food waste usually falls between 1:1 and 2:1. This optimal range varies depending on the specific properties of the substrates and the operational environment of the digester. Ratios of 1.5:1 or 2:1 are frequently advocated, striking an effective balance between the microbial inoculum from sewage sludge and the rapidly degradable organic matter found in food waste. This balance is crucial for successful anaerobic digestion and enhanced biogas production.

Studies indicate that the optimum inoculum-to-substrate (I:S) ratio is about 20%, although this figure can shift based on the total chemical oxygen demand and volatile solids content of the feedstock (Slimane et al., 2014; De Vrieze et al., 2015). Table 2.9 summarised the effect of I:S ration in AD process. By optimizing the S/I ratio, the digestion time can be reduced, while organic loading rates (OLRs) and hydraulic retention times (HRTs) improve. Furthermore, an optimized I:S ratio enhances the buffering capacity of the system, leading to a substantial increase in methane output. It is clear that co-digesting food waste (FW) and sewage sludge (SS) significantly advances the anaerobic co-digestion (AcoD) process and maximize its benefits.

Table 2.9
Effect of I:S Ratio in AD process from previous studies

Authors	I:S ratio	Key Findings	Impact of AD performances
Hinds et al., (2018)	Low I:S ratio	Insufficient microbial population	Reduced methane (CH_4) production.
	High I:S ratio	Rapid carbohydrate degradation → excessive VFA accumulation	Process instability and inhibition of methanogenesis

Slimane et al., (2014); De Vrieze et al.,(2015)	Optimum I:S ratio, 1:1 – 2:1 (~20% inoculum)	Depends on COD and volatile solids content	Improved digestion efficiency

2.6.3 Operational Mode and Configuration of Anaerobic Digesters

The efficiency of anaerobic digestion (AD) depends on the digester's design and mode of operation. Digesters can operate in batch, continuous, or semi-continuous modes, which influence waste retention time, mixing, and biogas production. Proper design and operation—including control of temperature, mixing, and feeding—ensure microbes function effectively and maximize methane output. Selecting the appropriate mode and configuration is essential for a stable and efficient digestion process.

2.6.3.1 Batch and Continuous Operation

The co-digestion of food waste (FW) and sewage sludge (SS) was studied using both batch and continuous operation modes. El-Mashad and Zhang (2010) reported methane production rates of approximately 293 mL CH_4 per gram of volatile solids (VS) with a hydraulic retention time (HRT) of 20 days, and 311 mL CH_4 per gram of VS at an HRT of 30 days in a batch process. Therefore, a larger reactor volume is essential for achieving a maximum degradation rate during batch operations. In contrast, Zhang et al. (2007) and El-Mashad and Zhang (2010) noted that the reactor volume tends to be smaller in continuous operations because digested substrate is removed regularly by adding appropriate amounts of feedstock at set intervals. Additionally, the optimum HRT of the digester is crucial for treatment effectiveness, as continuous loss of microbial populations—vital for the anaerobic co-digestion (AcoD) of food waste— can affect performance (Zhang et al., 2013). Both operation modes demonstrate significant improvements in methane production, with increases of 41% and 55%, respectively (Zhang et al., 2013). Figure 2.6 shows schematic diagrams of batch and continuous reactors.

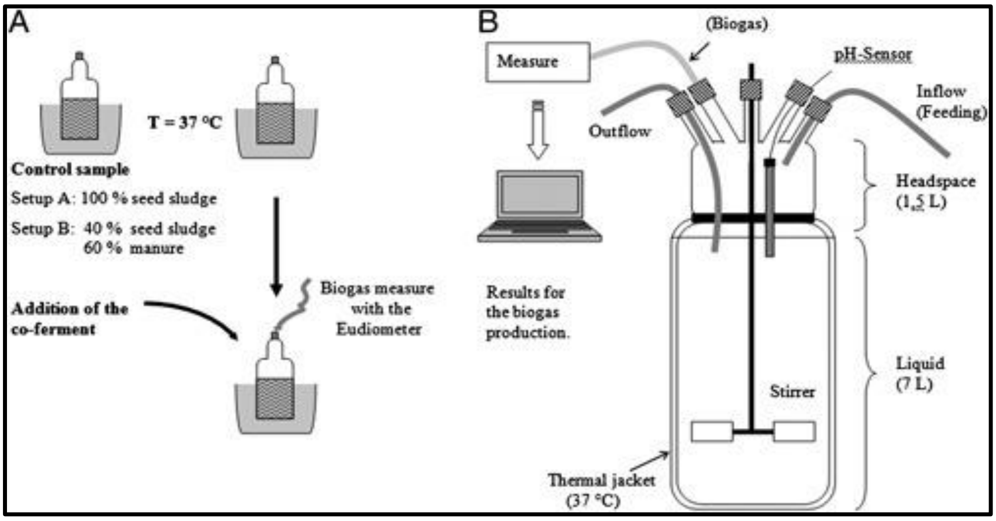


Figure 2.6 (A) Schematic diagrams of batch and (B) continuous reactors (Rojas et al., 2010)

2.7 Methane Gas Production

The anaerobic co-digestion of non-dairy creamer has been studied extensively, often in conjunction with other organic wastes. One study examined the co-digestion of expired non-dairy creamer with dairy manure. It found that while using 100% non-dairy creamer inhibited the anaerobic digestion process, a mixture containing 74% creamer yielded a cumulative methane production that was only 10.4% lower than the control group. This indicates that non-dairy creamer can serve as a viable co-substrate for methane production, but its proportion in the mixture must be carefully managed to prevent process inhibition (Zhu et al., 2011). Another study showed that co-digesting coffee solid waste with sewage sludge achieved methane yields between 0.24 and 0.28 m^3 CH_4/kg volatile solids, along with significant reductions in total and volatile solids (Neves et al., 2006).

A previous study by Adebayo et al. (2013) analyzed three different ratios of cattle slurry (CS) and coffee waste (CW) in samples A, B, and C, which were set at 1:1, 3:1, and 1:3 for anaerobic co-digestion. The findings revealed that sample A, with a 1:1 ratio, exhibited the highest methane yield at 77.55%. In comparison, samples B and C, with ratios of 3:1 and 1:3, yielded 73.71% and 75.37%, respectively. However, the 3:1 ratio was identified as the optimum ratio for biogas production under mesophilic conditions, attributed to its higher lignin content, which tends to slow down the digestion activity of microorganisms.

According to Girotto et al., (2017), chemical pre-treatment, used successfully for organic substrates such as food waste, is one of the best-performing pre-treatments compared to alkaline, oxidative, steam explosion, and thermo-chemical ones. H_2SO_4 pre-treatment led to an increased total methane potential during the anaerobic digestion of spent coffee grounds. H_2SO_4 was applied with different loadings, namely 2, 4, 6, and 8% w/w for 24 h, to assess the efficiency of the process and the optimal amount of the basifying solution applied. The highest concentration of H_2SO_4 (8% w/w) led to the best anaerobic digestion performances (392 mLCH$_4$/gVS) as a consequence of the slightly higher lignin degradation with 24% higher than that of the untreated substrate, and of the higher dissolved organic carbon concentration. Table 2.10 summarized the methane gas production from previous studies.

Table 2.10
Methane Gas Production from previous studies.

Authors	Substrate/Condition	CH_4 production	Key Findings
Zhu et al., (2011)	Non-dairy creamer + dairy manure	~10.4% lower than control	High creamer ratio inhibits AD; moderate proportion viable for methane production
	100% non-dairy creamer	Inhibited methane production	Excess substrate causes process inhibition
Neves et al., (2006)	Coffee waste + sewage sludge	0.24–0.28 m³ CH_4/kg VS	Stable methane yield with significant solids reduction
Girotto et al.,(2017)	Spent coffee grounds + H_2SO_4 pre-treatment (8% w/w)	392 mL CH_4/g VS	Highest methane yield due to improved lignin degradation and DOC release
	Cattle slurry : coffee waste (1:1)	77.55% methane yield	Highest methane yield among tested ratios
Adebayo et al.,(2013)	Cattle slurry : coffee waste (3:1)	73.71% methane yield	Optimum for biogas stability due to lignin content
	Cattle slurry : coffee waste (1:3)	75.37% methane yield	Slightly lower performance due to substrate imbalance

CHAPTER 3
METHODOLOGY

3.1 Sample Collection

The inoculum used in this study was thickened sewage sludge collected from the Titiwangsa 2 Sewage Treatment Plant operated by Indah Water Konsortium (IWK) in Kuala Lumpur (coordinate of location: 3.18336, 101.71230). This source was selected because it contains an active microbial population and is partially stabilized, making it appropriate for anaerobic digestion experiments and representative of real wastewater treatment conditions. The substrates used were slurry mixtures of non-dairy creamer and coffee waste, supplied by the Indah Water Research Centre (IWRC), Kuala Lumpur. These wastes originated from a beverage manufacturing facility in Shah Alam and were chosen due to their high organic content and biodegradability, which can enhance methane production during co-digestion. Their selection was also recommended by IWRC as suitable food-related industrial wastes for energy recovery studies. All samples were collected as shown in Plate 3.1 by using pre-cleaned containers (sampling bottles) to avoid contamination and were transported to the Faculty of Applied Sciences laboratory for subsequent analysis and testing.

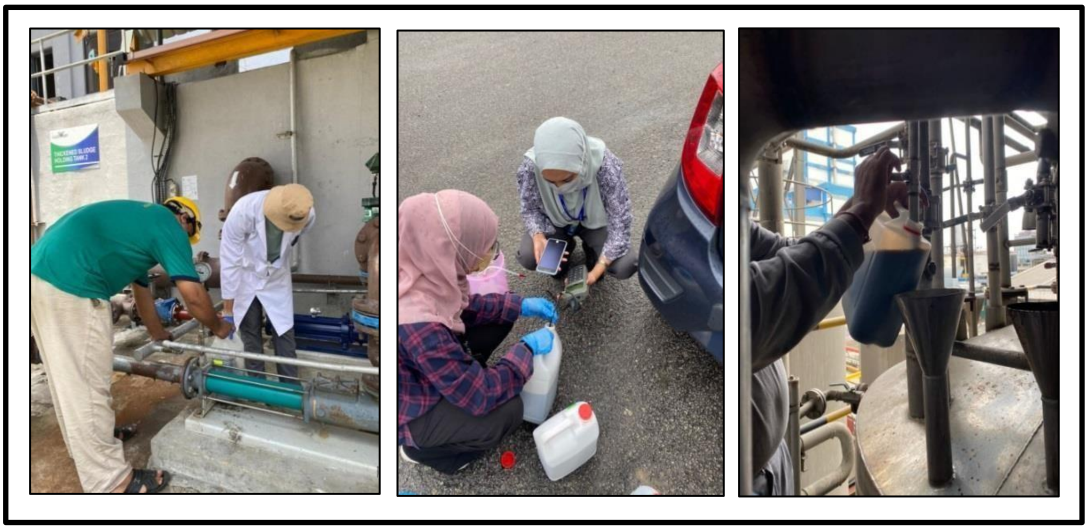


Plate 3.1 Sampling of Thickened Sludge and Food wastes
(Coffee and Non-Dairy Creamer)

3.2 Inoculum and Substrate Preparation

Inoculum (IWK Sludge) and substrates (food wastes) was separately prepared with deionized water with the ratio of 1:1 to achieve proper consistency and helps maintain a stable environment by diluting the substrate, which can prevent rapid acidification and ensure a balanced pH level, crucial for methanogenic activity ((Rocamora et al., (2021); Yanyang Li et al., (2018); and Corsino et al., (2021)). Before any optimization, the substrates and inoculum were neutralized to pH 6.8-7 by using either sulphuric acid (H_2SO_4) or sodium hydroxide (NaOH). The neutralization of pH was done in order to supports a diverse microbial community, which is essential for efficient anaerobic digestion. At this pH, methanogenic bacteria thrive, enhancing methane production (Zhou et al., (2016) and Wang et al., (2021)). The mixture was stored in a chiller at approximately 4°C for no more than 3 days before further experimental work to minimise biological degradation and maintain its original characteristics (Dauptain et al., 2021; Astals et al., 2020).

3.3 Sample Characterization

The characterization of each raw material, which are both substrates and inoculum was analyzed such as moisture content (MC), pH, chemical oxygen demands (COD), total solid (TS), volatile solid (VS), total suspended solid (TSS), volatile suspended solid (VSS), volatile fatty acids (VFA) and alkalinity (AL). Thus, all these parameters were measured according to standard methods.

3.3.1 Moisture Content (MC)

Empty crucibles were accurately weighed using a laboratory weighing balance. The moisture content of the waste sample was determined by placing 10 g of the sample into a crucible. The sample was then dried in an oven overnight at a temperature of 105°C. After drying, the samples were cooled in a desiccator and reweighed. The moisture content was calculated as the percentage weight loss before and after drying. The moisture content of the samples was determined using the equation below (3.1) (APHA, 2017):

$$\% \mathrm{MC} = \frac{wi - wf}{wi - w} \; x \; 100\% \tag{3.1}$$

Where,

Wi = weight samples before heating (g)

Wf = weight samples after heating (g)

W = weight of empty crucible (g)

3.3.2 pH

The sample was mixed with deionized water with the ratio that roughly 1:1. Thus, the pH of the mixture was measured by using a pH meter (APHA, 2017).

3.3.3 Chemical Oxygen Demands (COD)

The COD reactor was turned on and preheated to 150°C. Safety shield was placed in front of the reactor. The vial was held at 45^{o} angle. The sample was pipetted into the vial by 2.0ml (for the ultra-low and low range) or 0.2ml (for the high range and high range plus). The vials were capped tightly. The exterior of the COD vials was rinsed with the deionized water and wiped with the clean towel. The vial was held by the cap and over a sink. It was inverted gently several times to mix the content. The vial was placed in the preheated COD reactor. The blank sample was prepared by repeating the above steps, substituting 2.0ml (for the ultra-low and low range) or

0.2ml (for the high range and high range plus) deionized water for the sample. The exterior of the walls of all the vials were wiped and cleaned before heated simultaneously in digestion reactor for 2 hours. The reactor was turned off and the vials were cooled to about 120^{o}C. The vials were inverted several times while still warm. The vials were placed into a rack and cooled at room temperature. The experiment was continued to the colorimetric determination to measure COD. The program selected was 435 COD HR/HRP. The start button was activated, and the vial exterior was cleaned with a damp cloth followed by a dry one to eliminate fingerprints or smudges. The blank vial was placed in the cell holder and the ‘Zero’ function was selected, resulting in a display of 0 mg/L COD. The standard vial was then inserted into the holder, and the ‘Read’ function was pressed. Subsequently, the sample vials were measured by placing them in the holder and pressing ‘Read.’ To verify calibration, the blank vial was reinserted, and ‘Read’ was pressed again (HACH, 2021).

3.3.4 Total Solid (TS)

The weight of the empty crucible was measured and 10g of sample was placed in the crucible and weighed. Then, the crucible was placed in the oven at temperature 105^{o}C for 24 hours. The next day, the crucible was placed in the desiccator and the weighed of the crucible and sample was measured until constant readings. The total solid can be determined by using equation below (3.2) (APHA, 2017):

$$\% \, TS = \frac{w^1}{w2} \; x \; 100\% \tag{3.2}$$

Where,

$w1$ = weight of air-dried sample (g)

$w2$ = weight of dried sample after heating (g)

3.3.5 Volatile Solid (VS)

The weight of empty crucible was measured and recorded. The sample was placed in the crucible and heated in the oven for 4 hours at temperature of 550°C. After four hours, the crucible was cooled in the desiccator and the weight of the sample was measured. After that, the crucible was pre-heated in the muffle furnace for 15 minutes. The volatile solid can be determined using equation below (3.3) (APHA, 2017):

$$\% VS = \frac{w1}{w2} x\ 100\% \tag{3.3}$$

Where,

$w1$ = weight of dried sample after heating in the oven (g)

$w2$ = weight of ash after pre-heated in the furnace (g)

3.3.6 Total Suspended Solid (TSS)

A 50 mL sample was prepared for analysis, and the total suspended solids (TSS) apparatus was arranged. The filter paper and its aluminum pan were dried in an oven at 105 °C for 1 hour, and then cooled in a desiccator to room temperature before being weighed. This initial weight was recorded as B. Next, the filter paper was placed in a funnel, moistened with distilled water, and connected to a vacuum pump. The 50 mL sample was poured through the filter paper so that the solids were trapped on it. After filtering, the paper was carefully removed with tweezers, placed back in the pan, and dried again in the oven at 103–105°C for at least one hour. Once cooled in a desiccator, it was weighed again (recorded as A). The difference between the two weights (A–B) gave the amount of suspended solids in the water sample can be determined by using equation below (3.4) (Kashani et al., 2019).

$$\% \text{ TS}S = \frac{(A-B)\ X1000}{50ml}\ x\ 100\% \tag{3.4}$$

Where,

A = weight of empty filter paper, aluminium pan and sample after heated at 1 hour (g)

B = weight of empty filter paper with aluminium pan (g)

3.3.7 Volatile Suspended Solid (VSS)

The empty crucible was heated in furnace at 550^{o}C for 1 hour. The crucible was placed in a desiccator. The weight of the crucible was recorded. The crucible from TSS method was weighed and the reading was recorded (B). The crucible with sample (from TSS analysis) was burned for 15 minutes. The crucibl (3.5) mples was cooled at room temperature. The reading of crucible with sample was recorded (A) can be determined by using equation below (3.5) (Kashani et al., 2019):

$$\% \, VSS = \frac{(B-A)\, X1000}{50ml} \; x \; 100\% \qquad (3.5)$$

Where,

A = weight of crucible with continues sample after heated at 15minutes (g)

B = weight of crucible with continues sample from TSS (g)

3.3.8 Volatile Fatty Acids (VFA) - (Esterification method)

The instrument (770 Volatile Acids program) began with the preparation of a blank containing 0.5 mL of deionized water and a sample containing 0.5 mL of filtrate or supernatant. Each solution was mixed with 1.5 mL of ethylene glycol and 0.2 mL of 19.2 N sulfuric acid, and then heated in a boiling water bath for 3 minutes before being cooled to 25 °C. Subsequently, 0.5 mL of hydroxylamine hydrochloride, 2.0 mL of 4.5 N sodium hydroxide, 10 mL of ferric chloride–sulfuric acid solution, and 10 mL of deionized water were added sequentially with thorough mixing. After a second 3-minute reaction period, the blank was used to calibrate the instrument, and the prepared sample was analyzed to determine the volatile acid concentration, expressed as mg/L acetic acid, HOAC (HACH method, 2015).

3.3.9 Alkalinity (AL) - Digital Titrator

In the alkalinity test, 10 mL of the sample was transferred into a 250 mL Erlenmeyer flask and diluted with deionized water if the total volume was less than 100 mL. A digital titrator equipped with a 1.600 N H_2SO_4 cartridge (digit multiplier 10) was prepared by releasing air bubbles and cleaning the delivery tip. The titrant was gradually added to the sample while swirling, and the pH was continuously monitored until it reached the endpoint of 4.5. The pH probe was rinsed and cleaned after each measurement. To calculate the concentration, the total number of digits used was multiplied by the digit multiplier as shown in Table 3.1: Total digits used × digit multiplier = mg/L as $CaCO_3$ Total alkalinity (HACH method, 2015).

Table 3.1
Sample Volumes and Digit Multipliers

Range (mg/L as CaCO3)	Sample Volume (mL)	Titration cartridge	Digit Multiplier
10-40	100	0.1600 N H_2SO_4	0.1
40-160	25	0.1600 N H_2SO_4	0.4
100-400	100	1.600 N H_2SO_4	1
200-800	50	1.600 N H_2SO_4	2
500-2000	20	1.600 N H_2SO_4	5
1000-4000	10	1.600 N H_2SO_4	10

3.4 Optimization of Anaerobic Digestion (AD) of IWK Sludge and Food Waste

The optimization was conducted in 125 mL serum bottles. Under optimized conditions, the substrate and inoculum for the biochemical methane potential (BMP) test were introduced at a constant pH of 6.8 -7, temperature of 37^{o}C and HRT at 30 days. The working volume was kept at 70% of the total volume capacity of the bottle, while the remaining 30% was used as headspace for gas accumulation as shown in Figure 3.1 (Ramatsa et al., 2015). Before sealing with septa and aluminium caps, the nitrogen gas (N_2) was promptly purged in the headspace of the bottles for 3 minutes to remove oxygen and create anaerobic conditions for the AD process (Ebner et al., 2016).

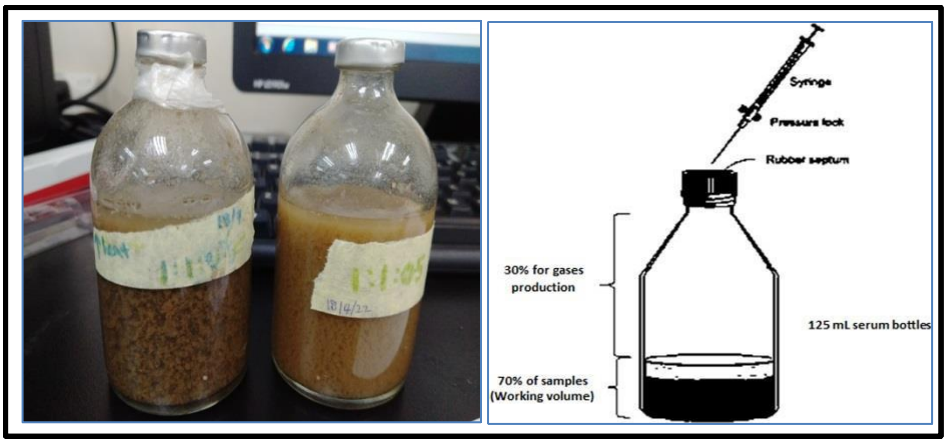


Figure 3.1 125 mL of Serum Bottles Set Up
(Ramatsa et al., 2015)

3.4.1 Control and Blank sample preparation

Blanks and controls play a crucial role in BMP tests by accounting for methane production from the inoculum, which could otherwise result in an overestimation of the substrate's methane potential. Proper preparation and use of blanks help ensure the accuracy and reliability of the BMP results (Olieveira et al., (2022) and Holliger et al., (2016)). In AD process, a "blank" typically refers to a setup that contains only the inoculum without any additional substrates or treatments. It serves as a baseline for BMP test which to measure the natural biogas production from the inoculum itself, without any influence from added materials or conditions ((Montalvo et al., (2018); Bhatnagar et al., (2020); Dong et al., (2024)). While, the control setup usually includes the substrate without any additional treatments or modifications. It serves as a standard to compare the effects of experimental treatments (Montalvo et al., (2018)). In this study, IWK thickened sludge was used as the blank. Non-dairy creamer and coffee waste used as control. The control involved using each substrate in separate serum bottles. For both blank and control were prepared without any pH adjustments.

3.4.2 Optimization of Inoculum to Substrate (I:S) on methane production

The optimization of the inoculum-to-substrate (I:S) ratio is crucial for maximizing methane production in anaerobic digestion processes. In this study, IWK thickened sludge was used as the inoculum, providing the microbial community, while coffee waste and non-dairy creamer waste were used as substrates, supplying the organic matter for degradation. The I:S ratio determines the balance between the microorganisms (inoculum) and the available organic material (substrate). If the ratio is too low, there may not be sufficient microorganisms to effectively degrade the substrate. Conversely, very high inoculum content may lead to inefficient resource utilization. In this study, different I:S ratios were evaluated using a biochemical methane potential (BMP) assay, including 1:1, 2:1, 1:1:1, 2:1:1, and 3:1:1. Table 3.2 summarizes the preparation of these I:S ratios for methane production analysis.

Table 3.2
I:S Ratio on Methane Production

I:S ratio	Inoculum	Substrates	
	IWK Thickened sludge	Coffee Waste (CW)	Non-dairy creamer waste (NDCW)
1:1	Present	Present	Not included
1:1	Present	Not included	Present
2:1	Present	Present	Not included
2:1	Present	Not included	Present
1:1:1	Present	Present	Present
2:1:1	Present	Present	Present
3:1:1	Present	Present	Present

3.4.3 Optimization of Inoculum to Substrate (I:S) on Methane Production at Different Pre-Treatments Method

For this study, the best I:S ratio from the 3.4.2 which is optimization of I:S on methane production was selected ratio of 2:1. This I:S ratio indicates the ratio of IWK thickened sludge to coffee waste and the ratio of IWK thickened sludge to non-dairy creamer waste. This I:S ratio was performed with different pre-treatments method. Thus, the pre- treatments chosen in this study were thermal pre-treatments at 90^oC (3 hours) and 160^oC (1 hour) and the chemical pre-treatments by using acid (2M H_2SO_4) and alkali (2M NaOH). Thus, the pre-treatments are summarizing in Table 3.3

Table 3.3
I:S Ratio on Methane Production with Different Pre-Treatments

I:S ratio	Samples			Pre-treatments			
	Inoculum	Substrates		Thermal		Chemical	
	IWK Thickened sludge	Coffee Waste (CW)	Non-dairy creamer waste (NDCW)	High Heat (160^{o}C at 1 hours)	Low Heat (90^{o}C at 3 hours)	2M H_2SO_4	2M NaOH
2:1	Present	Present	Not included	Not included	Not included	Present	Not included
2:1	Present	Present	Not included	Not included	Present	Not included	Not included
2:1	Present	Present	Not included	Present	Not included	Not included	Not included
2:1	Present	Present	Not included	Not included	Present	Present	Not included
2:1	Present	Present	Not included	Present	Not included	Present	Not included
2:1	Present	Not included	Present	Not included	Not included	Not included	Present
2:1	Present	Not included	Present	Present	Not included	Not included	Present
2:1	Present	Not included	Present	Not included	Present	Not included	Present

Detailed breakdown of the thermal and chemical pretreatment methods for IWK sludge, coffee waste, and non-dairy creamer (NDC) waste, based on the parameters provided. Thermal pretreatment methods for sludge, coffee waste, and non-dairy creamer (NDC) waste involve both low- and high-temperature processes. In the low-temperature thermal pretreatment, the waste is first heated to 90°C for 3 hours in oven or temperature-controlled reactor under atmospheric pressure. After heating, the sample is rapidly cooled to room temperature using a water bath to prevent microbial reactivation. This process disrupts cell walls and extracellular polymeric substances (EPS) in sludge (Park et al. (2020); and Zhang et al. (2014)), enhancing hydrolysis; breaks down lignocellulosic structures such as cellulose and hemicellulose in coffee waste (Park et al. (2020b); and solubilizes carbohydrates and proteins in NDC (Wang et al. (2024)). In the high-temperature thermal pretreatment, samples are similarly prepared but heated to 160°C for 1 hour under pressure in a high-pressure reactor or oven to prevent boiling. The heated samples are then quenched to halt further reactions. This method significantly reduces particle size and increases soluble chemical oxygen demand (sCOD) in sludge by breaking down complex organics, degrades lignin and releases fermentable sugars in coffee waste, and hydrolyzes fats and stabilizers into simpler compounds in NDC.

Chemical pretreatment methods include acid and alkali treatments. Acid pretreatment involves mixing the waste with 2M sulfuric acid at a 1:5 acid-to-waste ratio, stirring continuously at room temperature for 24 hours, followed by neutralization to pH 6.5–7.5 using sodium hydroxide to avoid inhibition in downstream processes (Wang et al. (2024b)). This treatment hydrolyzes proteins and lipids in sludge into amino acids and fatty acids (Mao et al. (2015), removes hemicellulose and reduces phenolic inhibitors in coffee waste (Park et al. (2020c), and breaks down corn syrup and stabilizers into monosaccharides in NDC (Wang et al. (2024b). Alkali pretreatment is performed by mixing the waste with 2M sodium hydroxide at a 1:5 ratio, stirring for 24 hours at room temperature, and then neutralizing the pH to 6.5–7.5 using hydrochloric acid. This method solubilizes lignin and humic substances in sludge to improve biodegradability, disrupts lignin-cellulose bonds in coffee waste to enhance cellulose accessibility, and saponifies fats while improving carbohydrate solubility in NDC.

3.5 Scale-up of Optimized Anaerobic Digestion (AD) conditions in 40 L Digester (Pilot Scale)

Upscaling optimized anaerobic digestion (AD) conditions from laboratory to pilot scale (40 L digester) was carried out following established anaerobic digestion operational guidelines and standard methods for biochemical methane production experiments (Angelidaki et al., 2009; APHA, 2017). The schematic diagram and the picture of the 40 L pilot-scale digester is shown in Figure 3.2 and Plate 3.2. The system is equipped with a controllable impeller for mixing, a gas sampling port, feed inlet, heater blanket, and liquid discharge port, as summarized in Table 3.4. Fresh thickened sludge was manually fed into the digester through the inlet port. The pH was maintained between 6.8 and 7.0 to ensure optimal microbial activity. Prior to operation, nitrogen gas was purged for 3–7 minutes through the gas sampling port to establish anaerobic conditions. A 3 L gas sampling bag was then connected to collect biogas. The digester temperature was maintained at 37°C using a heating blanket to ensure mesophilic digestion conditions.

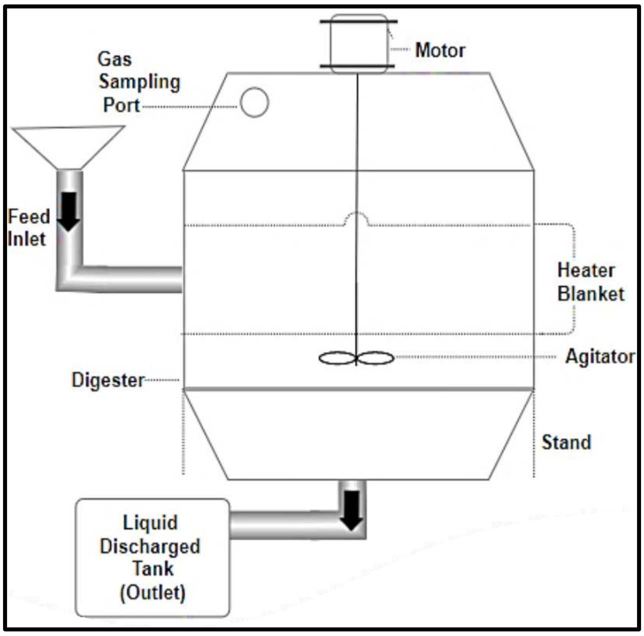


Figure 3.2 Schematic diagram of 40L digester (Pilot Scale)

Plate 3.2 Picture of 40L digester (Pilot Scale)

Table 3.4
Detailed features and description of 40L pilot digester

Features	Pilot Scale Design
Material	Stainless steel 304SS
Diameter at centre (mm)	±390
Height from stirrer to bottom (mm)	±250
Water level	HWL: ±310 mm
	LWL: ±300 mm
Detail	Piping detail
	OD: ±30 mm
	Pump detail
	Flow rate: 35L/min
Volume	40L
Factor plant to pilot scale	±13,000
Stirrer Motor	110-130 Watt
Torque	High torque, 4-fold transmission reduction
Agitation speed	varied 100-450rpm
Temperature	Ambient temperature
pH	5-8
Unit	1

3.5.1 Acclimatization

The acclimatization process was conducted in a 40 L upscale digester, as shown in Figure 3.2, located at the Indah Water Research Centre (IWRC) in Kuala Lumpur. Acclimatization is a critical adaptation process that ensures stable anaerobic digestion by allowing microbial communities to adjust to new environmental conditions. In anaerobic digestion, acclimatization enables microbes from the sludge to adapt to variations in parameters such as retention time, pH, and temperature within a closed digester system. This step is essential before initiating any further processing.

In this study, acclimatization began in early September 2021 and lasted approximately 1.5 months. A mixture of 15 L of IWK thickened sludge and 15 L of water (1:1 ratio) was prepared, yielding a total working volume of 30 L and 10L remaining for biogas production (Syed Sabeer Ali et al., 2022; Kougias et al., 2013). During the first week, the digester was stirred for at least 2 hours daily at a low stirring speed (3–4 Hz) to minimize microbial stress (Vavilin et al., 2007; Battista et al., 2016).

Key parameters, including pH and temperature, were maintained at pH 6.8-7 under mesophilic conditions at 37°C. Biogas composition was monitored at least three times per week. The analysis continued for 30–40 days, with stabilization assumed when daily biogas production decreased to less than 1% or when the cumulative methane production graph exhibited a constant trend, indicating complete degradation of organic substances and insufficient nutrient availability was identified as a limiting factor for microbial digestion and methanogen activity (Jingura & Kamusoko, 2017).

3.5.2 Intermittent Feeding of IWK Sludge as an inoculum (HRT: 21, 14 and 7 days)

Intermittent feeding was initiated following the completion of the acclimatization process in the pilot digester. Intermittent feeding involves introducing fresh inoculum into the digester in discrete batches. In this study, approximately 30% of the digester's working volume (30 L) was manually fed with 9 L of fresh thickened sludge, as shown in Plate 3.3. Prior to feeding, the sample was purged with nitrogen gas (N_2) for 3–5 minutes to maintain anaerobic conditions. The thickened sludge was retained in the digester for varying hydraulic retention times (HRT) of 21, 14, and 7 days, with continuous stirring at a minimal rotational speed.

After each HRT period, 9 L of liquid (equivalent to the fed volume of 30%) was discharged from the digester. The inlet and outlet samples were characterized for parameters including pH, total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), volatile fatty acids (VFA), moisture content, and alkalinity. Biogas produced during digestion was collected in 3 L gas bags and monitored at least three times per week using a portable gas analyzer and gas chromatography (GC-FID). The intermittent feeding process was conducted over a period of approximately four months to assess biogas yield trends across different HRT conditions.

Plate 3.3 40L pilot digester of AcoD process at IWRC, KL

3.5.3 Continuous Feeding of IWK Sludge as an inoculum (HRT: 10 and 7 days)

The continuous feeding of IWK Sludge as an inoculum in 40L pilot digester was conducted under two different hydraulic retention times (HRT):10 days and 7 days, following the cessation of intermittent feeding. In a real plant, continuous feeding with sludge is standard practice. For this study, the 40L pilot digester was utilized as a scaled- down model before applying the process to a full-scale plant. Therefore, continuous feeding was selected to better replicate the operations of a real plant. Approximately 30L of thickened sludge was used in this process. During the experiment, a feeding tank with a stirrer, liquid discharged tank, and a peristaltic pump were prepared, as illustrated in Figure 3.3 and Plate 3.4. This setup differed from the intermittent feeding process, which did not require a feeding tank or pumps.

To prevent the sludge from settling and clogging the tubing, the feeding tank was purged with nitrogen gas for 3-5 minutes, and the stirrer inside the tank was operated at a low speed. Samples from the inlet and outlet were analyzed for various parameters, including pH, TS, VS, TSS, VSS, VFA, moisture content, and alkalinity. For the 10-day HRT, the peristaltic pumps for both the feed and liquid discharge were set to deliver 2.08 mL/min, corresponding to 3L/day. During the 7-day HRT, the flow rate was increased to 4.28L/day for both pumps. A 3L gas bag was used to collect and store the biogas, which was measured after 24 hours.

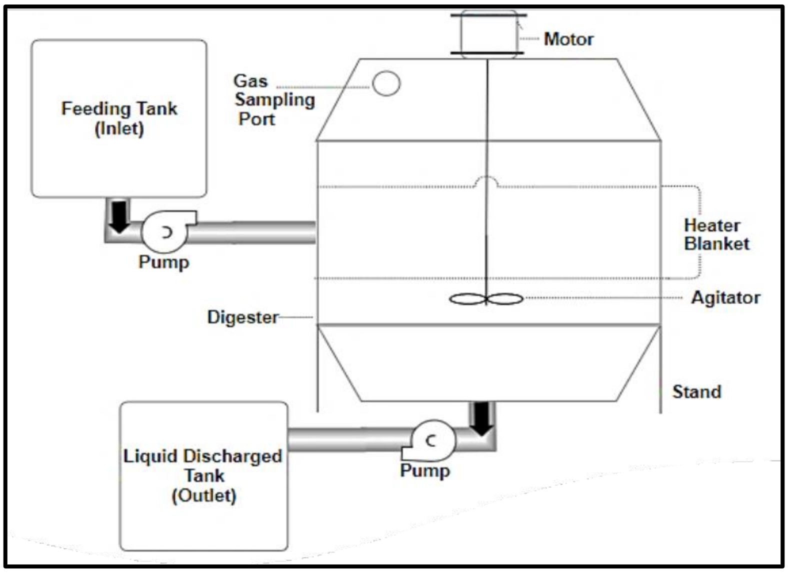


Figure 3.3 Schematic diagram of 40L pilot digester for continuous feeding

Plate 3.4 Set-up of the continuous feeding of IWK Sludge

The selection of 10 and 7-day HRTs was likely intended to optimize the anaerobic digestion process for enhanced methane production. A longer HRT (10 days) provides more time for microorganisms in the digester to break down the organic material in the sludge. This additional time allows a higher percentage of the organic matter to be converted into biogas, particularly methane, as the microbes have more opportunity to degrade complex compounds. Generally, longer HRTs tend to yield higher methane production, provided the organic loading rate does not exceed the system's capacity. On the other hand, a shorter HRT (7 days) can be used to accelerate the digestion process, testing whether a quicker digestion period still results in substantial methane production while increasing system throughput and efficiency. Although shorter HRTs may slightly reduce methane yields due to less time for microbial digestion, optimized conditions (such as adjusted temperature and feeding rates) can still produce relatively high methane outputs. Table 3.5 presents the working volume applied for different hydraulic retention times (HRTs) during the continuous feeding of IWK sludge as the inoculum. By comparing these two HRTs, the study aims to identify the optimal balance between retention time and methane production efficiency. The goal is to find the HRT that maximizes methane output while considering practical factors such as digestion efficiency, system size, and cost-effectiveness in a real-world-plant.

Table 3.5
Working Volumes for Different HRTs

HRT (days)	Daily Feed Volume (L/day)	Flow rate (mL/min)	Working Volume (L)
7	3.0	2.08	30
10	4.28	~2.97	

In anaerobic digestion, intermittent feeding involves adding the substrate in periodic batches, leading to variable microbial activity and uneven biogas production, but it is simple and flexible for small-scale or experimental systems. Continuous feeding, on the other hand, delivers substrate steadily, promoting stable microbial activity, consistent biogas output, and higher efficiency, making it ideal for large-scale operations, though it requires more equipment and careful control.

3.5.4 Continuous Feeding of Pre-treatments and Anaerobic Co-digestion of 40L pilot Digester at IWRC (HRT: 30, 10 and 7 days)

The optimized conditions obtained from the biochemical methane potential (BMP)-lab scale analysis was tested on 40L upscale digester which is placed at Indah Water Research Centre (IWRC), Titiwangsa Kuala Lumpur. From the 40L volume of the digester, 30L was the working volume and the remaining will be for the biogas production. From the results of BMP pre-treatments and co-digestion of IWK Sludge and Food waste, the BMP test with a ratio of 2:1 IWK Sludge: Coffee waste by Acid treatment (2M H_2SO_4 at pH 7) was chosen to be applied into the 40L pilot digester. For the start-up of the co-digestion process in this study, the HRT 30 days with low OLR, 1L/day was suggested (Abd Nasir et al., 2018). A longer HRT, such as 30 days, is recommended during the start-up phase to allow the microbial community to adapt to new substrates without being overwhelmed. This approach helps in maintaining process stability and preventing the washout of essential microorganisms (Dennehy et al., (2017); Shaojie et al., (2020) and Sousa et al., (2021)). The low OLR will give a chance for microbes to adapt with new environment and to avoid organic matter overload. In this process, about 20L of thickened sludge was mixed with 10L of coffee waste by ratio 2:1. The initial pH of mixed thickened sludge and coffee waste was observed. The pH of the coffee waste is too alkaline, pH 11-12 and the pH of the sludge is 6.8 (Plate 3.5). So, to maintain the optimum condition of anaerobic condition, the adjustment of pH was done by adding 2M H_2SO_4 until getting the pH 6.8-7 (Plate 3.6).

Plate 3.5 pH reading of coffee waste (left) and IWK Sludge (right)

Plate 3.6 pH reading of the AcoD of 2:1, IWK Sludge: CW by using 2M H_2SO_4

The feeding tank was purged with nitrogen gas for 3 minutes and the stirrer inside the feeding tank was continuously stirred with minimum rpm, to avoid the sample settling down and causing the tubing stuck. The slower rpm of the peristaltic pump was contributed to stuck issues of feed and liquid discharged tubing. Thus, the mixture of food waste and IWK sludge was maintained at 37^{o}C. The feed and liquid discharged sample was characterized such as the pH, TS, VS, TSS, VSS, VFA, moisture content, and alkalinity. The peristaltic pump of the feed and liquid discharged was set up to get 0.6944mL/min which is equal to 1L/day. The 3L gas bag (Plate 3.7) was used to store the gas and the biogas was measured after 24 hours by using a portable gas analyzer and GC-FID. The continuous feeding of the 40L pilot digester was continuously proceeding with HRT: 10 days by 3L/day and HRT: 7days by 4.28L/day. Thus, different HRT was performed in order to observe the performances of the digester of AcoD of IWK Sludge:CW in the production of methane.

Plate 3.7 3L gas bag used as a gas collector

The variation in hydraulic retention times (HRTs) between continuous feeding of sludge alone and co-digestion of sludge with chemically pretreated coffee waste is mainly attributed to differences in substrate biodegradability. Sludge is more readily biodegradable, allowing efficient anaerobic digestion at shorter HRTs of 7–10 days. In contrast, coffee waste contains complex lignocellulosic components that are more difficult to degrade, even after chemical pretreatment, which slows down the digestion process. As a result, a longer HRT of up to 30 days is required to ensure sufficient contact time for microbial breakdown and stable reactor performance in the co-digestion system.

3.6 Analysis of Methane Generation

In this study, methane generation was carried out and analyzed using two methods: gas chromatography with a flame ionization detector (GC-FID) and a portable gas analyzer.

3.6.1 Gas Chromatography- Flame Ionization Detector Analysis (GC-FID)

Gas Chromatography-Flame Ionization Detector (GC-FID) Agilent 7890A (30m x 320µm x 0.25µm) was used as the instrument for analysis of methane gas concentration in biogas which was located at Basic Instrumental Analysis (BIA) Laboratory, Block A, Faculty of Applied Science (FSG), UiTM Shah Alam. The standard gas was injected for 3 minutes before the analysis began. The gas sample was taken from the gas bag through the rubber septum by using the GC pressure lock syringe by injecting it through the injection port of the instrument. The items of GC- FID were set up as shown in (Table 3.6). The carrier gas that was used in this experiment is hydrogen gas. Thus, the analysis was processed for 3 minutes. The standard methane gas with a concentration of 30% was used for instrument calibration. Kalanatarifard (2015) stated that the reading of the sample must be taken daily to observe and monitor methane production. Gas concentrations were confirmed by matching retention times and peak areas with known gas standards for CH_4 (British Oxygen Co.). Thus, the concentration of methane gas produced was determined using an equation of the 'GC External Standard Method' shown in (3.6):

$$Cp(s) = \frac{(s)}{ap(std)} \times ((std)) \tag{3.6}$$

$C_{p(s)}$ = concentration of the component (p) in the sample

$C_{p(std)}$ = concentration of the standard in the reference

$a_{p(s)}$ = area of the peak of the component (p) in the sample chromatogram

Table 3.6
GC-FID Setting (Costa et al., 2012)

Parameters	Set Up
Column (Capillary)	Temperature:100 ^oC Flow: 3mL/min
Carrier Gas Flow	Hydrogen Gas
Front Inlet	Mode: Split injection Temperature: 150 ^oC Pressure: 14.770psi
Back Detector	Temperature: 250 ^oC H_2 Flow: 30mL/min Air Flow: 350mL/min Makeup Flow (N_2): 28.5mL/min Column + Makeup: 31.5mL/min Flame: ON
Oven	Temperature: 100 ^oC, Max (320 ^oC)

3.6.2 Portable Gas Analyzer

A portable gas analyzer is a compact, handheld device designed to measure the concentration of various gases in air. It operates using sensors that detect specific gas molecules and display the measured concentrations on a digital screen. Such analyzers are widely used for environmental monitoring, industrial safety, and research applications. The types of gases detected depend on the specific model and sensor configuration.

In this study, a multi-gas portable analyzer was employed, capable of simultaneously detecting methane (CH_4), carbon dioxide (CO_2), oxygen (O_2), sulfur dioxide (SO_2), and balance gas. Methane, carbon dioxide, oxygen, and balance gas were recorded as percentages (%), while sulfur dioxide was measured in parts per million (ppm). The instrument used was the ATEX-certified Geotech BIOGAS 5000 gas analyzer, which features a robust design suitable for anaerobic digestion (AD) sites, along with self-testing and self-monitoring functions during startup and simplified calibration procedures.

The portable gas analyzer is equipped with several components, including connector A, an inlet port, a static pressure port (white), a differential pressure port (blue), connector B, a gas outlet port (yellow), connector C, and protective dust caps. The instrument configuration is illustrated in Plate 3.8.

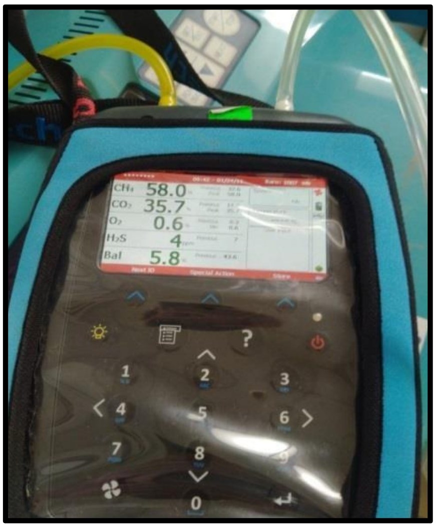


Plate 3.8 the portable gas analyzer features

3.6.3 Methane Yield

The methane yield was daily monitored until the end of the retention time of the AD process. Hence, methane production was recorded in a unit of ppm which later was converted into a volume of methane (mL). Thus, the methane yield was calculated to observe and measure the volume of methane produced by knowing the quantity of the organic matter added. Then, the methane yield was determined by using the equation shown in (3.7) below:

$$CH_4 \text{ Yield (mL } CH_4\text{/gVS)} = \frac{\text{(Cumulative volume of CH4 (mL CH4))}}{\text{Mass of original VS content (gVS)}} \qquad (3.7)$$

3.7 Flow chart of AcoD process in this study

Thus, the summary of the co-digestion between the IWK sludge and food wastes process conducted in this study were shown in Figure 3.4:

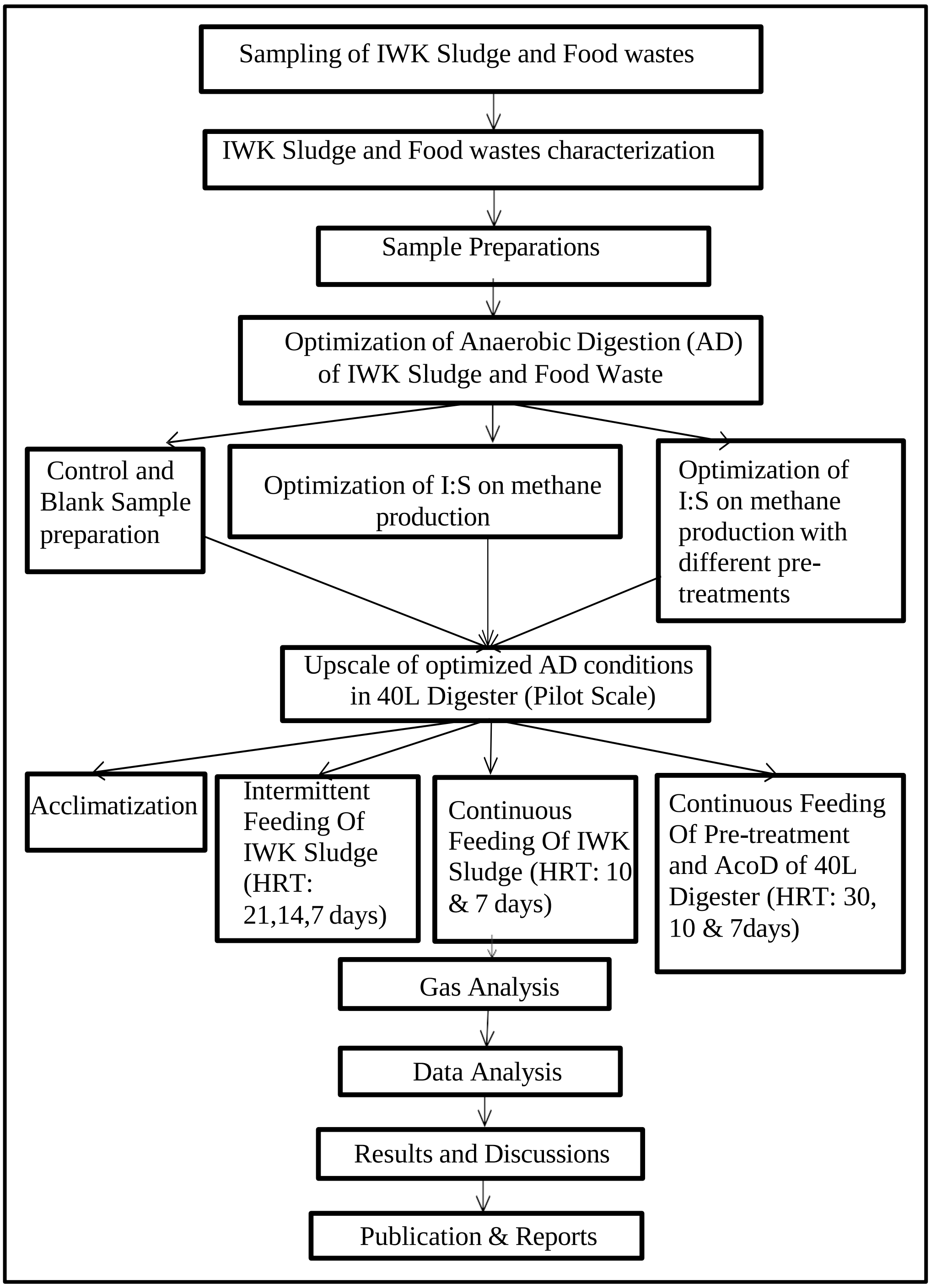


Figure 3.4 Flow chart of AcoD process in this study

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Baseline of Substrate and Inoculum Characterization

In this study, thickened sludge that was used as inoculum was collected from the IWK Titiwangsa 2, Sewage Treatment Plant (STP), which treats domestic wastewater for the areas of north-eastern Kuala Lumpur and Ampang-Ulu Klang conurbation areas in Selangor. Meanwhile, the food waste (substrate) samples were collected at Nestle Sri Muda Factory, Shah Alam. The collected samples were then analyzed for various parameters such as the chemical oxygen demand (COD), pH, total suspended solids (TSS), volatile suspended solid (VSS), volatile fatty acid (VFA), alkalinity (AL), total solid (TS), volatile solid (VS) and moisture content (MC). Due to highly concentrated thickened sludge and food wastes, dilution was done at dilution factor of 10–100. The collected samples were degassed to deplete the residual biodegradable organic material present in them. Raposo et al., (2011) prescribed, that degassing should be protracted until no significant methane production is observed: typically, 2–5 days of incubation. The average characterization results of all samples are summarized in Table 4.1:

Table 4.1
The characterization of substrate and Inoculum

Parameters	Unit	Type of samples		
		Coffee Waste (CW)	Non-Dairy Creamer Waste (NDCW)	Thickened Sludge (S)
pH	-	11.27 ± 0.26	5.38 ± 0.06	6.82 ± 0.09
Chemical Oxygen Demands (COD)	mg/L	21747 ± 1171.7	7850 ± 2293	20770 ± 896.88
Total Solid (TS)	%	1.92 ± 0.32	3.95 ± 0.31	3.36 ± 0.92
Volatile Solid (VS)	%	63.67 ± 5.95	57.18 ± 8.81	70.09 ± 7.08
Total Suspended Solid (TSS)	mg/L	12703 ± 830.32	10920 ± 877.44	26760 ± 497.59

Volatile Suspended Solid (VSS)	mg/L	9736.67 ± 259.29	9590 ± 260.58	21066.7 ± 741.92
Volatile Fatty Acid (VFA)	mg/L	308 ± 24.06	246.33 ± 22.94	347.67 ± 23.71
Alkalinity (AL)	mg/L	2314.33 ± 43.32	1453.33 ± 132.04	2314.33 ± 43.32
Moisture Content (MC)	%	98.06 ± 0.81	96.04 ± 1.49	97.33 ± 0.30

The pH for thickened sludge, and coffee waste (CW) was neutral to alkaline with 6.82 ± 0.09 and 11.27 ± 0.26 respectively. Meanwhile, the non-dairy creamer waste (NDCW) showed an acidic pH of 5.38 ± 0.06. Both low pH (≤5, acidic) and high pH (≥10, alkaline) are unfavorable for anaerobic digestion because they can disrupt the delicate balance of microbial processes involved in the breakdown of organic matter. However, previous studies revealed that the effluent from coffee processing can vary depending on several factors, such as the type of processing method used (wet, dry, or semi-washed). Wet processing (fully washed) typically results in coffee effluent with a pH ranging from 4.5 to 5.5**,** making it mildly acidic and produced high concentration of organic waste and suspended solids (Navitha and Kousar., 2018; Sahana et al.,2018). The pH of coffee waste can sometimes be alkaline (i.e., greater than 7) rather than acidic. During anaerobic digestion (AD), the microbes degrade the most in the pH range of 7-8 (Zakarya et al., 2018). Organic waste degradation is slow at low pH conditions. A decrease in pH value may be produced by the production of carbon dioxide gas and organic acid during the degradation of organic matter. The optimum pH for methanogenic bacteria activities is 6.8-7.2 (Turovskiy et al., 2006; Cioblaa et al., 2012). The digester should be maintained at a pH near 7.0 to enhance biogas production. In contrast, a low pH would create the accumulation of volatile fatty acids (VFA) which results in the inhibition of the digestion process (Parkin, 1986 & Pearse et al., 2012).

The CW produced higher chemical oxygen demands (COD) with 21747 ± 1171.7 mg/L which can reach up to 50,000 mg/L (Treagust et al., 2008) compared to thickened sludge, 20770 ± 896.88. Toxic chemicals such as tannins, alkaloids (caffeine), and polyphenolics can be found in coffee wastewater in small amounts. Caffeine concentrations greater than 350 mg/L can inhibit certain biological processes, thereby increasing the COD (Wongphoom et al, 2022). Coffee waste is rich in organic compounds such as lipids, hemicellulose, and cellulose by 26-33%.

These components are not easily degradable and contribute to high COD levels (Dinsdale et al., 1996). The higher the COD values, the higher the potential of substances to produce methane. A higher COD value indicates a larger amount of biodegradable organic matter, which enhances the potential for methane production during anaerobic digestion. Simply put, the greater the COD, the more substrate is available for methanogenic microbes to convert into methane, resulting in increased yields (Oklahoma State University Extension, 2016).

NDCW had lower COD level which are 7850 ± 2293. The previous studies by Kusum Lata and Arumugan., (1999) cited that the COD of dairy wastewater effluent was around 2000-3500 mg/L. NDCW generally have lower biodegradability, leading to less oxygen consumption by microorganisms or during chemical oxidation which generates less COD compared to more complex organic wastes like dairy or coffee wastewater, which contain proteins, fats, and other substances that rapidly break down and consume more oxygen (Ahmad et al., 2019).

Total Solid (TS) measures the suspended and dissolved solids content in water. The CW has the greatest TS value, with 5.61 ± 1.43%, followed by thickened sludge, 3.36 ± 0.92% and1.15 ± 0.26 % by NDCW. This shows that NDCW is low in solids. The lower the TS, the lower the yield of biogas are achieved in the plant, as can be seen that the NDCW contains only 1.15% as solids, and the rest is water. The volatile solid (VS) represents the quantity of organic matter that are available for biodegradation. Volatile solids (VS) of NDCW, CW, and thickened sludge at a range of 57-70% of organic matter content which is important for AD performances. Nevertheless, the total and volatile suspended solid (TSS) and (VSS) results for the CW, NDCW, and thickened sludge were 12703 ± 830.32 and 9736.67 ± 259.29, 10920 ± 877.44and 9590 ± 260.58, 26760 ± 497.59 and 21066.7 ± 741.92 mg/L. The VSS is a rough measure of solid concentration in samples. Takashinaet al., (2018), Tokumura et al., (2008) and Peerez et al., (2007) found that the TSS for CW and NDCW was 2700-2800 mg/L and 28-2500 mg/L.

Volatile fatty acid (VFA) gives information on the process status of AD as for pH, alkalinity, and COD (Boe et al., 2007). To maintain stable AD process, the VFA concentration should ideally be kept below about 400 mg/L (Sharma et al., 2022). Accumulation of VFA may interrupt the digester performance which leads to acidification (Yu et al., 2014). The digestion performance is strongly dependent to the initial characteristics before the digestion process even started. The VFA of CW, NDCW and thickened sludge were 308 ± 24.06, 246.33 ± 22.94 and 347.67 ± 23.71 mg/L respectively. Alkalinity (AL) is a very important parameter in the AD process. AL helps to avoid the failure of the digester and causes the inhibition of methanogenic bacteria activities by preventing the pH drop. The alkalinity results show differences in buffering capacity among the substrates, with CW having the highest value (2314.33 ± 43.32 mg $CaCO_3$/L), followed by thickened sludge (1990 ± 145.26 mg $CaCO_3$/L), and NDCW the lowest (1453.33 ± 132.04mg $CaCO_3$/L). Higher alkalinity improves process stability by maintaining pH and preventing acid accumulation, while lower alkalinity may increase the risk of instability during anaerobic digestion.On the other hand, the moisture content (MC) for the sludge and food waste samples is normally in the range of 96 - 98% which shows the water content of each sample was high (Labatut, 2011).

4.2 The optimization of Anaerobic Co-digestion (AcoD) parameters

The biochemical methane potential (BMP) test is the most popular method for determining the maximal methane production of a specific substrate (Raposo et al., 2011). Currently, BMP testing is considered the most reliable technique for assessing maximum methane yield, which is a crucial parameter when evaluating the feasibility and optimization of a full-scale anaerobic digestion (AD) plant. This includes considerations for co-digestion and pre-treatment strategies (Angelidaki et al., 2009; Lesteur et al., 2010; Carrere et al., 2016; Koch et al., 2016; Sol and Lansing, 2013; Ward, 2016). Additionally, BMP tests can be used to estimate the kinetic constant of the rate-limiting step, such as the hydrolysis rate for highly particulate substrates (Batstone et al., 2002, 2003; Batstone and Jensen, 2011; López et al., 2015). Understanding this is essential for achieving optimal anaerobic digester design and operation. The duration of BMP tests conducted on substrates with high levels of particulate matter can range from 20 days to over 100 days (Raposo et al., 2012).

4.2.1 Effects of inoculum-to-substrate (I:S) ratio on methane production

Operating parameters significantly influence the anaerobic co-digestion (AcoD) process, affecting both the quality and quantity of biogas production (Chulalaksananukul et al., 2012). These parameters vary depending on the type of waste and operational strategy, including substrate composition, co-substrate mixing ratio, reactor configuration, and environmental conditions such as temperature, hydraulic retention time (HRT), organic loading rate (OLR), pH, and nutrient availability. Anaerobic digestion is generally categorized into three temperature regimes: psychrophilic (4–25°C), mesophilic (30–40°C), and thermophilic (50–60°C) (Sch nürer et al., 2009; Bramstedt, 2016). Among these, mesophilic conditions are most widely applied, as they provide stable microbial activity and efficient digestion performance, although the operating temperature is often influenced by local climatic conditions (Mahanta et al., 2005).

For sludge-based digestion systems, optimal performance is typically achieved under mesophilic conditions (30–40°C) (Babaei et al., 2020; Sitorus et al., 2013). In addition, the pH of anaerobic digestion systems is generally maintained within the optimal range of 6.5 to 7.2 to support methanogenic activity (Babaei et al., 2020; Sibiya et al., 2014; Syaichurrozi et al., 2018). In this study, key operational conditions such as temperature and pH were maintained at controlled levels to ensure stable reactor performance. Among the various operational parameters, the inoculum-to-substrate (I:S) ratio and hydraulic retention time (HRT) are particularly critical, as they are often not fully optimized in anaerobic co-digestion studies involving IWK-thickened sludge and food waste for methane production. The I:S ratio plays a key role in maintaining microbial balance and preventing volatile fatty acid (VFA) accumulation, while HRT determines the sufficient contact time required for effective substrate degradation and biogas production.

Figure 4.1 presents Cumulative methane production for blank, control samples and all different inoculum-to-substrate (I:S) ratios. The blank sample consisted solely of IWK-thickened sludge (inoculum), which represents the baseline microbial activity without the addition of an external substrate. In contrast, the control samples, consisting of coffee waste (CW) and NDCW, were used to evaluate the methane production potential of the substrates under the tested conditions. The results indicated that the blank sample achieved the highest methane production on day 6, reaching 13.913 ± 0.12 mL CH_4/gVS, which reflects active microbial activity within the inoculum. In comparison, both control samples exhibited very low methane yields (≤1 mL CH_4/gVS), suggesting limited biodegradability of the substrates in the absence of an effective digestion system. It is also observed that only three lines are clearly distinguishable in Figure 4.1. This is attributed to the substantial difference in methane production between the blank and control samples. When all data are plotted on a single y-axis scale, the low methane yields of CW and NDCW are compressed near the baseline and tend to overlap, reducing their visual distinction. Consequently, fewer lines appear prominent despite multiple datasets being included.

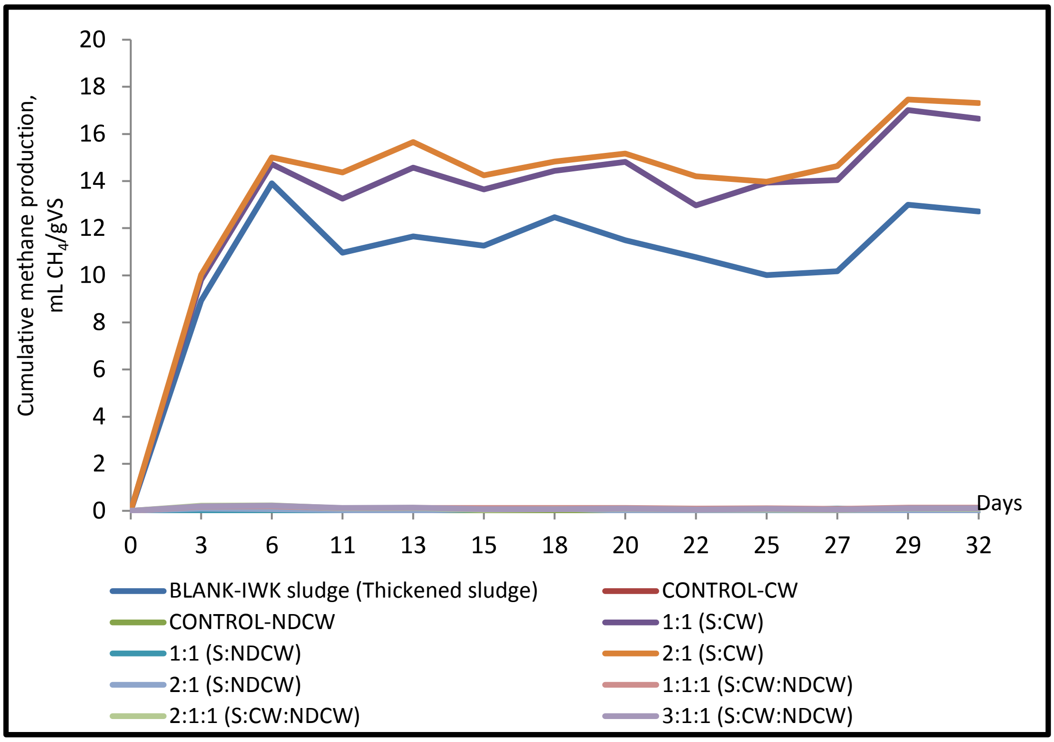


Figure 4.1 Cumulative Methane Production for all different Inoculum-To-Substrate (I:S) Ratio

In anaerobic digestion, the inoculum serves as the source of active microorganisms responsible for initiating and sustaining the process, while the substrate represents the organic material undergoing degradation. In some cases, such as thickened sludge, the material can function both as an inoculum and as a substrate due to its residual biodegradable content and microbial population. For example, a study evaluated the biochemical methane potential (BMP) test for thickened sludge using anaerobic granular sludge at different inoculum/substrate (I:S) ratios. The results showed that the cumulative methane yield was 51.4 mL CH_4/g VS added at an I:S ratio of 1:1.5. (Lim & Fox, 2013). However, the optimal inoculum-to-substrate (I:S) ratio may vary depending on several factors, such as the characteristics of the sludge and the desired quality of the biosolids. Table 4.2 and Figure 4.1 display the volume of biogas accumulated at different ratios. Results showed that methane yield interacted significantly with the chosen parameters.

Figure 4.1 shows the effect of the inoculum-to-substrate (I:S) ratio on methane production at mesophilic conditions with a pH range of 7-7.08. In all tested ratios, the highest volume of methane accumulated was observed at a ratio of 2:1 of IWK thickened sludge: coffee waste (S: CW) with a hydraulic retention time (HRT) of 32 days, yielding 17.31 ± 0.15 mL CH_4/g VS. This was followed by 1:1 (S: CW) at 16.643 ± 0.17 mL CH_4/gVS. In contrast, the control treatments—Control-CW (0.013 ± 0.0007 mL CH_4/g VS) and Control-NDCW (0.005 ± 0.0003 mL CH_4/g VS)—produced negligible methane, emphasizing the necessity of an active inoculum and balanced substrate interactions. Moreover, all systems incorporating NDCW consistently exhibited poor methane yields, suggesting limited biodegradability or possible inhibitory effects as shown in Table 4.2.

Table 4.2
Cumulative methane production of AcoD with variation of inoculum-to-substrate (I:S) ratio

Inoculum -to- Substrate (I:S) ratio	Type of wastes	pH of Digestion Mixture		Cumulative methane production (mL CH_4/gVS)	Methane Yield (m^3)
		Initial	Final		
	Blank-Sludge	6.79	8.05	12.72 ± 0.16	6.9×10^{-4} ± 0.822
	Control-CW	11.04	9.05	0.013 ± 0.0007	$5.8752\times10^{-8} \pm 4.11\times10^{-8}$
	Control-NDCW	5.44	5.71	0.005 ± 0.0003	$9.39\times10^{-2} \pm 1.22\times10^{-8}$
1:1	S: CW	7.03	7.47	16.643 ± 0.17	1.02×10^{-3} ± 0.315
1:1	S: NDCW	7.1	5.66	0.011 ± 0.0004	$7.8089\times10^{-7} \pm 3.32\times10^{-8}$
2:1	S:CW	7.08	7.56	17.31 ± 0.15	1.04×10^{-3} ± 0.621
2:1	S: NDCW	7	5.36	0.017 ± 0.0007	$4.186\times10^{-1} \pm 5.67\times10^{-8}$
1:1:1	S: CW: NDCW	7.06	5.83	0.141 ± 0.0005	$9.9255\times10^{-6} \pm 4.69\times10^{-8}$
2:1:1	S: CW: NDCW	7.04	5.8	0.085 ± 0.002	5.4954×10^{-6} ± 0.049
3:1:1	S: CW: NDCW	7.03	5.74	0.111 ± 0.002	6.9041×10^{-6} ± 0.049

Table 4.2 indicates that the initial pH of NDCW was acidic (pH 5.44), whereas the CW used in this study exhibited an alkaline pH (11.04), in contrast to the typically acidic nature of coffee residues reported in the literature (Mussatto et al., 2011). This deviation may be attributed to differences in processing conditions or prior treatment of the substrate. The alkaline nature of CW likely enhanced the buffering capacity of the system, thereby improving process stability. Consequently, the S:CW mixtures maintained near-neutral pH values (approximately 7.0–7.1), which are optimal for methanogenic activity, and exhibited less pronounced pH fluctuations throughout digestion. This buffering effect would mitigate the accumulation of volatile fatty acids (VFAs), preventing acidification and sustaining methanogenic activity.

In contrast, treatments containing NDCW showed a more substantial decline in pH (approximately 5.3–5.8), suggesting insufficient buffering capacity and the accumulation of VFAs during acidogenesis. Such acidic conditions are known to inhibit methanogenic archaea, thereby disrupting the balance between acidogenic and methanogenic phases of anaerobic digestion (Angelidaki et al., 2011). As a result, these treatments produced lower methane yields, highlighting the critical role of pH stability and buffering capacity in optimizing anaerobic co-digestion performance.

These observations are in agreement with previous studies highlighting the importance of substrate composition and pH stability in anaerobic digestion. Yadvika et al., (2004) emphasized that maintaining near-neutral pH is critical for optimal methane production. Likewise, Mata-Alvarez et al., (2014) reported that co-digestion enhances system buffering capacity and overall process efficiency. While studies such as Campos-Vega et al., (2015) often describe coffee waste as acidic and potentially inhibitory, the present findings demonstrate that when coffee waste is alkaline, it can instead improve digestion stability and methane yield.

In comparison with existing literature, this study provides stronger evidence that the physicochemical properties of substrates—particularly pH—play a decisive role in anaerobic digestion performance. The superior results observed for S:CW mixtures can therefore be attributed not only to improved substrate synergy but also to the beneficial buffering capacity of alkaline coffee waste. Overall, the findings reinforce the importance of careful substrate selection and characterization to maximize methane production and ensure stable digestion processes.

Selecting an appropriate inoculum-to-substrate (I:S) ratio is a key factor in anaerobic digestion, as it helps prevent volatile fatty acid (VFA) accumulation and enhances methane production efficiency. The data presented in Table 4.2 show that anaerobic co-digestion (AcoD) of non-dairy creamer waste (NDCW) at different I:S ratios (1:1, 2:1, 1:1:1, 2:1:1, and 3:1:1) resulted in consistently low methane yields. Across all conditions, methane production remained below 1 mL CH_4/gVS, indicating limited process performance. Increasing the inoculum proportion did not significantly improve methane generation, suggesting that higher microbial loading alone was insufficient to enhance substrate conversion. This may be attributed to substrate limitations or inhibitory compounds present in NDCW. In comparison, the control sludge showed relatively better methane production, indicating that NDCW may have reduced biodegradability or inhibitory effects on digestion. These results highlight that both excessively high and low I:S ratios can negatively affect digestion efficiency. High I:S ratios may cause microbial imbalance and inefficient substrate utilization, while low ratios may limit microbial activity and reduce methane formation (Ma et al., 2019). Therefore, careful optimization of the I:S ratio is essential for stable and effective anaerobic digestion.

Hydraulic retention time (HRT) also plays an important role in digestion performance. It refers to the period the substrate remains in the reactor and is influenced by operating temperature and feed characteristics. The experiments were conducted under mesophilic conditions, which are commonly preferred due to their operational stability and lower energy demand. Literature reports that mesophilic digesters typically operate at stable HRTs ranging from 15 to 35 days (El-Hadj et al., 2007), while thermophilic systems generally require shorter retention times of approximately 14 to 30 days (Petersson et al., 2009; Tyagi et al., 2011). Although longer HRT can improve substrate degradation and methane yield, it also increases reactor volume requirements and operational costs, particularly for complex substrates such as lignocellulosic waste (Huertas et al., 2020). On the other hand, insufficient HRT may result in incomplete digestion, leading to VFA accumulation and process instability (Syed et al., 2006; Tahri et al., 2022). However, shorter retention times can enhance process efficiency and reduce capital investment, provided that reactor stability is maintained (Huertas et al., 2020). Many studies indicate that the optimal hydraulic retention time for mesophilic anaerobic digestion is approximately 15–30 days, as it offers a good balance between methane production, process stability, and overall system efficiency. The best HRT was around 15-30 days.

4.2.2 Pre-treatment of Co-digestion of IWK Thickened Sludge and Food Wastes

In this study, the BMP test for pre-treatments and co-digestion of IWK Sludge and food wastes was performed in triplicate in 125 mL serum bottles with a working volume of 70% while 30% of the remaining serum bottle volume is for the gas production. The serum bottles contained inoculum and substrate are required to achieve an initial inoculum-to- substrate ratio of 2:1(VS-basis). Blank and control assays containing only inoculum and substrate were used to correct for the background methane potential of the inoculum and substrates. Several types of IWK thickened sludge and food waste pre-treatment were prepared, as tabulated in Table 4.3. The initial and final pH of the BMP test was analyzed using a benchtop pH meter. The BMP test was conducted within an HRT of 32 days at 37^{o}C.

All pre-treatment with different inoculum-to-substrate (I:S) ratio for the BMP experiment achieved almost constant methane generation after the retention time of 29 days. The BMP assays are considered complete when daily production falls below 1% or when the cumulative graph indicates a stable trend of methane production, suggesting that organic substances have fully degraded. The microorganisms no longer have enough food for digestion purposes. Figure 4.2 shows the cumulative methane production of blank, control, and pre-treatments of IWK sludge and food wastes. Methane production increased initially as the inoculum adapted to the substrate, reaching a peak before day 7. After day 7, production declined and stabilized, indicating reduced methanogenic activity likely due to substrate depletion and/or accumulation of inhibitory intermediates such as volatile fatty acids. It displays that methane production drastically increased at the first six days of digestion; however, the trend decreased at around 15-20 days due to the depletion of readily biodegradable substrates. As the digestion progresses, most easily degradable organics are consumed during the early active phase, leaving behind more complex compounds that are harder for microbes to break down. This results in reduced microbial activity and a lower rate of methane production as the process moves into the stabilization phase. There was a constant production of methane from day 20 until day 32. It can be concluded that approximately 20 days are needed to digest all substrates to produce methane. The highest cumulative methane production was from the 2:1(IWK Sludge: coffee waste) by acid treatment, 2M H_2SO_4 which is 18.493 ± 1.101 mL CH_4/gVS, followed by 2:1 (IWK Sludge: coffee waste) by low heat and acid treatment, (90^oC, 180 min + 2M H_2SO_4) by 4.176 ± 0.084 mL CH_4/gVS and others. The lowest methane production range was from the ratio of 2:1 (IWK sludge: NDCW) either by alkali, high heat, or low heat treatment was ≤ 1 mL CH_4/gVS, as shown in Table 4.3.

Table 4.3

Parameters of pre-treatments and anaerobic co-digestion of IWK sludge and food waste

Inoculum -to- substrate (I:S) ratio	Type of wastes	Pre-treatments	HRT (Days)	pH of Digestion Mixture		Cumulative methane production (mL CH_4/gVS)	Methane Yield (m^3)
				Initial	Final		
	Blank-Sludge	-		6.79	8.05	12.72 ± 0.16	$0.00069 \pm 1.04\times10^{-5}$
	Control-CW	-		11.04	9.05	0.013 ± 0.0007	$5.8752\times10^{-7} \pm 4.11\times10^{-8}$
	Control-NDCW	-		5.44	5.71	0.005 ± 0.0003	$1.9481\times10^{-7} \pm 1.22\times10^{-8}$
		Acid, 2M H_2SO_4		7.04	8	18.493 ± 1.101	$0.0012 \pm 8.51\times10^{-5}$
		Low Heat, 90°C, 180min		7.31	7.27	2.134 ± 0.071	$0.00013 \pm 5.43\times10^{-6}$
		High Heat, 160°C, 60min		8.38	7.2	2.586 ± 0.135	$0.00016 \pm 1.01\times10^{-5}$
2:1	S: CW						
		Low Heat + Acid	32	7.03	8.4	4.176 ± 0.084	$0.00026 \pm 6.33\times10^{-6}$
		High Heat +Acid		7.02	8	0.458 ± 0.004	$2.8273\times10^{-5} \pm 2.91\times10^{-7}$
		Alkali, 2M NaOH		7	5.65	0.168 ± 0.0008	$5.7564\times10^{-6} \pm 2.74\times10^{-8}$
2:1	S: NDCW	High Heat + Alkali		7.02	5.58	0.158 ± 0.004	$5.3861\times10^{-6} \pm 2.09\times10^{-7}$
		Low Heat, 90°C, 180min		7.05	5.29	0.105 ± 0.006	$3.5988\times10^{-6} \pm 3.12\times10^{-7}$

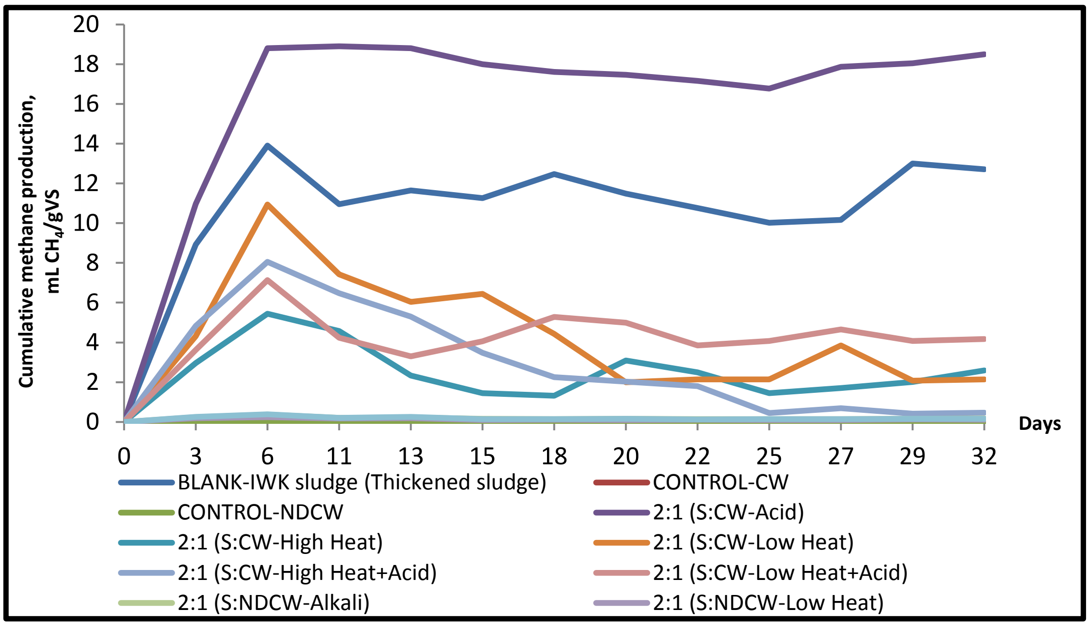


Figure 4.2 Cumulative methane production of IWK sludge and food wastes for all treatments

Table 4.3 summarizes that the sludge (blank) produced 12.72 mL CH_4/gVS followed by control; coffee waste, (CW) and non-dairy creamer waste, (NDCW) by 0.013 ± 0.0007 and 0.005±0.0003 mL CH_4/gVS. Thickened sludge can generate methane, and greater gas production can be achieved through pre-treatment and co-digestion with food waste. However, the previous batch of pre-treatment and co-digestion of the BMP test of sludge with NDCW by a ratio of 2:1 showed a low methane production compared to CW. pH of the NDCW before the AD process was around 3-5 which is acidic. A narrow operating range of 6.5–7.6 is often recommended since pH influences the microorganism enzymes and can change their configurations and influence the kinetics of the reactions (Parkin et al., 1986; Remigi et al., 2006). A low pH can bring about an accumulation of VFA, which inhibits digestion (Parkin et al., (1986) and Pearse et al., (2018). As a result, unstable operations can develop as the VFA production rate exceeds the methanogenic VFA utilization rate.

A drop in pH slows down the microbes that convert VFAs into methane. As a result, VFAs accumulate, methane production decreases, and the process becomes unstable, which was observed in IWK sludge with NDCW after 32 days as shown in Table 4.3. Huang et al. (2019) observed that non-dairy coffee whitener (NDCW) is composed of 60-65% glucose or corn syrup, along with 2-5% sodium caseinate, in addition to stabilizers and emulsifiers. Additionally, it contains approximately 1.2% minerals, 8.5% protein, 32.5% fat, and the highest percentage, 58%, is carbohydrates. So, the acidic pH would inhibit and disrupt microbial activities during the AD process.

4.3 Methane production in a 40L Pilot Scale Digester

4.3.1 Acclimatization of IWK Sludge in a 40L Pilot Scale Digester

Acclimatization of anaerobic digestion is a process in which the microbes from the sludge can adapt and be familiar with the new environment with different parameters such as the retention time, pH, and temperature in a digester-closed system. As we know, acclimatization is the first and crucial step before proceeding with any process. In this study, the acclimatization was started in early September 2021 within one and a half months by preparing 15L of IWK thickened sludge and 15L of water (1:1) with a total working volume of 30L. During the first week of acclimatization, the digester was stirred for at least 2 hours daily with minimum rpm (3-4hrz) to avoid the microbes being stressed.

Figure 4.3 shows the methane gas started to generate starting from day 7 until day 21. A reduction in methane (CH_4) concentration was observed between days 21 and 28 during the acclimatization phase. This decline can be attributed to a temporary imbalance in the anaerobic microbial community, particularly the inhibition of methanogenic archaea. Such inhibition is commonly associated with the accumulation of volatile fatty acids (VFAs), which can lower the system pH and disrupt methanogenic activity. Additionally, the slight presence of oxygen (O_2) detected during this period suggests possible air intrusion, further contributing to the suppression of strictly anaerobic methanogens. Similar trends have been reported in previous studies, where transitional phases in anaerobic digestion systems often result in temporary methane reduction due to VFA accumulation and microbial adaptation delays. For instance, Gerardi et al., (2003) noted that fluctuations in microbial activity during start-up or acclimatization can significantly affect methane yield. Likewise, Angelidaki et al., (2003) and colleagues highlighted that process instability, particularly due to organic overloading or environmental stress, can inhibit methanogenesis before system recovery occurs. Following this unstable phase, the system gradually stabilizes as methanogens adapt, VFAs are metabolized, and optimal anaerobic conditions are restored, leading to a subsequent increase in methane production. But constant trend for methane was seen on day 42 onwards. This observation shows that microorganisms need time to adapt and perform their biological functions. At the end of the acclimatization process, from day 30 until 45, it was assumed that the microbes reached their stable acclimatized state. The graph shows a reduction in biogas yield values on day 42-45. This implies that the acclimatization process has been effectively finalized. The acclimatized sludge from the digester is set to be utilized as a seed for the upcoming pilot study.

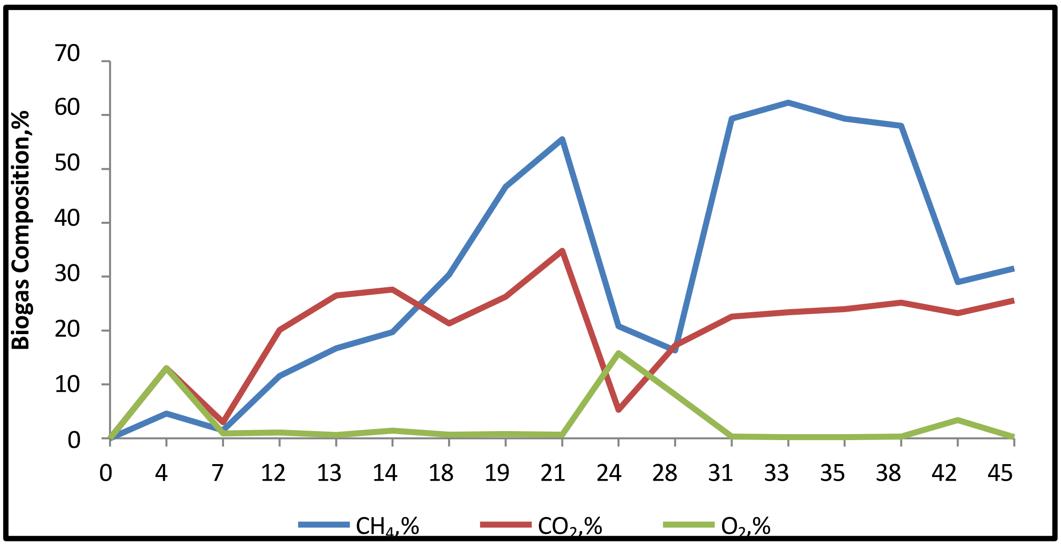


Figure 4.3 Cumulative Biogas Composition (%) during Acclimatization Process

4.3.2 Intermittent Feeding of Thickened Sludge; HRT: 21, 14 and 7 Days

Intermittent feeding is a feeding strategy in anaerobic digestion where the substrate is provided to the microbial community in a discontinuous manner, with periods of feeding followed by periods of no feeding. This is in contrast to continuous feeding, where the substrate is provided to the microbial community constantly and steadily. In this study, approximately 30% of the pilot digester's working volume (30 liters) consisted of IWK sludge and co-substrate mixture. It helps maintain safe and stable operation. This allows enough headspace for biogas collection, reduces the risk of overflow from foaming or scum, and ensures better mixing between microbes and the substrate. The pilot digester was filled with 9 liters of freshly thickened industrial waste (IWK) sludge. After feeding, nitrogen gas (N_2) was purged into the digester for 3 to 5 minutes to remove any oxygen before introducing the substrate. The thickened sludge was retained in the digester for a hydraulic retention time (HRT) of 21 days, during which the digester was continuously stirred at a low mixing speed.

At the end of the 21 days, approximately 9 liters of thick sludge were discharged from the digester, representing 30% of the total fed volume. Both the fresh thickened sludge and the residue released from the digester were analyzed for various parameters, including pH, total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), volatile fatty acids (VFA), moisture content, and alkalinity. The results of this characterization are presented in Table 4.4. Additionally, the biogas produced was collected in a 3-liter gas bag, and gas samples were monitored at least three times a week using a portable gas analyzer and Gas Chromatography (GC-FID). Methane production was measured at least three times per week to ensure adequate tracking of production variations while maintaining a practical sampling schedule, allowing for reliable analysis without excessive effort as commonly practiced in anaerobic digestion studies (Angelidaki et al., 2009; Holliger et al., 2016).

Table 4.4
Characterization of IWK Sludge for Intermittent Feeding before and after Anaerobic Digestion at different HRTs

Type of samples	HRT (Days)	pH	COD (mg/L)	TS (%)	VS (%)	VFA (mg/L)	AL (mg/L)	MC (%)	TSS (mg/L)	VSS (mg/L)	Methane Yield (%)		
											Min	Max	Avg
Feed (inlet)	0	6.83	16640	3.12	67.43	403	1330	96.5	4920	2990			
Liquid discharged (outlet)	7	7.32	15520	2.17	59.34	206	1550	91.2	3740	2050	26.5	62.4	52.3
COD Reduction, %	-	-	6.7	-	-	-	-	-	-	-			
Feed (inlet)	0	6.8	17640	2.62	69.44	418	1670	97.1	4110	2070			
Liquid discharged (outlet)	14	7.76	12530	2.1	59.04	262	2880	86.7	3730	1890	44.7	64.2	54.1
COD Reduction, %	-	-	22.04	-	-	-	-	-	-	-			
Feed (inlet)	0	6.85	17680	2.51	71.36	338	4104	97.1	4870	3680			
Liquid discharged (outlet)	21	7.88	13290	1.3	64.63	197	4470	90.4	3180	2290	47.5	65.2	55.4
COD Reduction, %	-	-	24.83	-	-	-	-	-	-	-			

During intermittent feeding, all residues discharged from the digester displayed similar characteristics, regardless of different hydraulic retention times (HRT). Data characterization of the thickened sludge before and after the anaerobic digestion at HRT of 21, 14, and 7 days is presented in Table 4.4. Significant reductions in chemical oxygen demand (COD) and volatile fatty acids (VFA) were observed. Meanwhile, TSS and VSS are used to assess feedstock characteristics, monitor degradation efficiency, and evaluate process performance. A decrease in VSS over time typically indicates successful breakdown of organic matter and methane production. The COD reduction (%) for HRT's of 7, 14 and 21 days showed a slightly decreased by 6.7, 22.04 and 24.83%. The VFA concentrations at HRTs of 7, 14, and 21 days were 206, 262, and 197 mg/L, respectively. The rise at 14 days indicates enhanced acidogenesis and accumulation of intermediate products, whereas the decrease at 21 days suggests effective VFA uptake by methanogens for methane production. This pattern is consistent with the methane yield trend, where process stabilization at later stages reflects improved microbial balance. Overall, the low VFA levels (<300 mg/L) confirm stable digestion conditions without acidification, in agreement with previous studies linking VFA reduction to efficient methanogenic activity and process stability (Appels et al., 2008; Chen et al., 2008).The reduction in volatile fatty acids (VFA) observed in this study, with levels decreasing to 190–200 mg/L, aligns well with previous research indicating successful anaerobic digestion, where methanogens effectively consume VFAs. Table 4.4 shows the minimum and maximum reading of methane production by using a portable gas analyzer. The minimum and maximum values indicate the range of methane production observed, while the average value represents the overall performance of the system. This approach provides both variability and a representative estimate of methane yield under the experimental conditions.

The IWK sludge maintained a pH range of 6.8–7.8 across all HRTs (7, 14, and 21 days), which is suitable for microbial activity and supports stable fermentation (Hwang et al., 2004). The pH of the outlet residue after digestion for all three HRTs ranged from 7.5 to 7.9, indicating stable digester performance. Data showed the COD value of the feed for all three HRTs was higher than that of the residue, with a difference of 16000-17000 mg/L. A COD above 10,000 mg/L is generally considered optimal for anaerobic digestion performance (Darwin et al., 2013). While higher COD can lead to increased biogas production, the methane content of the biogas may be lower due to the presence of other gases, such as carbon dioxide (Tauber et al., 2019). Regarding total solids (TS), the feed contained approximately 2-3% TS, while the residue showed reductions of 0.52%, 0.95%, and 1.21% TS for HRTs of 14, 7, and 21 days, respectively.

Higher TS can promote a greater methane production rate; however, excessive TS may inhibit the process and lead to instability (Liotta et al., 2014). The optimal TS and volatile solid (VS) content varies depending on the substrate type and characteristics, operating conditions, and specific goals of the process. Before digestion, the VFA levels in the feed for all three HRTs ranged from 330 to 400 mg/L, with alkalinity levels between 1300 and 4100 mg/L. After digestion, the VFA levels decreased to a range of 190 to 200 mg/L, and the alkalinity increased to between 1550 and 4400 mg/L. Although the alkalinity range observed in this study (1550–4400 mg/L) is lower than that reported in previous studies, such as Zhang et al. (2020), it remains within an acceptable range for system performance, which noted alkalinity between 2500 and 6000 mg/L, and it still falls within functional limits provided that the pH remains stable. Furthermore, the low VFA-to-alkalinity ratio observed in the system (<0.3) indicates stable anaerobic digestion conditions and meets the stability criterion proposed by Ripley et al. (1986) indicating balanced microbial activity and overall process stability. Higher VFA concentrations can drive a higher methane production rate; however, excessive VFA levels can also lead to process inhibition and instability (Al Sulaimi et al., 2022). In conclusion, the system remained stable, evidenced by lower VFA levels, higher alkalinity, and a VFA-to-alkalinity ratio below 0.3, indicating efficient and well-balanced anaerobic digestion without process instability.

The intermittent feeding with varying hydraulic retention times (HRT) was carried out for nearly four months to examine biogas yield patterns. If the HRT is not adequately extended, it can lead to disturbances in the digester, as this may result in the loss of gradually increasing bacteria vital for hydrogen production. A short HRT may reduce substrate degradation efficiency due to insufficient digestion time. Shorter HRTs are typically chosen for hydrogen-producing bacteria, which have a faster growth rate compared to hydrogen- consuming methanogens. In this study, the digester was run at hydraulic retention times (HRT) of 21, 14, and 7 days. Figure 4.4 shows that biogas production changed depending on the HRT. The average methane production for HRTs of 21, 14, and 7 days ranged from 50–60%, 45–65%, and 25–65%, respectively, as shown in Figure 4.4, with overall methane trends of approximately 48–60% across all HRTs. This stable methane production may be influenced by minor O_2 presence in the AD system, likely caused by air leakage or oxygen ingress during feeding, sampling, or imperfect reactor sealing, indicating partially incomplete anaerobic conditions. However, no lag phase was observed at the beginning of the process, as the substrate already contained readily biodegradable organic matter (Zhou et al., 2012), supporting immediate microbial activity and methane formation.

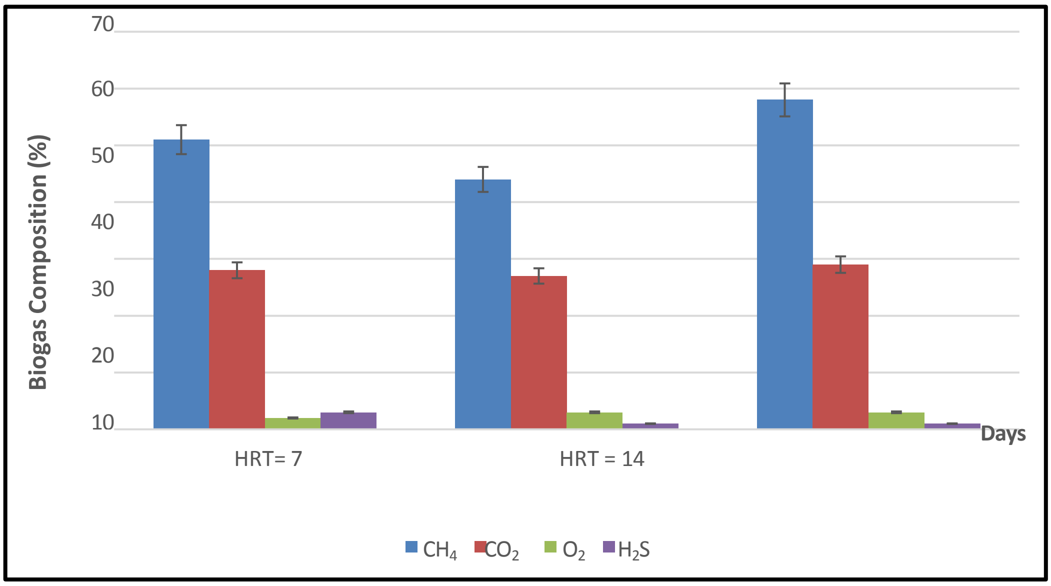


Figure 4.4 Biogas Composition (%) of Intermittent Feeding of Thickened Sludge by AD with HRT of 21, 14 and 7 days

Methane production was found to be highest at an HRT of 21 days, as this period provides sufficient time for microorganisms to fully degrade organic matter and convert volatile fatty acids into methane, ensuring stable system performance. At an HRT of 7 days, methane yield was lower because the sludge passed through the reactor too quickly, resulting in incomplete degradation and insufficient time for methanogens to grow and function effectively. Meanwhile, methane production at 14 days HRT was lower than that at 21 days indicating an overall increase in methane yield with longer retention time as digestion. Overall, the results indicate that a 21-day HRT offers optimal conditions for microbial activity and methane generation compared to shorter retention times. In summary, the 21-day HRT provided the most favorable conditions for methane production due to sufficient time for complete substrate degradation and stable microbial activity, whereas shorter HRTs (14 and 7 days) resulted in lower methane yields because of incomplete digestion and potential biomass washout, as widely reported in anaerobic digestion studies (Angelidaki et al., 2009; Holliger et al., 2016).

4.3.3 Continuous Feeding of Thickened Sludge at Different HRT s

The trends observed in the continuous feeding system are consistent with those from the batch (intermittent feeding) experiments, where longer hydraulic retention time (HRT) resulted in higher methane production. This study was further extended from intermittent to continuous feeding to better represent real-scale anaerobic digestion processes commonly applied in wastewater treatment plants and to assess system performance under more practical and stable operating conditions. In the continuous system, the 10-day HRT showed better performance than the 7-day HRT, highlighting that sufficient retention time is essential for effective degradation of thickened sludge and efficient progression of anaerobic digestion stages. The improved methane production at longer HRT is attributed to enhanced contact time between microorganisms and substrate, which promotes more complete organic matter breakdown and methane formation. In contrast, the shorter HRT likely resulted in biomass washout and incomplete digestion, leading to lower methane yield.

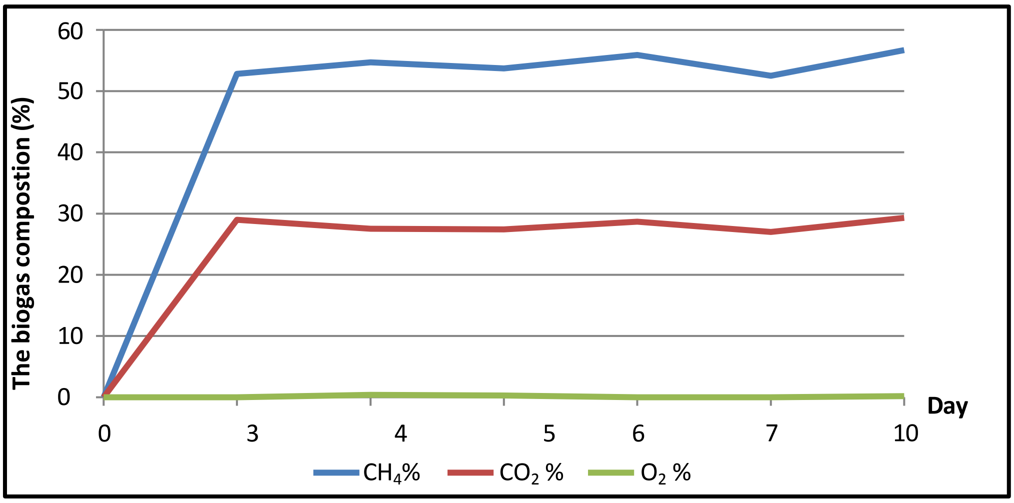
These findings are supported by previous studies which report that insufficient HRT can compromise reactor stability and methane production due to reduced microbial retention and limited substrate conversion efficiency (Angelidaki et al., 2009; Holliger et al., 2016). Liotta et al. (2014) also emphasized that shorter retention times may inhibit methanogenic activity, particularly in high-solid systems that require longer digestion periods for effective treatment. Overall, results from both batch and continuous systems confirm that HRT is a key operational parameter in anaerobic digestion, where longer retention times enhance microbial stability and methane production. The table 4.5 summarizes the performance of a continuous anaerobic digestion system treating IWK sludge at two hydraulic retention times (HRTs), 7 and 10 days. At an HRT of 7 days, treatment efficiency was limited, with COD decreasing slightly from 17,840 mg/L to 16,140 mg/L, corresponding to a low removal efficiency of 9.53%. However, a relatively high VS reduction of 50.83% indicates that a portion of the biodegradable organic matter was still effectively degraded. The methane yield remained relatively stable, ranging from 54.3% to 58.7% with an average of 56.4%, suggesting consistent biogas quality despite limited COD removal. In contrast, at an HRT of 10 days, overall performance improved markedly, with COD reduced from 21,550 mg/L to 10,670 mg/L, achieving a significantly higher removal efficiency of 50.49%. Although VS reduction was slightly lower at 43.77%, it remained within an acceptable range for anaerobic digestion, while methane yield showed a similar trend, averaging 54.4%.

Overall, increasing the HRT enhanced organic matter degradation, particularly in terms of COD removal, due to longer substrate–microorganism contact time and improved digestion efficiency. These findings are in line with previous studies on sewage sludge digestion, which commonly report COD removal efficiencies of 40–70% and methane contents of 55–65%. The results at 10 days HRT fall within this reported range, whereas the 7-day HRT appears insufficient for effective stabilization. This indicates that longer retention times are necessary to achieve optimal treatment performance of IWK sludge in continuous anaerobic digestion systems, even though methane composition remains relatively consistent across both conditions.

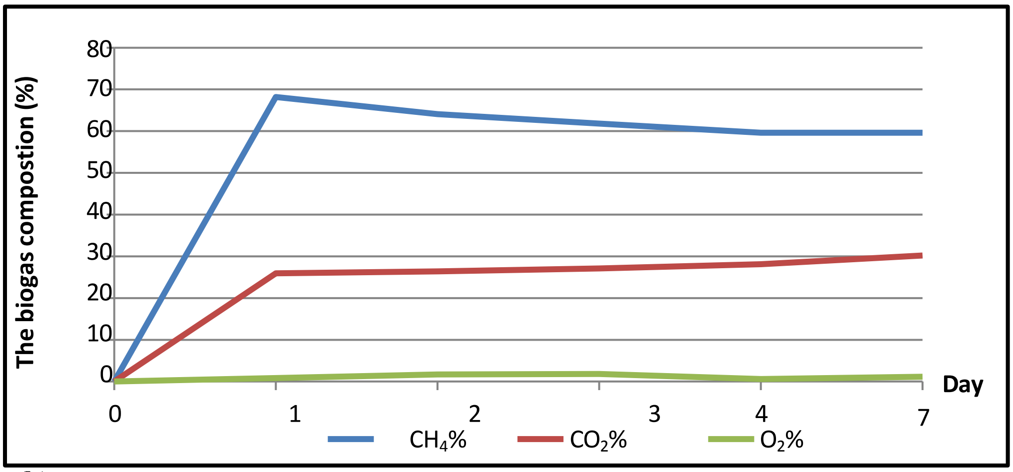
Table 4.5
Characterization of Inlet and Outlet Samples of Continuous Feed within HRT 7 & 10 Days

Type of samples	HRT (Days)	pH	COD (mg/L)	TS (%)	VS (%)	VFA (mg/L)	AL (mg/L)	MC (%)	TSS (mg/L)	VSS (mg/L)	Methane Yield (%)		
											Min	Max	Avg
Feed (inlet)	0	6.83	17840	3.12	67.43	403	1330	96.5	5850	3570			
Liquid discharged (outlet)	7	7.98	16140	2.17	59.34	206	1550	91.2	3740	2050	54.3	55.3	54.2
COD, VS Reduction, %	-	-	9.53	-	50.83	-	-	-	-	-			
Feed (inlet)	0	6.78	21550	2.51	75.11	677	1750	97.3	5710	3750			
Liquid discharged (outlet)	10	7.55	10670	0.59	54.31	321	3920	89.8	4590	1770	54.5	56.7	55.6
COD, VS Reduction, %	-	-	50.49	-	43.77	-	-	-	-	-			

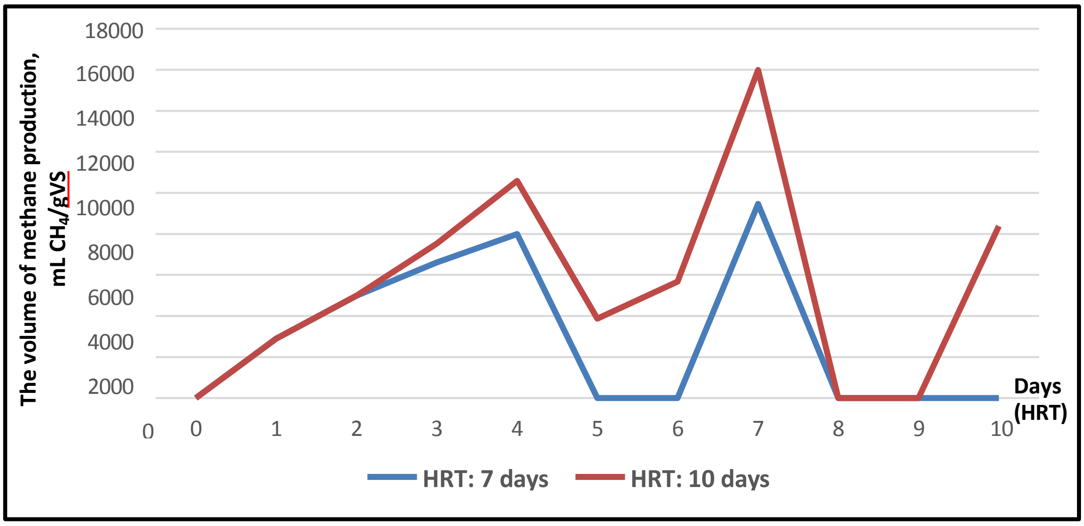
From the results, Figure 4.5 (c) shows the cumulative methane production for both HRT, 10 and 7 days. Both graphs (a) and (b) illustrate that methane gas concentrations rose to 50-54% and 50-58% respectively from day 0 to day 3, followed by a slight decrease to 45-53% on day 7, remaining stable thereafter. Previously observed, during acclimatization and intermittent feeding, methane gas levels began to drop on day 7, a trend that was quite similar for continuous feeding. Thus, it can be assumed that on day 7 of digestion, there is insufficient food or organic matter for microbes to degrade; therefore, the digester should be fed with new thickened sludge. In this study, two types of HRT were performed, first by HRT for 10 days and followed by HRT for 7 days. The longer HRT was used to assess biogas production under a lower organic loading rate (3 L/day). The effect of reducing HRT from 10 to 7 days was also evaluated, and methane production remained stable at 7 days, indicating no significant impact on gas yield under these conditions.



(a)



(b)



(c)

Figure 4.5 (a) Biogas Composition (%) at HRT of 7 (b) Biogas Composition (%) at HRT of 10 days and (c) The Cumulative Methane Production at HRT of 10 & 7 days by Continuous Feeding.

Based on Figure 4.5(c), the cumulative methane production by continuous feeding of thickened sludge within HRT 7 days shows the highest methane accumulated by 9463.23 mL CH_4/gVS compared to HRT 10 days, 8377.45 mL CH_4/gVS. Nonetheless, an earlier investigation conducted by Remigi et al. (2006) indicated that short HRTs are typically not utilized for methane generation. This could be due to insufficient contact time to allow for the degradation of the substrate by the microbial population. It has also been reported that operation of the methanogenic stage at HRTs lower than 8–10 days may lead to instability of the methanogenic process (Ge et al., 2011; Zamanzadeh et al., 2013 and Bouallagui et al., 2003). However, pH significantly affects performance in anaerobic digestion. Table 4.5 presents a comprehensive analysis of the initial and final digestion of thickened sludge at hydraulic retention times (HRT) of 10 days and 7 days. Except for methane production, there are no discernible characterization differences among the various HRTs. The feeding of thickened sludge was at an optimum pH value than the outlet by 6.8 and 7.5-7.9, which is a good range as the fermentative microorganisms are less sensitive (Hwang et al., 2004).

The COD value of the feed was higher than the outlet by a range of 16000-21000 mg/L. The suitable range of COD for effective anaerobic digestion should be as high as 10,000 mg/L (Darwin et al., 2013). The feed has a total solids (TS) content of approximately 2-3%, while the outlet has a TS content of about 1-2%. It suggested that the hydraulic retention time (HRT) and the daily percentage of sludge feeding flow rate are directly related to the effectiveness of digestion process.

4.3.4 Continuous Feed by Co-digestion of Thickened Sludge and CW

Co-digestion of thickened sludge with coffee waste (CW) under continuous feeding provides a balanced nutrient supply, improves organic matter breakdown, and enhances methane production compared to sludge alone. This approach supports stable microbial activity and minimizes process instability, offering a more efficient and sustainable method for biogas generation.

4.3.4.1 Effect of Hydraulic Retention Time (HRT) on Anaerobic Co-Digestion of 2 M H_2SO_4 Acid-Pretreated Sludge and Coffee Waste at Different Feeding Rates

The subsequent experiments proceeded using an inoculum-to-substrate ratio of 2:1, combining IWK sludge and coffee waste subjected to acid treatment with 2M H_2SO_4, and hydraulic retention times (HRT) of 7, 10, and 30 days.

Plate 4.1 shows continuous anaerobic digestion system used for co-digestion of IWK sludge and coffee waste. The co-digestion of IWK sludge and coffee waste was carried out in a continuous anaerobic digestion system to better represent real-scale wastewater treatment operations, where continuous feeding is more commonly applied than intermittent systems. This approach also allowed the assessment of reactor stability and methane production under steady operating conditions. A 30-day HRT was selected initially to ensure sufficient microbial adaptation and to reduce process disturbances during the introduction of the mixed substrate. The results indicated that increasing HRT improved biogas and methane production, which is mainly due to enhanced contact time between microorganisms and substrate, allowing more complete organic matter degradation. This highlights the importance of sufficient retention time for stable performance in systems treating complex waste.

Plate 4.1 The Feeding Tank equipped with a stirrer

When compared with intermittent feeding of IWK sludge alone, the continuous co-digestion system with coffee waste showed better overall performance, attributed to improved nutrient balance, higher biodegradability, and more stable reactor conditions. Coffee waste provided readily degradable organics that enhanced microbial activity, while sludge contributed buffering capacity, resulting in a more balanced digestion environment. In contrast, intermittent sludge feeding is more susceptible to fluctuations in substrate loading, which may reduce process stability and efficiency.

These observations are consistent with previous studies reporting that longer HRT enhances methane yield and system stability by improving microbial retention and reducing biomass washout in anaerobic digestion systems (Angelidaki et al., 2009; Holliger et al., 2016). Similarly, Liotta et al. (2014) noted that shorter HRT can restrict methanogenic activity, particularly in high-solid or complex substrates that require longer degradation periods. In addition, the continuous feeding strategy promoted more stable operation by ensuring a constant substrate supply and enabling gradual microbial adaptation, which is essential for maintaining steady-state conditions in full-scale applications. However, due to the highly alkaline nature of coffee waste (pH 11–12), pH adjustment to 6.8–7 was necessary to maintain optimal methanogenic activity and prevent inhibition. Overall, the findings demonstrate that HRT, co-digestion, and continuous feeding significantly improve methane production efficiency and operational stability compared to intermittent mono-digestion of IWK sludge.

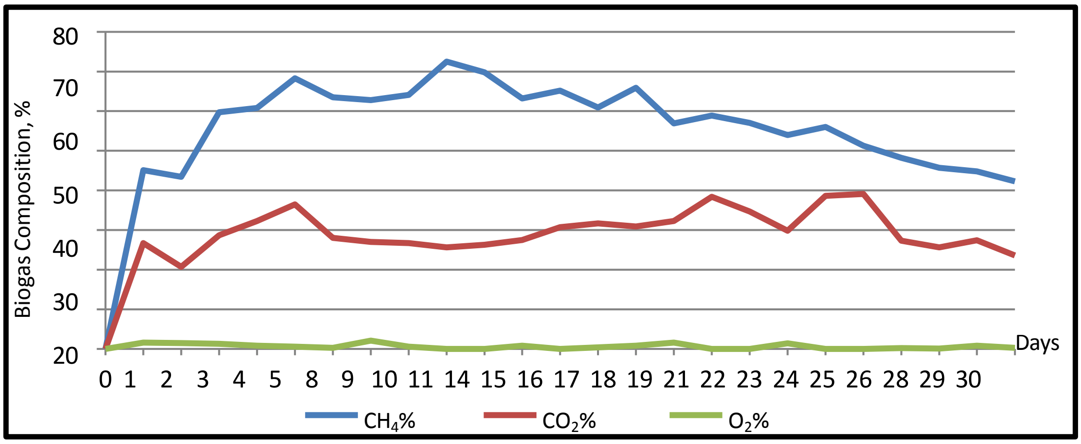
Table 4.6
Characterization of Influent and Effluent Streams in Continuous Anaerobic Co-Digestion of Acid Pretreated IWK Sludge and Coffee Waste (2:1) under HRTs of 30, 10, and 7 Days

Type of samples	HRT (Days)	pH	COD (mg/L)	TS (%)	VS (%)	VFA (mg/L)	AL (mg/L)	MC (%)	TSS (mg/L)	VSS (mg/L)	Methane Yield (%)		
											Min	Max	Avg
Feed (inlet)	0	7.04	23800	7.42	68.76	1578	2380	93.7	28530	21580	42.7	54.7	47.2
Liquid discharged (outlet)	7	7.74	17400	4.01	52.79	375	3750	90.9	18290	14650			
COD,VS Reduction, %	-	-	26.89	-	21.52	-	-	-	-	-			
Feed (inlet)	0	7.05	28550	3.59	63.61	1812	2430	94.8	22410	18210	53.4	57.9	55.4
Liquid discharged (outlet)	10	7.98	12540	1,58	55.43	322	3670	86.4	15700	8640			
COD,VS Reduction, %	-	-	56.08	-	46.52	-	-	-	-	-			
Feed (inlet)	0	7.05	31580	5.67	61.33	1970	3100	94.9	32320	23530	42.3	72.5	57.6
Liquid discharged (outlet)	30	8.12	12130	2.12	53.73	315	4710	88.2	18950	14010			
COD,VS Reduction, %	-	-	61.59	-	61.07	-	-	-	-	-			

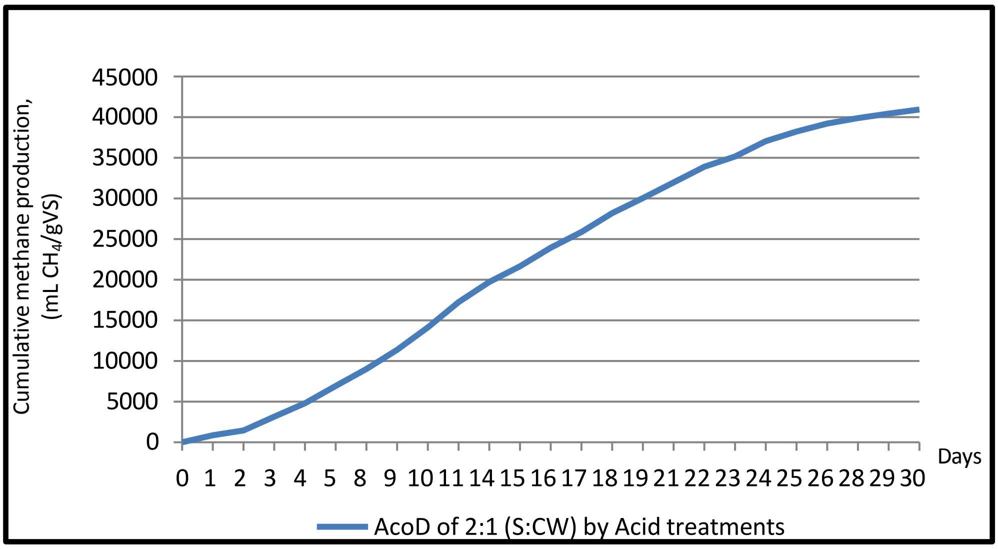
The initial pH of the slurry for HRT 30, 10, and 7 days was adjusted accordingly in between the range 7-7.05 by using 2M H_2SO_4 as shown in Table 4.6. The pH level of the co-digestion sample before digestion, which ranges from 6.8 to 7.4, is ideal because fermentative microorganisms are less sensitive in this range (Hwang et al., 2004). A study examined the co-digestion of food waste with waste-activated sludge, revealing that the optimal pH range is between 7.0 and 8.0 (Qian et al., 2017). All three hydraulic retention times (HRTs) demonstrate consistent digester performance, as seen by the final pH falling within the range of 7.74-8.12. The pH after digestion (outlet residue) of all three HRTs indicates stable digester performance. From the data, the COD value of feed of all three HRTs before digestion was higher than the residue by a range of 23000-31000 mg/L. The COD value of feed before digestion at HRT of 30 days showed the highest COD by 31000 mg/L. The good range of COD for anaerobic digestion performances should be more than 10,000 mg/L (Darwin et al., 2013).

Higher COD can lead to higher biogas production, but the methane content of the biogas can be lower due to the presence of other gases (carbon dioxide) (Tauber et al., 2019). The chemical oxygen demand (COD) measures the oxygen required for reactions with oxidizing agents in a sludge sample (American Public Health Association, 1965). In anaerobic digestion, COD typically reflects the number of organics present in sludge. Next, the total solid, TS of feed is around 3-7% compared to a residue that reduced 1-4% TS for HRT 7, 10, and 30. Increased total solids (TS) can enhance methane production rates; however, excessive TS levels may lead to inhibition and instability (Liotta et al., 2014). The ideal levels of TS and volatile solids (VS) can vary based on the nature and traits of the substrate being processed, the operating conditions, and the specific objectives of the procedure. The VFA levels in the feed of all three HRTs before digestion ranged from 1500 to 1900 mg/L, while the alkalinity levels ranged from 2300 to 3100 mg/L. After digestion, the VFA was in the range of 310-370mg/L and the AL was 3600-4700mg/L. The low VFA concentration (310–370 mg/L) and high alkalinity (3600–4700 mg/L) indicate that the digester operated in a stable condition. The strong alkalinity helped neutralize acids formed during digestion, preventing VFA accumulation and pH reduction. This suggests that methanogenic bacteria were active and effectively converted intermediates into methane, maintaining a stable process. Higher VFA can lead to a higher methane production rate, but excessive VFA can cause process inhibition and instability (Al Sulaimi et al., 2022).

Figure 4.6(a) depicts the daily biogas production as a percentage from day 0 to day 30 of the digestion process. Based on Figure 4.6(a), the methane content at an HRT of 30 days ranged from 40–70%, indicating enhanced digestion performance under longer retention time, meanwhile, Figure 4.7(a) and (c) show the methane produced for HRT 10 days was at a range of 54-62% and 42- 54% for HRT 7 days. It can be observed that the methane production drastically increased on the first 2 days of digestion, and started to decline on day 7. Within a 30-day HRT, the highest daily methane production was recorded on day 11, at 72%. On day 5, within a 10-day HRT, the maximum daily methane production was 57.9%, while on day 2, within a 7- day HRT; the highest daily methane production was 54%. Figure 4.6(b) shows cumulative methane production was constant around day 25-30 within HRT 30 days. The cumulative methane production remained constant for days 8–10 of HRT 10 days and 5-7 days of HRT 7 days, as shown in Figure 4.7(b)–(d). Thus, a constant trend of methane production indicates the organic substances were fully degraded. The microorganisms no longer have enough nutrients for digestion purposes. The methane production accumulated by HRT 30 days was the highest at 40936.7 mL CH_4/gVS followed by HRT 10 days and 7 days with 13921.07 and 7461.88 mL CH_4/gVS. As a measure that is related to the loading rate, a shorter HRT corresponds to a higher loading rate. According to Kim et al., (1965), shorter HRTs are known to be associated with VFA acidification, which could bring inhibitory effects. Generally, mesophilic digestion can be accomplished within 15–30 days (Mao et al., 2015).



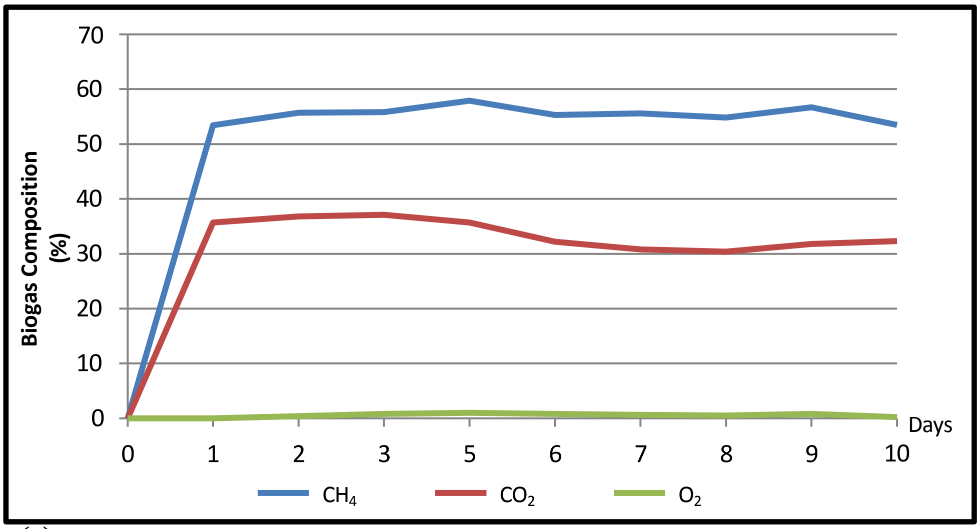
(a)



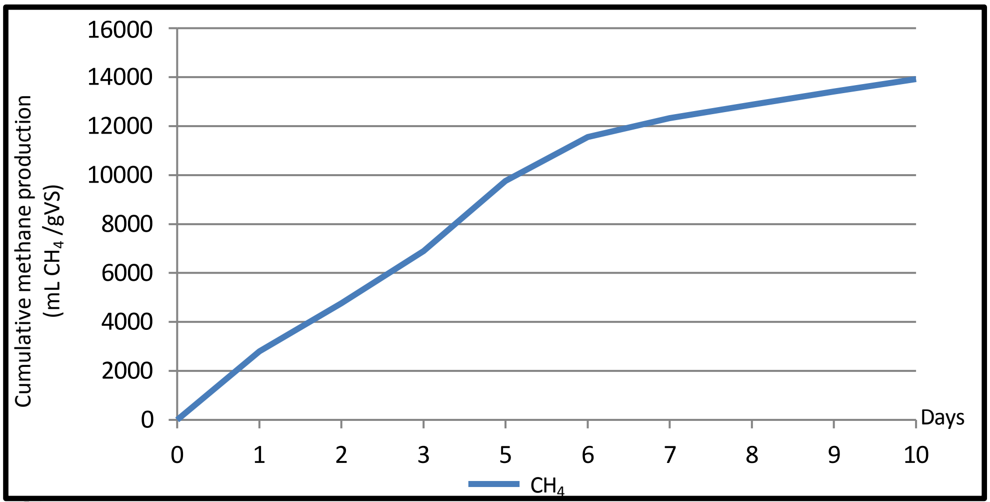
(b)

Figure 4.6 (a) Graph of Biogas Composition (%) and (b) The Cumulative Methane Production at HRT 30 days by Continuous Feeding of 2:1 (S: CW, Acid treatments)

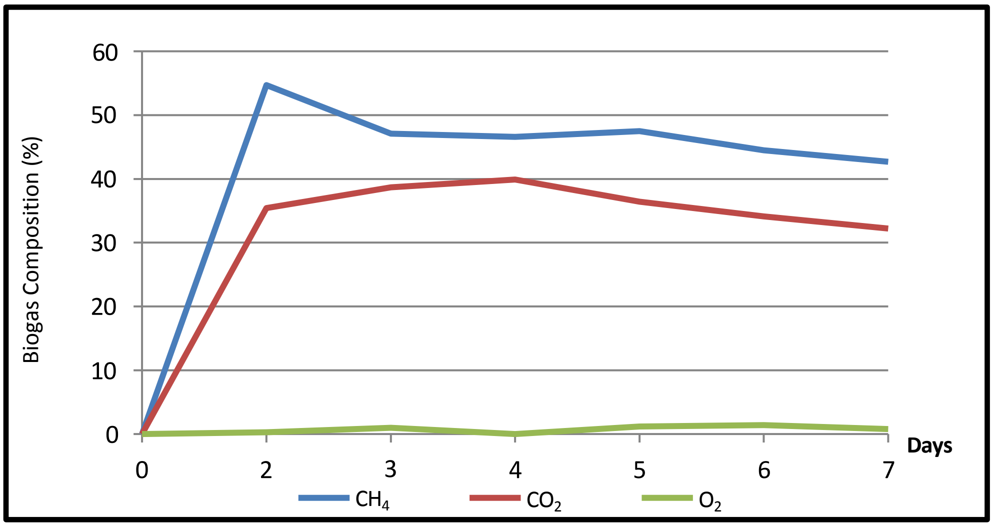
Figure 4.7(a–c) shows the biogas composition (%), while Figure 4.7(b–d) depicts the cumulative methane production for the 10- and 7-day HRT systems. The data suggest that shorter HRTs, which correspond to higher organic loading rates compared to the 30-day HRT, compromise process stability. This instability is linked to digester overloading, where accelerated hydrolysis and acidification lead to the accumulation of volatile fatty acids (VFAs), which in turn inhibit methanogenic activity and disrupt anaerobic digestion (Franke-Whittle et al., 2014). In continuous systems, the organic loading rate reflects the daily substrate input, and excessive loading has been shown to negatively impact performance. Similar observations were made by Rincon et al. (2007), who reported that higher loading rates caused pH drops and process imbalance, even when biogas production increased. These findings underscore the importance of maintaining an appropriate HRT and organic loading rate to achieve stable digestion and efficient methane production. In conclusion, the best HRT is 30 days for anaerobic co-digestion between the IWK Sludge and coffee waste which indicates more stable digester performance. The results indicate that both HRT and co-digestion significantly affect biogas production. The highest methane yield was obtained at 30 days HRT (40,936.7 mL CH_4/gVS), compared to 10 days (13,921.07 mL CH_4/gVS) and 7 days (7,461.88 mL CH_4/gVS). In addition, co-digestion at a 2:1 (IWK sludge: coffee waste) ratio improved methane production due to better substrate balance and biodegradability.


Biogas Composition (%)
70
60
50
40
30
20
10
0
Days
0 1 2 3 5 6 7 8 9 10
CH4
CO2
O2

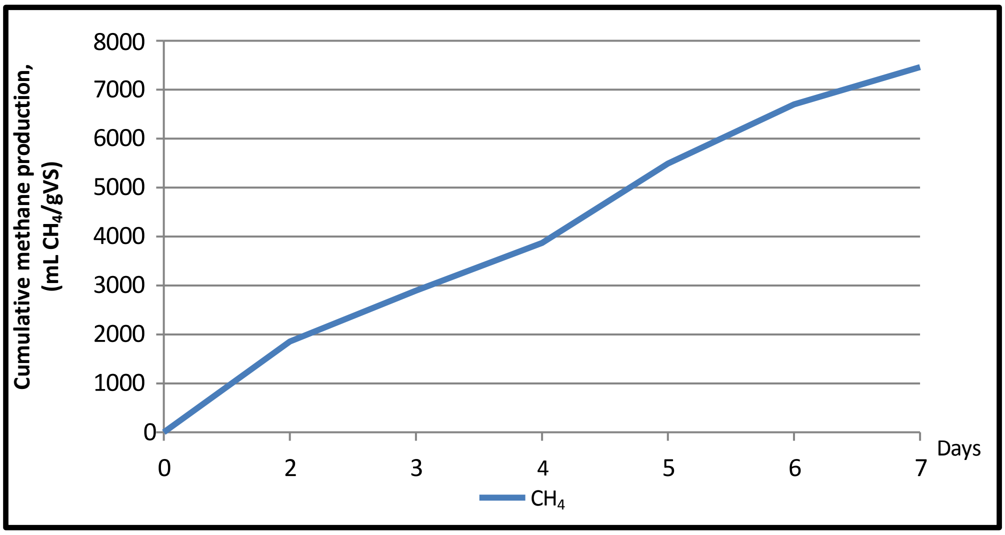
(a)


Cumulative methane production (mL CH4 /gVS)
16000
14000
12000
10000
8000
6000
4000
2000
0
Days
0 1 2 3 5 6 7 8 9 10
CH4

(b)


Biogas Composition (%)
60
50
40
30
20
10
0
Days
0 2 3 4 5 6 7
CH4
CO2
O2

(c)



(d)

Figure 4.7 (a-c) Biogas composition (%) and (b-d) the cumulative methane production at HRT 10 & 7 days by continuous feeding of 2:1 (S: CW, Acid treatments)

4.4 Comparison of Cumulative Methane Production By Mono-Digestion of IWK Sludge and Co-Digestion with Coffee Waste (CW) at different Hydraulic Retention Time (HRT) with and without Pre-treatment Methods

The study evaluates cumulative methane production from mono-digestion of IWK sludge and co-digestion with coffee waste (CW) under different hydraulic retention times (HRT), both with and without pre-treatment.

4.4.1 Mono-digestion of IWK Sludge and Co-digestion with CW at 7 and 10 days HRTs with and without Pre- treatment

HRT is a very important parameter for hydrogen and methane production in continuous mode. Very low HRT can cause the washout of the reactor, which means all microorganisms are washed out of the reactor, and the process stops (Parajuli et al., 2022). Figure 4.8 presents the cumulative methane production from monodigestion of IWK sludge and co-digestion with coffee waste (CW) at different hydraulic retention times (HRTs), with and without acid pre-treatment (2M H_2SO_4) in a 40 L pilot-scale digester. At an HRT of 7 days, the acid-treated co-digestion system (2:1 sludge to CW) produced a lower methane yield (7461.88 mL CH_4/gVS) compared to monodigestion of IWK sludge (9463.23 mL CH_4/gVS). This indicates that, under short retention time conditions, monodigestion of IWK sludge achieved higher methane production than the co-digestion system.

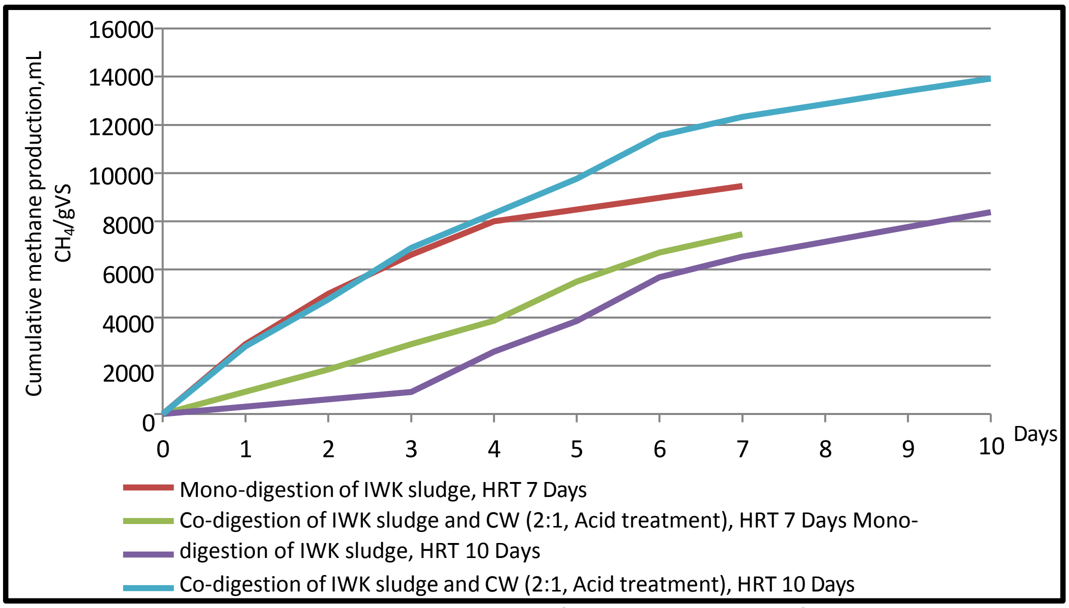


Figure 4.8 Cumulative Methane Production of Mon-Digestion of IWK Sludge and Co-Digestion with CW within HRT of 7 and 10 days with and without Acid Treatment

Comparable findings have been reported in previous studies, where monodigestion of sewage sludge demonstrated equal or higher methane yields than co-digestion under suboptimal operating conditions. Sillero et al. (2022) reported that sludge monodigestion produced higher methane yields than co-digestion when process parameters were not fully optimized, particularly at elevated loading conditions. Similarly, Laetitia et al. (2019) observed that monodigestion of sludge can outperform co-digestion when instability arises from substrate mixing and imbalance. Dai et al. (2016) further noted that although co-digestion generally enhances methane potential through improved nutrient balance, its performance is highly dependent on maintaining suitable substrate ratios, as improper conditions may lead to reduced process efficiency.

In contrast, other studies have shown that co-digestion can enhance methane production when properly optimized. Li et al. (2014), for example, reported increased methane yield from co-digestion of sewage sludge and food waste due to improved carbon-to-nitrogen balance and enhanced biodegradability. This highlights that the performance of co-digestion is strongly influenced by operational conditions and substrate characteristics. Overall, these findings suggest that while co-digestion has the potential to improve methane production, its effectiveness depends on maintaining stable operating conditions, particularly HRT and substrate composition. Under the conditions investigated in this study, monodigestion of IWK sludge demonstrated superior performance at short HRT.

Research by Qiao et al. (2015) found that a high organic loading rate combined with easily degradable substances in coffee waste could result in instability in the process. It might account for the limited methane production noted during a hydraulic retention time (HRT) of 7 days. Sulphuric acid treatment of coffee waste can enhance methane production in anaerobic digestion. The hydrolysis of coffee waste with sulphuric acid can produce reducing sugars, which can be used as a substrate for methane production (Oi et al., 1989). The shorter hydraulic retention time (HRT) and lower organic loading rate (OLR) contributed to the reduced methane production. Ranade et al. (1989) found that shorter HRT results in the accumulation of volatile fatty acids (VFAs). Using an HRT longer than 10 to 15 days enables better utilization of digester components, leading to more efficient biogas production. Another study investigated the effect of HRT on biogas production from coffee waste and found that HRT of 20 days resulted in the highest methane yield of 0.24 L CH_4 g VS^{-1}(Widjaja et al., 2019). The reduced methane content observed in the co-digestion of IWK sludge with coffee waste may be associated with operational constraints during the feeding process, particularly pump clogging. Coffee waste is characterised by high viscosity and a tendency to form agglomerates, which can increase the likelihood of blockages in the feeding line and digester inlet (Qiao et al., 2015). Such interruptions may result in irregular substrate feeding, reduced effective organic loading, and limited substrate–microorganism contact. As a result, these conditions can adversely affect the stability of the anaerobic digestion process and subsequently reduce methane production.

However, an excess loading also contributed to lower methane production. Feeding the reactor for about 4.29L/day of the sample within 7 days of HRT will result in overloading in the reactor was marked by a decline in pH and methane yield (Rittmann et al., 1981). The type of food waste itself also may affect the whole digestion process, for instance, coffee waste being lignocellulosic biomass (lignin, cellulose, tannin, and hemicellulose) (Pujol et al., (2013); and Davila et al., (2013)) which this degradation is strongly influenced by HRTs. The sulphuric acid treatment can be used to remove inhibitors, such as caffeine and tannin, which can improve the digestibility of the substrate and increase the methane yield. Generally, the lignocellulosic wastes require a longer retention time for the digestion of anaerobic microbes to take place (Yadvika et al., 2004).

Figure 4.8 shows the anaerobic co-digestion (AcoD) of IWK Sludge and Coffee waste by a ratio of 2:1 by acid treatment (2M H_2SO_4) recorded the highest methane production by 13921.07 mL CH_4/gVS within HRT of 10 days. Figures 4.9 and 4.10 indicate that the greatest COD (56.08%) and VS (46.52%) reductions occurred with co-digestion of IWK sludge and coffee waste at a 2:1 ratio, pre-treated with 2M H_2SO_4, under a 10-day HRT. In comparison, mono-digestion of sludge achieved lower reductions of 50.49% (COD) and 43.77% (VS). As shown in Figure 4.11, this condition also resulted in a 66.17% increase in methane production, which was higher than that obtained at 7-day HRT. This leads to higher methane yields compared to mono-digestion of individual waste. This is due to the synergistic effects of different substrates, which can enhance methane production (Xingxing et al., 2022; Jessica et al., 2020; Kim & Kang., 2015). Meanwhile, the mono-digestion of IWK sludge itself displays the highest COD and VS reduction (%) than the AcoD of IWK Sludge and Coffee waste by a ratio of 2:1 by acid treatment (2M H_2SO_4) within HRT of 7 days by 29.53% and 50.83% with the methane increment (%) of 21.15%. Research has shown that high COD removal is directly proportional to high methane production and microbial biodegradation activities, indicating that an increase in COD removal leads to an increase in methane production rate. The improved COD removal is linked to higher biogas and methane production, emphasizing the importance of reducing COD for enhanced biodegradability of organic matter and increased methane production during the anaerobic digestion process (Choeisai et al., 2014; Al-Sulaimi et al., 2022 and Hallaji et al., 2018).

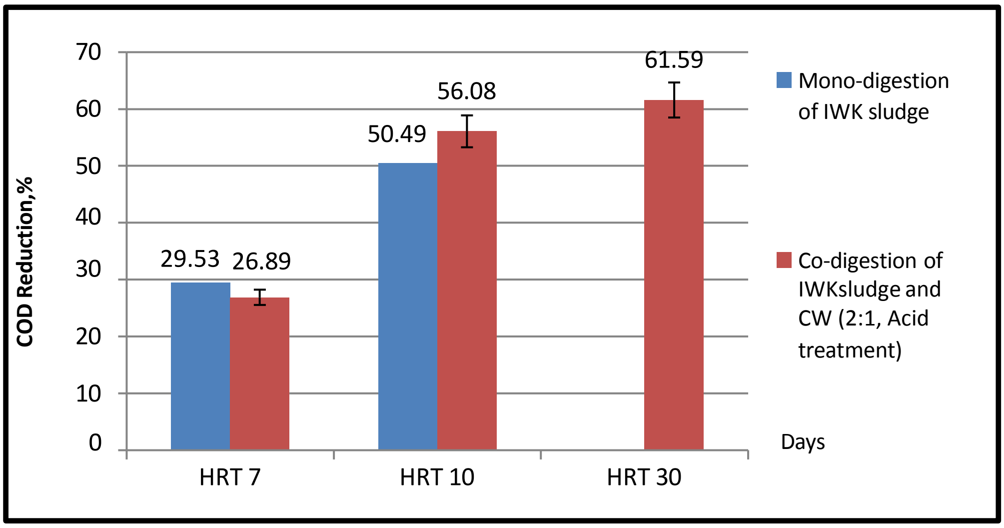


Figure 4.9 COD Reduction (%) of Mono and Co-Digestion of IWK Sludge and CW

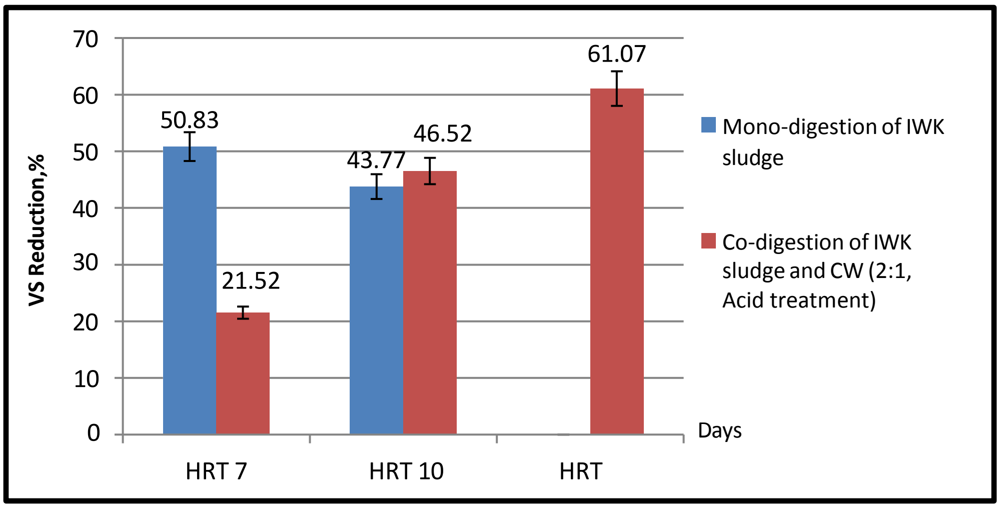


Figure 4.10 VS Reduction (%) of Mono and Co-Digestion of IWK Sludge and CW

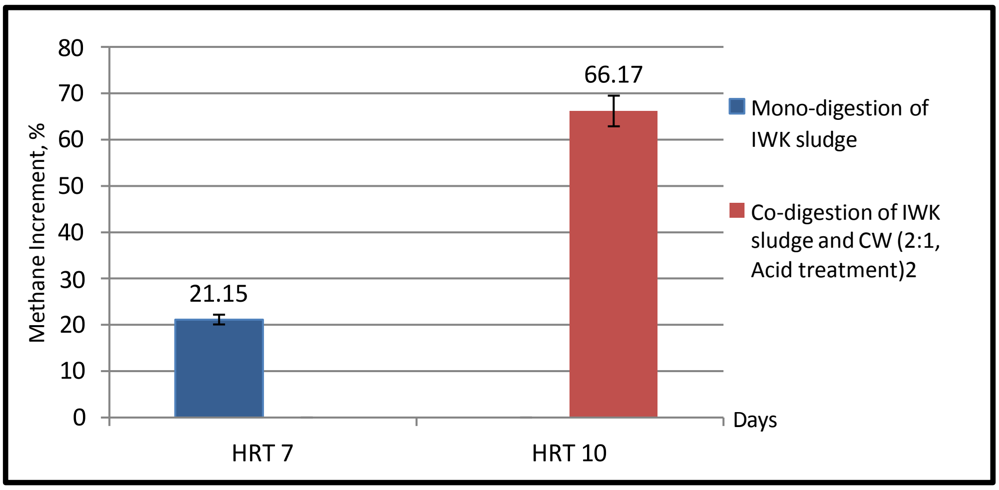


Figure 4.11 CH_4 Increment (%) of Mono and Co-Digestion of IWK Sludge and CW

A longer HRT leads to increased methane production, providing more time for the substrate to be broken down and stabilized, allowing for proper interactions with the bacteria within the digester (Bhatt and Tao., 2020). In this study, an HRT of 10 days produced more methane, and the methanogenesis process had a longer culture doubling time compared to an HRT of 7 days. Generally, the methanogens have a longer regeneration time compared with the hydrolysis acidogenesis bacteria. For example, one study found that coffee waste co-digested with sewage sludge had a methane yield of 0.24-0.28 m^3 CH_4 (STP)/kg VS(initial), and 76-89% of the theoretical methane yield was achieved (Neves et al., 2006). To avoid being washed out from the reactor, the HRT must be long enough to retain the methanogens. Ma et al. (2013) demonstrated that the anaerobic reactor failed when the hydraulic retention time (HRT) was shorter than 2 days, which is insufficient for the microorganisms to reach their growth potential. The longer the HRT, the higher the methane yield and the higher the volatile solids (VS) removal efficiency in the co-digestion of food waste and sewage sludge (Cheong et al., 2022).

A low accumulated methane production was observed by mono- digestion of IWK Sludge without pretreatment with 8377.45 mL CH_4/gVS within HRT of 10 days compared to co-digestion of IWK sludge and CW with acid treatment (2M H_2SO_4). This is because sulphuric acid treatment of coffee waste can enhance methane production in anaerobic digestion by improving the hydrolysis of carbohydrates, removing inhibitors, and producing reducing sugars (Oi et al., 1981). Furthermore, sulphuric acid treatment can be used to remove inhibitors, such as caffeine and tannin, which can improve the digestibility of the substrate and increase the methane yield (Widjaja et al., 2019). A study by Sreekrishnan et al., (2004) cited that the longer HRT will require a large digester volume, increasing the overall operational cost, while a shorter HRT will remove the active bacterial population. From the results, feeding the reactor for about 3L/day of the co-digestion of IWK sludge and coffee waste within 10 days of HRT shows a stable anaerobic digestion performance compared to mono-digestion. As informed, the anaerobic co-digestion can result not only in a higher methane yield (which is just caused by the higher methane yield of food waste compared to raw sewage sludge) but also in accelerated methane production. This finding corresponds very well with results revealed by Kim et al. (2003), who observed the highest methane production rate in co-digestion of sewage sludge with food waste in 30–40% VS under mesophilic conditions. Generally, mesophilic digestion can be accomplished within 15–30 days (Mao et al., 2015).

Overall, the pretreatment and co-digestion of IWK sludge and CW can be an alternative way to enhance methane production. The HRT can affect methane production in mono-digestions of sludge and co-digestion of sludge and food waste in anaerobic digestion. However, the effect of HRT can depend on several factors, such as the type and characteristics of the waste being treated, the operating conditions, and the specific goals of the process. Therefore, further research is needed to fully understand the effect of HRT on methane production in mono-digestion of sludge compared to co-digestion of sludge and food waste in anaerobic digestion.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The anaerobic digestion process proved to be an effective and sustainable approach for treating biodegradable sewage sludge while simultaneously generating renewable energy in the form of biogas. The system successfully converted sewage sludge and coffee waste into bioenergy, with methane composition ranging from 50–70%. All research objectives were successfully fulfilled through both laboratory- and pilot-scale investigations. For the first objective, optimization of the inoculum-to-substrate (I:S) ratio and hydraulic retention time (HRT) using BMP tests in 125 mL serum bottle reactors identified an optimal I:S ratio of 2:1 (IWK sludge: coffee waste), which produced the highest methane yield of 17.31 mL CH_4/gVS. Regarding the second objective, chemical pre-treatment with 2M H_2SO_4 was shown to enhance substrate biodegradability and increase methane yield to 18.49 mL CH_4/gVS under optimal conditions. For the third objective, successful scale-up to a 40 L continuous stirred tank reactor (CSTR) was achieved, where stable methane production of 40,936.7 mL and a COD removal efficiency of 61.6% were recorded under mesophilic conditions using GC-FID and a portable gas analyzer. Overall, the results confirm that integrating co-digestion with acid pre-treatment significantly improves methane production and process stability, demonstrating its potential for large-scale anaerobic digestion applications.

5.2 Recommendations

The BMP test of the pre-treatment and co-digestion process revealed that non- dairy creamer waste (NDCW) produced significantly less methane than coffee waste, even after several pre-treatments were applied. It is supported by the characterization of the NDCW, which showed that the sample was too acidic, with a pH ranging from 3 to 5. NDCW is not recommended in the pilot-scale study, as it may disrupt the entire anaerobic co-digestion process, regardless of the types of pre-treatment used. For a 125mL serum bottle BMP test (lab scale), a working volume of 70% is commonly used, with the remaining space left for biogas accumulation. The test is usually performed at 35-37^{o}C and pH 7-7.5 using IWK sludge as an inoculum. An inoculum-to-substrate ratio of 2:0.5 on a VS basis is recommended, especially for inhibitory substrates. There are still opportunities to explore other pre-treatment methods, such as psychochemical and thermal treatments, through BMP testing. Modifying the substrate-to-inoculum (S/I) ratio of IWK sludge combined with coffee wastewater and non-dairy creamer wastewater (NDCW)—for instance, using ratios of 3:1:0.25 or 4:1:0.25—could be advantageous in future research by lowering the proportion of NDCW. This helps reduce microbial inhibition from excessive amount of biodegradable organic matter introduced into the digester from the feed mixture especially from NDCW or volatile fatty acids, promoting more efficient digestion. Furthermore, co-digestion with other fat- and carbohydrate-rich food wastes, such as kitchen waste, should be explored, as it balances nutrients, dilutes inhibitory substances, fosters microbial synergy, and enhances both process stability and methane production.

The addition of potassium permanganate ($KMnO_4$) for the future work may enhance the anaerobic digestion (AD) process by improving the breakdown of organic matter. $KMnO_4$ acts as a secondary oxidizing agent that helps decompose complex organic substrates into simpler compounds, which methanogens can more easily convert into methane. It also promotes hydrolysis and acidogenesis by breaking down larger organic molecules, such as lipids, carbohydrates, and proteins, into smaller molecules, including fatty acids and alcohols. Additionally, $KMnO_4$ helps maintain anaerobic conditions by removing traces of oxygen that could inhibit the growth of methanogenic archaea. It is crucial to control the concentration of $KMnO_4$ carefully, as it is an oxidizing agent that can inhibit methanogenic activity and disrupt the anaerobic environment when used in excessive amounts.

The conversion of methane (natural gas) into liquid methane, also known as liquefied natural gas (LNG), is primarily used to transport and store natural gas in a more compact and manageable form. Methane is the primary component of natural gas, and it is typically found as a gas at atmospheric pressure and temperature. Liquefying it reduces its volume by about 600 times, making it easier and more economical to store and transport, especially over long distances where pipelines are not feasible.

This recommendation addresses technical issues related to the digester system and its design. A recurring problem is clogging during continuous feeding, particularly when a peristaltic pump connected to the digester. The sludge samples we collected are typically raw and often contaminated with dust, plastic particles, and fabric residue that are not entirely removed during the collection process. This contamination leads to persistent clogging at the inlet and outlet ports of the digester, especially at the outlet. As a result, the liquid discharged does not flow out properly. The L-shaped design of the outlet tubing complicates the cleaning of clogs, which significantly prolongs the time required to restore normal operations. This issue negatively affects both anaerobic digestion and methane production. To mitigate these problems, we recommend briefly blending the sludge samples before pouring them into the feeding tank, which should help reduce clogging. Redesigning the inlet and outlet ports of the digester: the inlet port should be located at the bottom. In contrast, the outlet port should be higher up in the digester, below the gas sampling port. This revised design expected to be more efficient than the previous configuration.

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APPENDICES

APPENDIX A

A1 Sampling of Raw Inoculum

Figure A1 Sampling of raw inoculum

A2 Sewage Treatment Plant (STP) Titiwangsa 2

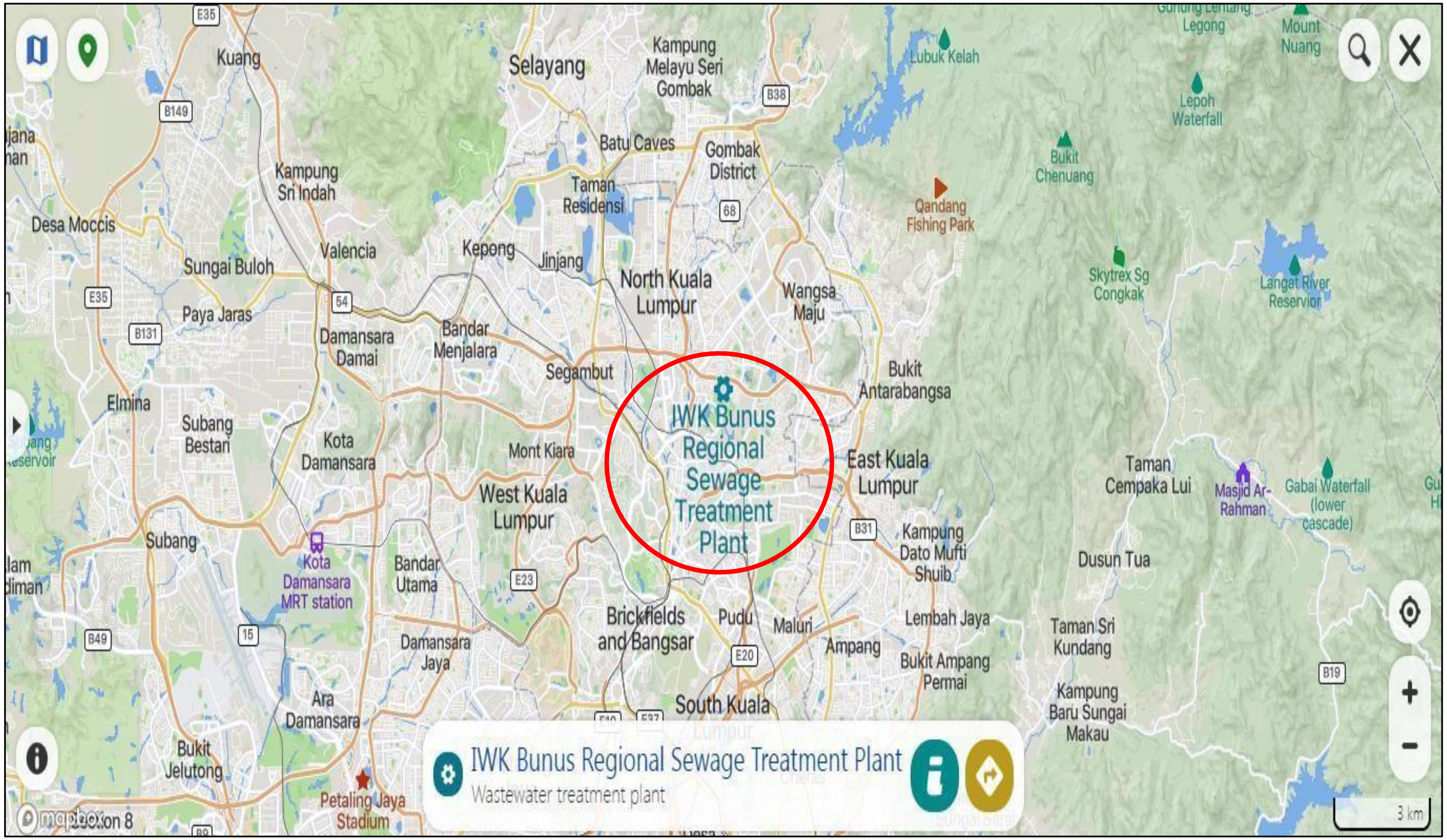


Figure A2 Google maps of STP, Titiwangsa 2, KL

A3 Sampling of Raw Substrates

Figure A3 Sampling of Raw substrates

A4 Sampling Point

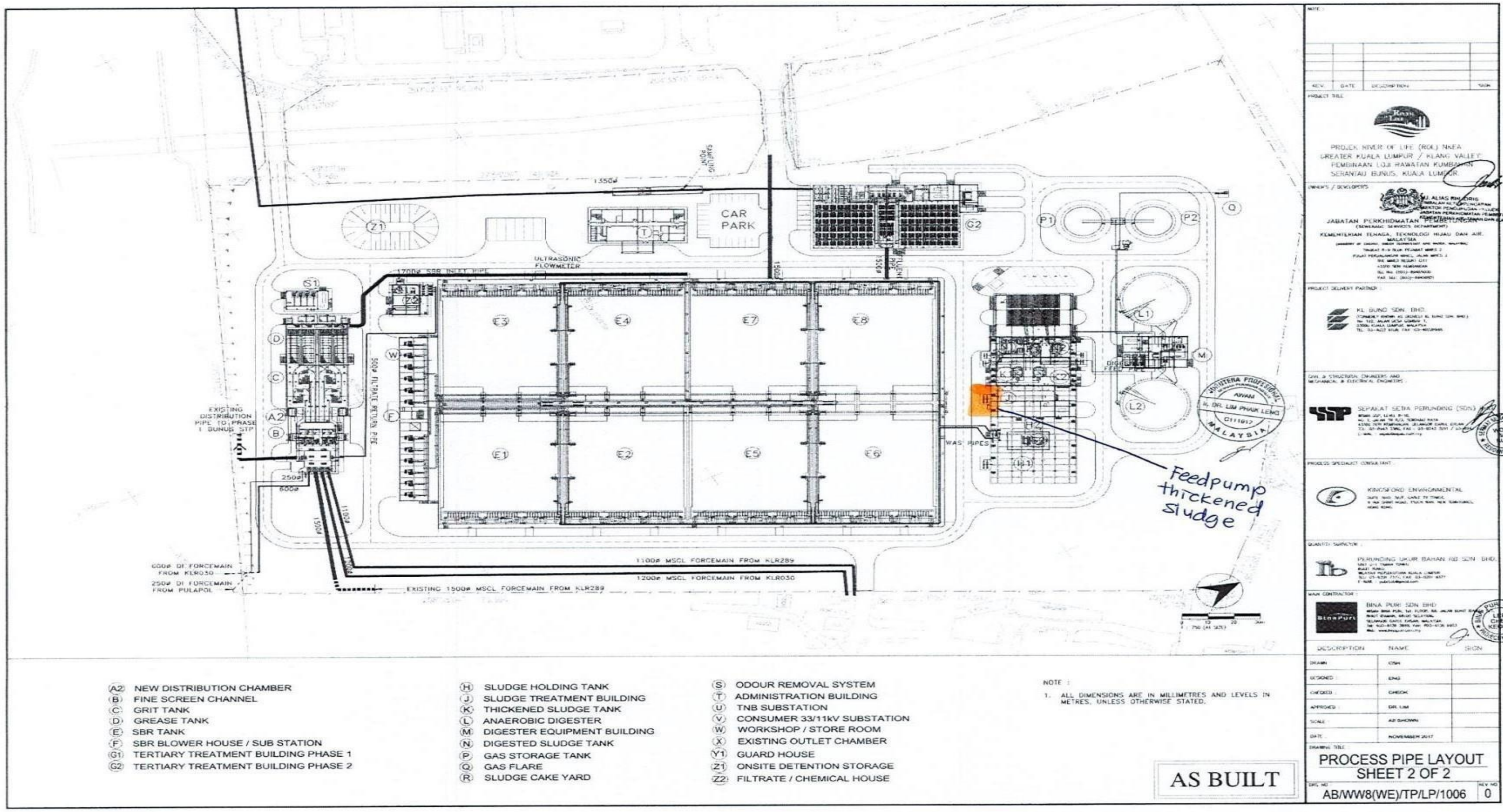


Figure A4 Sampling Point

APPENDIX B

Characterization of Inoculum and Substrates

B1 Vial for COD Test

Figure B1 COD test vials

B2 Dilution of Substrates

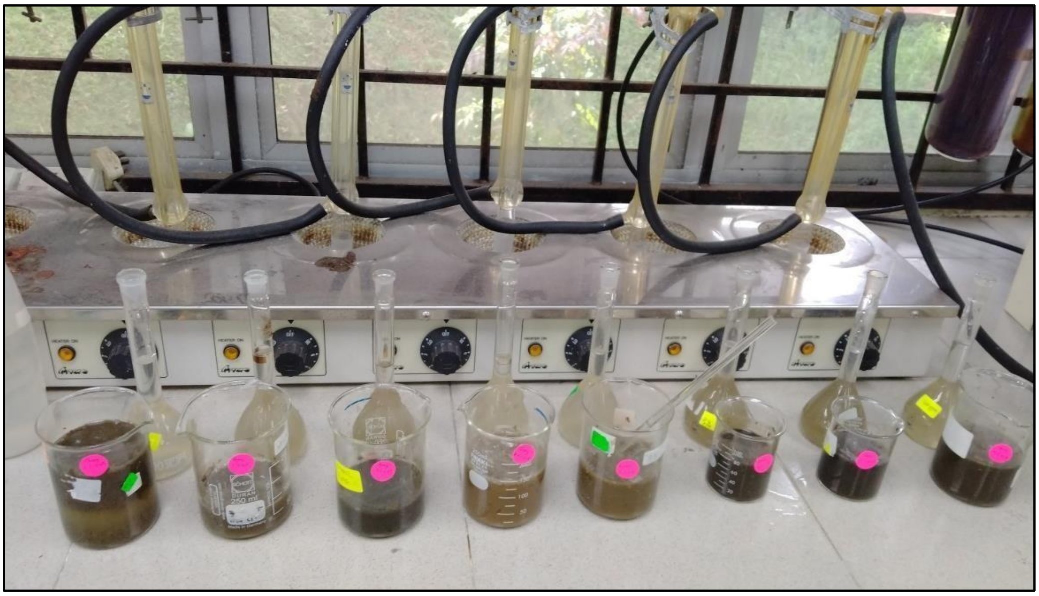


Figure B2 Dilution of substrates

B3 Moisture Content analysis

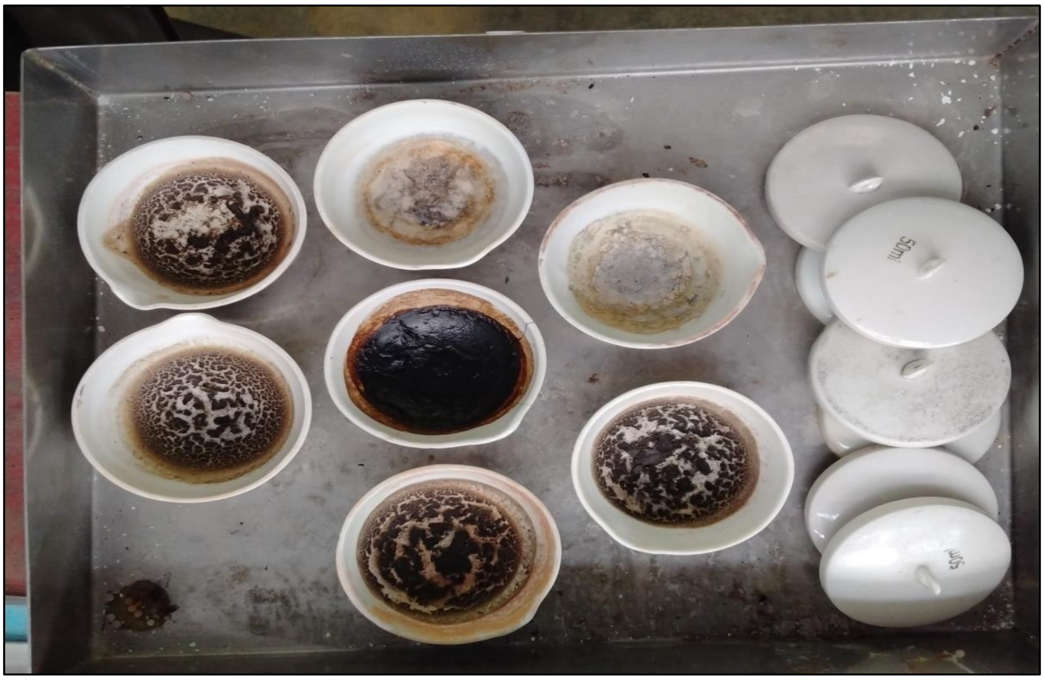


Figure B3 Moisture Content analysis

B4 Total Solid (TS) and Volatile Solid (VS) analysis

Figure B4 TS and VS analysis

B5 pH test of Samples

Figure B5 pH test of samples

B6 Alkalinity Test by HACH method

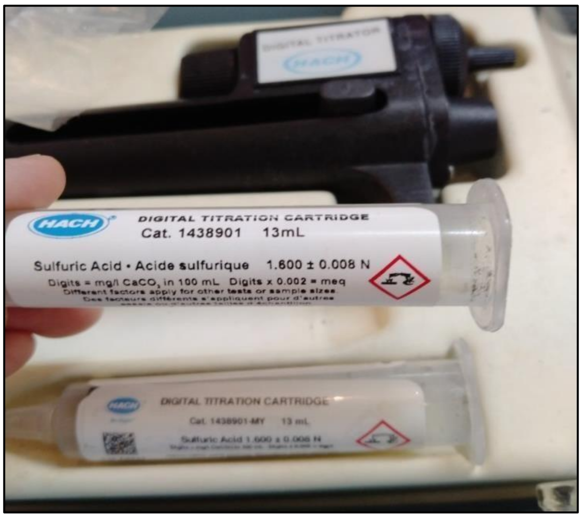


Figure B6 Alkalinity test by HACH Method

B7 Volatile Fatty Acid (VFA) by HACH method

Figure B7 VFA test analysis

B8 Total Suspended Solid (TSS) and Volatile Suspended Solid (VSS)

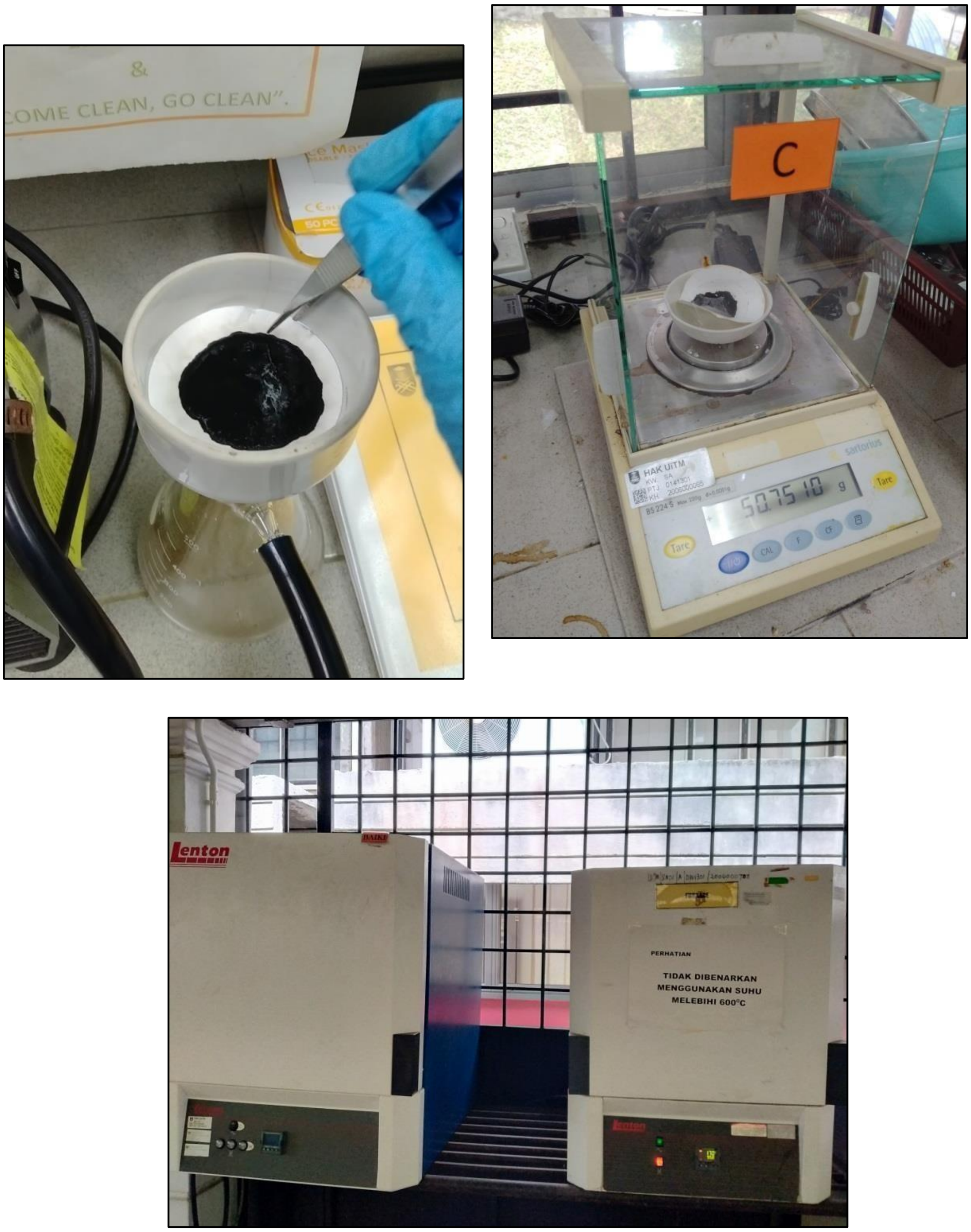


Figure B8 TSS and VSS test analysis

APPENDIX C

Laboratory Batch Test (Biochemical Methane Potential, BMP)

C1 Batch Reactor

Figure C1 Batch reactors with substrates

C2 Purging Process with Nitrogen, N_2 gas

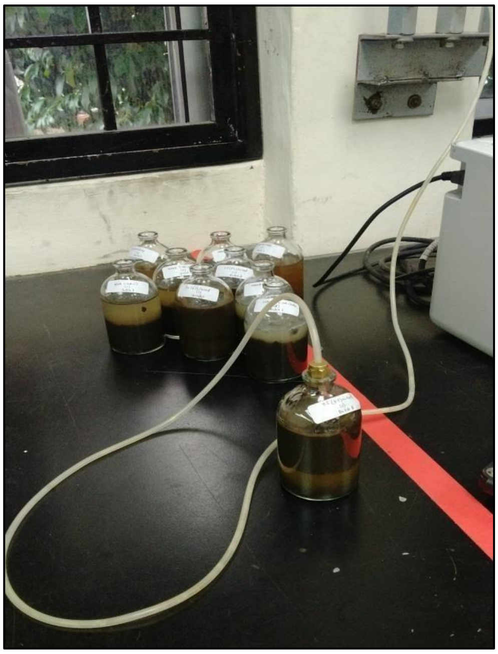


Figure C2 Purging process

C3 Laboratory Batch Setup

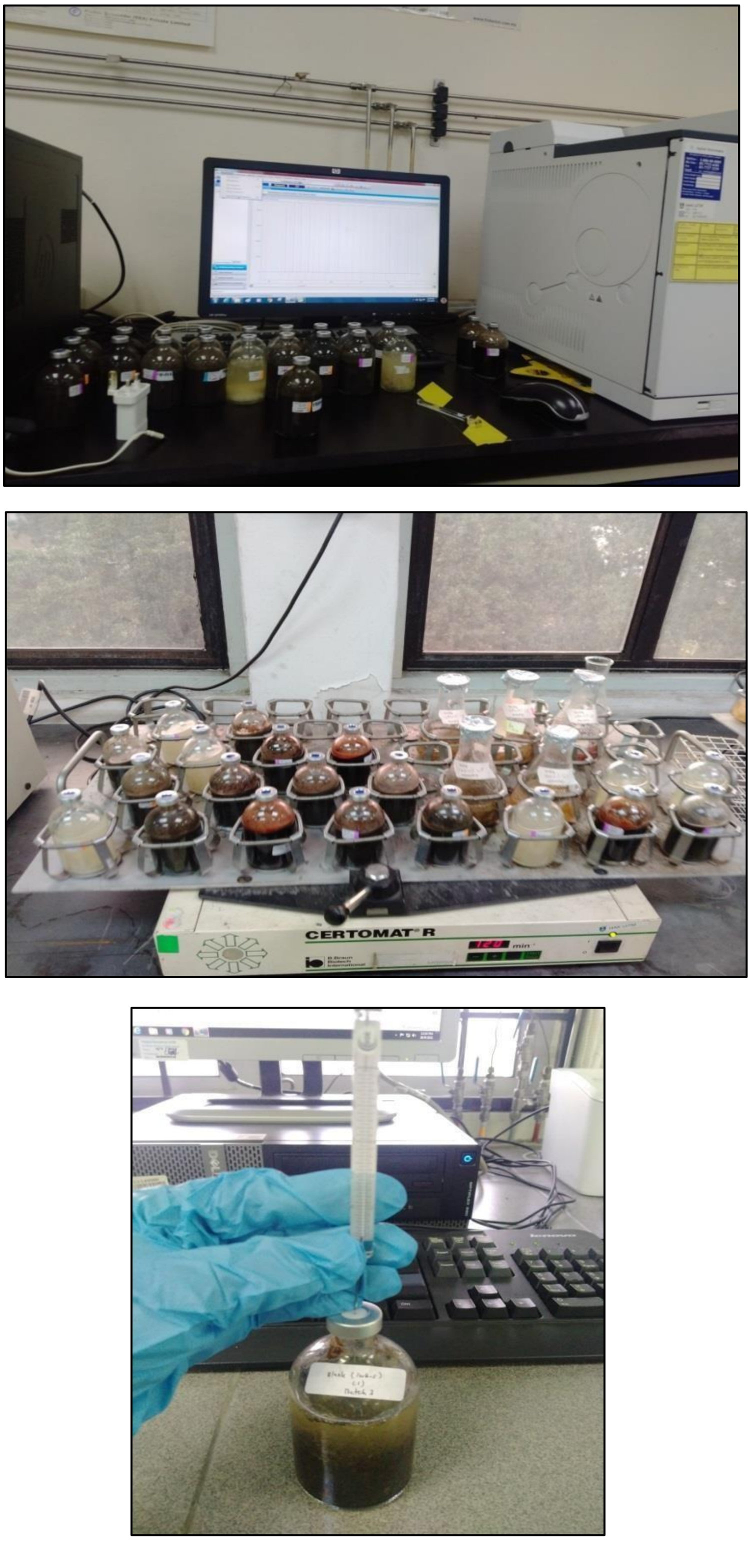
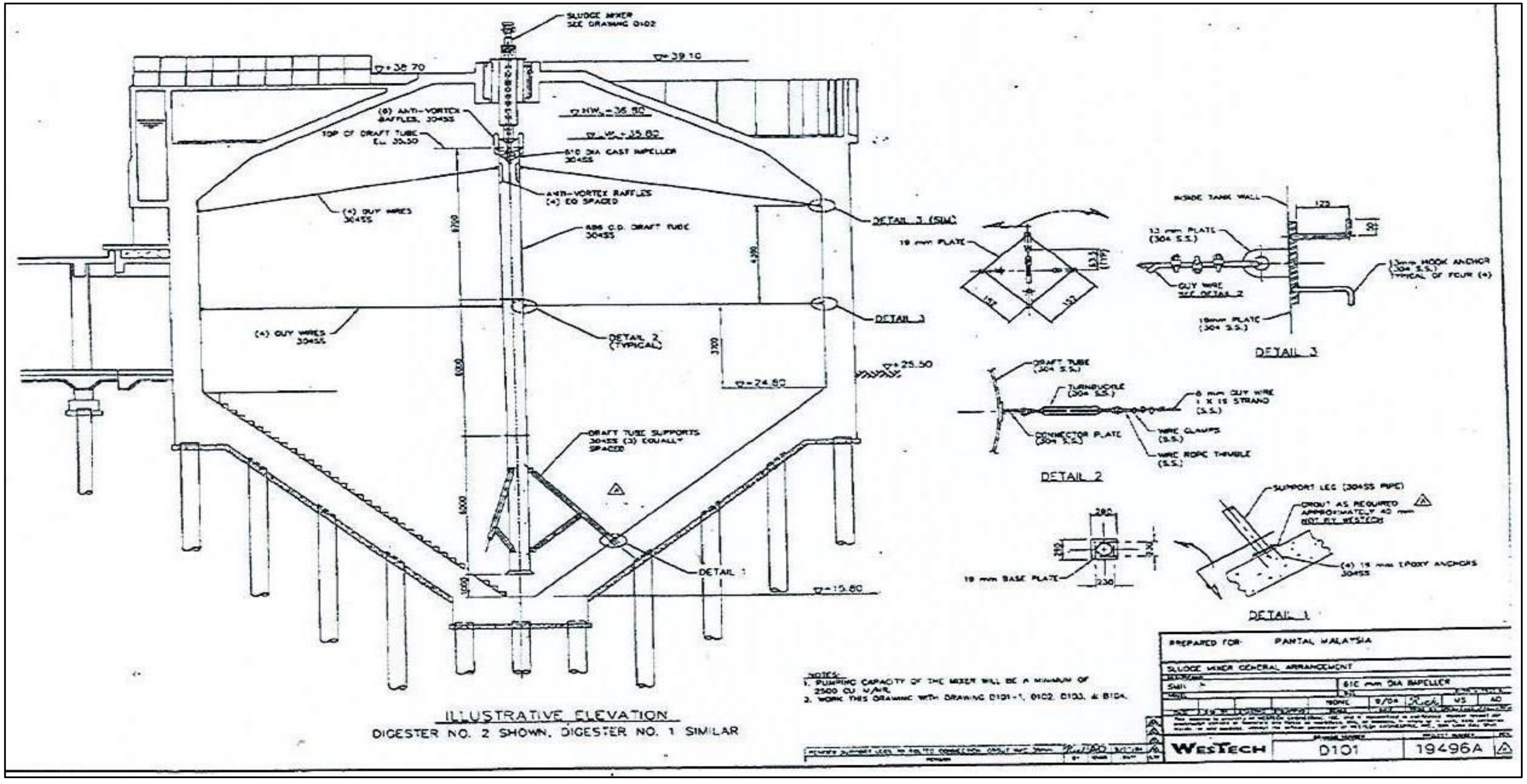


Figure C3 Laboratory Batch Setup

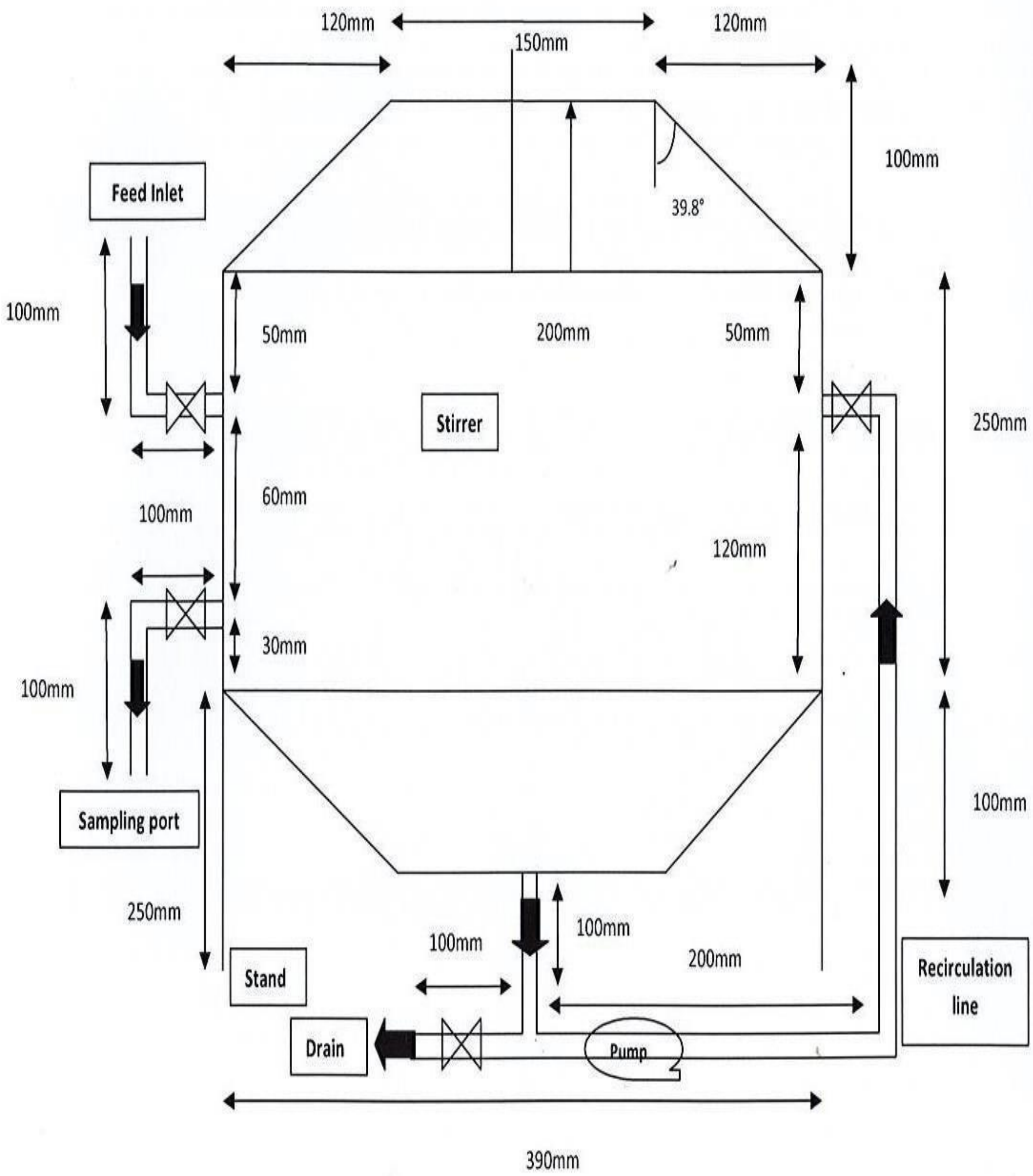
APPENDIX D

Others Attachement
D1 Pilot Scale Design-Full Dimensions



D2 Design of pilot scale plant (Anaerobic Digester Unit)

The pilot scale studies were run using pilot reactor of 40 L in volume equipped with impeller where it speed can be controlled.



D3 Site Visit at Sewage Treatment Plant (STP) Pantai Dalam, KL

Figure D3 Site Visit at Pantai Dalam, KL STP

APPENDIX E

Pilot Scale Study

E1 Sampling for Pilot scale study

Figure E1 Sampling for pilot scale study

E2 Anaerobic Pilot Digester

Figure E2 Anaerobic Pilot Digester

E3 Pilot Scale Study Setup

Figure E3 Pilot scale process setup

APPENDIX F

F1 Results of GC-FID Analysis

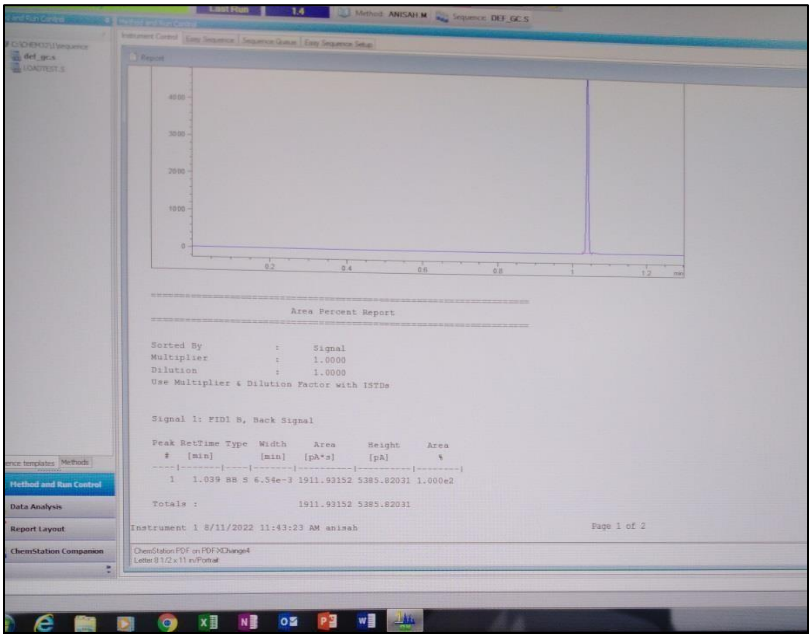


Figure F1 Results of GC-FID analysis

AUTHOR'S PROFILE

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LIST OF PUBLICATION

N.A.A. Latif., Z.M. Zaki., F. Buyong. Estimation of methane production via anaerobic co- digestion of food waste and sludge by biochemical methane potential (BMP) test. *Malaysian Journal of Analytical Sciences Vol 26 No 2 (2022): 241 – 250*. https://mjas.analis.com.my/mjas/v26_n2/html/26_2_6.html