LIPID INHIBITION AND FOAMING IN ANAEROBIC DIGESTION OF FOOD PROCESSING WASTEWATER

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Chair : Associate Professor Rozita Omar, PhD
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High-strength wastewater is a suitable substrate for the anaerobic digestion (AD) to generate a higher yield, as it is a high organic matter source. However, several backlashes, such as foaming and scum formation incidents, lead to unstable operation and reduced digester working volume, decreasing microbial activity and biogas yield. This research was carried out to identify the effect of protein and lipids on scum formation and foam in one-stage and two-stage AD. Besides that, the scum reduction efficiency using natural oils and biosurfactants was also assessed. The one-stage process was carried out through a batch test in 1L and 10 L bottles. Meanwhile, the two-stage AD was through a continuous process at a hydraulic retention time (HRT) of 0.5 days, a temperature of 35-38°C, and a pH of 7 in 30 L fermenters. The inoculum was anaerobic sludge (AS) and cow manure (CM) for one-stage AD, while for the two-stage only AS was used. The substrate was synthetic wastewater (SW) and food processing wastewater (FPW). Antifoams such as natural oils (rapeseed oil (RO), palm oil (PO)) and biosurfactants (Rhamnolipid (RL) and tea saponin (TS)) were employed in the two-stage reactor to combat the scum formation. Performance of the AD process was analyzed by COD, protein and lipid removal efficiencies, total volatile fatty acid (VFA), ammoniacal nitrogen (AN) concentration, pH, and biogas accumulation. The foam was analyzed using an aeration-based foaming assay and the scum reduction efficiency through volume. This research shows that protein-induced foaming in one-stage AD was not present in two-stage AD. Foaming was also not observed in one-stage and two-stage digesters fed with FPW. On the other hand, scum was only present in two-stage AD treating FPW. The fatty acid analysis revealed that scum consists mainly of saturated and α-linoleic fatty acids. This shows that the scum in the digester is indeed from lipid inhibition. The best performance was at 6g/L lipid at COD removal of 93.8%, AN of 52.9 mg/L, total VFA of 4.44 g/L and biogas of 0.364 L/L/D in Tank 2, day 4. The 0.5% v/v RO could suppress scum in two-stage AD by 32%, while 0.5% v/v PO only suppressed by 7% with a biogas accumulation of 0.201 L/L/D and 0.220 L/L/D significantly lower than blank (0.364 L/L/D). The biosurfactants, RL and

TS, increased the hydrolysis and acidogenesis; however, scum was not successfully suppressed. A reduction of biogas in Tank 2 of two-stage AD (methanogenesis phase) was observed in both applications of natural oil and biosurfactants, indicating a detrimental effect.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

INHIBITASI LEMAK DAN BUIH DALAM PENCERNAAN ANAEROBIK MENGGUNAKAN AIR SISA PEMPROSESAN MAKANAN

Oleh

SHARIFAH NAZURA BINTI SYED SABEER ALI

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Air sisa berkekuatan tinggi adalah substrat yang sesuai untuk pencernaan anaerobik (AD) untuk menjana hasil yang lebih tinggi, kerana air sisa berkekuatan tinggi merupakan sumber bahan organik yang tinggi. Walau bagaimanapun, beberapa serangan balas, seperti berbuih dan insiden pembentukan kekam, membawa kepada operasi yang tidak stabil dan mengurangkan isipadu kerja penghadam, mengurangkan aktiviti mikrob dan hasil biogas. Kajian ini dijalankan untuk mengenal pasti kesan protein dan lemak pada pembentukan kekam dan buih dalam AD satu peringkat dan dua peringkat. Selain itu, kecekapan pengurangan kekam menggunakan minyak asli dan biosurfaktan juga dinilai. Proses satu-peringkat telah dijalankan melalui ujian kelompok dalam botol 1L dan 10 L. Sementara itu, AD dua- peringkat dilakukan melalui proses berterusan pada masa pengekalan hidraulik (HRT) selama 0.5 hari, pada suhu 35-38°C, dan pH 7 dalam penapai 30 L. Inokulum adalah enap cemar anaerobik (AS) dan baja lembu (CM) untuk AD satu peringkat, manakala untuk dua peringkat hanya AS digunakan. Substrat yang digunakan ialah air sisa sintetik (SW) dan air sisa pemprosesan makanan (FPW). Antibuih seperti minyak asli (minyak rapeseed (RO), minyak sawit (PO)) dan biosurfaktan rhamnolipid (RL) dan teh saponin (TS) telah digunakan dalam reaktor dua peringkat untuk memerangi pembentukan kekam. Prestasi proses AD dianalisis dengan COD, kecekapan penyingkiran protein dan lemak, jumlah lemak meruap asid (VFA), kepekatan nitrogen ammonia (AN), pH, dan pengumpulan biogas. Buih itu telah dianalisis menggunakan ujian berbuih berasaskan pengudaraan dan kecekapan pengurangan kekam melalui isipadu. Penyelidikan ini menunjukkan bahawa pembuihan yang disebabkan oleh protein dalam AD satu peringkat tidak dijumpai dalam AD dua peringkat. Pembuihan juga tidak diperhatikan dalam penghadam satu peringkat dan dua peringkat yang diberi FPW. Sebaliknya, kekam hanya terdapat dalam AD dua peringkat merawat FPW. Analisis asid lemak mendedahkan bahawa kekam terutamanya terdiri daripada asid lemak tepu dan α-linoleik. Perkara ini menunjukkan bahawa kekam dalam penghadam memang daripada perencatan lipid. Prestasi terbaik

adalah pada 6g/L lipid pada penyingkiran COD 93.8%, AN sebanyak 52.9 mg/L, jumlah VFA sebanyak 4.44 g/L dan biogas sebanyak 0.364 L/L/D dalam Tangki 2, hari ke-4. 0.5% v/v RO boleh menyekat kotoran dalam AD dua peringkat sebanyak 32%, manakala 0.5% v/v PO hanya ditindas sebanyak 7% dengan pengumpulan biogas sebanyak 0.201 L/L/D dan 0.220 L/L/D ketara lebih rendah daripada kosong (0.364 L/L/D). Biosurfaktan, RL dan TS, meningkatkan hidrolisis dan asidogenesis; walau bagaimanapun, kekam tidak berjaya ditindas. Pengurangan biogas dalam Tangki 2 AD dua peringkat (fasa metanogenesis) diperhatikan dalam kedua-dua aplikasi minyak asli dan biosurfaktan, menunjukkan kesan yang memudaratkan..

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LIST OF ABBREVIATIONS

UPM	Universiti Putra Malaysia
°C	degree celsius
α	Alpha
β	Beta
%	Percentage
AD	Anaerobic Digestion
FPW	Food Processing Wastewater
RL	Rhamnolipid
TS	Tea saponin
RO	Rapeseed Oil
PO	Palm Oil
WAS	Waste Activated Sludge
FA	Fatty Acid
AN	Ammoniacal Nitrogen
COD	Chemical Oxygen Demand
HCL	Hydrochloric Acid
FOG	Fat, Oil and Grease
AS	Anaerobic Sludge
CM	Cow Manure
LCFA	Long Chain Fatty Acid
VFA	Volatile Fatty Acid
WTP	Wastewater Treatment Plant
V/V	Volume/Volume

CHAPTER 1

INTRODUCTION

1.1 Background

The wastewater treatment method has become crucial in overcoming environmental pollution in these urbanization times. Wastewater sources, including domestic households, municipal communities, or industrial activities, must undergo treatments before being discharged into the environment. However, wastewater remains one of the major pollutants in our inland waterways. Implementing more advanced designs in wastewater treatment technologies is required to satisfy tighter regulatory. Water treatment is typically based on physical, chemical, and biological processes. Today's wastewater treatments are much more technologically advanced than in previous years, and new, improved methods are going in the right direction (Ma et al., 2024).

Anaerobic Digestion (AD) has shown promising advancements over the years in the biological treatment processes. AD is a route of organic matter degradation to reduce dependence on fossil fuels. It also benefits as a constant energy provider with minimal impact on the environment, generation of high calorific value gases, and lower energy consumption. Thus, AD is widely applied in various complex wastewater treatment plants, including municipal wastewater treatments, chemical and agricultural industries, and sewage treatments (Cremonez et al., 2021; Demirel & Yenigün, 2002). Typically AD has four metabolic phases involving acid-forming and methane-forming bacteria. They are generally kept in a single system in conventional methods for a delicate balance. However, problems arise as both groups of bacteria have different environmental and nutritional needs. Besides that, one-stage AD also leads to acidification with high loads of organic substrate, resulting in severe process failure (Janesch et al., 2021; Moeller & Zehnsdorf, 2016). The two-stage anaerobic digestion system was introduced as a physical separation of two groups of bacteria with the notion of enhancement in process stability and control (Demirel & Yenigün, 2002). Many studies have reported that two-stage AD is an exciting alternative for better overall process performance leading to higher energy yield and degradation rates (Li et al., 2019; Mahat et al., 2021).

High-strength wastewater is a good substrate for AD to generate a higher yield as it is a high organic matter source. However, several backlashes exist, such as foaming and lipid inhibition incidents. Lipids are a major component in waste streams from edible oil refineries, palm oil mills, slaughterhouses, wool scouring, dairy industries, and food processing wastewater (FPW). This study uses high-strength wastewater from the food processing industry as a substrate. Lipids included in the wastewater consist mainly of neutral fats and long-chain fatty acids (LCFAs). Even though emulsified lipids are commonly treated through wastewater treatment, the exact behavior in biological treatment processes is still not well understood (Chipasa & Mędrzycka, 2006). In a two-stage AD, the treatment of high-strength wastewater containing a high amount of lipids and proteins poses some challenges, such as the formation of scum, foaming, and sludge flotations (Mahat et al., 2021)

Scums are defined as slow degraded organic matter formed on a digester's surface, generally consisting of metastable viscous liquid. Scum formation leads to physical, biological, and economic failures if left untreated. They often cause pipe blockage, reduce reactor surface area and interfere with mixing equipment. Some causes of scum formation are improper mixing, temperature fluctuations, and organic overloading (Thaku & Barjibhe, 2019). Besides that, one of the significant causes of scum in AD is the accumulation of LCFA in the degradation process of lipids (Affes et al., 2017; Kim et al., 2004; Rasit et al., 2015). LCFAs usually contain an even number of carbon atoms, typically 14 –24, among which 16- and 18-carbon LCFAs are common in waste. LCFAs are degraded through the β-oxidation pathway to acetate and H_2. Then it is converted to methane. However, β-oxidation of LCFA is a relatively slow reaction and is hindered by the low solubility of LCFAs, causing process inhibition and instability (Kim et al., 2004; Rasit et al., 2015). It results in the accumulation of intermediates on the surface of the digester. LCFA and magnesium and calcium ions in the reactor produce extensive scum inhibiting the contact between substrate and microorganism, resulting in lower methane production and COD removal (Tagawa et al., 2002). Scum is still a significant problem in wastewater treatment plants treating a high-lipid substrate, and very few solutions have been researched. Some examples are enzymatic pretreatment using lipases and mechanical methods (Soares et al., 2019) and the usage of natural oil such as coconut and rapeseed oil using municipal solid waste in one stage of AD (Thakur et al., 2019). These approaches have shown promising results; however, enzymatic pre-treatment is utterly expensive, and the usage of natural oil has not been tested in a two-stage system.

Besides that, high organic content in the substrate often triggers foaming to occur in the digester. It leads to unstable operation and a reduction in digester working volume, thus decreasing microbial activity and biogas yield (He et al., 2017). Studies have shown that the primary causes of foaming incidents in the digester are caused by unstable operating conditions such as temperature fluctuations, inadequate mixing, and inconsistent loading rates (Ganidi et al., 2009; Lindmark et al., 2014). Other studies have suggested that the accumulation of surface-active agents such as proteins, lipids, volatile fatty acids (VFA), and extracellular polymeric substances (EPS) in the digester causes foaming(Ganidi, 2011; Ganidi et al., 2009). Among all the causes studied, the organic loading rate and composition of the substrate play an essential role in inducing foaming. In AD, the substrate contains varieties of compounds, out of which proteins and lipids are the most prominent.

In the market, chemical-based defoamers and antifoams are available as solutions, but they may not be suitable for every application, especially for biological processes. Research conducted by Kougias et al. (2015) concluded that rapeseed oil is a cheap and environmentally friendly antifoam for treating foam formation in manure anaerobic digesters. It efficiently suppressed foaming by 51% in a protein-rich substrate and 52% in a lipid-rich substrate. It was also stated that the co-digestion of manure and rapeseed oil stimulated the LCFA degradation resulting in minimum lipid inhibition. There is a substantial possibility of reducing scum in two-stage AD by implementing rapeseed oil (RO) as a solution, but further research is required to verify its effectiveness.

The usage of surfactants for the reduction of scum/foam is related to their ability to reduce surface tension and improve emulsification activity in a process. Oleate, one of the typical long-chain fatty acids (LCFA) in AD, was reported to have low solubility and thus quickly accumulates on the surface of the digester resulting in restricted bioavailability and accessibility to anaerobic microbes(Eftaxias et al., 2021). Besides that, Dereli et al. (2014) also demonstrated that the particle size of LCFA influences its bioavailability. The researchers discovered that LCFA precipitates were biodegradable in anaerobic incubation; nevertheless, increasing particle size decreased the methane generation rate, indicating difficulties for microorganisms to reach the substrate. The usage of biosurfactants such as rhamnolipid and saponin can enhance the solubility of the substrate and improve the hydrolysis process in the acidogenesis phase (Li et al., 2017; Tan et al., 2015; Zhou et al., 2013). It was also reported that rhamnolipid could enhance the high abundance of acid-forming bacteria and was the primary cause for improving organic matter solubilization, emulsification, and hydrolysis, which was advantageous to VFA synthesis. A previous study made clear that biosurfactants could improve the digestion process in the acidogenesis stage in the treated of waste activated sludge (WAS) (Huang et al., 2015), but the effect of biosurfactants on the treatment of high lipid wastewater in a two-stage process is still unclear. The usage of bio-surfactants is also inexpensive as compared to enzymatic pre-treatment due to high operating cost and energy demand (Liew et al., 2020)

In conclusion, when using high-strength wastewater, the digester has severe operational issues such as lipid obstruction and foaming. To understand the issue more deeply, it is essential to understand its source properly. Generally speaking, proteins and lipids are the most frequently cited causes of scum and foam formation in prior studies; nevertheless, it is challenging to assess the impacts of proteins and lipids separately because there are numerous complex components in a single system. Biodegradable and environmentally friendly solutions are essential in overcoming scum and foam however, more research needs to be conducted for the use in two-stage AD (2).

1.2 Problem Statement

Scums and foams are dominant in wastewater rich in proteins and lipids due to their hydrophobic properties. The correlation of proteins and lipids to scums and foams is scarcely discussed due to multiple complex substances in a single system. As a result, the solutions to solving scum and foam in AD are more complex because the root of the problem is still unknown. FPW contains high concentrations of proteins and fats from processed foods like nuggets, sausages, and breaded products. Besides that, a wider range can be researched since FPW has a high protein and lipid content.

Scum formation is a serious operational problem faced in many industries treating high-strength wastewater, but very little research has been conducted on the causes and solutions of scum in two-stage AD. A study conducted by Rasit et al., 2015 reviewed the methods for overcoming lipid inhibition in AD. Some of the methods mentioned are enzymatic hydrolysis, adsorbent addition, feeding sequences, easy degradation substrate addition, and saponification. However, it was pointed out that a detrimental effect was present on the microorganism leading to sludge accumulation. Besides that, the financial estimation was also higher due to the higher cost and usage of advanced types of equipment (Chatterjee & Mazumder, 2019). Other conventional methods, such as adding chemical additives and oleic acid, were also suggested. However, studies have demonstrated a slight inhibitory effect in dairy cattle manure digesters. Rapeseed oil, a cheap and environmentally friendly antifoam, has shown promising results in the foam and scum reduction efficiency of manure and single-stage AD, but further investigation is required (Kougias et al., 2015; Thaku & Barjibhe, 2019).

Biosurfactants have been implemented in many ways, such as in agriculture, to improve the apparent solubility of chemical pollutants while providing excellent wettability and uniform distribution of fertilizer application in the soil (Fakruddin, 2012). Besides that, its use in the degradation of oil in (Waste Activated Sludge) WAS for the ability to increase hydrocarbon solubility and reduce interfacial tension between two aqueous solutions (Nakhla et al., 2003; H. Zhang, Xiang, et al., 2009). Furthermore, the addition of rhamnolipid (RL) in (WAS) improved the solubility and VFA accumulation; however, inhibition of methanogenesis was observed Huang et al., 2015a).
Theoretically, natural oil can reduce the foam and scum in manure digestion through a bridging mechanism. Rapeseed oil (RO) advanced as a cost-effective approach to reduce foam due to its fatty acid composition. However, the implementation of two-phase digestion fed with FPW is still unclear. Palm oil, on the other hand, has good emulsification ability and excess lipase enzyme that can potentially reduce scum(Tambun et al., 2019). The fatty acid composition is also promising since its lower linoleic acid percentage can be inhibitory (Templer et al., 2006). Thus, further investigation is required to investigate the effect of palm oil and rapeseed oil in two-stage AD treating FPW. Biosurfactants have an excellent potential to reduce scum formation in two-stage AD due to their capability to improve solubilization, emulsification, and hydrolysis; however, some studies have reported inhibition of methanogenesis due to VFA accumulation (Huang et al., 2015; Li et al., 2019). In contrast, another study revealed that the addition of rhamnolipid, a biosurfactant, during the acidogenic phase improved methane generation during the methanogenic phase with a

dosage of 0 to 0.04 g/g total suspended solid (TSS)(Xu et al., 2021). Hence, further research is required to shed light on this topic.

1.3 Research Objectives

This research investigates the causes and potential solutions of scum formation and foaming in one-stage and two-stage AD treating food processing wastewater (FPW). The objectives of the study are

1. To evaluate the effect of high protein and lipid on foam and scum production in one-stage and two-stage AD.
2. To investigate the scum reduction efficiency of natural oils and biosurfactants in two-stage AD treating FPW.

1.4 Scope of study and limitations

The scope of this study focuses on the effect of high protein and lipid wastewater in one-stage and two-stage AD. Besides that, this study also focuses on finding a solution for scum formation in two-stage AD. The one-stage and two-stage systems were fed with synthetic wastewater rich in protein and FPW to determine the system's performance and the volume of scum produced. It was observed that one-stage AD had no production of scum when fed with high protein and FPW; thus, the scum produced was counteracted for two-stage AD only by deploying natural oils and biosurfactants as a cheap and environmentally friendly solution. Our study used rapeseed and palm oil as natural oil, rhamnolipid, and tea saponin as biosurfactants. The performance of the system was evaluated through only selected analysis related to the effect of scum and the addition of natural oil and biosurfactants such as biogas production, total volatile fatty acid, COD, ammonia concentration, protein, and lipid. The research could not be conducted with synthetic wastewater rich in lipids without protein due to constricted sources and the high price of the chemical sodium oleate that acts as a synthetic lipid.

1.5 Significant of Research

This research can provide insight into the effect of protein and lipid on scum and foam and distinctly differentiate the basis of foam and scum. Since lipids and protein are frequently found together in a system, it is yet known how they affect foam and scum. However, given the differences in characteristics between protein and lipid, they may affect foam and scum in various ways. Further research is required in order to differentiate their effects efficiently. If the root cause of the issue can be identified, the remedy to foam and scum can be understood more clearly. The findings of this research will rebound to society as various industries have faced similar problems when treating high-strength wastewaters in not only anaerobic processes but aerobics and WAS as well. The demand for an effective and environmentally friendly solution is rising daily. Thus, this study can help future researchers to have a better understanding of this topic.

1.6 Thesis outline

The introductory chapter provides the background of research on scum and foam. It also explains the problem statement objectives and research gap for the effects of protein and lipid on foam and scum in one-stage and two-stage AD by implementing natural antifoams for scum reduction. This chapter also explains the limitation of the study. Chapter 2 highlights the relevant literature review on past research carried out over the years for scum and foam in AD. It also emphasizes the theoretical paradigms for the topic area for future studies. Chapter 3 explains and justifies the research methodology and introduces the origin of the material used, such as FPW, cow manure, anaerobic sludge, and synthetic wastewater. Chapter 4 presents the descriptive results and statistical analysis of the research objectives, while Chapter 5 summarises the overall outcome of the research.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Anaerobic digestion (AD) has gained interest as a cost-effective and sustainable treatment technology. The AD process is related to the degradation and balance between microbial activity and the absence of oxygen, undergoing four significant processes: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Kadam & Panwar, 2017). The low energy requirement and capital investment make AD an easy alternative for various industries and wastewater treatment plants worldwide. There is a considerable weightage of implementing AD in various industries, such as producing biogas that can reinstate fossil fuels and enforce positive energy recovery. Even though AD offers a great advantage to the industry, there is plenty of room for process improvement. Persistent foaming is AD's most common process failure (He et al., 2017). Besides that, when implementing a lipid-rich substrate in AD, lipid inhibition is a primary disadvantage that causes several problems, such as reduced digester working volume and blockage of pipes (Soares et al., 2019).

2.2 Introduction to Anaerobic Digestion

The AD process is a sophisticated dynamic system that involves chemical and biological processes in a multistage. AD has been identified as the most environmentally sustainable method for treating organic wastes because it not only allows the separation of environmentally friendly materials from landfills but also for the generation of bioenergy and prospective by-products such as soil biofertilizer and biogas production (Foresti et al., 2006). Instead of composting the organic waste, AD allows organic matter to biodegrade without oxygen, producing a digested product that can be used as a nutrient source. AD enables the treatment of high organic loading wastes to reduce volume and load while producing biogas as a by-product. Biogas can generate heat, electricity, and vehicle biofuels (Awe et al., 2017).

2.3 Anaerobic Digestion Processes

Anaerobic digestion occurs in four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis; the anaerobic digestion process is dependent on interactions between the various bacteria capable of carrying out the phases, as shown in Figure 2.1 (Rasit et al., 2015). All wastes are loaded concurrently in single-stage batch reactors, and all four phases occur consecutively in the same reactor.

The first process is hydrolysis, which converts substrates into simple compounds. This process mainly converts carbohydrates to sugars, lipids, and proteins to long-chain fatty acids and amino acids. Hydrolytic microorganisms secreting different hydrolyzing enzymes such as cellulase, cellobiose, and hydrolases carry out hydrolysis reactions. Esterase, glycosidase, and peptidases are examples of hydrolases present in hydrolysis (Cirne et al., 2007; Merlin Christy et al., 2014). Forced by extracellular enzymes secreted by bacteria that are either obligate or facultative anaerobes, hydrolyzing reactions are divided into two stages. The first phase involves bacterial colonization, in which hydrolytic bacteria adhere to the surface of solids. Bacteria on the particle surface release enzymes and produce monomers that can be used by the hydrolytic bacteria and the other bacteria. The bacteria will degrade the particulate surface at a constant depth per unit of time in the second phase(Dahiya et al., 2015).

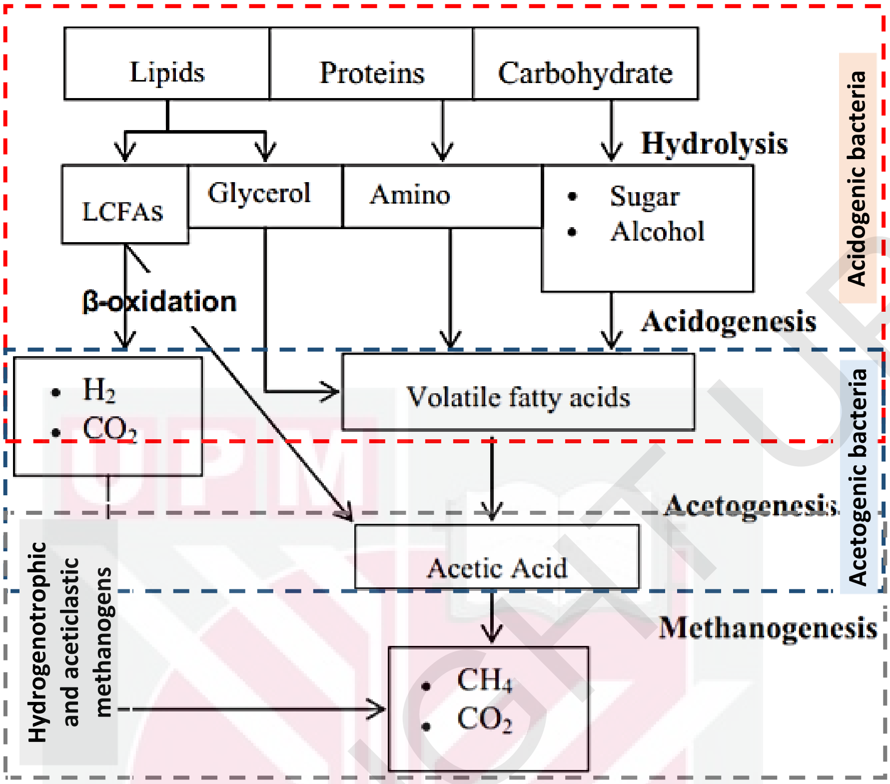

Figure 2.1: The metabolic pathway of lipid, protein and carbohydrate in AD.

In the next stage, which is the acidogenesis stage, long-chain fatty acids and amino acids produced by hydrolysis are used as a substrate for fermentative microorganisms to produce organic acids such as acetic, propionic, butyric, and other short-chain fatty acids, alcohols, H_2, and CO_2 or by anaerobic oxidizers during acidification of sugars (Kalyuzhnyi et al., 2000). Hydrolytic and acidogenic microorganisms grow at a rate ten times that of methanogens. In liquid-phase digestion, acidogenesis is usually the fastest reaction in the anaerobic conversion of complex organic matter. The conversion from organic substances to organic acids lowers the system's pH, which benefits acidogenic and acetogenic bacteria that prefer a slightly acidic environment with an optimal pH of 4.5 to 5.5 (Demirel & Yenigün, 2002).

Although increasing the thermal performance can improve hydrolysis and acidogenesis, it has the opposite effect on the acetogenesis stage. When the system is heated to increase the performance of hydrolysis and acidogenesis bacteria, volatile acid production can cause overload resulting in slower acetogenic reactions. Besides that, the methanogenic bacteria's ability to convert the volatile acids increases pH and causes inhibition in the acetogenesis and methanogenesis stages (Babaei & Shayegan, 2019; Gaby et al., 2017). Studies showed that all bacteria that β-oxidize long chain fatty acids (LCFA) belong to one of two families: *Syntrophomonadaceae* within the class of *Clostridia* or *Syntrophaceae* within the subclass of *Deltaproteobacteria*. It has been proven that LCFA degradation is the rate-limiting step in lipid degradation during AD and can inhibit the process at a high concentration (Ziels et al., 2015)

The third process is acetogenesis, where an increasing amount of hydrogen in the liquid causes the accumulation of electron sinks that cannot be absorbed directly by methanogenic bacteria. It must be degraded further by the hydrogen-producing acetogenic bacteria(Merlin Christy et al., 2014). Acetogenic bacteria are strict anaerobes that require an optimum pH of about 6, isolated primarily from anoxic environments, and use the acetyl coenzyme A pathway. They grow slowly and are sensitive to shifts in organic loadings and environmental changes. They require lengthy adjustment periods to new environmental conditions (Anukam et al., 2019).

Methane can be produced in two ways: cleaving acetic acid molecules to produce carbon dioxide and methane or reducing carbon dioxide with hydrogen. The last process is methanogenesis, the most critical stage in AD (Trisakti et al., 2017). The operating conditions, such as raw material composition, hydraulic retention time, temperature, mixing rate, and pH, significantly impact the methanogenesis stage. Overloading the digester, temperature changes, or introducing a large volume of oxygen can reduce methane production (Irvan et al., 2016).

2.4 Stages in Anaerobic Digestion

The stages in AD can be divided into one stage and two-stage. The two-stage is mainly as an alternative for a more efficient digestion (Li et al.,2014).

2.4.1 One-stage Anaerobic Digestion

The most basic system is the one-stage anaerobic digestion. It allows hydrolysis/acidogenesis and acetogenesis/methanogenesis to occur in the same reactor. A balanced anaerobic digestion process necessitates that the degradation rates in both phases be of equal size (Angelidaki et al., 1998). Numerous research has been conducted over the years to improve the process efficiency of conventional AD, such as the implementation of pre-treatments and adjustments in operating conditions for a more stable process (Azizi et al., 2019; Han et al., 2017; Zhang et al., 2016). This implementation has shown promising results; however, the conventional one-stage AD methods have several limitations. The two major communities of microorganisms, acidogens, and methanogens, are kept in a single system for a fine balance because they have vastly different nutritional requirements, growth kinetics, and sensitivity to environmental situations (Gioannis et al., 2017). This can result in inefficient hydrolysis and acidogenesis processes, reducing digestion performance and biogas recovery (Maspolim et al., 2016). Another drawback of conventional AD of organic solid waste is the slow rate of hydrolysis, which converts high molecular weight organic matter into simple compounds. Thus, long sludge retention times (SRT) are required, necessitating a large digester volume, which results in high operating and capital expenses for conventional AD (Han et al., 2017).

2.4.2 Two-Stage Anaerobic Digestion

A two-stage AD system can be used, with hydrolysis and acidogenic fermentation (stage 1) physically separated from methanogenesis (stage 2). This configuration allows for the establishment of optimal conditions for the acidogens and methanogens, optimizing the specific metabolic functions of each group, thus resulting in a better operating condition. The main advantages of the two-stage AD process over the single-stage AD process are superior stability under high organic loading rates, lower SRT requirement, higher bioenergy production efficiency, and a faster conversion rate(Akgul et al., 2017; Kang et al., 2016; Li & Jin, 2015).

Research conducted on the digestion of municipal solid waste and food-processing wastewater revealed that the two-stage AD has a higher methane yield and lower reactor volume compared with one-stage AD. It was also concluded that the two-stage AD performed better (Aslanzadeh et al., 2014). The proportion of carbon converted into biogas in the two-stage AD process was significantly higher than in the one-stage process. The kinetics of methanogenesis in the two-stage AD was also faster. Furthermore, the two-stage process generated higher content of CH_4 than the one-stage process. There was excellent resistance to change in influent concentration in both hydrogenotrophic and acetotrophic bacteria (Pham Van et al., 2020).

2.5 Challenges in Anaerobic Digestion

AD is a widely used process for converting biogenic material into energy. In recent years, there has been a significant increase in understanding of the microbial process for anaerobic digestion. However, some issues remain unresolved. The most common drawbacks of the biogas process are floating layers (Jiao et al., 2022), foaming(Krohn et al., 2022) , and over-acidification (Pedersen & Nyord, 2023).

2.5.1 Floating layers

Inadequate mixing, filamentous bacteria development, and fibrous substrate feeding contributed to floating layer production. The build-up of organic components in the layer and little intermediate exchange within the layer resulted in the production of volatile fatty acids (VFA), which impeded the microbial breakdown process in the floating layer(Lienen et al., 2013). Several disadvantages occur due to the floating layer occurrence in the digester. The digester's active volume is reduced, resulting in inefficient gas recovery (Lienen et al., 2013). Furthermore, pressure build-up in the digester can cause gas mixing devices to become clogged, gas pipelines to become fouled, and even the roof of the digester to be damaged. The economic costs of energy loss, manpower overtime, and cleaning costs was also a drawback due to floating layer problems (Ganidi et al., 2009)

2.5.2 Foaming

Foam in AD is a complex three-phase phenomenon caused by surface active materials such as solids constituents in the sludge and gas produced within the digester (Subramanian & Pagilla, 2015). In general, foam is a crude gas dispersion in a liquid. Most foam comprises a gaseous phase, with the liquid phase occurring between the gaseous bubbles in the lamellae. In biogas plants, two types of foam differ in size and difficulty of elimination. Foam with a large bubble diameter is easily combated through a reduction in food/substrate, as well as the use of mixing equipment such as stirrers. On the other hand, foam with tiny bubbles causes more problems because it is very stable and difficult to remove (Moeller et al., 2012).

2.5.3 Over-acidification

Over-acidification is a disruption in the AD process characterized by a decrease in digester pH due to fatty acid accumulation due to a disrupted methanogenesis step (Lienen et al., 2013) and an increase in acidogenic bacteria activity. Over-acidification is caused primarily by a biogas system overload with high organic loading rates or inhibitors in the digester, such as ammonia, sulfides, heavy metals, or phenolic compounds (Chen et al., 2008). This reduces the digester's buffering capacity(Appels et al., 2011). The pH variations significantly impact microbial growth and metabolic processes in AD; a pH drop below optimal levels caused by high VFA concentrations could result in lower biogas yield and, eventually, financial losses. It has previously been stated that methanogenesis bacteria are highly pH dependent and can only tolerate a limited pH range between 6.5 and 7.2, whereas the ideal pH for acidogenesis-bacteria survival and function could be between 4 and 6.5 (Xu et al., 2020). Thus, a pH lower than the stated value could cause over-acidification (7). Nonetheless, the emergence of the acidification phenomenon in AD reactors inhibits methanogenesis and disrupt the hydrolysis and acidogenesis steps (Li et al., 2009).

Over-acidification has also led to a decrease in digester performance fed with livestock manure. Excessive acidification in a full-scale AD reactor fed with livestock manure and agricultural residues significantly reduced daily electricity generation from 13.5 MWh/day to 6.9 MWh/day, corresponding to decreased biogas production and subsequent energetic utilization. The concentration of VFAs reached its highest value of 9196 mg/L during the study and had the most severe acidification (Moeller & Zehnsdorf, 2016).

2.6 Foaming in Anaerobic digestion

Several factors contribute to the deteriorating performance of a digestion system. Foaming is one of the problems that cause unstable operating conditions leading to disturbance in microbial activity and biogas production. Persistent foaming is commonly formed in the biogas reactor or pre-storage tank(Kougias et al., 2013). Besides that, persistent foaming also actively decreases the digester working volume and forms dead zones(Ganidi et al., 2009). These problems ultimately result in increased operational expenditures due to income losses, human resources over time, and maintenance costs (Kougias, Boe, et al., 2013). Moreover, foaming causes adverse effects on the environment due to the overflowing of digesters and loss of methane in the liquid phase from oversaturation(Kougias, Boe, et al., 2013).

Foaming in aerobic and anaerobic digestion is one of the food industry's most common operational problems for protein and lipid wastewater treatment (Boe et al., 2012). Foaming causes deterioration of the treatment process performance, mechanical failure, and maintenance costs. Wastewater from food industries such as food processing, palm oil mills, and abattoir are high in oil and grease, and protein induces severe foaming in their biological treatment system even though preceded by a pre-treatment system utilizing polymer flocculation (Muhammad Rahman, 2021).The treatment at mesophilic temperature and a high organic loading rate induced foaming (Moeller et al., 2018). Full-scale anaerobic digestion facilities opted for operation adjustments such as increasing operation temperature, which increases operating cost, or lowering organic loading rate at the expense of lower treatment throughput (Lindorfer & Demmig, 2016). The former can cause less stable operation; meanwhile, the latter can negatively impact the productivity of the products in such processing plants unless an additional wastewater treatment system is installed. Other strategies include the treatment of feed, the addition of antifoams, changes in mixing and feeding strategy, and installing nozzles and overflow pipes (Lindorfer & Demmig, 2016).

Pure liquids cannot foam unless a surface-active material is present, as the bubble produced is not stable. Thus, it bursts almost immediately. However, in a dilute aqueous solution where surface-active material is present, as the air-liquid interface expands and the equilibrium at the interface is disturbed, a restoring force is set up that tries to re-establish this equilibrium. This results from elasticity in the film so that it is less likely to break; hence, foam is established (Pugh, 1996). Surface-active agents include particles, polymers, specifically adsorbed cations, or anions from inorganic salts that can cause foaming even at low concentrations (as low as 10^{-9} M). Wastewater produced from food processing is high in lipids, protein, and carbohydrates, depending on the product's nature. These materials are hydrolyzed into LCFA, VFA, and amino acids and act as surface-active agents. Elevated levels can decrease surface tension, thus enhancing foaming potential(Boe et al., 2012; Lindorfer & Demmig, 2016). Moreover, an amino acid produced from protein hydrolysis in the anaerobic digestion process can stabilize the foam formed (Kougias, Boe, & Angelidaki, 2015).

2.6.1 Potential causes of foaming in AD

2.6.1.1 Microbial strain

The microbial processes in anaerobic systems are hydrolysis, acidogenesis, acetogenesis, and methanogenesis, which are processes carried out by combining multiple microbial strains to form a syntrophic interaction. However, such interactions can be easily disrupted, resulting in process imbalance (X. Guo et al., 2014). Sewage sludge is frequently used as a substrate in anaerobic digesters; thus, many *Gordonia* sp. and *Microthrix parvicella* bacteria are introduced into the system. Operating conditions such as temperature and loading rates increase the amount of *Gordonia* sp. and *M. parvicella* in the digester resulting in digester foaming (Garg et al., 2021). The foaming characteristics of these bacteria are related to their hydrophobic and structural properties. They have a hydrophobic cell surface that causes foaming to stabilize. *Gordonia* sp. is the most common foam-forming bacteria from the mycolate group that presents a high hydrophobicity characteristic (Guo et al., 2015; Petrovski et al., 2011). At the same time, *M. parvicella* sp is the most common foam former that consumes lipids found in food waste digesters. Due to its filamentous structure and hydrophobic surface, easy uptake of LCFA is possible with mixing (Kragelund et al., 2007; P. H. Nielsen et al., 2002). Besides their structural characteristics, these bacteria also produce surfactants in different amounts, causing foam stabilization (C. Jiang et al., 2021). The foam stabilization could occur through the reduction of surface tension and restrict the drainage of liquid in the foam surface, similar to hydrophobic particles (Heard et al., 2008).

2.6.1.2 Surface Active Agents

In anaerobic co-digestion systems, the feeding substrates contain various compounds. Among these compounds, proteins, acetic acid, and lipids have been classified as foaming agent that coats the bubbles in the digester resulting in stable foam formation.

Proteins are well known as one of the industry's most common classes of foaming agents. The high protein concentration at the liquid interface leads to the formation of aggregates and interactions. Proteins stabilize the foam and emulsions by forming a viscoelastic, immobile, adsorbed layer(Wilde et al., 2004). The mechanism of protein foam formation is based on the hydrophilic and hydrophobic characteristics of the protein, thus creating a protein film that holds the bubble in place(Foegeding et al., 2006). In a solution, proteins are commonly charged. At pH 7, basic amino acids in the proteins are positively charged; however, acidic amino acids are negatively charged. The ratio of the amino acids determines the net charge, thus contributing to foam stabilization in a system (Boe et al., 2012).

In the case of lipids, lipids are complex compounds that are highly hydrophobic. Due to their hydrophobic characteristic, they often attach to solid particles. The most common lipids found in wastewater processes are fats and oils. In AD, fats and grease are hydrolyzed into simpler compounds such as glycerol and fatty acids (Awe et al., 2018). However, due to their surface-active properties, lipids can potentially cause foaming. The hydrophobic end of the lipids is attracted to the air phase, and the hydrophilic end binds to the liquid phase leading to a decrease in surface tension, which leads to foaming when air bubbles are produced in the feedstock(Long et al., 2012). A study by Boe et al. (2012) reported that Na-oleate, classified as lipid, increased the tendency for foaming and foam stability in water and manure. It was highlighted that manure digesters with high protein and lipids have a high potential for foaming problems.

Volatile fatty acids are another type of surface-active agent that can promote foaming. The overloading of substances in the digester causes the accumulation of volatile fatty acids, such as acetic acid. Acetic acid is the predominant acid, accounting for approximately 85% of the volatile acid content in an anaerobic digester (Mahat et al., 2021). Ganidi et al. (2011) found that volatile fatty acids were potentially not essential component of AD foams. However, many researchers have identified acetic acid as a cause of foaming (Wongfaed et al., 2020) . Methanogenic bacteria are the only bacteria that utilize acetic acid in AD but have slow growth rates. Thus, causing an excess of acetic acid in the process. The accumulation of VFAs in the digester reduces surface tension and enhances foaming from the properties of free polic ends that demonstrate surfactant properties causing the gas bubbles to accumulate in the gas-liquid interface (Boe et al., 2012; He et al., 2017; Subramanian & Pagilla, 2014).

2.6.1.3 Unstable operating conditions

Many studies have reported that unstable operating conditions such as organic overloading, temperature fluctuation, and inadequate mixing have caused foaming in the anaerobic digestion system. Operational problems may occur during anaerobic treatments of effluents with high-fat content, such as sludge production with low methanogenic activity, enhanced foam formation, flotation, and biomass washout (Jeganathan et al., 2006; W. Li et al., 2018; Nakhla et al., 2003). Research carried out by Ganidi et al. (2009) shows evidence that some of these parameters contributed to the stabilization and initiation of foaming in an anaerobic digestion system.

The organic loading rate of the AD system is one of the primary causes of foam formation in AD (Ganidi et al., 2009; Kougias et al., 2014). High organic compounds in the digesters lead to the accumulation of excess surface-active agents such as lipids, proteins, and detergents that are not fully degraded by the bacteria. This can promote foaming to occur (Kougias et al., 2013; Lienen et al., 2014). Besides that, the increase in organic content in the system also promotes the activated sludge content, thus increasing the filamentous bacteria in the system (Pagilla et al., 1997). Shock or inconsistent loading rates temporarily causes high gas production rates and high ratios of carbon dioxide to methane content in the digester gas, inducing foam. Furthermore, an experiment conducted by Ganidi et al. (2011) revealed that an organic loading of 2.5 kg VS m^{-3} was a critical threshold for foam initiation for sludge originating from a non-foaming digester.

Besides, a system without a mixing component has a relatively higher chance of producing foaming (Lindmark et al., 2014; Stroot et al., 2001). Inadequate mixing causes inverse solid profile, dead zone formation, and reduction in reactor volume. This results in lower biogas production in shorter and longer periods. Besides that, inadequate mixing also leads to high sedimentation (McMahon et al., 2001), which could have led to the formation of scum and foam that can limit the release of biogas (Lienen et al., 2013). Thus, anaerobic digestion mixing becomes an essential aspect mainly while the process runs in a high organic loading rate and short hydraulic retention time (McMahon et al., 2001). An experiment conducted by Stroot et al. (2001) revealed that in lower or no mixing conditions, a foam layer developed after several hours of feeding. This layer did not interrupt biogas production but increased the process's working volume. Even though there are many advantages to mixing in AD, the mixing conditions must be optimized according to the digester requirements. Reducing dead zones and maintaining uniform viscosity and shear distribution is vital without affecting microbial flocs or the syntrophic interactions between bacteria during the AD process (Singh et al., 2021). Mixing equipment requires proper examinations and knowledge regarding the geometry of the impellers and the bacterial adaptions in such environments. This relationship between the microbial community and shear force in the system is hard to derive, thus causing detrimental effects on the system, such as lower biogas yield and loss of digester working volume (Singh et al., 2020).

Temperature fluctuations in AD are another crucial factor that should be considered as one of the causes of foam formation. Drastic temperature differences cause acute disturbances to the performance of the systems. This also causes unstable operations impacting the growth and metabolism of the microorganism(Rademacher et al., 2012; Shin et al., 2010). Generally, AD is carried out in two conditions: mesophilic at approximately 30-40°C and thermophilic at 55-60°C. Mesophilic conditions are more prone to have stable operations and are frequently adopted in the industry for better biogas production (S. K. Cho et al., 2013). Besides that, mesophilic processes have lower energy requirements and less sensitivity to shock load or toxic substances (Fernández Rodríguez et al., 2012).

In terms of foaming, thermophilic conditions have been reported to have lesser foaming properties attributed to the lower surface tension and viscosity of sludge, thus increasing foam drainage (Hayta et al., 2001; Dohányos et al., 2004). The primary factor that should be considered when foaming occurs at mesophilic temperature is that the foam matrix is lower than the temperature in a bulk phase, causing an increase in the growth of more *Nocardia* species in the foam matrix (Ganidi et al., 2009).

2.6.2 Mechanism of Foam Formation

Foam is defined as a gas dispersion in a liquid containing a high proportion (approximately 95%) of gas. The liquid phase is contained within a thin film between the gas bubbles. However, foam can only be produced after a specific concentration of a surface-active compound is exceeded. Surface-active compounds have amphiphilic properties, meaning their molecules acquire hydrophilic and hydrophobic functional groups. Surface-active compounds are innocuous at low concentrations (Moeller et al., 2012).

Initially, the foam may be considered as a temporary dilute dispersion of bubbles in the liquid. However, the structure gradually changes upon aging, and the bubbles transform into polyhedral gas cells with thin flat walls. The polyhedral are almost regular dodecahedra, and the interconnecting channels consist of a thin film of liquid between foam bubbles called lamella (Figure 2.2). Junction points of the interconnecting channels are known as Plateau borders (PB) (Pugh, 1996). Foams undergo two critical processes. In a slow process, water drainage is the flow of water from the foam phase driven by gravity and curvature, induced by a pressure gradient known as water drainage. The second is bubble rupture or coalescence, when lamella separates two bubbles producing fewer, more giant bubbles or a single bubble, and headspace rupture lower the total foam volume. The rate at which foams form and bubbles rupture determines the foam volume of the treatment system(Pelton, 2002).

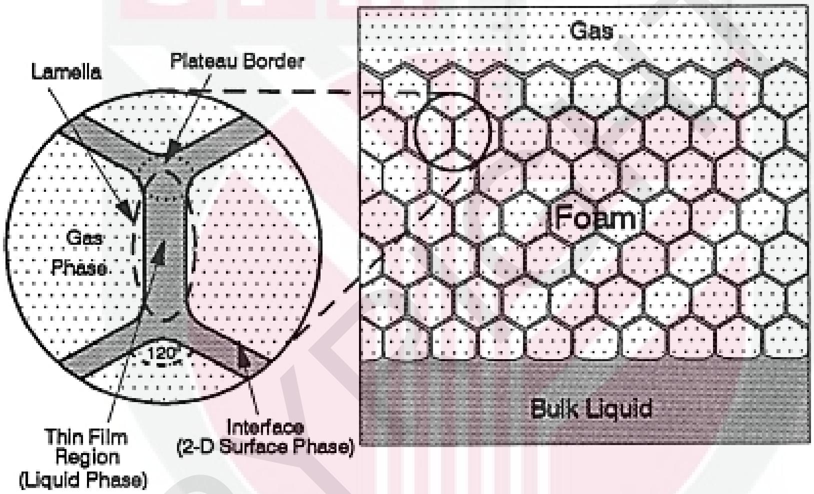

Figure 2.2: The structure of foam

2.6.3 Classification of Foam

The foams from biogas-waste plants were classified into two sizes, which are small and large bubbles (Moeller, Görsch, et al., 2012). It is simple to prevent foam with a reasonably high bubble diameter using mechanical disrupters and starvation. Foam with microscopic bubbles, in contrast, presents more challenges because it is incredibly stable and difficult to remove. The method of generating these foams and more comprehensive information on their chemical characteristics is not yet accessible (Moeller et al., 2012). The causes of the various bubble sizes and the mechanism underlying foam generation are not yet understood (Moeller, Görsch, et al., 2012).

2.6.4 Types of Foam in Anaerobic Digestion

The size of bubbles and their stability is the most common visual classifications of type of AD foam by plant operators in the case of uncontrolled foam structures in AD. Foams with large bubbles have a shorter lifespan than foams with smaller bubbles, which appear more stable (Moeller et al., 2012). The two types of foam categorized in AD digesters are conventional foaming and rapid expansion. Foaming bubble formation is similar in both cases, which is gas surrounded by liquid and solids/surfactant, but in the case of rapid expansion, it is gas holdup due to physical factors such as increased gas pressure in the digester that eventually is released by the sludge (Subramanian & Pagilla, 2015). Based on the filament, particulate, and surface-active material thresholds, whether these two types of foaming events are mutually exclusive or correlated is unknown.

2.6.4.1 Conventional Foaming

Conventional foaming is when the foam accumulates at the gas/liquid interface. It involves liquid, surface active agents as solids and gases (biogas) as shown in Figure 2.3. In the AD digesters, the three-phase foam bubbles are formed and stabilized by a coating of surface-active compounds, making them stable. CO_2 and methane are formed as biogas diffuses into existing bubbles and is entrapped during mixing, thus triggering foaming to occur on the surface (Campbell & Mougeot, 1999). This type of bubble formation necessitates high concentrations of surfactants, such as proteins and lipids that are highly hydrophobic compounds. (Marinova et al., 2002).

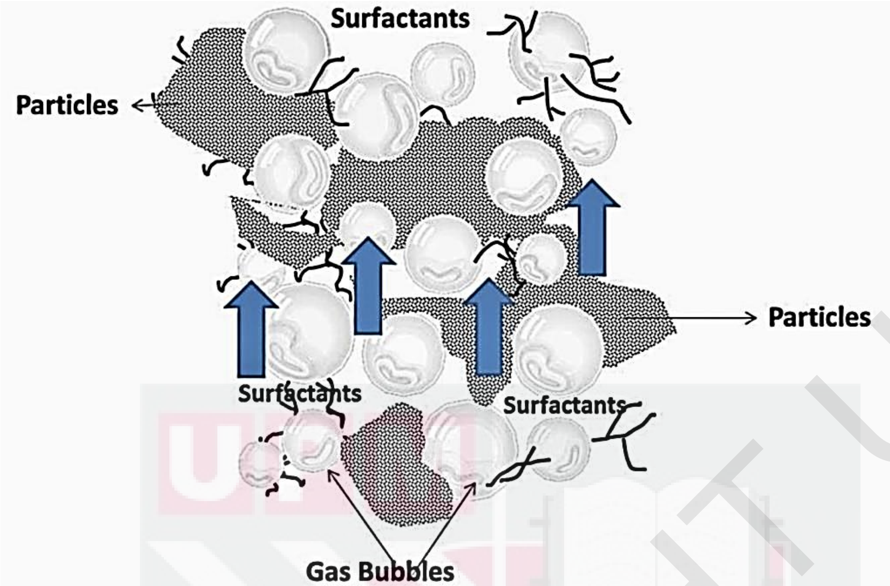

Figure 2.3: The schematic diagram of three-phase foam stabilized by surface-active agents (surfactants) (Subramanian & Pagilla, 2015).

2.6.4.2 Rapid Expansion Foaming

Pressure differences and gas saturation in the digester produce such rapid expansion bubbles. The digester's solid particles and cell-wall fragments may serve as nucleation sites. The conventional definition of foaming includes the stabilization step, in which surface-active agents form a coating around the gas bubbles creating a more stable foam. However, in this case, the foam is stabilized by the interaction of surface-active material and particles at the same time, thus resulting in a shorter lifespan of foam. The digester contents appear to increase rapidly in volume, resulting in digester overflows. The total volume of the digester can contract or expand in response to the amount of gas in the digester (Shen et al., 2015; Subramanian & Pagilla, 2015).

2.7 Lipid inhibition in Anaerobic Digestion

Lipids have shown promising advancements over the years in AD. It is a good substrate for producing higher amounts of methane than protein and carbohydrate substrates (Slopiecka et al., 2022). Studies on AD for high-strength lipid wastes have reported numerous negative impacts from lipid inhibition during the AD process, including inhibition by LCFAs, inhibition of acetolactic and methanogenic bacteria, transport limitations between soluble organic matter and microbial cells, sludge flotation, digester foaming, and other operational challenges (Ganidi et al., 2011; Mahat et al., 2021; Slopiecka et al., 2022). These problems ultimately increase operational expenditure and manpower requirements (Hu et al., 2018).

2.7.1 Lipid Degradation in Anaerobic Digestion

Hydrolytic bacteria in AD degrades the lipid in the first stage to convert triglycerides and long-chain fatty acids (LCFA) into free fatty acids and glycerol. For carbohydrates and proteins that can be easily degraded, simpler monomers such as sugars and amino acids exist. However, lipids undergo a β-oxidation pathway where coenzyme A breaks down LCFA into hydrogen and acetate, as shown in Figure 2.1. This pathway is a rate-limiting step in the process that causes the accumulation of LCFA on the surface of the digester. This phenomenon results in the accumulation of viscous organic matter on the digester's surface, known as scum(Thaku & Barjibhe, 2019). The difference between the rates of hydrolysis of lipids which occurs quickly, and β-oxidation of LCFAs results in process imbalance, thus disrupting microbial activity (Ma et al., 2015; Ziels et al., 2016).

2.7.2 Introduction to scum

Scums are often theorized as the same foam compound in previous research; however, in this study, scums and foams are two different compounds that were analyzed. Scums are viscous metabolic substances that accumulate on the surface of the digester. They have low density and are often white or brownish in color(Thaku & Barjibhe, 2019). Scum is typically produced in main settling tanks, and mechanical methods such as surface skimmers with water sprays are usually used to remove scum and other floating objects from the digester's surface (Gömeç, 2006). Because of the presence of insoluble components with floating qualities, such as fats and lipids, high suspended solids can also enhance the formation of scum layers and foaming (Gömeç, 2006). Scums cause several disadvantages to the digester, such as blockage of pipes, low biogas yield, and continuous release of organic matter in the effluent (Soares et al., 2019; Thakur et al., 2019). In a study, UASB reactors were used to treat fat and other low-density materials. Scum builds, and a layer of floating materials forms on the liquid surface of the settling compartment and within the GLSS device, reducing the digester's efficiency (Pereira et al., 2009). The difference between scum and foam are classified in Table 2.1.

Table 2.1: Summary of differences between foam and scum

Characteristics	Foam	Scum
Definition	Crude gas dispersion in a liquid	Viscous metabolic substances that accumulate on the surface of the digester
Phase	A liquid film encloses the gas phase	Liquid phase
Stability	Less stable, can be removed through mixing	More stable, requires physical removal methods
Viscosity	The viscosity of foam is strongly dependent on the quality of the foam	High viscosity
Formation	Gas production with the presence of surface-active agents such as proteins and lipids	Lipid inhibition

2.8 Methods to overcome lipid inhibition in AD

2.8.1 Feeding sequence

Studies have suggested that adjusting a feeding sequence when treating the substrate with LCFA can benefit microbial adaptation. This allows the microorganism to increase its tolerance to the substance in stages. This method maximized COD removal, and methane yield increased significantly (Ziels et al., 2017). However, the system performance deteriorated drastically when higher organic loading rates were used. It resulted in lipid inhibition, foam production, and fat, oil, and grease (FOG) accumulation in sludge (Jeganathan et al., 2006). Besides that, there were also signs of reduction in treatment efficiency with different variations in feeding sequences due to the accumulation of LCFA, biomass washout, and the effect of LCFA on microbial cell walls (Cirne et al., 2007; Samarasiri et al., 2016). Therefore, to implement a feeding sequence to reduce lipid inhibition in AD, it is crucial to assess an optimal feeding sequence to achieve a more stable operation (Ziels et al., 2017).

2.8.2 Enzymatic Hydrolysis

Enzyme hydrolysis offers a significant advantage in overcoming lipid inhibition in AD. Hydrolysis involves degrading insoluble organic matter such as lipids, carbohydrates, and proteins. It is carried out by hydrolytic microorganisms such as *Clostrida, Streptococci, Bifidobacteria, Enterobacteriaceae*, and *Bacteriocides* that break down the complex compounds into simple monomers such as fatty acids, sugars, and amino acids in anaerobic conditions(Weiland, 2010). However, the hydrolysis phase becomes a rate-limiting step due to the cross-linkage of glycan and peptide strands in cell walls(Lin et al., 2009). Enzymes in the hydrolysis stage increase the solubility of LCFAs, thus allowing a better methane conversion rate. It also resulted in higher COD removal and biogas production in poultry wastewater using enzyme hydrolases (Valladão et al., 2007). The usage of lipase in treating high lipid wastewater in an upflow anaerobic sludge blanket (UASB) reactor was efficient in reducing scum by 1.8 times, proving that lipase increases the solubility of LCFA in the digester without affecting the quality of the effluent(Soares et al., 2019). Besides that, lipase was favored to reduce lipid inhibition to improve the degradation of fatty acids up to 55% for high Fat, Oil, and Grease (FOG) wastewater, enhanced COD removal, and increased biogas production. However, it has been pointed out that the enzyme can be hard to extract and works better when treating vegetable oils rather than animal fats (Liew et al., 2020; Polizelli et al., 2013).

2.8.3 Ultrasonication

Ultrasonication is one of the most effective physical processes for disintegrating substrates in the AD process. Compression and rarefaction are produced in this sophisticated process as ultrasonic waves move through the medium. It then results in the development of microbubbles, which steadily grow over time in cycles until they reach a varying diameter at which they violently implode. This process is known as cavitation(Salama et al., 2019). Sonicated substrates released more organic carbon into the solution than unsonicated substrates, according to research on the effects of ultrasonication on FOG. The authors concluded that the dissolution of LCFAs in the aqueous phase, which subsequently increased biomethane synthesis, was most likely the cause of the rise in organic load. Another approach was used by Saifuddin & Fazlili, (2009), which was ultrasonication to treat palm oil mill effluent with a high lipid concentration. After 30 minutes of ultrasonic treatment at 20 kHz and 100 W, a maximum SCOD/TCOD ratio of 29% was reached, indicating enhanced biodegradability of the soluble organic material during the treatment. Even though the outcomes of the ultrasonication treatment did not lead to a consistent conclusion, the effectiveness of combining ultrasonication with other treatments has been established. Maximum hydrolysis of 78% was attained at dairy wastewater treatment using ultrasound and an enzyme as a catalyst to remove fat.

In contrast to these promising findings, Li et al. (2013) observed that ultrasonication had a detrimental impact. They investigated how ultrasonication affected two substrate types - FOG and kitchen trash even though co-digestion with FOG increased the amount of biomethane produced, ultrasonic pre-treatment of FOG before co-digestion did not increase the yield of biomethane.

2.8.4 Chemical Pre-treatments

Alkali pre-treatment is commonly applied in AD. The first reactions during alkali pre-treatment are solvation and saponification, which cause solids to swell (Carlsson et al., 2012). As a result, the specific surface area increases, and anaerobic microbes can easily access the substrates. The COD solubilization is then increased by simultaneous reactions such as saponifying uronic acids and acetyl esters and neutralizing various acids formed by particle breakdown (Kim et al., 2003). When alkali methods were used for pre-treating substrates, the biomass consumes some of the alkalies, so higher alkali reagents may be required to achieve the digestion time (Hendriks & Zeeman, 2009).

2.8.5 Antifoams

Antifoams are surface-active solid substances that can decrease the surface tension of the solvent. Generally, its function is to disrupt the stability of the liquid films in the foam, increasing the liquid drainage rate and thus enhancing foam destruction (Marinova et al., 2002). Antifoams are commonly used in the industry as a solution to overcome foaming. Chemical antifoam is a "fast" action antifoam typically consists of oil (polar and non-polar), hydrophobic solid particles, or a mixture of both. Most foam-control agents for aqueous systems are formulated of several functional components. 'Carrier oils' is the base liquid comprising 75-90% of the formulation. It can lower surface tension, but its primary purpose is to carry 'active ingredients to reach the layer of surface-active material and spread on the foam surface. Silicone oil is a particularly effective antifoam ingredient due to its low surface tension, thermal stability, inertness, and total insolubility in water. Its pure form shows little foam-inhibiting ability (Höfer & Ecosiris, 2012). Thus, natural antifoams have gained interest by many researchers over the years. Further details were discussed in subsection 2.9

2.9 Natural Oils as Antifoams

Natural oils such as canola/rapeseed oil, sunflower oil, and coconut oils are the common antifoaming agents that have been studied by previous researchers with positive outcomes (Kougias et al, 2015; Thakur et al., 2019). There is positive outcomes in defoaming efficiency and overall digester performance which are discussed in the following subsections

2.9.1 The foam rupture mechanism

The bridging-dewetting mechanism is theorized for the foam film rupture of this antifoam. The solid particle first comes in contact with the two opposite surfaces of the foam film, forming a 'solid bridge' between them. If the solid particle is sufficiently hydrophobic, it is dewetted by the liquid, and the three-phase contact lines come in direct contact with each other – the foam film gets perforated at the particle surface(Karakashev & Grozdanova, 2012). This type of antifoam is called a 'fast' antifoam, where defoaming takes less than a minute. It is postulated that the antifoam globules enter the foam films' surface, facilitate the lamella's thinning, and destroy these films soon after the globule entry via the bridging-dewetting mechanism described above (Denkov, 2004).

The defoaming mechanism of oil-based antifoams without the addition of hydrophobic substance proceeds via water drainage but is faster compared without the antifoam addition. The oil globules are suggested first to escape the foam films into the neighboring Plateau border, get trapped there, and be compressed by the walls of the shrinking PBs as a result of water draining from the foam and eventually entering the walls of the PBs, causing the rupture of the neighboring films (Denkov et al., 2000; Marinova et al., 2012).

Digester foaming often occurs due to the hydrophobic characteristics of lipids in the digester in the form of salt. A study by Thaku & Barjibhe, 2019 revealed that scum levels could be reduced by 32% with the addition of canola oil due to its defoaming potential. The digester also had elevated biogas production and methane yield. This could be correlated with the reduction of foam in the digester when it cooperated with natural oils.

2.9.2 Rapeseed Oil

Rapeseed oil when digested with manure supplemented with proteins (gelatine) or lipids (Na-oleate), rapeseed oil positively affected the bio-methanation process at all tested concentrations. Oleic acid had lower defoaming effectiveness than rapeseed oil than the other tested defoamers. Because rapeseed oil contains saturated (i.e., palmitic acid) and polyunsaturated-like linoleic acids in addition to monounsaturated fatty acids like oleic acid, this discrepancy could be related to the chemical features of the antifoam. As a result, a combination of various fatty acids may have improved rapeseed oil's propensity to destabilize foam by 40% in protein reactors. (Kougias et al 2015).

2.9.3 Palm Oil

Palm oil consists of mainly palmitic acid (16:0) at 39.3-47.5% and Oleic acid (18:1) at 36-44%, as shown in Table 2.2. This composition seems promising as palmitic acid has a high emulsification activity that can potentially reduce scum formation. Besides that, crude palm oil also contains excess lipase enzymes that can help in the hydrolysis stage (Tambun et al., 2019). Besides that, the fatty acid linoleic acid is also reported to be more inhibitory than oleic acid (Templer et al., 2006). Thus, the lower linoleic acid composition in palm oil is beneficial in this case.

Table 2.2: The fatty acid composition of different natural oils.

Fatty Acids	Chain	Rapeseed	Corn	Sunflower	soybean	palm
Oleic acid	18:1	51-70%	20-42.2%	14-39.4%	17-30%	36-44%
Palmitic acid	16:0	2.5-7%	8.6-16.5%	5-7.6%	8-13.5%	39.3-47.5%
Linoleic acid	18:2	15-30%	34-65.6%	48.3-74%	18-59%	9-12%
Linolenic acid	18:3	5-14%	0.05-2%	0.05-0.3%	4.5-11%	0.05-0.5%
Myristic Acid	14:0	0.05-0.2%	0.05-0.3%	0.05-2%	0.05-2%	0.05-2%

2.9.4 Sunflower Oil

Sunflower oil was investigated for its defoaming efficiency in manure digestion fed with raw cattle manure and glucose as a substrate. The research findings were that sunflower oil successfully broken-down foaming in the digester. However, the fatty acid composition of sunflower oil (Table 2.1) makes it a bad candidate for methane-producing digester, as linoleic acid causes inhibition (Templer et al., 2006). Besides that, oleic acid is also an essential component in natural oils that help rupture foam in the digester (Kougias et al., 2013). In sunflower oil, the oleic acid composition is significantly lower.

2.9.5 Coconut Oil

Thaku & Barjibhe, 2019 investigated the effect of coconut oil addition to scums in one-stage anaerobic digestion; the result was a reduction of only 1%. The author concluded that adding coconut oil showed moderate methane and biogas production results. However, it did not have a significant impact on the scum levels.

2.9.6 Summary of Explanation

Many researches have been conducted over the years on using natural oils in anaerobic digestion. The literature summaries for different types of natural oils are discussed in Table 2.3.

Table 2.3: The summary of the explanation for natural oils

Natural Oils	Explanation	Reference
Rapeseed Oil	Dosage of 0.5% v/v reduced foam in manure digester. The fatty acid composition of rapeseed oil can reduce scum levels.	Kougias et al., 2015
Palm Oil	High emulsification activity and excess lipase enzyme can potentially reduce scum formation.	Tambun et al., 2019

Table 2.3: Continued

Coconut Oil	The addition of coconut oil in single-stage AD showed moderate methane production; however, no significant impact on scum.	Thakur et al., 2019
Sunflower Oil	The oleic acid composition is significantly lower, whereas the linoleic acid composition is higher, thus causing methane inhibition.	Templer et al., 2006

2.9.7 Advantages and Disadvantages of Using Natural Oils

The long-term use of vegetable oils or biodiesel was utilized successfully in many plants to combat foaming. The advantages and disadvantages are briefly explained in Table 2.4.

Table 2.4: Advantages and disadvantages of the usage of natural oils

Advantages	**Disadvantages**
Low material cost	Poor dispersion
Less expensive for long-term usage	A higher dosage is required for a longer application time.
It can reduce surface tension on the surface of the digester	High viscosity
Low toxicity	Easily metabolized
Microorganisms can achieve adaptation relatively fast, thus not causing disturbance to the digester.	
Easily obtainable	

Vardar-Sukan, 1998 reported that the usage of natural oils was inefficient since they have high viscosity, are easily metabolized, and have poor dispersion, but this was not the case (Kougias et al., 2013); the application of natural oil significantly reduced foaming and surface tension of the digester.

2.10 Surfactants as Natural Emulsification Agents

The industry has various types of surfactants, such as surfactin, glycolipids, and saponins. However, rhamnolipids and saponin have shown promising advancements in AD processes.

2.10.1 Introduction to Surfactants

Surfactants are surface-active materials that contain both hydrophobic and hydrophilic groups. It is now extensively studied as it is a good alternative for increasing the performance of a digestion system. Surfactants are generally divided into two groups which are chemical surfactants and biosurfactants, or divided into cationic, anionic, non-ionic, and zwitterionic surfactants (Wang & Mulligan, 2009). Some examples of chemical surfactants are sodium dodecyl sulfate (SDS), sodium, dodecyl benzene sulfonate (SDBS), linear alkylbenzene sulfonates (LAS), and Tween 80(S. Jiang et al., 2007; Madamwar et al., 1991; Sharifi et al., 2014; Sun et al., 2019). However, the chemical surfactant can be toxic to the environment and quickly accumulate at the water interface. The toxicity of chemical surfactants causes inhibition to methane-producing bacteria by 50% (Nie et al., 2017), thus beginning the emergence of biological surfactants with lesser environmental toxicity, higher biodegradability, and efficiency. It has also been proven to improve methane production in a digester by improving hydrolysis and acidogenesis (Y. Wang et al., 2021). Besides that, chemical surfactants can change sludge's structural properties to the denaturation of proteins unfolded in the chain expansion (Bhuyan, 2010). There are many categories of biosurfactants, as shown in Figure 2.4.

Glycolipid biosurfactants (BS) have excellent surface-active capabilities such as emulsifying, dispersing, solubilizing, foaming, penetrating, wetting, self-assembly, and unique biological features. Glycolipid bio-based substances have been studied as ecologically friendly BSs or functional bio-based substances with superior interfacial and biological parameters(Morita et al., 2016). One of the most common glycolipids biosurfactants used is rhamnolipid, a low molecular weight biosurfactant that's useful for lowering surface and interfacial tension (Jahan et al., 2020). Surfactant molecules effectively reduce intermolecular interactions between solvent molecules when they replace water or oil molecules along the contact, lowering surface or interfacial tension (Dobler et al., 2016).

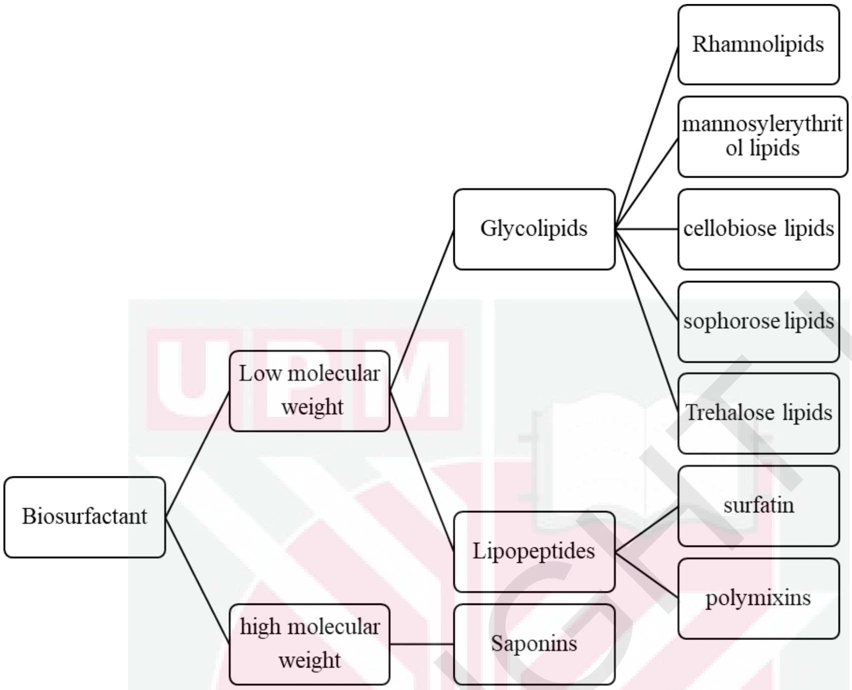

Figure 2.4: The categories of bio-surfactants (Jahan et al., 2020; Morita et al., 2016).

Surfactants will initially partition into the interface, lowering the system's free energy by (a) lowering the energy of the interface and (b) removing the hydrophobic parts of the surfactant from water contact. Subsequently, as the surface area of the surfactants increases, and the surface tension decreases, the surfactants begin aggregating into micelles, lowering the system's free energy by decreasing the contact area of the surfactant's hydrophobic parts with water (Volkering et al., 1997). The characteristics of different categories of bio-surfactants are shown in Table 2.5. The bio-surfactants used in this study are rhamnolipid and tea saponin. The high surface activity of the biosurfactants enhances the solubilization of organic matter and improves hydrolyzation (Huang et al., 2015). The presence of surfactant molecules at air-water interfaces reduces the surface tension of the solution. Surfactant also creates micelles, which are small aggregates of surfactant molecules. Single molecules can be found in aqueous solutions at low concentrations. Surfactant molecules cluster together beyond a specific concentration, called the critical micelle concentration (CMC), forming aggregates of 20-200 molecules (Volkering et al., 1997). This phenomenon causes the reduction of surface tension in the interface; thus, it is likely to reduce scum formation.

Table 2.5: The characteristics of surfactants.

Biosurfactants	**Glycolipids**	**Lipopeptides**	**Saponin**
Origin	Microorganism	Microorganism	Plants
Structure	covalently bonded with carbohydrates and consist of monosaccharides, disaccharides, and polysaccharides	seven amino acids where its hydrophobic tail is composed of a 13 to 15-carbon long chain	Saponins consist of an aglycone unit linked to one or more carbohydrate chains
Examples	rhamnolipids, sophorolipids, and trehalolipids	Surfactins, polymixins	Tea saponin
Properties	excellent surface and biological activities	antimicrobial, antibacterial, and antiviral properties	Surface active properties

Other properties of biosurfactants are that they are capable of self-assembly and the formation of micelles. This increases their specificity and enables them to have morphologically distinct structures from one another, such as sphere-shaped, rod-shaped, and worm-shaped micelles. They are more efficient than chemical surfactants because their critical micelle concentration value is much lower, which allows them to reduce surface tension even more and more effectively. This indicates that less biosurfactant is required for a maximum reduction in surface tension. From Table 2.4, the suitable bio-surfactants for this research are glycolipids and saponin.

2.10.2 Rhamnolipid (RL)

Glycolipids are the most commonly studied because they show many advantages in terms of biodegradability, toxicity, structural diversity, surfactant activity, and stability in extremes of temperature, pH, and salt concentration (Shi et al., 2021). There are contradicting effects of rhamnolipid in AD, several studies have suggested that the addition of rhamnolipid in the digester has caused an inhibition in methane production and lowered methane production (Johnravindar et al., 2022), but a study conducted by Xu et al., 2021, revealed that the inclusion of RL during the acidogenic phase improved methane generation during the methanogenic phase in two-phase AD of WAS. The cumulative methane output rose from 100.4 mL to 168.9 mL with an increase in RL dose from 0 to 0.04 g/g TSS.

2.10.3 Tea Saponin (TS)

Tea saponins are a type of saponin that are natural non-ionic surfactants found in the roots, stems, leaves, blossoms, and seeds of Camellia plants. They have been shown to improve the bioavailability of microorganisms(Yu & He, 2018). Tea saponin appears to inhibit methanogenesis and perhaps interfere with interspecies hydrogen transfer between the anaerobic methanogens (Guo et al., 2008; Prabhudessai et al., 2009). However, Hiroki et al. (2004) revealed that adding saponin improved anaerobic digestion, particularly by boosting the rate of acidogenesis. The high degradation rate of saponin supports the theory that it was gradually degraded during digestion(X. Huang et al., 2015a). This finding is consistent with saponin being easily biodegraded into its hydrolysate (Mølgaard et al., 2000).

2.11 Summary of literature

Foaming and scum are operational severe problems faced by many AD biogas plants. Common causes of foam and scum are usually caused by process instability, such as temperature fluctuations, improper mixing, and organic overloading. However, surface-active agents such as proteins and lipids are the more prominent cause of scum and foam. According to the literature study, proteins and lipids are the two substances most frequently found in high-strength wastewater; thus, it is essential to comprehend how they affect scum and foam independently. Besides that, biosurfactants and natural oils are an environmentally friendly approach in overcoming scum and foam. Based on previous studies, the most suitable natural oil used were rapeseed oil and palm oil, whereas for the biosurfactant, rhamnolipid, which is the most common type of glycolipid, and saponin used.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter aims to explain the rationale for interpreting data according to the one-factor-a-time approach. There is various instrumental and experimental design that was implemented in this research in order to achieve the results of this study. The chapter also represents the depth of understanding of factors needed for implementing data analysis for sampling. Besides that, this chapter also provides the flowchart of the overall methodology for the chosen variables (Figure 3.1). The characterization of substrate and inoculum was the focus of this study's preliminary phase. The effect of protein and lipid on the anaerobic biodegradability of food processing wastewater (FPW) and synthetic wastewater were analyzed with the scum and foam production. A laboratory-scale two-stage anaerobic digester was used to investigate its efficacy in treating FPW and synthetic wastewater at different protein and lipid concentrations. The investigation method involves the use of domestic anaerobic sludge from the Indah Water Konsortium (IWK) as a seed with synthetic wastewater and food processing wastewater from a food processing industry to reduce the organic substances in the effluent. The scum reduction efficiency of various antifoams was investigated in this study.

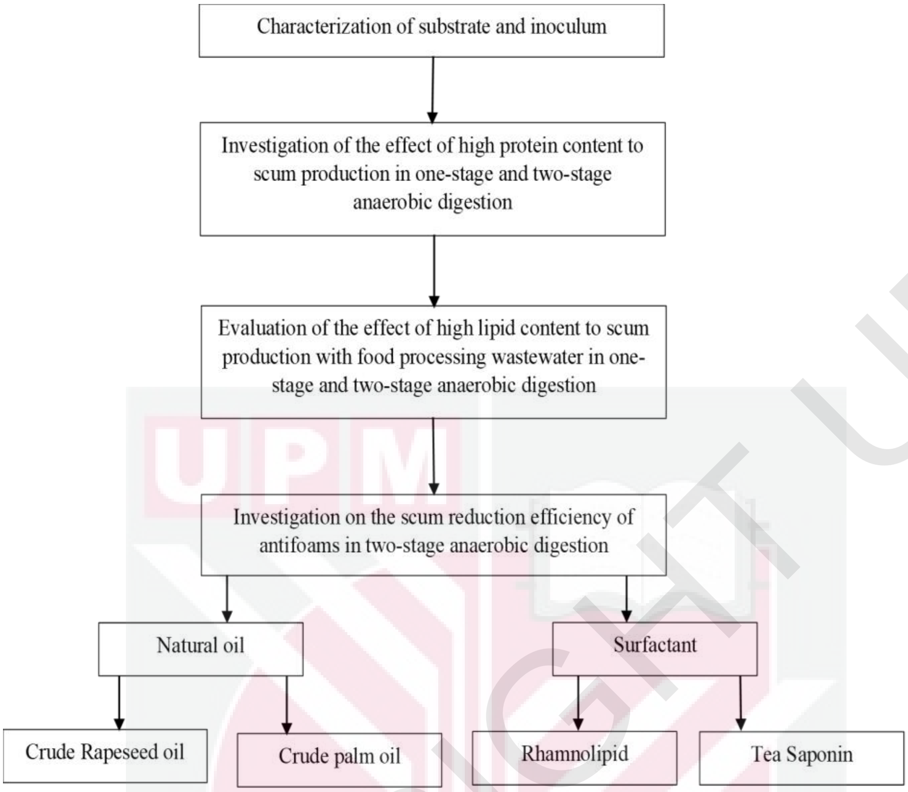

Figure 3.1: The overall research flowchart

3.2 Materials

The raw materials used in this study were sourced from a local industry, a municipal wastewater treatment plant, and a farm in UPM. At the same time, the chemicals were purchased from local suppliers. The details are explained in the subsequent subsections below.

3.2.1 Inoculum

Two types of inoculums were used in the preliminary study via one-stage batch anaerobic digestion (AD). The purpose of using two types of inoculums was to identify the effect of protein to foaming in the anaerobic digester. It was reported the addition of gelatine as a protein source induced foaming tremendously in manure digestion (Boe et al., 2012). Proteobacteria, Bacteroidetes, and Methanosaeta were found in large numbers in the bacterial and archaeal communities from the anaerobic sludge employed in this work, according to high-throughput 454 pyrosequencing of total DNA carried out in a previous study

(Mahat et al., 2021). Proteobacteria has functional significance for the degradation of active pollutants, while methanosaeta is an arecheal community of important methanogens to produce methane (Khan et al., 2019)

3.2.1.1 Anaerobic sewage sludge

The anaerobic sewage sludge used in this study was obtained from an anaerobic sludge Tank, sampling point digester 3 of the municipal sewage treatment plant Indah Water Konsortium, located in Kuala Lumpur, as shown in Figure 3.2. The digester runs at mesophilic temperature (35-38°C) with a flow rate of 300 – 400 m^3/day. Figure 3.2 shows a collection of the anaerobic sludge (AS) and the collected sample (Figure 3.3).

Figure 3.2: Anaerobic sludge Tank, sampling point digester 3, municipal wastewater treatment plant (WTP), Indah Water Konsortium.

Figure 3.3: AS collected from anaerobic sludge Tank Digester 3.

3.2.1.2 Cow Manure (CM)

CM used in this research was collected from a waste collection Tank in the UPM farm located in Serdang, Selangor, as shown in Figure 3.4. There was no treatment at the site prior to this collection Tank at the farm. The CM was filtered using a kitchen sieve to remove solid content upon collection. The filtered sample was diluted with distilled water at a 1:1 ratio and kept at a temperature of 2-4°C. The CM was acclimatized before conducting the research, according to Subsection 3.4.

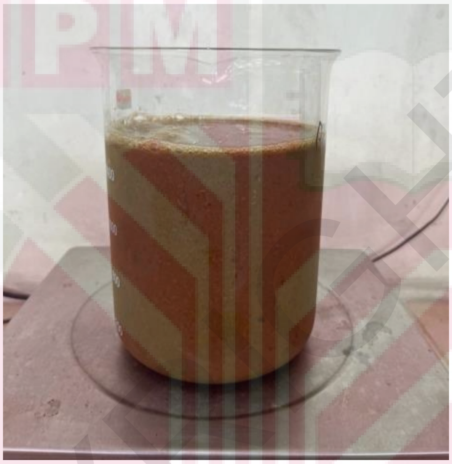

Figure 3.4: CM collected from waste collection Tank in UPM farm

3.2.2 Substrate

3.2.2.1 Synthetic Wastewater (SW)

The SW used in this research was prepared with yeast extract (Marmite®) and meat extract (Bovril®). The SW was used as a substitute for food processing waste as the ingredients are similar. However, SW has low lipids and fats content, thus becoming a good substrate for this research. The ingredients of the products are presented in Table 3.1; they had a brownish appearance, as shown in Figure 3.5.

Figure 3.5: SW prepared from yeast extract (Marmite®) and meat extract (Bovril®)

Table 3.1: Composition of meat and yeast extract for synthetic wastewater

Ingredients Meat Extract	Per 100 g
Protein Carbohydrates -sugars Total Fat -saturated fat Sodium Potassium	13.3 g 24.4 g 0.7 g 0.1 g 0.1 g 3510 mg 1200 mg
Yeast Extract	
Protein Carbohydrates -sugars Total Fat Sodium Fiber Thiamin Riboflavin Niacin Folic acid Vitamin B12	38.4 g 19.2 g 0.5 g 0.1 g 4.3 g 3.1 g 5.3 mg 7.0 mg 160 mg 2500 µg 15 µg

The synthetic wastewater also contained micronutrients that are beneficial to archaea. Previous studies on anaerobic digestion also used yeast extract as the synthetic wastewater medium and have proven that yeast extract is a good biostimulant for anaerobic microorganisms (Piao et al., 2019; Wagner et al., 2012). The stock solution for the synthetic wastewater was prepared using a 1:1 ratio of Marmite and Bovril at 470 g in 2 L distilled water. The chemical oxygen demand (COD) was measured and used as an indicator in the preparation of the experiments.

3.2.2.2 Food Processing Wastewater (FPW)

The substrate used in this research was collected from Ramly Food Processing Sdn. Bhd., located at Batu Caves, Selangor, Malaysia (Figure 3.6). Ramly Processing Sdn. Bhd. Located in Selangor is a food processing facility. In order to treat their wastewater, firstly, they use chemical pre-treatment, such as polymer, to coagulate the oil and grease (O&G) particles from the wastewater. The pre-treated wastewater is next processed using dissolved air floatation (DAF) to remove suspended particles, oil and grease, and other insoluble contaminants from water slurries. Finally, the water slurries are sent through a sequencing batch reactor (SBR) to reduce organic contaminants for biological treatment before being released into water sources.The rich lipid and protein content produced in the food processing industry makes it a suitable substrate for this research. It was stated that lipid-rich wastewater has a high potential to produce methane compared to carbohydrate and protein-rich wastewater(Abomohra et al., 2022). The raw sample was collected from the waste collection Tank, as shown in Figure 3.7. Before the substrate was used, any solidified matter was removed through filtration using a kitchen sieve to avoid pipe clogging and accumulation of organic matter in the digester. The wastewater was then kept at a temperature of 2-4°C in a chiller (Hi-Ten) to minimize the degradation of organic matter prior to conducting the experiments. As much of the FPW was used in this study, several batches were taken as needed. Therefore, each batch was first characterized for chemical oxygen demand (COD), fat, oil, grease, and protein content. The substrate was diluted according to the required COD for each part of the research.

Figure 3.6: Raw food processing wastewater collection from Ramly Food Processing Sdn Bhd.

Figure 3.7: Raw food processing wastewater

3.2.3 Chemicals and reagents preparation

The reagents used in this research are chemical mixtures that can facilitate a reaction for specific analysis. Before experimenting, the prepared chemicals and reagents are listed in Table 3.2 below.

Table 3.2: The list of chemicals and reagents

Chemicals	Brands	Country	Purpose
Sulphuric Acid (H_2SO_4)	R&M Chemicals	United Kingdom	
Hydrochloric Acid (HCl)	R&M Chemicals	United Kingdom	
N-Hexane (C_6H_{14})	R&M Chemicals	United Kingdom	
Potassium Dichromate ($K_2Cr_2O_7$)	Systerm	Malaysia	Standard potassium dichromate
Silver Sulphate (Ag_2SO_4)	Systerm	Malaysia	
1,10-Phenanthroline-ferrous sulphate-complex	Systerm	Malaysia	Ferroin indicator solution
Mercury (II) Sulphate ($HgSO_4$)	R&M Chemicals	United Kingdom	

Table 3.2: Continued

Ammonium iron II sulfate (($(NH_4)_2Fe(SO_4)2.6H_2O$)	Systerm	Malaysia	Standard Ferrous Ammonium Sulfate titrant (FAS)
Sodium Hydroxide (NaOH)	R&M Chemicals	United Kingdom	
di-Sodium Tetraborate $Na_2B4O_7.10H_2O$	R&M Chemicals	United Kingdom	Borate Buffer Solution
Boric acid (granular) H_3BO_3	R&M Chemicals	United Kingdom	Indicating boric acid solution
Methylene red	R&M Chemicals	United Kingdom	Indicating boric acid solution
Methylene Blue	R&M Chemicals	United Kingdom	Indicating boric acid solution
Ethanol	R&M Chemicals	United Kingdom	Indicating boric acid solution
Anhydrous Sodium Sulphate	Systerm	Malaysia	Oil & Grease
Buffer solution pH 4.0, 7.0 and 10.0	Systerm	Malaysia	pH meter (Calibration)

3.3 Anaerobic Digesters Configuration and Operating Conditions

There are two types of anaerobic digesters used; one-stage and two-stage digesters. One-stage digesters were used to identify the scum and foam tendency when fed with SW and FPW using two inoculums. Meanwhile, only one inoculum, activated sludge, was used in the two-stage digester.

3.3.1 One-stage Anaerobic digester

The one-stage digestion experiment was carried out as a batch process (Figure 3.9). 1 L and 10 L Scott bottles with a working volume of 80% were used. The system's temperature was regulated using a water bath (Memmert, Germany) to maintain mesophilic temperature (35-38°C). Mesophilic conditions are more prone to have stable operations and are frequently adopted in the industry for better biogas production. Besides that, mesophilic processes have lower energy requirements and less sensitivity to shock load or toxic substances (Rodríguez et al., 2012). After every feeding, the digester was sparged with nitrogen gas (N_2) for 30 seconds before being sealed tightly with parafilm and a rubber stopper to keep it in anaerobic condition. The other end of the tubing was clipped before inserting inside the inverted measuring cylinder to collect the biogas using the water displacement method, as shown in Figure 3.8. Distilled water with a pH of 2 was used as a solution to trap the biogas and avoid any dissolvent of the biogas composition.

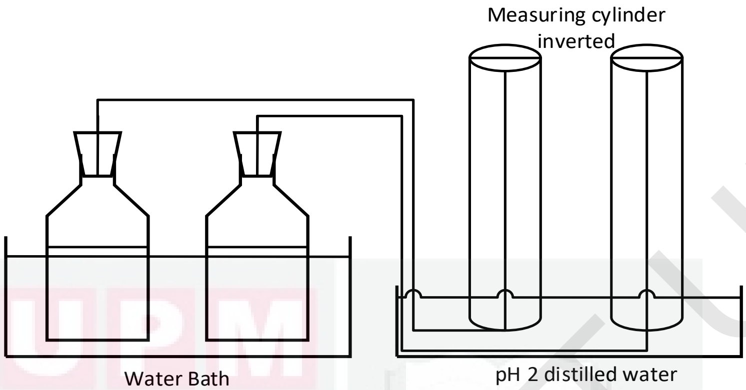

Figure 3.8: Schematic diagram of one-stage anaerobic digestion system using water displacement method

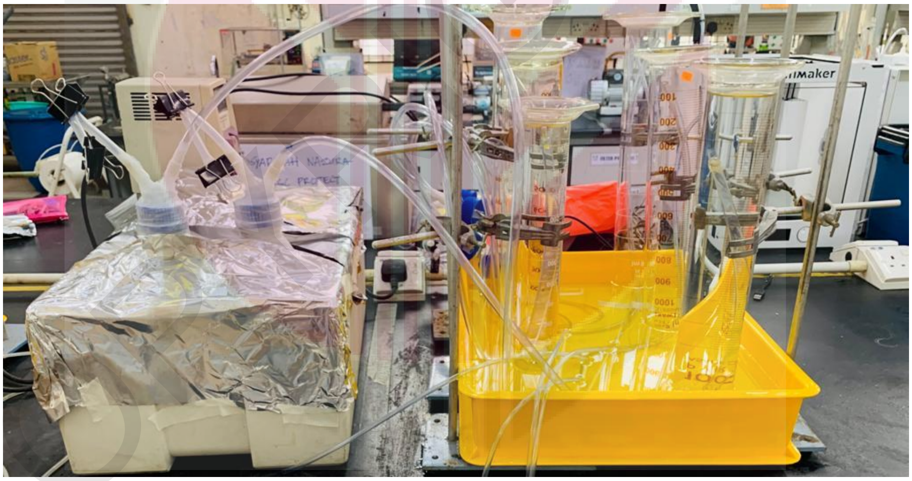

Figure 3.9: Experimental set-up of one-stage anaerobic digestion using water displacement method

3.3.2 Two-stage Anaerobic Digester

Figure 3.10 and 3.11 depicts the schematic diagram of a submerged Dynamic Anaerobic Membrane Bioreactor (DAnMBR) (Mahat et al., 2021). However, this research did not use the membrane as the focus was on scum production in Tank 1. The up-flow anaerobic two-stage reactor has a total working volume of 30 L and is made up of two cylindrical Plexiglas vessels. The working volume is divided into two parts. The first stage is a 10 L acidogenic reactor (AR) with 20 cm diameter x 44 cm height dimensions.

Meanwhile, the second stage is a methanogenic reactor (MR) measuring 30 cm in diameter by 46 cm in height and holding a volume of 20 L. The headspace height of both reactors is 3 cm. The substrate was fed using a peristaltic pump (Longer Pump, BT600-2J). The sampling process from Tank 1 and Tank 2 was done daily after 24 hours of feeding. A submerged heater (Eco Aquarium Heater) was used to maintain and control the system's temperature (35-38°C). Depending on the hydraulic retention time, the influent was fed intermittently for 8 hours per day at a flow rate ranging from 10 to 30 L/day hydraulic retention time (HRT) since intermittent feeding has shown better performance compared to continuous feeding (Alkarimiah et al., 2011). The HRT was gradually decreased at 0.8, 0.6, and 0.5 (resultant OLR of 0.18, 0.26, and 0.75 g COD/L.day, respectively) to allow inoculum adaptation (20). The experiment was initiated after the inoculum achieved a steady biogas production and COD removal of 90%. The experiment was conducted on a duration of 4 days that is the maximum number of days required before the scum is required to be physically removed to avoid blockage in the digester. Besides that, as per observation, scum occurs on top of the digester within few hours of feeding.

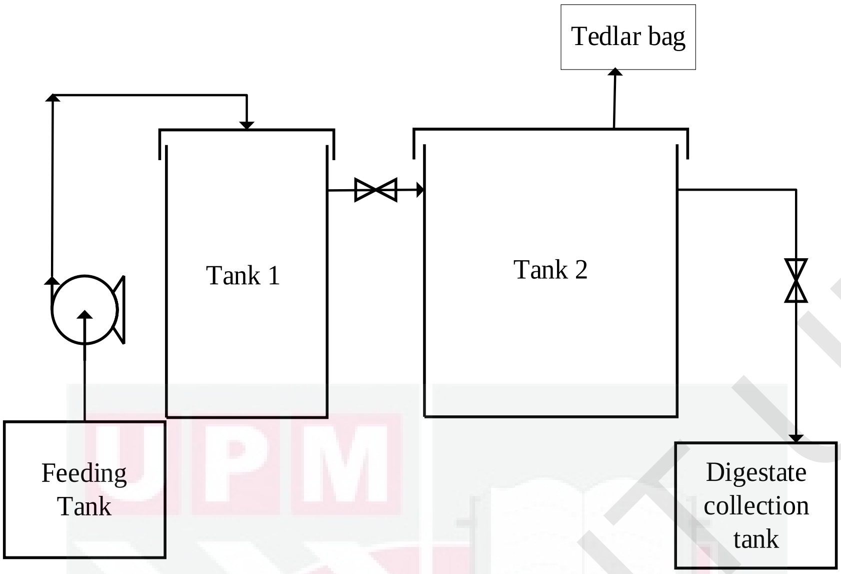

Figure 3.10: Schematic diagram of two-stage anaerobic digester

Figure 3.11: Experimental set-up of two-stage anaerobic digester

3.4 Acclimatization of Inoculums

The acclimatization of inoculums is a crucial part of a stable operating condition. A study conducted by Rajput & Sheikh (2019) revealed that acclimatized sludge has a better process performance compared to sludge used directly from Tanks. There was an increase in biogas yield, buffering capacity, and volatile solid removal.

3.4.1 Acclimatization of Inoculums in One-Stage AD

The experimental procedure to conduct the study for batch one-stage AD was through the acclimatization process of inoculums as the setup explained in Section 3.3.1 above. The inoculums (AS and CM) collected were acclimatized through a series of steps. Firstly, 400 mL of inoculum was collected and added with 400 mL of water at mesophilic temperature (35-38°C) to allow the bacteria to digest the previous supernatant. The substrate-to-inoculum ratio used in this study was 1:1, which recorded the highest COD, BOD, TS, and VS removal in a previous study (Mahat et al., 2020). Low biogas collected (around 10 mL/day) and COD removal of above 90% indicates the success of this step which took around a week. In the next step, each inoculum was fed with SW as the substrate at different COD concentrations to allow the bacteria to adapt to a new environment. Since the pH of SW was between 4.5 and 5.5, the pH was altered by adding 3 N NaOH to maintain a pH of 7. Hydrolysis occurs five times faster at neutral pH (Duong et al., 2019). The acclimatization started at COD of 1500 mg/L and then increased to 2500, 3500, and 4500 mg/L. The COD was adjusted by using the dilution of a stock solution prepared in section 3.2.2.1. The acclimatization phase lasted for 15 days. Once the COD removal was 90% at the highest organic loading (4500 mg/L COD) and the biogas production achieved 50% of the digester working volume (above 400 mL), the acclimatization phase deemed completed. This process was repeated multiple times to ensure the digester performance was stable.

3.4.2 Acclimatization of inoculum in two-stage AD

The raw sludge was first let to be acclimatized in a Scott bottle with a working volume of 0.8L and water with a 1:1 ratio before introducing SW. The SW was introduced at first in a low COD concentration (2000 mg/L) in the Scott bottle. Once it reached a COD removal percentage of 90%, the sludge was transferred to the two-stage digester. The reactor was fed with SW at a slow increasing rate for the sludge to adapt to the new environment. 2000 mg/L COD of SW was fed first with an HRT of 0.5 days, then increased to 3500 and 5000 mg/L COD once 90% COD removal was achieved at each increment. The low 2000 mg/L COD of SW was used as that was the observable positive outcome of biogas production in the digester. Lower COD resulted in less biogas due to low organic content. The experiment on the effect of protein on scum and foam was conducted (Subsection 3.5) once the reactor had a stable operation with a COD removal of 90% in the effluent.

3.5 Effect of protein on foaming and scum in AD

The effect of protein concentration on scum and foam production was assessed by introducing SW and gelatine as the protein source. The study was conducted through a one-stage and two-stage anaerobic digestion system. CM was only used in one-stage AD, while AS was used in both one-stage and two-stage digesters. The reason was that CM was used to differentiate and observe the foaming tendency with AS, as a previous study mentioned that CM produces prominent foaming with a high protein concentration substrate (Kougias et al., 2013). The protein alteration in this study was using gelatine.

3.5.1 Effect of Protein on Foaming and Scum in one-stage AD

The foaming tendency experiment was conducted through a batch process with a digester working volume of 0.8 L. The protein concentrations investigated in this research were 3, 9, 15, and 21 g/L. The temperature of the system was maintained at a mesophilic temperature (35-38°C) using a water bath. The experiment was conducted in triplicate for an accurate result.

3.5.2 Effect of Protein on Foaming and Scum in two-stage AD

The digestion was carried out in a two-stage digester through a continuous process. The system was fed with SW and gelatine according to the required protein concentration intermittently at a hydraulic retention time (HRT) of 0.5 days. The system's temperature was set to mesophilic temperature (38-40°C). The pH of the substrate was kept constant at pH 7, and the system's alkalinity was monitored throughout the experiment. The protein concentration examined in this part of the research was 3, 6, 9, 12, and 15 g/L. At the highest protein concentration (15 g/L) it is observed that the anaerobic sludge in the system was floating on day 3 of feeding. Thus, the experiment was stopped at this concentration of protein to avoid further digestion problems. These concentrations were chosen for this experiment as these are the standard protein concentrations found in the food waste and processing industries (22).

3.6 Effect of lipid to foaming and scum in one- and two-stage AD

The effect of lipid concentration on scum and foam production was assessed by introducing FPW into the digesters. The study was conducted through a one-stage and two-stage anaerobic digestion system with 10L and 30L working volumes, respectively. AS was used as an inoculum in the digesters. The duration was set to 4 days as, according to observation. The concentration of the lipid was assessed at 2, 4, 6, 8, and 10 g/L as these were the achievable concentrations for lipids using FPW. Beyond 10 g/L, the COD and scum levels were too high for the digester.

3.6.1 Acclimatization of one-stage digester inoculum using FPW

The effect of lipid concentration on scum and foaming was started with the acclimatization of anaerobic sludge to FPW. The FPW has low pH. Thus, the pH was increased to 7.0 using 3 N NaOH. COD of FPW fed started from 1500 mg/L and then increased to 2500, 3500, and 4500 mg/L was used for acclimatization. The COD of FPW was adjusted by the addition of water. Each COD concentration was deemed completed once the fed COD reached a removal percentage of 90% and the biogas volume was above 50% of the digester working volume. The acclimatization process in this part of the research was conducted using a 10L digester. The increase in digester working volume was because the production of scum in lipid-rich wastewater was not visible in a small bottle. Only traces of scum were observed; thus, the digester working volume was increased to have a better input.

3.6.2 Acclimatization of two-stage digester inoculum using FPW

For the next part where FPW was needed to be introduced. The FPW was fed into a two-stage reactor and progressively added together with SW until the feed contained 100% FPW. The pH of the raw FPW was about 3.0 - 4.5, thus, 3.0 N NaOH was used to raise the pH of the feed solution to roughly 6.8 - 7.0 before feeding it into the reactor. The influent combination of SW and FPW had an average COD content of 3000 mg/L, and OLR was determined to range between 2.0 and 2.5 g COD/L.d using HRT of 0.5 days throughout the first half of the acclimation period. Increasing the average influent COD to 4500 mg/L resulted in an OLR of 2.50-3.50 g COD/L.d, which was followed in the second half. The acclimatization phase was 15 days long. The treatment phase began immediately after the digester established a stable state with 90% COD elimination. The different sample of FPW was prepared by dilution of the raw sample. The dilution was carried out according to the COD of the initial raw sample. The presence of scum in the digester was manually skimmed off to avoid blockage of pipes. This was carried out at least once a week, depending on the extent of scum presence (33).

3.7 Effect of antifoams on scum and foam in AD

The antifoam was applied in the two-stage digester for four days. There are two categories of antifoams: natural oils (rapeseed oil and palm oil) and biosurfactants (Rhamnolipid and Tea saponin). The antifoams were dosed by mixing them with the substrate prior to feeding.

3.7.1 Natural Oils

Natural oils are concluded to have good scum reduction capability. A study by Thakuri et al. (2019) concluded that the higher oil concentration resulted in higher defoaming and descuming potential. In this research, crude rapeseed oil and crude palm oil were used in the two-stage AD digester. The operating conditions of the digester were similar to section 3.6. The lipid concentration chosen as blank for this part of the research was 6g/L since it had the highest biogas production. Higher dosages of natural oils had a high defoaming efficiency based on the study by Kougias et al. (2014), which used manure digestion. However, this study aims to investigate the effects of natural oils on two-stage AD inoculated with AS

3.7.1.1 Rapeseed Oil (RO)

The rapeseed oil (RO) used in this research was crude rapeseed oil obtained from golden arawana, Shanghai, China. In the two-stage digester, the operating conditions were similar to those in section 3.6. The applied dosage was 0.25 and 0.5% v/v. RO is a combination of monounsaturated fatty acids such as oleic acid and saturated and polyunsaturated-like linoleic acids. As a result, the combination of various fatty acids improved rapeseed oil's propensity to destabilize foam in aqueous solution. Thus, fatty acid in natural oils plays a crucial role in the defoaming property. Theoretically, the higher the dosage of rapeseed oil used, the higher the foam and scum removal efficiency was reported. However, when 0.5% dosage was applied, the biogas volume reduced significantly, indicating an inhibition in Tank 2. A lower dosage (0.25% v/v) was applied later on to improve the performance of the Tank 2.

3.7.1.2 Palm Oil (PO)

As previously mentioned, the fatty acid structure in natural oils is the main contributor to the scum and foam reduction ability. Hence, palm oil was used in this research as it had palmitic acid, oleic acid, and low linoleic acid composition. However, there was no significant reduction in scum; however, there was inhibition in Tank 2, so a lower dosage was not implemented. The crude palm oil used in this research was collected from Able Perfect Sdn. Bhd. in Telok Panglima Garang.

3.7.2 Bio-surfactants

As an amphipathic substance, surfactants can improve hydrolysis efficiency by separating large sludge compounds and releasing the encapsulated hydrolase, allowing more material transfer for a better acidogenesis process(He et al., 2019). Two types of biosurfactants used in this research are Rhamnolipid (RL) and Tea saponin (TS).

3.7.2.1 One-stage Preliminary study of biosurfactants

Rhamnolipid with a dosage of 0.02, 0.04, and 0.06 g g^{-1} TSS and tea saponin with a dosage of 0.05, 0.10, 0.15 g g-1 DS was first experimented with using a preliminary test to analyze the inoculum's performance. The preliminary study introduced different rhamnolipid and tea saponin dosages into a one-stage digester at 0.8 L working volume. The inoculum used was AS, and the substrate was FPW at a 1:1 ratio. The study was conducted at 38-40°C and pH 7. The observations were biogas accumulation, protein removal, COD removal, Total VFA, and pH. Each concentration was carried out in duplicate for an accurate reading. The optimum dosage was then used for the two-stage AD digester. The rhamnolipid used in this study was obtained from Wuhan Golden Kylin Industry & Trade CO Ltd., China. It had a purity of 95% with a brown liquid appearance (CAS Number: 869062-42-0). The components of the rhamnolipid obtained were glycolic anionic biosurfactants produced by the fermentation process of *Pseudomonas aeruginosa* in rapeseed oil. The tea saponin was bought from the same company in powder form with a purity of 98%. The tea saponin was extracted from tea seed, making it a plant-based biosurfactant.

3.7.2.2 Two-stage AD with biosurfactants

The optimum dosage from the preliminary study was applied for the two-stage digester. This part of the research's operating conditions was similar to section 3.6. The lipid concentrations of the substrate (FPW) were maintained at 6 g/L.

3.8 Analytical methods

3.8.1 Chemical Oxygen Demand (COD)

COD is a measurement of the amount of oxygen in a sample that can be consumed in a reaction with oxidizing agents. COD typically reflects the number of organics present in anaerobic digestion. The COD reduction can be used to assess the efficiency of anaerobic digestion; COD reduction can reflect the amount of degradation occurring within an anaerobic digester, as it reflects the consumption of organics. COD was measured using the Standard Closed Reflux Titrimetric Method (5220 C), as described in the Standard Method (APHA, 2005). This procedure is applicable to COD values ranging from 40 to 400 mg/L, and sample dilution was required for concentrations that exceeded the measurable range.

The COD reflux reactor (HACH, DRB 200) was preheated until the temperature reached 150^{o}C prior to the analysis. After being removed from the chiller, the sample must be at room temperature before proceeding with the test. The samples are prepared in a test tube according to the appropriate dilution factor. 2.5 mL of samples (or 2.5 mL distilled water for blank preparation) were pipetted into each test tube, followed by 1.5 mL of potassium dichromate (Systerm). Each test tube was then gently mixed. Before digestion, 3.5 ml of sulfuric acid reagent was pipetted into each sample. The test tubes were then placed in the COD reactor for 2 hours at 150^{o}C. After two hours, the solution is transferred into a 250 mL conical flask for titration after being cooled at room temperature for 30 minutes. Next, 1-2 drops of ferroin indicator (Systerm) were added, followed by titration with 0.1 M ferrous ammonium sulfate (FAS) titrant until the color turned from yellow or light green to reddish-brown. Equation (3.1) depicts the calculation for COD concentration.

$$\text{COD (mg/L)} = \frac{(\text{A}-\text{B}) \text{ X M X } 8000}{\text{sample volume}} \qquad (3.1)$$

Where *A* is the volume of FAS titrated for blank (mL), *B* is the volume of FAS titrated for sample (mL), and *M* is the molarity of FAS which is calculated using Equation (3.2).

$$M = \frac{\text{Volume of potassium dichromate (K2Cr2O7)}}{\text{Volume of 0.1 M ferrous ammonium sulfate (FAS)}} \text{ x } 0.1 \qquad (3.2)$$

The reagent standard potassium dichromate was prepared by adding 500mL distilled water with 4.903 g of $K_2Cr_2O_7$ (Systerm, primary standard grade) and 167 mL of concentrated H_2SO_4 (R&M chemical, 95-98%) and 33.3 g $HgSO_4$ (R&M chemicals) after let cool the mixture was diluted to 1L. The FAS titrant was prepared by adding 39.2 g $(NH_4)_2Fe(SO_4)_2.6H_2O$ in distilled water with 20 mL of concentrated H_2SO_4 (R&M chemical, 95-98%). After the mixture cooled down to room temperature, it was diluted to 1 L. Lastly, the sulphuric acid reagent was prepared by adding Ag_2SO_4 (Systerm, technical grade) to concentrated H_2SO_4 (R&M chemical, 95-98%) at the rate of 5.5 g Ag_2SO_4 /kg H_2SO_4. Let the mixture dissolve overnight for 1 to 2 days.

3.8.2 Protein Analysis

Protein analysis was performed to measure the protein concentration in raw food processing wastewater, substrate, influent, and effluent. The protein analysis in this research was conducted according to the Bradford assay protocol. 0.25 mL sample was mixed with Bradford reagent (R&M Chemicals, RMstain). After 5 minutes, the absorbance of the liquid was measured using a UV-Spectrophotometer (Shimadzu, Model-1800) at an absorbance of 595 nm. Distilled water was used as blank. The protein concentration was calculated from the standard curve prepared before conducting the research, as shown in Figure 3.12. The absorbance of the samples was duplicated.

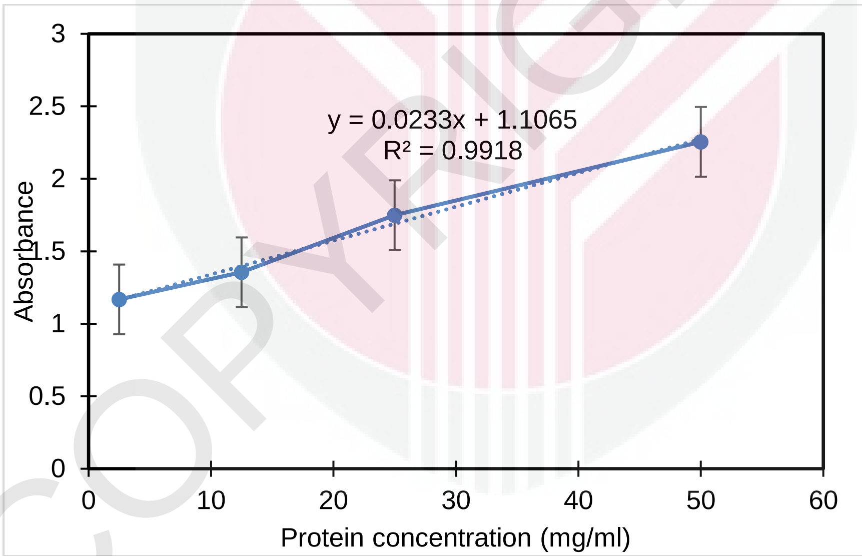

Figure 3.12: The protein standard curve

3.8.3 pH

Enzymatic activity or digester performance is influenced by pH. Acceptable enzymatic activity of acid-forming bacteria occurs above pH 5.0, but the sufficient enzymatic activity of methane-forming bacteria does not occur below pH 6.2.

Most anaerobic bacteria, including methane-forming bacteria, perform well within a pH range of 6.8 to 7.2. Although anaerobic digester efficiency is considered efficient within the pH range of 6.8 to 7.2, it is best when it is within the range of 7.0 to 7.2. pH values below six or above 8 are restrictive and somewhat toxic to methane-forming bacteria. A variation in the system's pH can alter the efficiency and cause a system failure. A high alkalinity level is required to maintain a stable pH(Martín-González et al., 2013). The pH in this research was maintained at an optimum range. The pH was measured using a pH meter (pH-80, HM Digital). Before experimenting, the pH meter was calibrated using pH 4, 7, and 10 buffer solution.

3.8.4 Ammoniacal Nitrogen (NH_3-N)

The ammoniacal nitrogen was measured using the APHA Method 4500-C as mentioned in the Standard APHA method. A sample volume of 50 mL topped up with distilled water up to 250 mL was placed in a beaker. 12.5 mL of borate buffer solution was added, and the pH was adjusted to 9.5 using 0.01 N sodium hydroxide (NaOH) or 0.01 N sulphuric acid (H_2SO_4). The solution was heated in a 250 ml round bottom flask with a heating mantle connected to a conical flask with 25 mL of indicating boric acid solution. The round bottom flask and conical flask were connected through a condenser to collect the distillate. The distillation process was carried out until 125 mL of distillate was collected. The distillate was then diluted with distilled water up to 250 mL and prepared for titration. The solution was titrated with 0.02 M H_2SO_4. The endpoint was determined upon reaching a pale purple color. The amount of sulphuric acid was recorded. The method was repeated with blank using distilled water. The NH_3-N was calculated using Equation (3.3).

$$NH_3\text{-N (mg/L)} = \frac{(A-B)\ X\ 280}{\text{sample volume (mL)}} \quad (3.3)$$

Where *A* is the volume of titrated 0.02 N sulfuric acids (sample), mL, and *B* is the volume of titrated 0.02 N sulfuric acids (blank), mL

The reagents in this analysis were prepared by first adding 500 mL sodium tetraborate (R&M Chemicals) (9.5 g powder in 1 L distilled water) and 88 ml 0.1 N NaOH and then diluted to 1 L. For the indicating boric acid solution, 20 g powder boric acid (R&M chemicals, granular) in 1 L with 10 ml mixed indicator solution. The mixed indicator solution is a combination of 200 mg methyl red (R&M chemicals. Standard grade) in 100 ml ethanol (R&M chemicals) and 100 mg methylene blue (R&M chemicals) in 50 ml ethanol (R&M chemicals). The final solution was diluted to 1 L.

3.8.5 Alkalinity

Alkalinity is defined as an ability of a system to resist massive changes in pH and maintain an optimum level. Sufficient alkalinity is essential for proper pH control. A fluctuation in alkalinity can be caused by the accumulation of organic acids that fail to be converted into methane, a slug discharge, and waste that inhibits sludge activity(Magdalena et al., 2019). The main species that contribute to alkalinity fluctuations are carbonate or bicarbonate systems. Two-point titration method was proposed as an indicator to monitor alkalinity ratios. A build-up of VFAs would be directly linked to the destruction of the bicarbonate buffering capacity. Total alkalinity (TA) and partial alkalinity (PA) would be related to bicarbonate alkalinity. The intermediate alkalinity (IA) difference is related to VFAs alkalinity (Ripley et al., 1986). The IA/PA ratio below 0.3 is required to maintain a steady operating condition(Martín-González et al., 2013). The alkalinity was calculated using Equation (3.4)

$$\text{Alkalinity} = \frac{\text{A X N X 50,000}}{\text{sample volume (mL)}} \tag{3.4}$$

Where, *A* is the volume of titrated 0.02 N sulfuric acids (mL), and *N* is the molarity of H2SO4 (0.02 N).

3.8.6 Fat, oil & grease (FOG) analysis

The Standard Oil and Grease (5520 B) Partition-Gravimetric method was used to determine the concentration of fat, oil, and grease (FOG) in the treated and untreated samples (APHA, 2005). A separating funnel was filled with 500 mL of samples (e.g. permeate, raw wastewater). The oil and grease in the samples were extracted using 20 mL N-hexane (R&M chemical, analytical reagent) and vigorously shaken for 2 minutes while alternately opening the cap to depressurize the funnel. After the supernatant bottom layer was eliminated, the separated upper layer containing the organic layer was retrieved through filtration. The organic layer was filtered through a filter paper (NICE, 101), filled with one spoon of anhydrous sodium sulfate (R&M chemicals, standard grade) to remove any excess moisture in the sample, into an empty ceramic dish using gravity filtration through a glass funnel. The organic solvents were then evaporated in a water bath at 85°C for 10 minutes, then drying in an oven at 102°C for about 10 minutes. The samples were removed and cooled in a desiccator until they reached room temperature. The weight of the beaker containing the oil residue was measured. Equation (3.5) was used to calculate the O&G content in the sample.

$$\text{O\&G (mg/L)} = \frac{\text{(A−B) X 1000}}{\text{sample volume (mL)}} \tag{3.5}$$

Where *A* is the final weight of beaker and *B* is the initial weight of the beaker with oil-residue

3.8.7 Total Volatile Fatty Acid (VFA) Concentration

The total volatile fatty concentration was calculated using the APHA method 1992 5560-C (APHA, 1992). 100 mL of the sample was diluted with 100 mL of distilled water. Next, 5 mL of concentrated sulphuric acid (R&M chemical, 95-98%) was added. The sample was transferred into a round bottom flask connected to a condenser. The distillate was collected up to a volume of 150 mL. The pH of the collected distillate was measured. The titrant used was 0.02 N NaOH. The titration's endpoint was when the sample's pH reached around 8 to 8.3. The total VFA was calculated as in Equation (3.6)

$$\text{Total VFA (mg/L)} = \frac{(A-B) \times 0.1 \times 60000}{100 \times 0.7} \qquad (3.6)$$

Where *A* is the initial volume of 0.02N NaOH, mL, and *B* is the volume of titrated 0.02 N NaOH, mL

3.8.8 Biogas Accumulation

The volume of biogas produced (daily and cumulative) was determined using the water displacement method with an inverted measuring cylinder (Figure 3.5). The volume of water displaced in the container equals the volume of gas. The gassing device was connected to the digester on one end and an inverted graduated cylinder containing water with a pH of 2. The gas volume calculated equals the amount of water displaced in mL (Kabaivanova et al., 2022).

3.8.9 Foaming Tendency

The foaming properties of the samples were determined using an aeration-based foaming assay, as shown in Figure 3.13. The foaming tendency (mL-foam/(mL-air min)) was calculated by dividing the volume of foam (mL) immediately after aeration by the airflow rate (mL/min) (Boe et al., 2012).

Figure 3.13: The aeration-based foaming tendency assay

3.8.10 Total solid and volatile solid

Solids analyses are crucial for monitoring compliance with regulatory agency wastewater discharge limitations as well as for controlling biological and physical wastewater treatment processes. Total solids refer to the material residue left in the vessel following a sample's evaporation and subsequent drying at a specific temperature in an oven, whereas volatile solid refers to the weight loss after ignition in the furnace at high temperatures.

The Standard Total Solids Dried at 103-105°C (2540 B), and Standard Fixed and Volatile Solids Dried and Ignited at 550°C (2540 E) as stated in the Standard Method (APHA, 2005) were the foundation for the measurement of TS and VS. Prior to analysis, the weighed, preheated evaporating dish was ignited cleanly for one hour at 550°C in a muffle furnace. A well-mixed sample's measured volume was pipetted into a plate that had already been pre-weighed. The sample was dried to a constant weight after being placed in an oven (Memmert, Interscience Sdn. Bhd.) for around 6 hours at a temperature of 103-105°C. Equation (3.7) and (3.8) was used to calculate the test results. Additionally, the TS result was further used to determine VS by igniting the sample in the oven for 30 minutes at a temperature of 550°C.

$$TS\ \frac{mg}{L} = \frac{(A-B) \times 1000}{\text{sample (mL)}} \qquad (3.7)$$

$$VS\ \frac{mg}{L} = \frac{(B-C) \times 1000}{\text{sample (mL)}} \qquad (3.8)$$

Where *A* is the weight of the empty dish (mg) , *B* is the weight of the dry sample with the dish after being ignited at 105°C, and *C* is the mass of the sample with the dish after being ignited at 550°C.

3.8.11 Scum reduction efficiency

The scum reduction efficiency was calculated using the method mentioned by Thaku & Barjibhe, 2019. The initial and final volume of scum was determined using the average of the circumference of the reactor at 4 different points as shown in Figure 3.14. The volume of scum before and after the addition of antifoams was recorded, and the percentage of scum reduction was calculated using Equation (3.9).

$$\text{Scum reduction} = \frac{\text{Volume before antifoam addition} - \text{volume after antifoam addition}}{\text{volume after antifoam addition}} \quad (3.9)$$

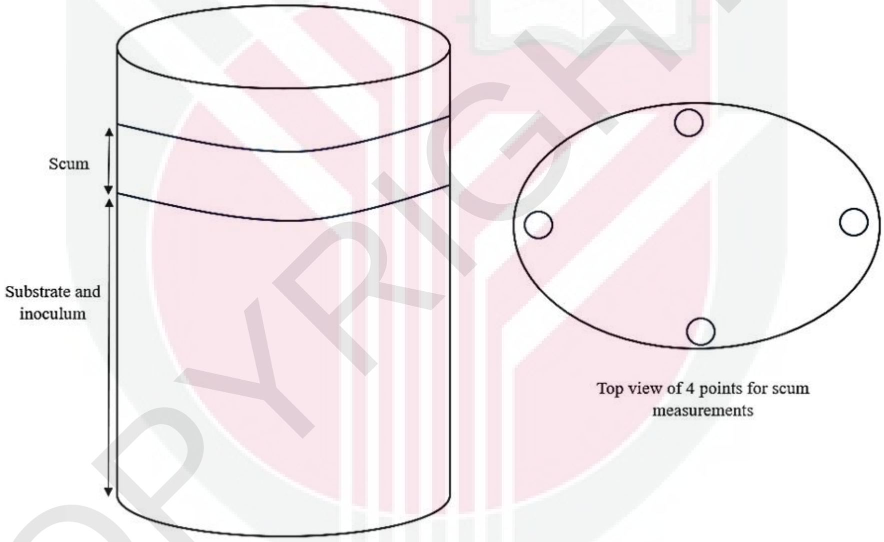

Figure 3.14: schematic diagram of 4 points measured in Tank 1 for scum

3.8.12 Fourier Transform Infrared Spectroscopy (FTIR)

Analyses using Fourier transforms infrared spectroscopy (FTIR) were utilized to identify the major organic function groups and identify their constituent components. In order to identify the functional groups, the samples (In liquid form) were sent for analysis using an FTIR spectrometer (Nicolet 5700, Thermo Electron Corporation, USA).

3.9 Statistical Analysis

3.9.1 Analysis of Variance (ANOVA)

The type of ANOVA used in this study was one-way ANOVA, a two-sample t-test generalization analysis. It is carried out to determine if two samples of a factor have the same mean through the t-test. The one-way ANOVA also examines if the means are statistically significant, and it tests the null hypothesis as shown in equation 3.10

$$Ho : \mu_1 = \mu_2 = \mu_3 = \ldots \mu_k \qquad (3.10)$$

Where *k* is the number of groups and *μ* is the group mean

3.9.2 Standard Deviation

The term "standard deviation" refers to measuring the data's dispersion from the mean. A low standard deviation implies that the data are grouped around the mean, whereas a large standard deviation shows that the data are more dispersed. The standard deviation was calculated using equation 3.11.

$$\sigma = \sqrt{\frac{\sum(xi-\mu)2}{N}} \qquad (3.11)$$

where *σ* is the population standard deviation, *N* is the size of the population, xi is each value of the population and *μ* is the population mean.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

This chapter elucidates the results of the objective of this research. The first sections explain the effect of protein on foam and scum in one-stage anaerobic digestion (AD) with synthetic wastewater (SW) and gelatine. The following section demonstrates the effect of protein on scum and foam production in two-stage AD with a similar substrate. Next, the effect of lipids was investigated in one-stage and two-stage AD using food processing wastewater (FPW) as a substrate. Finally, the effect of two types of antifoams, natural oils, and biosurfactants, were implemented in the scum reduction efficiency study of the two-stage AD with FPW as substrate. All the analyzed data were discussed according to the objectives of the research. This study also aimed to have distinctively differentiated foam and scum that is present in AD.

4.2 Characterization of substrate and inoculum

The purpose of material characterization is to identify and quantify the elements and compounds of a substance in order to assist in establishing its biocompatibility and required methodology for a more accurate result. The substrate (FPW) and inoculums (Anaerobic sludge and cow manure) were characterized by analyzing pH, biological oxygen demand, chemical oxygen demand (COD), ammoniacal nitrogen (AN), alkalinity, and total solid. Table 4.1 depicts the results of the characterized samples.

Table 4.1: The characterization of substrate and inoculums

Analytical Methods	Substrate		Inoculum	
	FPW	**SW**	**Activated sludge**	**Cow manure**
pH	4-4.5	4-5	7-7.5	6.5-7
Biological Oxygen Demand (BOD) mg/L	7,500-10,000	10,000-15,000	11,500	35,450
Chemical Oxygen Demand (COD) mg/L	15,000-19,500	25,000-30,000	27,360	86,250
Ammoniacal nitrogen (AN) mg/L	12.5-19	-	227	63
Alkalinity	0	0	1720	2620
Total Solid (TS) g/L	4.95-5.5	0	23.1	62.8
Volatile Solid (VS) g/L	3.4-4.6	0	16.2	56.1
VS/TS	0.68-0.84	0	0.702	0.892
Protein g/L	9-25	30	5-10	30-35
Oil & Grease (O&G) mg/L	9,000-12,000	0	0	0

From the results, it is noted that the pH of the substrate SW and FPW are almost similar, while the pH of inoculums (AS and CM) is neutral. The acidic pH of FPW and SW is due to the high organic acids that are present (Sundberg et al., 2013). Organic acids are known to be odorous (Brinton, 1998), and significant FAs were also noticed in our study. However, the common fatty acids are lactic and acetic acid, whereas, in FPW, there was abundant palmitic acid (Table 4.3). There was the absence of FOG in SW and high FOG content in FPW as SW was prepared with yeast and meat extract that lacks lipids, whereas FPW is processed food waste from animal origin.

For the inoculums, cow manure's COD, protein, and BOD were the highest, followed by AS. This result indicates that the organic content in cow manure (CM) is the highest. CM also has a high content of lignin (Park et al., 2019) compared to AS, which is consistent since cows are herbivores. The high lignin composition explains the higher composition of total solids in CM. The total solids of fresh cow dung have been reported to be around 17–21%, which is not beneficial for biogas production (Jeppu et al., 2022). One of the disadvantages of using CM as inoculum is persistent foaming in the digester (Kougias et al., 2014). The foaming caused can be due to the high protein concentration, which acts as a foam stabilizer (Kougias, Boe, et al., 2013). Besides that, the high solid content in CM makes it hard to digest.

4.3 Effect of protein to foam and scum in one-stage AD with SW

The effect of protein on foaming and scum in anaerobic digestion (AD) was investigated with one-stage AD. Two types of inoculums were used (CM and AS). The substrate for this part of the research is synthetic wastewater (SW) with gelatine as a protein source. The experiment was conducted through a series of batch tests. This experiment aimed to differentiate and observe the foaming tendency of two different inoculums when fed with the protein-based substrate.

4.3.1 Foaming Tendency and Scum Production

The foaming tendency of the sample treated anaerobically using AS and CM was calculated and plotted in Figures 4.1 and 4.2, respectively. The foaming tendency for AS digester was the highest at 1.0 mL-foam/(mL-air·min) at 21 g/L protein on day 3 followed by 9 g/L and 15 g/L at 0.77 and 0.73 mL-foam/(mL-air·min). Meanwhile, for the CM digester, the foaming tendency was the highest at 1.16 mL-foam/(mL-air·min) for 15 g/L protein on day 0 followed by 9 and 21 g/L on day 3. There was visible foaming observed in the AS digester on Day 1, 24 hours after feeding 21 g/L protein, as shown in Figure 4.3. The foaming tendency at 21 g/L was relatively high for the AS digester. It was suggested that the inefficient conversion of organic matter in the system causes the accumulation of hydrophobic materials at the interface, inducing foaming, as stated in another study (Yang et al., 2022). This is proven as the COD concentration was high at 7,987 mg/L on Day 3. High organic compounds in the digesters lead to the accumulation of excess surface-active agents on the surface, triggering foaming (Yang et al., 2021). Scum production in this part of the research was absent when inoculated with both CM and AS.

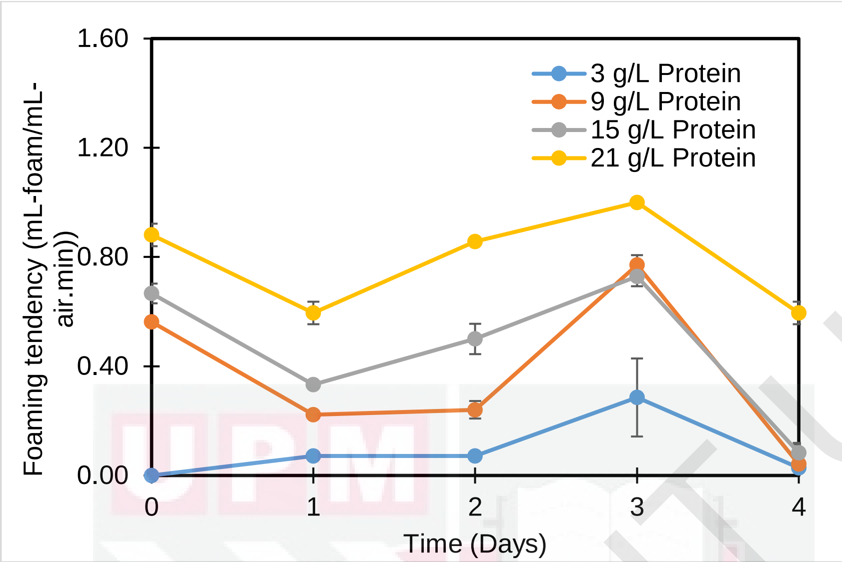

Figure 4.1: The foaming tendency at different protein concentrations for AS digester.

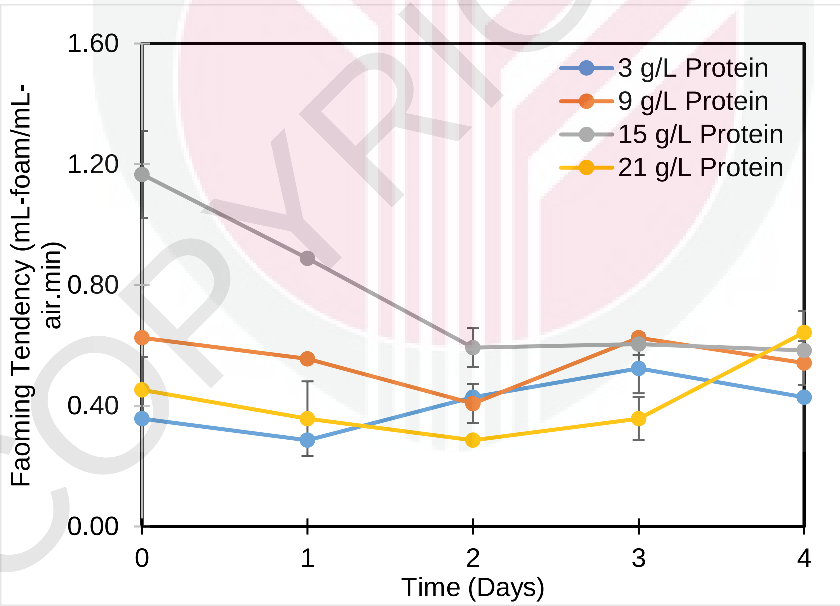

Figure 4.2: The foaming tendency at different protein concentrations for CM digester

Figure 4.3: Foaming in one-stage digester (21 g/L protein)

For the CM digester, the foaming tendency is seemingly higher than for AS digester, but the higher protein concentration resulted in a lesser foaming tendency. The decrease in foaming tendency in the CM digester could be due to the toxic effect of a high concentration of AN in the CM digester, which was the digestion product from the high initial protein in the digester. This observation is evident when on Day 2 and Day 3, the foaming tendency of CM digester was lower than AS digester at a substrate concentration of 21 g/L. The AN counteracts foam formation in the digester resulting in fewer gas bubbles(Kougias, Boe, et al., 2013). Another possible explanation is that, though both reactors were fed with the same feed composition, different inoculums resulted in different microbial communities in the two digesters (Kougias et al., 2014)

4.3.2 Percentage of Protein Removal

The protein removal efficiency for the reactor performance was investigated for activated sludge (AS) and cow manure (CM) as inoculum at different protein concentrations. The results are presented in Figures 4.4 and 4.5, respectively. More than 50% of the protein was depleted during the anaerobic treatment with AS digester and lower at 30% for CM digester. The initial protein concentration in the AS digester was 10 g/L for the added substrate protein concentration of 3 g/L, as the sludge also already contained protein, as noted in its characterization (Table 4.1). The protein was converted at a rate 2-4 g/L per day for 4 days. However, for the subsequent following substrate concentrations (9, 15, 21 g/L), the initial protein concentration in the system was higher at 11, 16, and 21 g/L, respectively, but the conversion was 1-3 g/L per day, slightly lower than substrate protein concentration of 3 g/L. It can be deduced that higher organic loading rates of protein in the digester reduce the protein degradation rates. The results are consistent with a previous study that proved that higher organic loading rates reduce process performance (Liu et al., 2017). It can be observed that the highest removal percentage for anaerobic sludge was at 3 g/L protein at 88.2%, followed by 66.9% and 54.7% for a protein concentration of 15 and 9 g/L, respectively.

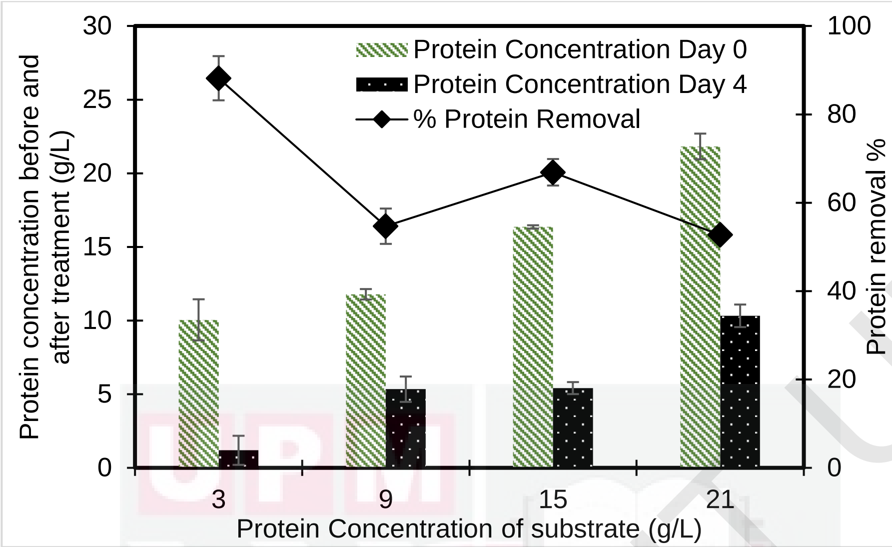

Figure 4.4: Protein removal for AS digester

The CM digester observed that the initial protein concentration of the inoculum was significantly high. It comprises not just protein that was not digested by the animal body but also cellular protein inside protein-rich microorganisms such as methanogens (Tasaki, 2021). As noted in Table 4.1, the protein content of CM was already at 30-35 g/L explaining the lower removal efficiency of CM, where the highest percentage observed was at a protein concentration of 3 g/L at 42.6%, followed by 31.1% and 29.9% at 9 g/L and 15 g/L, respectively. Higher protein concentrations resulted in a lower percentage of protein removal in this study. This could be due to more refractory proteins that the bacteria could not utilize for a shorter time (Yang et al., 2015). Proteins were also the most abundant macromolecule that remained in the digestate to treat food waste. Besides that, the presence of recalcitrant proteins found in meats and bones causes a slow degradation rate since they are challenging to hydrolyze (Ekstrand et al., 2022). Higher protein removal indicates higher biogas production from treatment efficiency. This study proved that an anaerobic sludge digester had a higher protein removal percentage, and higher biogas production was observed (Ahlberg-Eliasson et al., 2017).

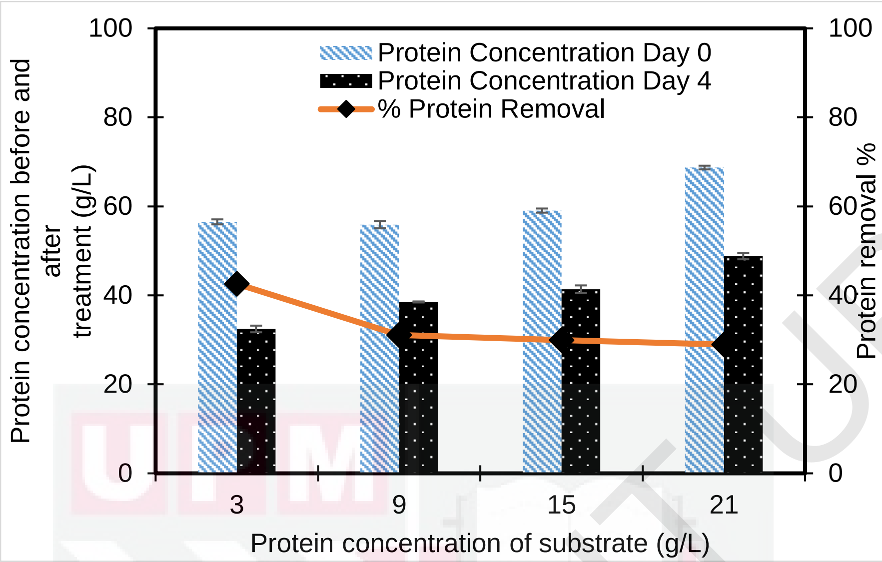

Figure 4.5: Protein removal for CM digester.

4.3.3 Percentage of COD removal

COD estimates the amount of oxygen in a sample consumed in a reaction with oxidizing agents. It reviews the organic content present in the digester and evaluates the efficiency of the digester's performance. The percentage of COD removal reflects the consumption of organic matter within the digester (Meegoda et al., 2018). This study's COD removal percentage is depicted in Figures 4.6 (AS digester) and 4.7 (CM digester). The results reveal that the highest percentage of COD removal for anaerobic sludge digester was 56% for 3 g/L protein and 61% at 9 g/L protein for manure digester. At 3 g/L protein concentration, it was noted that the COD percentage removal was the highest for anaerobic sludge digester but lowest for manure digester. This could be due to the difference in inoculum COD. Manure has an inoculum COD of almost three times more than anaerobic sludge. At the highest protein concentration (21 g/L), the COD removal was low for both digesters indicating unstable operation. High organic compounds in the digesters lead to the accumulation of excess surface-active agents that are not fully degraded by the bacteria. The initial COD of the 21 g/L protein was also higher than other concentrations, which could be due to more gelatin required to increase the protein concentration in the substrate. Shock or organic overload can cause temporary high biogas production and high ratios of carbon dioxide to methane content in the digester gas, triggering foaming. Over time, it leads to an increase in the population of acidogenesis bacteria that produces acids. However, the methanogenic bacteria could not consume the acids as quickly, resulting in the accumulation of acids. Consequently, this would reduce the pH of the digester and kill the methanogenic bacteria (A. J. Kang & Yuan, 2017). Low organic matter removal in high substrate concentration is widely regarded as the main limitation of sludge anaerobic digestion, which causes a large proportion of organic matter to remain in the digestate after the treatment (Ekstrand et al., 2022).

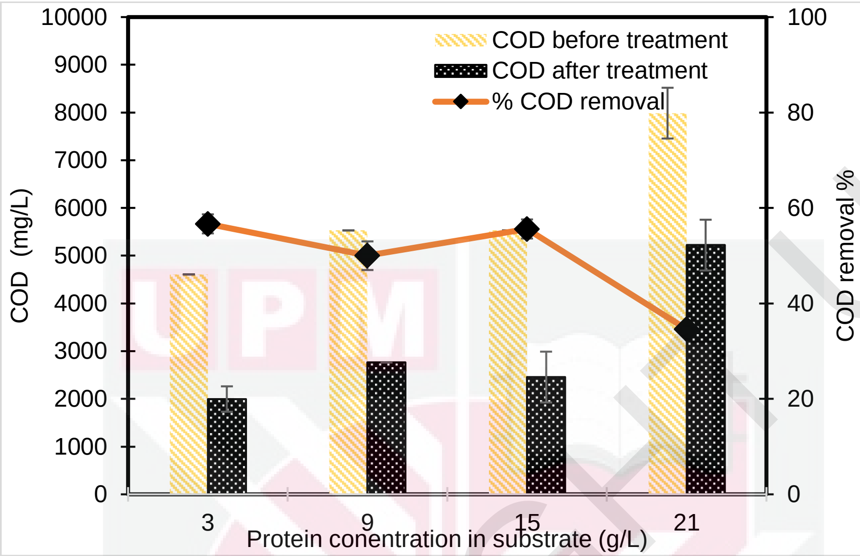

Figure 4.6: COD removal for AS digester.

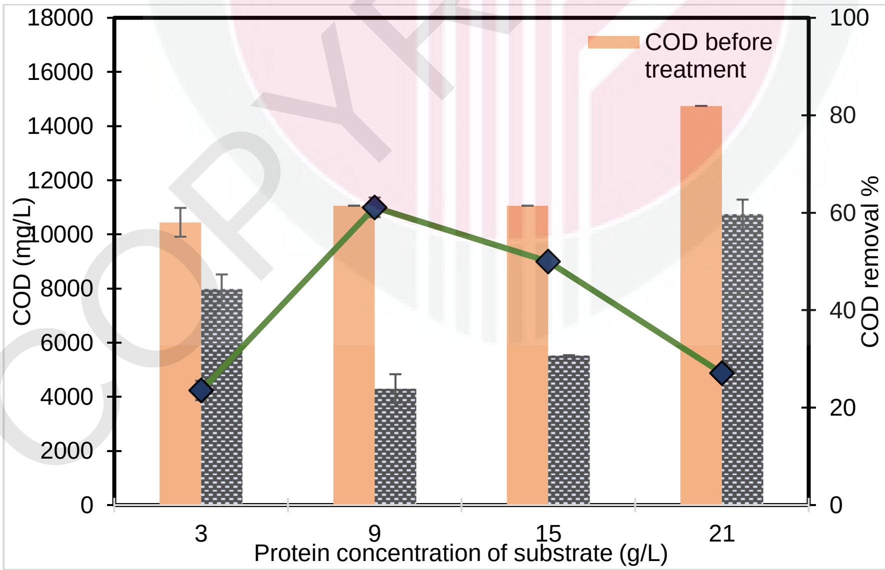

Figure 4.7: COD removal for CM digester.

4.3.4 Biogas Accumulation

The biogas composition varies for every system depending on the feed substrate composition and operating conditions of the digesters (Kuo & Dow, 2017). Figures 4.8 and 4.9 show the biogas production of AS and CM digesters, respectively. The biogas production in an anaerobic sludge digester increases at higher protein concentrations. The highest volume recorded was 1433 mL at 21 g/L, followed by 840 ml and 713 mL for 9 and 15 g/L protein, respectively. The lowest biogas production was recorded at 3 g/L protein because of organic matter depletion. There was no evidence of inhibition of biogas production in the anaerobic sludge digester at higher protein concentrations. Methanogenesis could have still occurred in the presence of acetoclastic methanogens that can adapt to high ammonium concentrations (Westerholm et al., 2016). The presence of these genus bacteria is familiar in mesophilic digesters. Interestingly, the methanogenic bacteria were observed to have higher resistance in digesters with high loading rates (Jiang et al., 2022). This could be the case for this study. The higher protein concentration resulted in higher biogas production.

The trend for biogas production in the CM digester increased to 15 g/L of protein concentrate in the substrate and reduced to 21 g/L. At 3 g/L the biogas production was 175 mL at day 4, which is relatively low. The low biogas production could be related to the slow degradation rate of CM digestion at the initial phase of the treatment for bacterial adaptation (Zamanzadeh et al., 2017) for this protein concentration. The highest observed biogas production for the CM digester was 840 mL for 9 g/L protein. After that, increased protein concentration resulted in a slight decrease in biogas production for 15 g/L but a severe reduction in 21 g/L. The typical amino groups in proteins have amine, ammonium ions, and potent methanogenic bacteria inhibitors produced during the microbial degradation of proteins, causing biogas production restrictions. This reinstates that a higher AN concentration causes process instability and system failure (Schnürer & Nordberg, 2008; Vítěz et al., 2020). Besides that, studies have proven that a high protein concentration, especially gelatin, reduces methane and biogas yield due to process inhibition in CM digestion. CM used in this research had an energy content of 89%, indicating a high potential to produce methane; however, extensive foaming observed in the CM digester led to decreased digester performance. It was reported that the foam covering the liquid surface prevents efficient gas-liquid transfer, thus decreasing biogas production (Ganidi et al., 2009) In conclusion, The CM digester had a higher foaming tendency than AS digester. Scum formation was absent in both the digester and the COD; biogas accumulation and protein removal efficiency were higher in the AS digester when compared to CM digester.

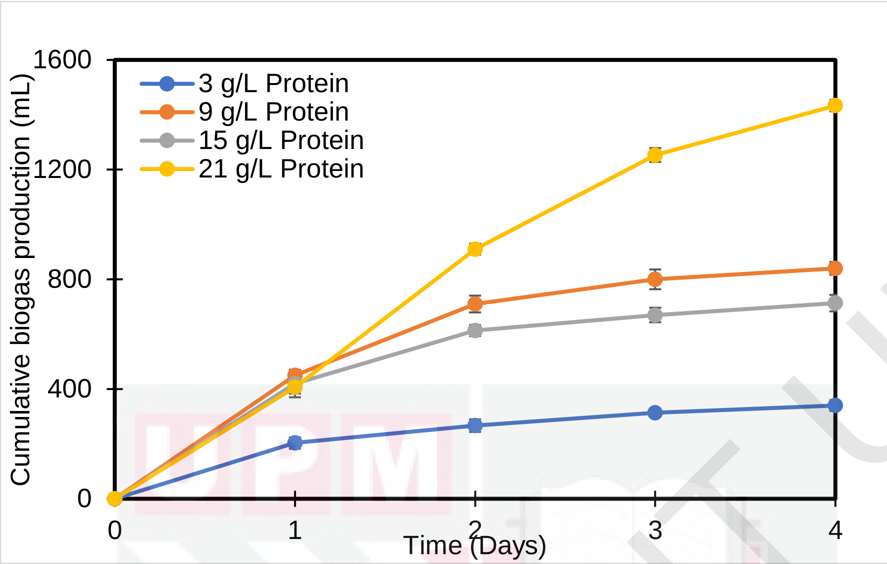

Figure 4.8: Cumulative biogas production at different protein concentrations for AS digester.

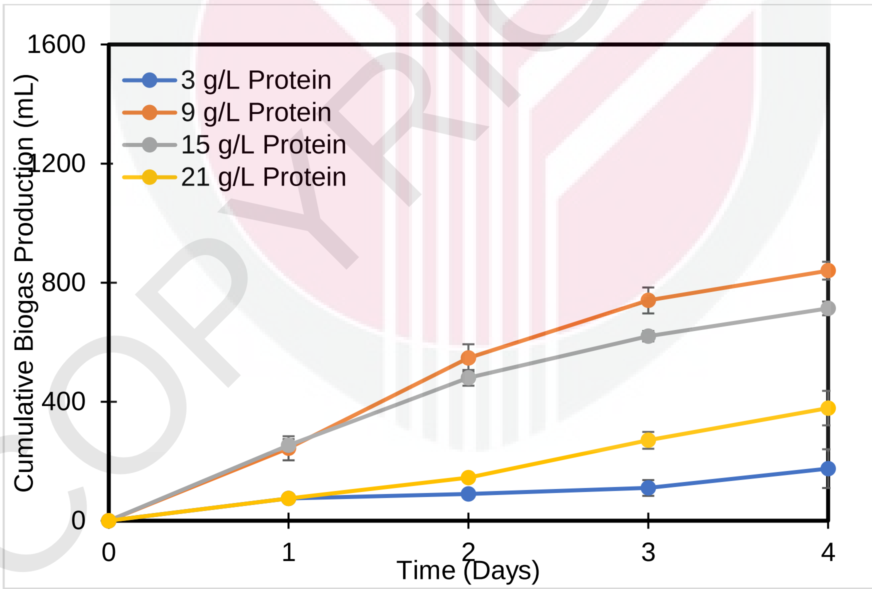

Figure 4.9: Cumulative biogas production at different protein concentrations for a CM digester

4.4 Effect of protein to scum and foam production in two-stage AD with SW.

For the two-stage digestion of protein-based substrate, the substrate used was SW inoculated with AS. AS was chosen as an inoculum for this section has it had better performance and lower foaming tendency. The experiment was conducted for 4 days, and the observations were foaming tendency, scum production, protein and COD removal efficiency, AN concentration, total VFA, and biogas accumulation.

4.4.1 Scum and Foam Production

It is common in a digester that has high concentrations of surface-active agents such as proteins and lipids to produce foaming (C. Jiang et al., 2018; Moeller et al., 2018; Moeller & Görsch, 2015). Proteins have been associated with foam in many biogas plants. In this research, the foaming tendency test revealed that foaming was not present in the collected samples. Also, there was no observable foam on the surface of the Tank 1 and Tank 2 digester. The absence of foaming in the digester could be due to the weak interaction at the water-air interface (J. Liu et al., 2022). A stable foam requires a protein with hydrophobic and hydrophilic groups on its surface and flexibility. Gelatin is created by acid or alkaline hydrolysis of collagen. The source of collagen, as well as its age and kind, determine its final qualities. Gelatin molecules can take a wide range of conformations in an aqueous solution under regulated temperature, pH, and solvent quantity, which could ultimately influence foam stabilization (Caetano et al., 2006).Gelatin's complex conformations could have contributed to the digester's absence of foam, demonstrating that protein-induced foam depends on the source of protein.

Besides that, scum is also a common operational problem in the digester. Typically, scums are found in a digester that treats high-strength wastewater, such as food waste and domestic wastewater, with a high concentration of organic substances (Pereira et al., 2009; Thaku & Barjibhe, 2019). However, when using a two-stage digester in this study, scum was absent in both Tank 1 and Tank 2 for all protein concentrations. The absence of scum in the digester supports previous findings that it is more correlated to lipid inhibition from the accumulation of long-chain fatty acid (LCFA) (Affes et al., 2017; Rasit et al., 2015). Furthermore, in a two-stage digester, the production of scum is still widely not understood due to the complexity of the process and the presence of various organic substances. The presence of multiple organic substances in the substrate makes it difficult to comprehend the role of protein in scum production since it has a hydrophobic characteristic and is a slow degrading substance compared to carbohydrates (Wilde et al., 2004, Fang, 2001). Thus, it can be ruled out that protein does not produce scum on the surface of the digester.

Although there was no scum and foam in the digester, there were traces of sludge floatation in Tank 1 when the two-stage digester was fed with high protein concentrations (12 & 15 g/L), as shown in Figure 4.10. One reported cause of sludge flotation is a high protein concentration, as studies have found that protein-grown sludge granules have poor sludge properties, such as low density, compactness, and settleability (Fang et al., 1994; B. Wang et al., 2018). The residue was present in Tank 1 and was carried into Tank 2. Besides that, the residue was also present in the effluent of the digester, as shown in Figure 4.11. From observation, the sludge clumped together and surrounded by a thin film. The presence of the residue required frequent removal as it caused blockage of pipes and disrupted the effluent flow.

Figure 4.10: Sludge residues on the surface of Tank 1

Figure 4.11: Sludge residues in the two-stage AD effluent

FTIR analysis was carried out to better understand the sludge residue's content (Figure 4.12). Three samples were 15 g/L protein wastewater (Sample SW), sludge residue for a two-stage reactor (Sample residue), and anaerobic sludge (Sample sludge) were analyzed. The most dominant peak for the analysis results is 3396 cm^{-1}, 3342 cm^{-1}, and 3403 cm^{-1} for samples SW, residue, and sludge, respectively. The peak is correlated to polysaccharides structure (stretching of O-H bonds), a hydroxyl functional group. The peaks 2884 cm^{-1} and 2817 cm^{-1} in sample SW, 2918 cm^{-1} in sample residue, and 2885 cm^{-1} in sample sludge indicate aliphatic C-H stretching (Isik et al., 2020). The broad shortened peaks of 2130 cm^{-1}, 2123 cm^{-1}, and 2130 cm^{-1} for samples SW, residue, and sludge, respectively, indicate the presence of alkynes C triple bond C stretching. The peak at 1644 cm^{-1} (sample SW), 1646 cm^{-1} (sample residue), and 1645 cm^{-1} (sample sludge) shows the existence of Amides I, stretching of C=O and C-N bonds which are protein secondary structures. The peaks of 1440 cm^{-1} (Sample SW), 1461 cm^{-1}(sample residue), 1440 cm^{-1} (sample sludge), and 1362 cm^{-1} (sample sludge) represent the existence of amides III (C-N stretching). Besides that, the peak of 724 cm^{-1} in sample SW, 722 cm^{-1} in sample residue, and 719 cm^{-1} in sample sludge indicate amide IV. The main components of the samples classified in the FTIR spectra are proteins from the detection of the amide functional group, and polysaccharides were also dominant from the peaks 3600-3000 cm^{-1} (Hong et al., 2021). From the results obtained, it can be concluded that the floating matter in the digesters is AS residue.

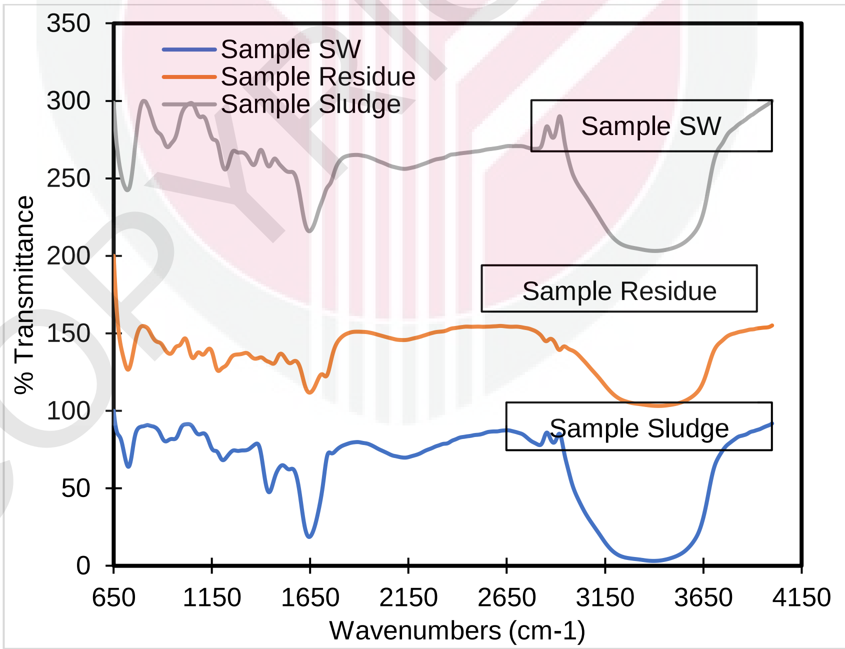

Figure 4.12: FTIR analysis of sample SW, residue, and AS

4.4.2 Percentage of Protein Removal

The protein removal efficiency for a two-stage anaerobic reactor was investigated at different protein concentrations. Figures 4.13 and 4.14 show the results of protein removal in Tank 1 and Tank 2, respectively. The highest percentage of protein removal was at protein concentrations of 3 and 6 g/L, achieving a 100% reduction in both Tanks 1 and 2. This result indicates that the protein was digested efficiently in the first three phases of the anaerobic digestion process. At a substrate concentration of 9 g/L, the protein removal efficiency slightly reduced to 93.5% in Tank 1 but stayed at 100% in Tank 2, indicating good performance. However, at higher substrate protein concentrations of 12 and 15 g/L, there were signs of reduction in digestion performance as the percentage of protein removal reduced to 34.3% and 37.2%, respectively, in Tank 1 and 71.9% and 46.9% in Tank 2. The reduction in protein removal indicates anaerobic process inhibition, possibly due to the high concentration of VFA reducing the microbial activity.

The reported high protein degradation suggests that a significant portion of the protein was indeed converted into soluble nitrogen compounds. However, there are limitations to the type of amino acid chains that can be detected in the Bradford Assay Protocol method. The Bradford assay protocol detects basic amino acid chains such as lysine and histidine, whereas the highest concentration of amino acids found in the degradation of gelatins are glycine, proline, glutamic acid, and alanine (Duong et al., 2019). The high percentage of protein removal could have left out these types of amino acids that may have still been present in the digester.

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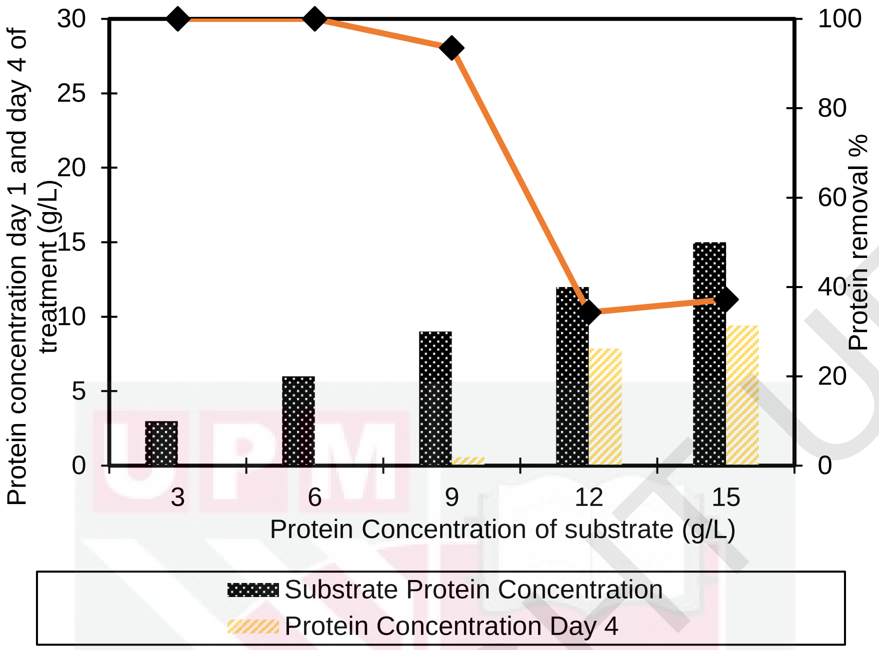

Figure 4.13: Percentage of protein removal for two-stage AD in Tank 1

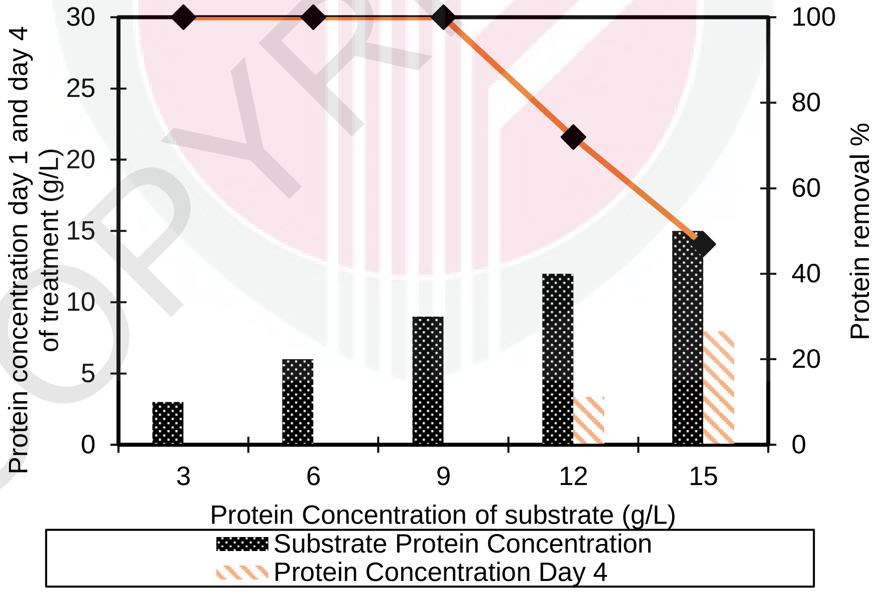

Figure 4.14: Percentage of protein removal for two-stage AD in Tank 2

4.4.3 Percentage of COD removal

For COD removal in Tank 1, the highest COD removal was at 9 g/L protein concentration of substrate at 61%, followed by 3, 6, 12, and 15 g/L, as shown in Figure 4.15. Whereas for Tank 2 (Figure 4.16), the highest COD removal was at 6 g/L protein concentration with a percentage of 92%. The COD removal percentage seemingly increased, above 53%, when treated with 3-12 g/L substrate protein concentration; however, it reduced when treated with 15 g/L protein concentration. It was also observed that the VFA was high even when the COD removal was above 90% (Figure 4.19). High VFA and low COD concentrations in the effluent imply incomplete organic matter breakdown and a potential imbalance in the anaerobic digestion process. Besides that, the standard COD method can be influenced by many factors, such as digestion time and reagent strength. It is also possible that the soluble COD was easily detectable as compared to slowly biodegradable COD(Jwara et al., 2022). For microbial activity to be at its peak, variables like temperature, pH, hydraulic retention time (HRT), and organic loading rate (OLR) must be carefully managed (Ganidi et al., 2011). Process imbalances and insufficient organic matter decomposition might occur when operating conditions differ. For instance, microbial activity may be hindered if the temperature is too low or the HRT is too brief (Liu et al., 2017;Gagliano et al., 2018).

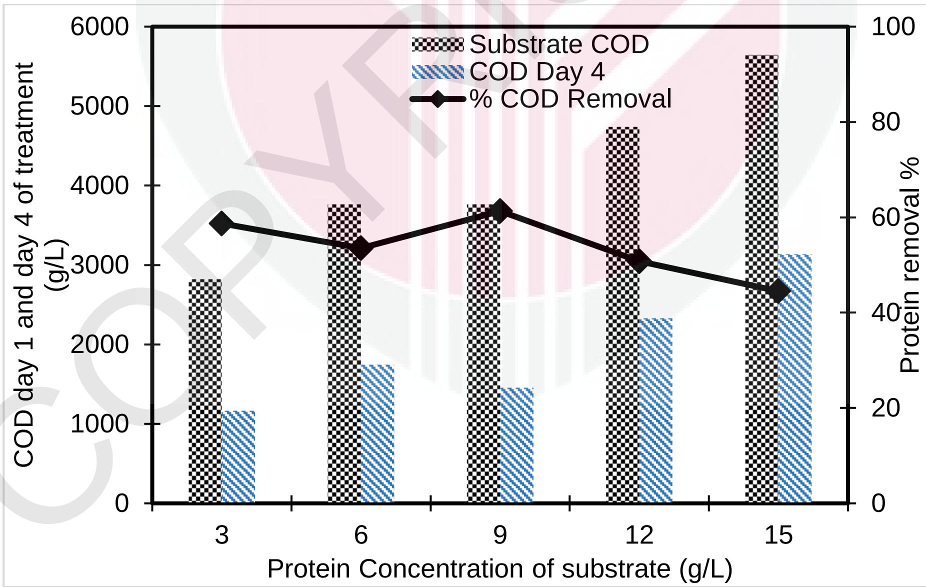

Figure 4.15: Percentage of COD removal for two-stage AD in Tank 1

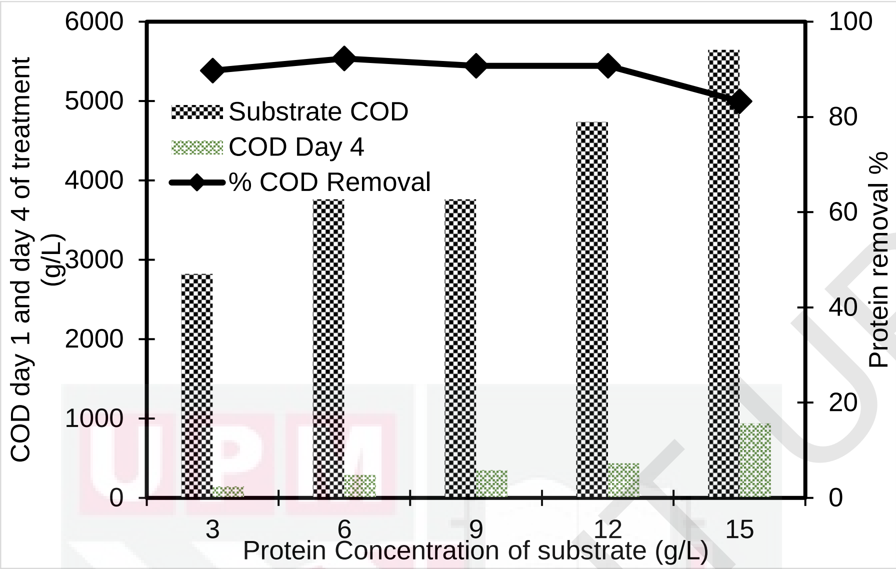

Figure 4.16: Percentage of COD removal for two-stage AD in Tank 2

4.4.4 AN Concentration, Total VFA concentration, and pH

This study observed that at high protein concentrations (12 & 15 g/L), the AN concentration increased above 200 mg/L. In Tank 1, the AN concentration was 278.6 mg/L and 219.4 mg/L on day 4, respectively, as shown in Figure 4.17. Meanwhile, in Tank 2 (Figure 4.18), the AN concentration was 219.2 mg/L for 12 g/L protein and 260.12 mg/L for 15 g/L protein. AN concentration of 50 to 200 mg/L benefits anaerobic processes because AN is required to synthesize amino acids and nucleic acids, which are essential for bacterial growth. It also assists in maintaining a neutral pH at a mesophilic temperature between 7.2 and 7.5. Most anaerobic digestion reactors are sensitive to ammonia, especially in the methanogenesis reactor. A study conducted with food waste revealed that ammonia at a concentration of 2 g/L is sufficient to cause disturbances in the process when pH is not controlled, which was the case for this study. While hydrolysis and acidification were less impacted, ammonia nitrogen disruptions in AD processes were primarily due to the suppression of methanogenesis. High ammonium concentrations' inhibitory effects on AD would cause VFA build-up and a drop in pH. These elements would inhibit *Methanosaeta* action and the acetoclastic pathway (H. Chen et al., 2016). The reactor system in this study may have experienced this inhibition, resulting in lower AN concentration than theoretically predicted from protein breakdown (Akindele & Sartaj, 2018).
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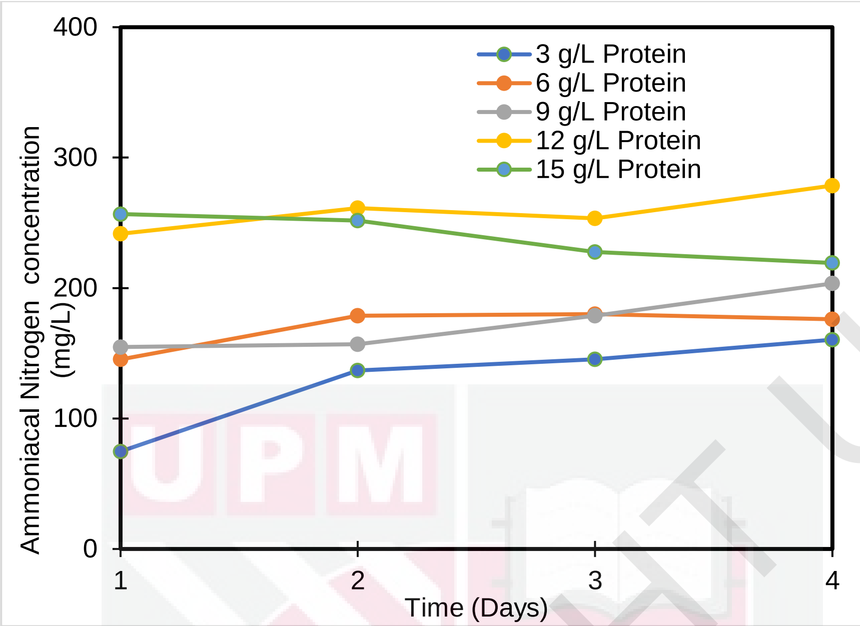

Figure 4.17: AN Concentration for two-stage AD in Tank 1

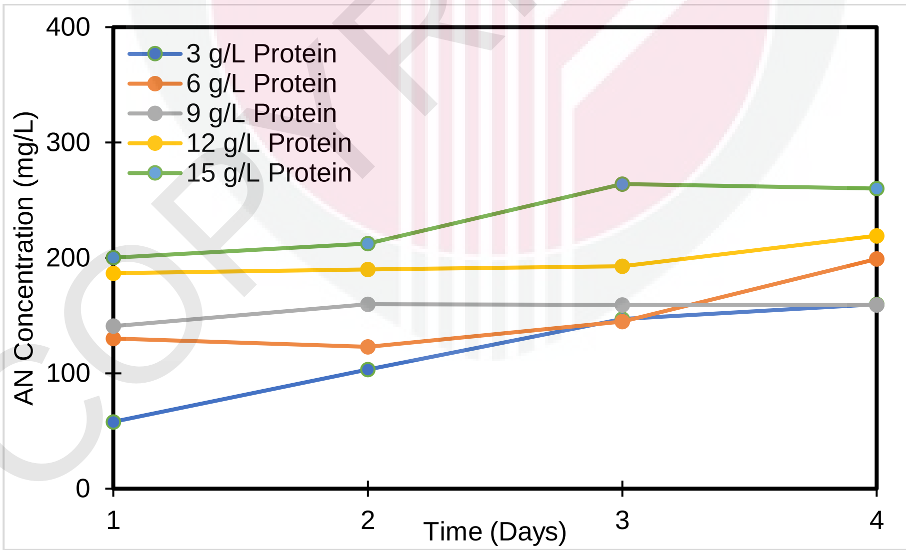

Figure 4.18: AN Concentration for two-stage AD in Tank 2

The biogas accumulation (Figure 4.23) results indicate that digestion has a detrimental effect when the AN concentration increases. AN, known as a methanogen inhibitor, may be a problem in anaerobic digestion, particularly in digestions that use protein-rich feedstocks as substrates (H. B. Nielsen & Angelidaki, 2008). AN could primarily affect anaerobic digestion by influencing acetate-using methanogenic archaea, hydrogen-using methanogens, and syntrophic bacteria at higher than 200 mg/L (Li et al., 2014). Among all microorganisms, methanogens are the most sensitive to AN. According to some researchers, hydrogenotrophic methanogens are more resilient than acetoclastic ones (Ariunbaatar et al., 2015; Schnürer & Nordberg, 2008; Yenigün & Demirel, 2013). Thus, this can relate to the reduction in biogas accumulation when the AN concentration increases for 12 and 15 g/L.

It was observed that the total VFA increased higher in Tank 1 (Figure 4.19) compared to Tank 2 (Figure 4.20) due to the production of organic acids by the acidogenic bacteria (H. Q. Yu & Fang, 2003). Even though the process was slowed down at higher protein concentrations in both tanks due to AN inhibition (Ariunbaatar et al., 2015), it was observed that the total VFA increased over time. Several researchers proposed that higher concentrations reduce the performance of anaerobes that utilizes propionic acid. Thus, propionic acid starts to accumulate. Propionic acid accumulation further inhibits the methanogens and increases the total VFA concentration in the reactor, causing an imbalance in the reactors due to acidification and reduced buffer capacity and the reduction of pH (Ariunbaatar et al., 2015; Banks et al., 2012; Zhang et al., 2013). Additionally, the presence or build-up of intermediates, such as acetic acid, during the anaerobic degradation of gelatine may slow down the rate of gelatine hydrolysis in a mesophilic, anaerobic environment (González et al., 2005).

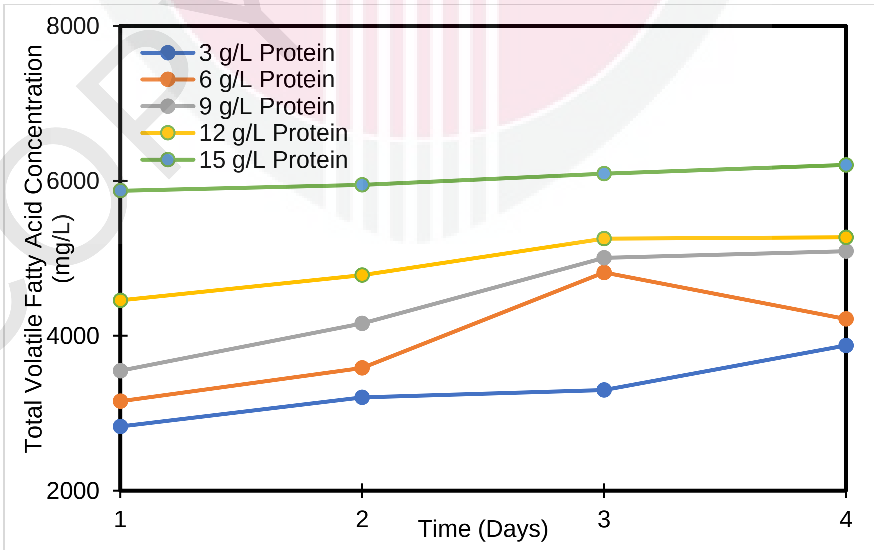

Figure 4.19: Total VFA Concentration for two-stage AD in Tank 1

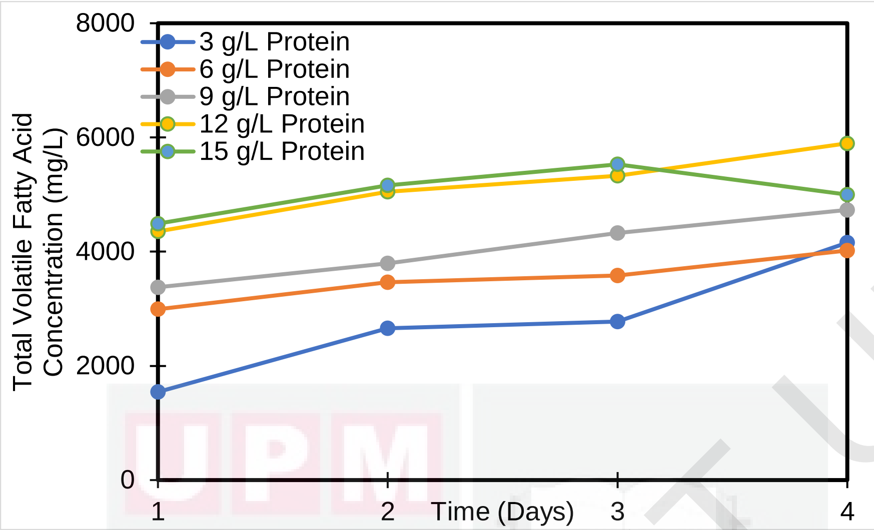

Figure 4.20: Total VFA Concentration for two-stage AD in Tank 2

The pH in tank 1 reduces over time, correlated with the production of VFA. At 9 g/L substrate protein concentration, the pH increased on day 3 and day 4 due to the drastic sparge in AN concentration for almost 100 mg/L and the static concentration of total VFA. In tank 2, the buffering capacity of the digestion remained neutral for each concentration over time, except there was a noticeable decrease in pH for 12 g/L protein concentration. This was related to the reduction in AN concentration and the drastic increase in total VFA. Besides, pH influences microbial activity and Total Ammonia Nitrogen (TAN) partitioning. The equilibrium concentration of ammonium and AN is pH-dependent. Under optimal operating conditions, digesters treating protein-rich waste have high pH values, often exceeding pH 8. Increasing the pH from 7 to 8 in mesophilic conditions decreases free AN concentration (Borja et al., 1996). In this study, the pH reading increased significantly when the protein concentration of substrate increased in both (Figures 4.21 and 4.22). This also proves that the pH increment is correlated to the AN concentration of the system when it exceeds 200 mg/L.

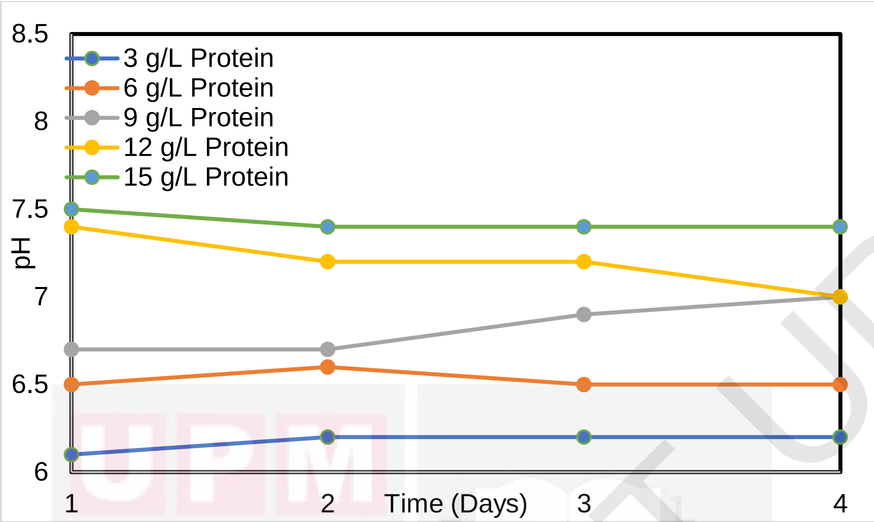

Figure 4.21: pH for two-stage AD in Tank 1.

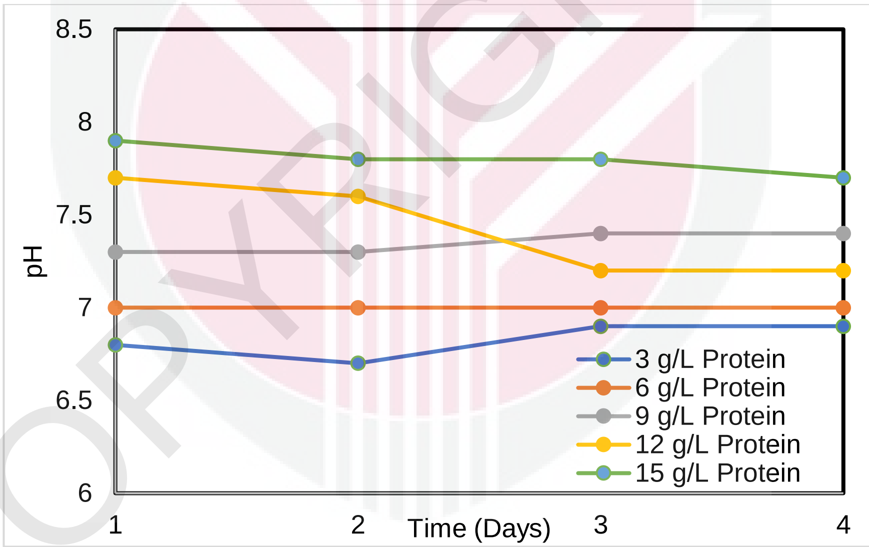

Figure 4.22: pH for two-stage AD in Tank 2.

Furthermore, as a base, AN neutralizes the organic acids produced by fermentative bacteria, assisting in maintaining the neutral pH conditions necessary for microbial activity and growth (Gallert et al., 1998). The AN concentration in tank 1 and tank 2 was high when fed with protein concentrations of 12 and 15 g/L, which increased the pH, causing the microorganism's toxicity and reducing the degradation rate.

4.4.5 Biogas Accumulation

The biogas accumulation increased steadily with an increase in protein concentration increment up to 12 g/L protein in the substrate (Figure 4.23). The highest biogas accumulation was at a substrate concentration of 12 g/L at 21,100 mL on day 4, while the lowest biogas accumulation was substrate concentration of 15 g/L at 8,120 mL. Biogas accumulation was drastically decreased when fed with a protein concentration of 15 g/L. As discussed previously, this could be due to the high concentration of VFA in the digesters. According to current research, methanogen inhibition and organic matter hydrolysis in alkaline circumstances can increase the benefits of VFA generation compared to acidic or near-neutral environments (Wang et al., 2009). In this instance, the high concentration of VFA reduced the pH of the digester, thus causing disturbance to the methanogens. Additionally, it has been noted that wastewaters containing protein have lower biogas yields, foaming, and poor effluent quality (Tanimu et al., 2015). The combination of protein and carbohydrates is the most effective anaerobic digestion. Additionally, it was noted that anaerobic digestion based only on the protein was the least effective because the rates of organic matter removal and methanation declined with increasing protein concentration due to propionic acid build-up, a low C/N ratio, and reduced microbial activity (Chen et al., 2008b; Wagner et al., 2012).

The low cumulative biogas production and poor biogas yield coefficient indicate low process efficiency and ineffective biogas generation. Several circumstances may have caused the discrepancy. Proteins' complex metabolic properties, such as their high molecular weight or interactions with carbohydrates, and the high protein concentration in the substrate could lead to an incomplete breakdown. Due to this partial breakdown, fewer proteins are converted into biogas (Li et al., 2022). The high VFA concentrations in the digester suggest that ineffective protein breakdown can lead to a build-up of VFAs. Acetic acid, propionic acid, and butyric acid are examples of VFAs that can build up and hinder methanogenesis while upsetting the equilibrium of the microbial community in the anaerobic digestion digester, which could be the case for this study (Kovács et al., 2013) By archaeal community analysis, *Methanosphaera* was the dominant species found present in a previous study of the same system (Mahat et al., 2021) The majority of methane produced—nearly 70% of it—is created by acetoclastic methanogens. They primarily fall under two groups: *Methanosarcina* and *Methanosaeta,* which in this study is lacking. The limited acetoclastic methanogens could result in lower biogas yield. The most common composition of biogas produced in AD are methane, carbon dioxide, and hydrogen. Nitrogen in the digester could also occur from the purging process at the experiment's beginning.

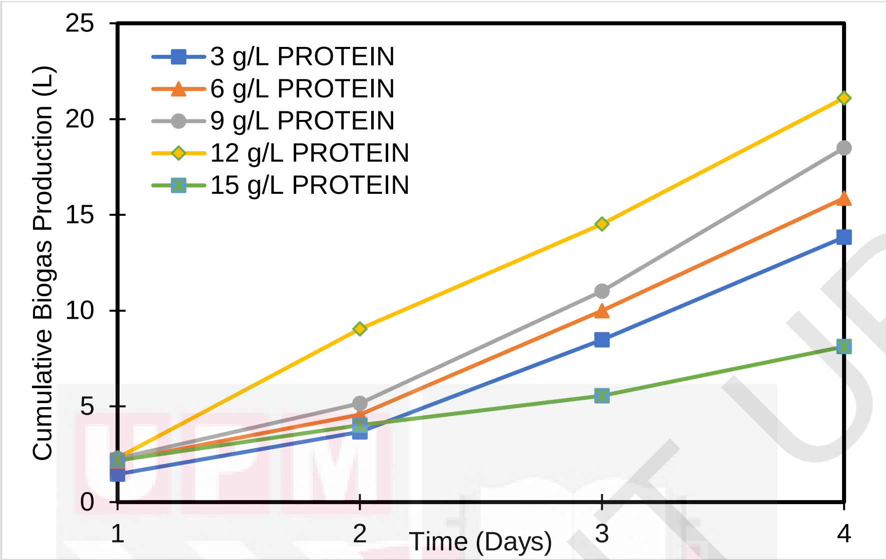

Figure 4.23: Biogas Accumulation in two-stage anaerobic digester

4.5 The effect of on lipid scum and foam in one-stage and two-stage AD with FPW

For this section, the experiment was conducted using FPW as a lipid source. The protein in the FPW was ruled out as previous sections proved that protein, even at high concentrations, does not cause scum production. There are two types of digesters: one-stage (10 L) and two-stage (30 L). The one-stage reactor was conducted through a batch test, while the two-stage was continuous. The inoculum used was AS.

4.5.1 Scum Production

Scum is defined as substances lighter than water, such as oil, grease, and fats, that float to the surface and form a scum layer. Scum build-up can effectively shut down a digester (Thakur et al., 2019). Figure 4.24 shows that the scum formation is directly proportional to the lipid concentration in FPW. The observation from this part of the experiment revealed that the scum formation increased as the lipid concentration increased. The scum also increased with days with is related to the accumulation of fatty acids (FA). It is previously validated that protein-rich wastewater did not initiate scum to form (Subsection

4.2.2); however, when fed with FPW with high lipid content, scum was produced in two-stage AD.

Surprisingly, scum was not observed in one-stage AD even when treated with high lipid concentration FPW. The absence of scum in one-stage AD can be due to the process of acidogenesis and methanogenesis co-occurring in a single system. Since, in one-stage AD, the processes are parallel, the methanogens can consume the lipid intermediates immediately, reducing the accumulation of FA (Cremonez et al., 2021b). Figure 4.25 depicts the microscopy view of scum produced in 10 g/L lipid concentration. From the image, the overall content was an organic matter, proven through the fatty acid analysis in section 4.5.1.1. There were also traces of bubbles that can be observed, revealing that there is foaming present below the surface of the scum, which can also be seen in Figure 4.26 scum production in the surface of Tank 1. In the case of prolonged feeding, the scum would continuously accumulate, causing blockage of pipes and disrupting the digester performances. Besides that, the liquid and gas transfer between the digester will also be disrupted.

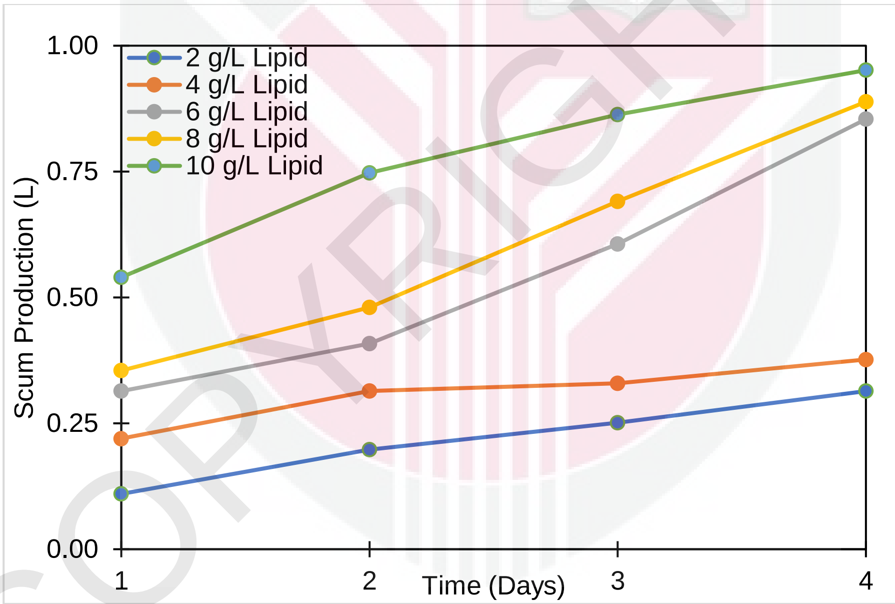

Figure 4.24: Scum Production in two-stage AD in Tank 1

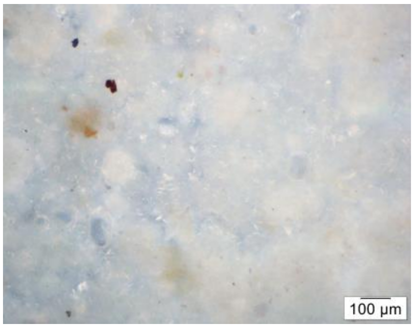

Figure 4.25: Microscopy analysis of scum (10 g/L lipid concentration)

Figure 4.26: Scum produced on the surface of Tank 1 (10 g/L lipid concentration

4.5.1.1 Fatty Acid Analysis of Scum and FPW

The characterization of scum produced at 10 g/L lipids is shown in Table 4.2. There are high amounts of proteins and lipids in scum. More detailed characterization was carried out on the scum and FPW for comparison. The fatty acids (FA) are characterized as saturated, monosaturated, and polyunsaturated. The saturated FA found in the sample is depicted in Table 4.3. Scum has a higher amount of saturated FA (C12, C16, and C18) than FPW, with palmitic acid as the most dominant. Besides that, lauric and stearic acids are also higher in scum than FPW. Monosaturated FA in Table 4.4 indicates that FPW has a higher value, similar to linoleic acid (Cis) in polyunsaturated FA in Table 4.5. Unsaturated long-chain fatty acids (LCFAs) are classified into Cis and trans, with trans being more toxic than cis(Chatgilialoglu et al., 2014). The saturated FA was present in a larger volume of scum, and unsaturated FAs were present in FPW. The lipid in AD is degraded into LCFA and glycerol. Generally, unsaturated FA in FPW undergoes degradation into shorter saturated FA through the β-oxidation pathway(Samarasiri et al., 2016). The higher amount of saturated FA in scum, especially stearic and palmitic acid, indicates that FAs are a rate-limiting step. Palmitic acid and stearic acid are the primary saturated LCFAs accumulated throughout the anaerobic digestion process, as stated in a study(Dasa et al., 2016). They are five times slower to decompose than unsaturated acids(Silvestre et al., 2014). This validates that the accumulation of scum over time in the digester is indeed from the degradation of lipids forming LCFAs.

Table 4.2: Characterization of scum

Analytical Method	Unit	Values
pH		4.5-6.5
Protein	g/L	125
Oil & Grease (O&G)	mg/L	140,000

Table 4.3: Saturated Fatty Acids in Scum and FPW

Fatty Acid methyl ester	Structure	mg/100 g sample	
		FPW	Scum
Lauric	C12:0	32.75	77.81
Myristic	C14:0	171.25	145.81
Palmitic	C16:0	655.34	1028.08
Stearic	C18:0	122.04	160.28

Table 4.4: Monosaturated Fatty Acids in Scum and FPW

Fatty Acid methyl ester	Structure	mg/100 g sample	
		FPW	Scum
Palmitoleic	C16:1	128.98	52.88
Oleic	C18:1	327.12	214.56

Table 4.5: Polyunsaturated Fatty Acids in Scum and FPW

Fatty Acid methyl ester	Structure	mg/100 g sample	
		FPW	Scum
Linoleic (Cis)	C18:2	320.68	103.70
α-Linolenic	C18:3	88.21	1636.50

4.5.2 Foaming Tendency

The foaming tendency test revealed that foaming did not occur in the digesters when fed with lipid substrate in the two-stage AD. The foaming was only observed once the scum (Figure 4.32) was skimmed off manually. This proves that foaming is present in two-stage AD but is not visible due to the scum. From observation, the foaming present in Tank 1 had little bubbles and was loosely apart (Figure 4.27). They were also susceptible to touch, meaning a slight movement caused all the bubbles to rupture, indicating low stability. A previous study indicated that the foaming present in a lipid-rich digester was a light bubble with a colorful reflection and was easy to expand with an increase in biogas production (Kougias et al., 2013). In a lipid molecule, the hydrophilic end of the amphiphile molecules turns towards the liquid phase, while the hydrophobic end turns towards the air phase. As a result of this structural positioning, monolayers form at liquid/air or liquid/surface interfaces, promoting foam formation (Zhang et al., 2003). From observation, there was also no evidence of scum formation on the surface of the digester when fed with protein-rich wastewater; the absence of scum in two-stage AD indicated that scum is related to lipid degradation inhibition. The presence of an intermediate known as LCFAs has been reported to inhibit and be the primary cause of process destabilization, causing an accumulation of viscous substances on the surface of the digester (Rasit et al., 2015).

Figure 4.27: Foam formation in Tank 1 two-stage AD fed with 10 g/L lipid

4.5.3 Percentage of COD removal

Several studies have shown good advantages when implementing two-stage AD compared to one-stage AD, especially when the digestion process is continuous. The highest percentage of COD removal (90%) for two-stage AD was at a substrate lipid concentration of 2 g/L followed by 4, 6, 8, and 10 g/L in Tank 1 (Figure 4.28). Meanwhile, the highest for Tank 2 was at 97%, for 2 g/L substrate lipid concentration, followed by 6, 4, 8, and 10 g/L as shown in Figure 4.29. Whereas the highest COD removal in one-stage AD was significantly lower at 79% for 2 g/L substrate concentration on day 4 of digestion, as shown in Figure 4.30. COD is an indirect measure of the amount of organic matter; thus, the percentage of COD removal in Tank 1 indicates good performance.

It can be seen that as the lipid concentration increases, the percentage of COD removal reduces. This difference could be because of higher organic matter content in the digester when fed with high lipid concentrations. When using FPW as a substrate, the increase in lipid concentration also indicates the increase of other organic matter, such as proteins and carbohydrates. The high organic content, for instance, in 10 g/L resulted in an initial COD of 6,586 mg/L, which is considerably high. On day 4, the percentage of removal was low for 10 g/L, possibly due to the accumulation of organic matter (scum) on the digester surface being very prominent. This could have led to a reduction in digester performance. Besides that, the results clearly indicate that one stage has a lower percentage of COD removal compared two-stage which was also proven through single-factor ANOVA analysis in Table 4.6. The results of the ANOVA analysis indicate a p-value of less than 0.05 which signifies that the difference between the percentage of COD removal between one-stage and two-stage is significant.

Table 4.6: Single Factor ANOVA analysis (Summary)

Groups	Counts	Sum	Average	Variance
Single stage AD	5	348.48	69.696	79.659
Two-stage AD	5	446.56	89.312	48.557

Table 4.7: Single factor ANOVA analysis

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	961.954	1	961.954	15.005	0.004717	5.3177
Within groups	512.866	8	64.108			
Total	1474.82	9				

Two-stage AD enables the selection and enrichment of diverse bacteria and archaea in an individual process stage while ensuring the overall process's stability, thus improving the removal of organic matter (Dareioti et al., 2021). Protein, lipids, and carbohydrates are hydrophobic substances (Kougias, Boe, et al., 2013) that can easily be attracted to the top of the digester when high hydrophobic compounds accumulate on the surface (scum). The undigested material will unlikely be carried to Tank 2, thus validating the reduction in biogas accumulation in two-stage compared to one stage that does not have scum.

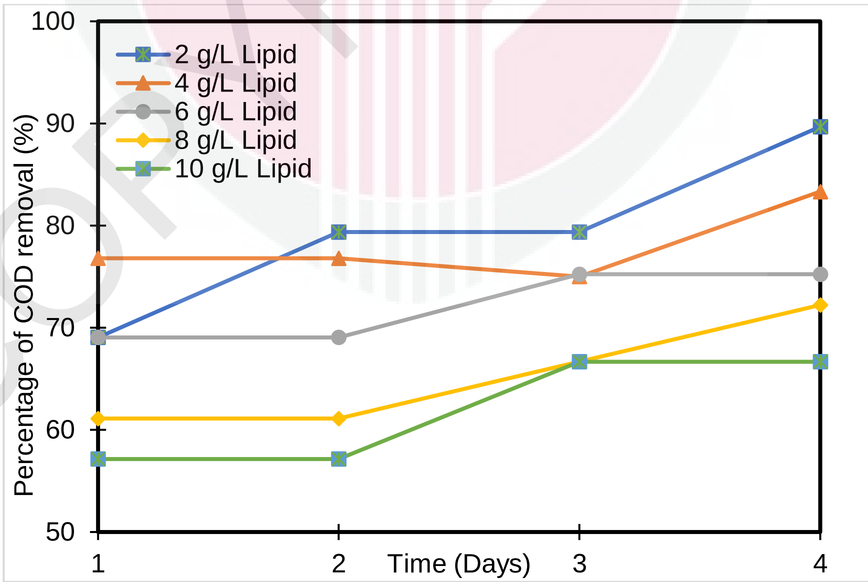

Figure 4.28: Percentage of COD removal of two-stage AD in Tank 1

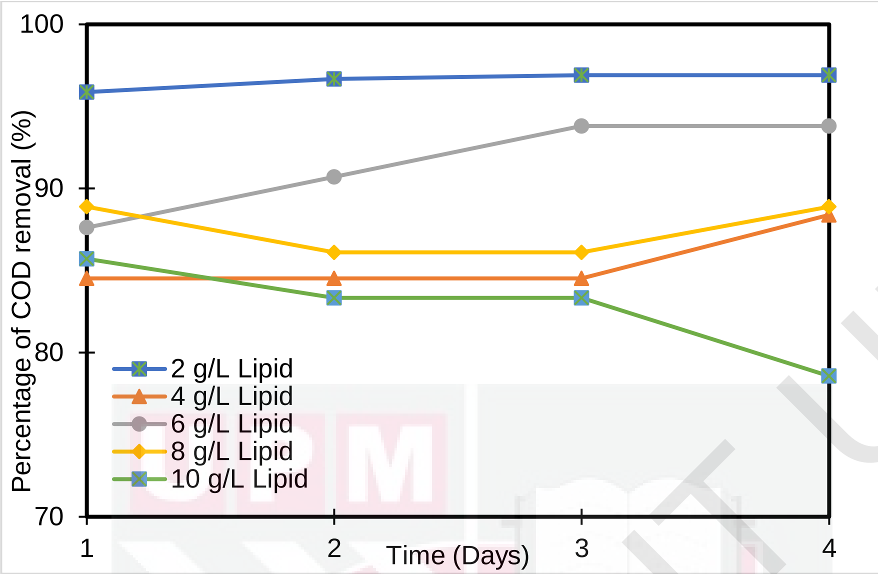

Figure 4.29: Percentage of COD removal of two-stage AD in Tank 2

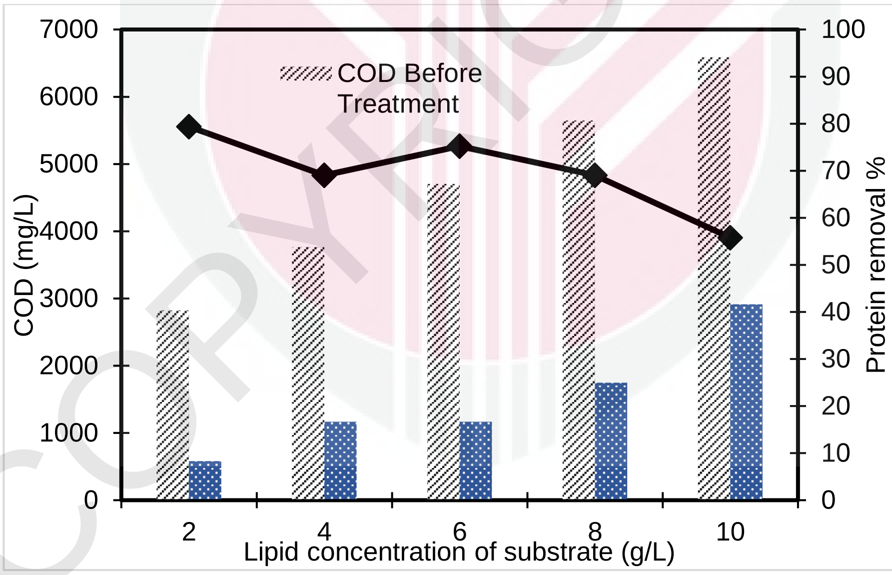

Figure 4.30: Percentage of COD removal of one stage AD

4.5.4 Protein removal and AN concentration

As the lipid concentration increases, the high percentage of protein in the substrate increases because the FPW used in this study was diluted according to the required lipid concentration, as shown in Table 4.8. The dilution was carried out from the stock solution.

Table 4.8: The FPW dilution of stock solution

Lipid Concentration (g/L)	Initial COD (g/L)	Initial protein (g/L)
2	2.82	8.41
4	3.76	19.03
6	4.7	24.8
8	5.64	28.7
10	6.59	39.7

The percentage of protein removal; increased over time in Tank 1 of the two-stage AD (Figure 4.31). The protein concentration in FPW is much higher compared to the treatment of SW wastewater. However, sludge floatation was not observed. This could be due to the difference in FPW and SW+ gelatine amino chains. FPW is from animal proteins; the common trend is rich in lysine (25-30 mg/g) and Trp and sulfur amino acids at 10-15 mg/g and 3-4 mg/g, respectively (Prandi et al., 2019) , while the chains in gelatine are glycine and proline(Regita et al., 2019). The highest removal achieved in Tank 1 was 100% at 2 and 6 g/L, while the lowest percentage of protein removal was 75% at the highest lipid concentration of 10 g/L. As the substrate protein concentration increase, as discussed earlier the ammonia inhibition, the protein removal efficiency slightly decreases due to the increase in AN concentration in the digester (Figure 4.32). The protein removal percentage in Tank 2 of the two-stage AD achieved 100% for all lipid concentrations. The AN concentration increased with lipid concentration in Tank 2 (Figure 4.33). The highest AN concentration was at Tank 2 when fed with 10 g/L lipid concentration at 77 mg/L on day 4. The lowest AN concentration was 2 g/L at 13.72 mg/L. From the results, it can be concluded that the protein concentration is correlated to the AN concentration.

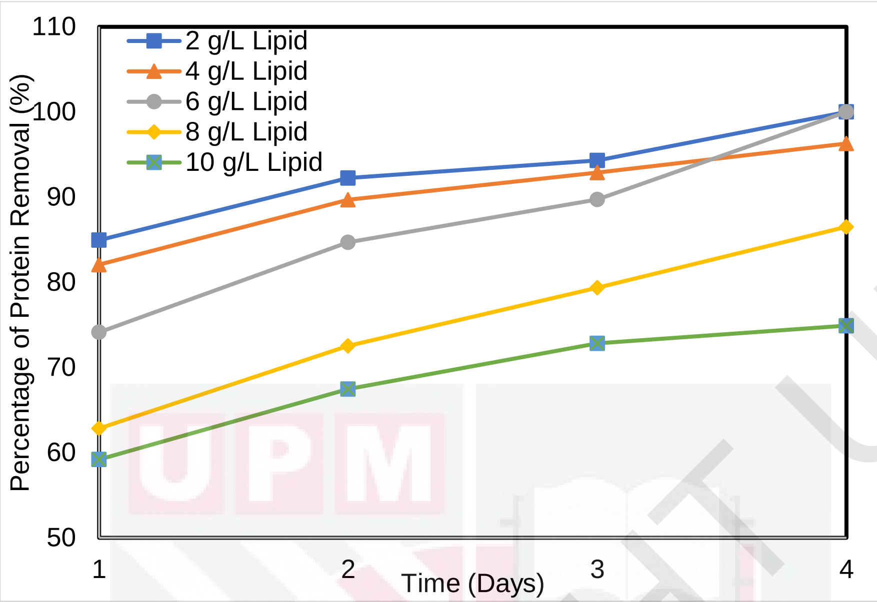

Figure 4.31: Percentage of protein removal of two-stage AD in Tank 1.

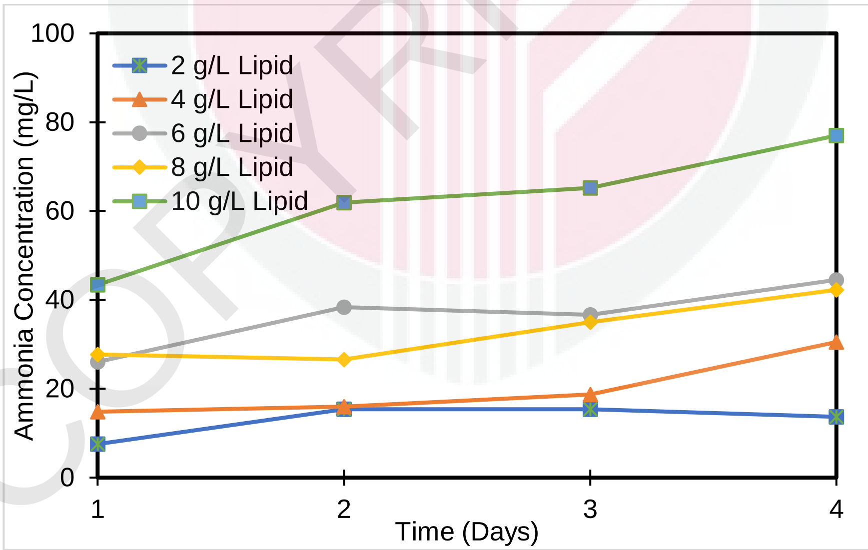

Figure 4.32: AN Concentration for two-stage AD in Tank 1.

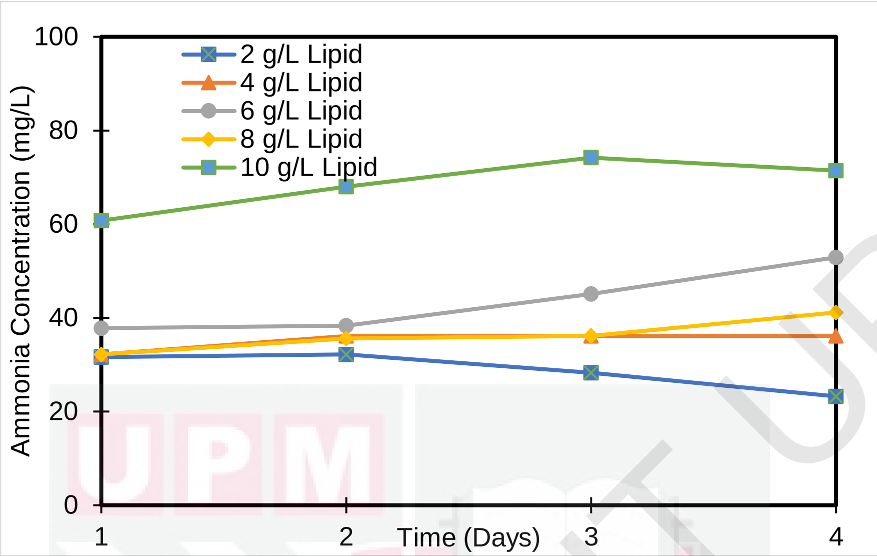

Figure 4.33: AN Concentration for two-stage AD in Tank 2.

For the one-stage AD, the 100% protein removal was only achieved with a lipid concentration of 2 g/L and gradually reduced after that, as shown in Figure 4.34. Only 70% removal was recorded when protein concentration was 10 g/L. The AN concentration increased to 6 g/L lipid concentration; however, there was a noticeable decrease in AN concentration at 8 g/L and 10 g/L lipid as depicted in Figure 4.35. The reduction of AN concentration can be related to the increase in total VFA, which resulted in a decrease in the pH of the digester, thus reducing the activity of methanogenic bacteria. The correlation of total VFA is discussed in the next subsection.

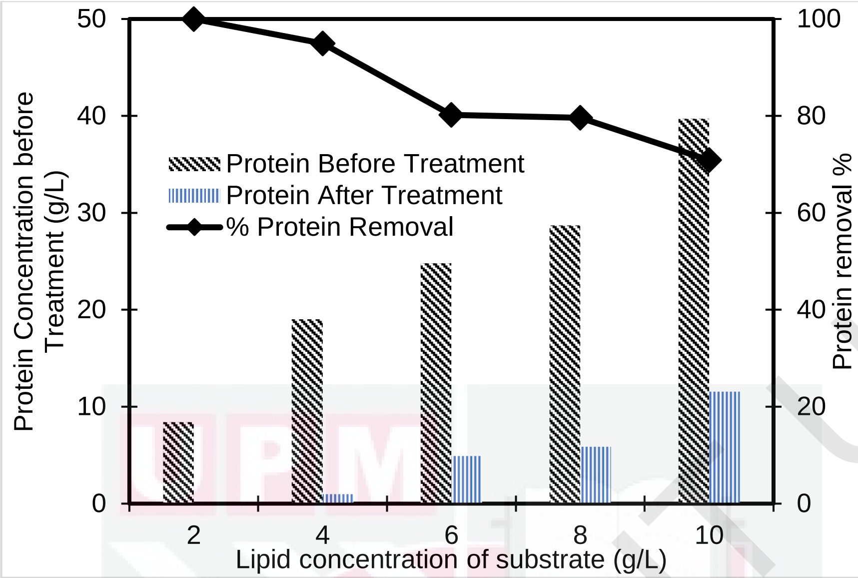

Figure 4.34: Percentage of protein removal of one stage AD.

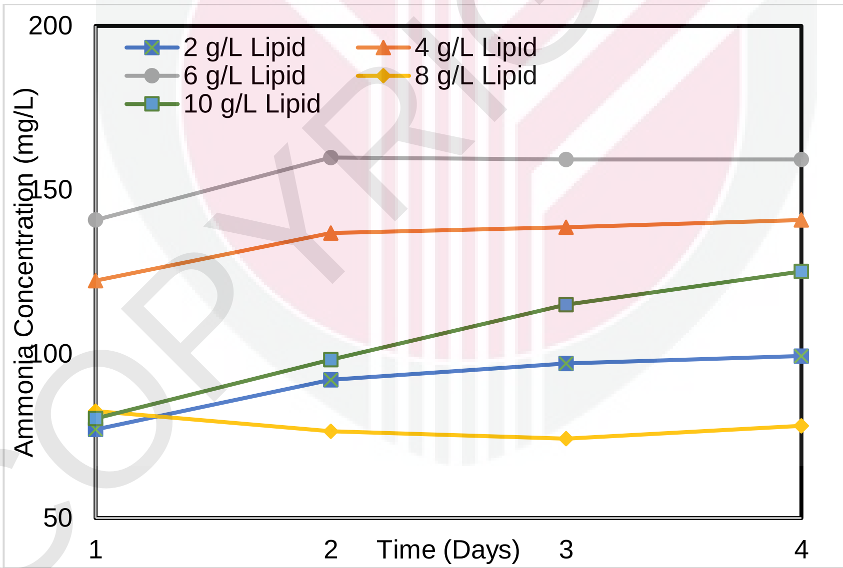

Figure 4.35: AN Concentration for one-stage AD

4.5.5 Total VFA Concentration and pH

The total VFA in the two-stage AD increased steadily in both Tanks 1 and 2 with an increase in substrate lipid concentration. The total VFA was higher in Tank 1 compared to Tank 2 as shown in Figures 4.36 and 4.37, respectively. This is due to the continuous hydrolysis and acidogenesis process in Tank 1. The pH in Tank 1 was the lowest when fed with 8 and 10 g/L lipid on day 2, which was 6.4 and 6.3, respectively. However, the VFA concentration increased significantly in Tank 2. The specific concentrations of intermediates produced during the acidogenesis stage may vary depending on the digester conditions; it has been reported that VFA concentrations can fluctuate significantly for digesters operating at different pH levels (Huang et al., 2015). The alkalinity ratio in the Tank 1 and Tank 2 is less than 0.4, indicating consistent buffering capability from ammonium bicarbonate due to FPW amino acids and protein content (Drosg et al., 2015).

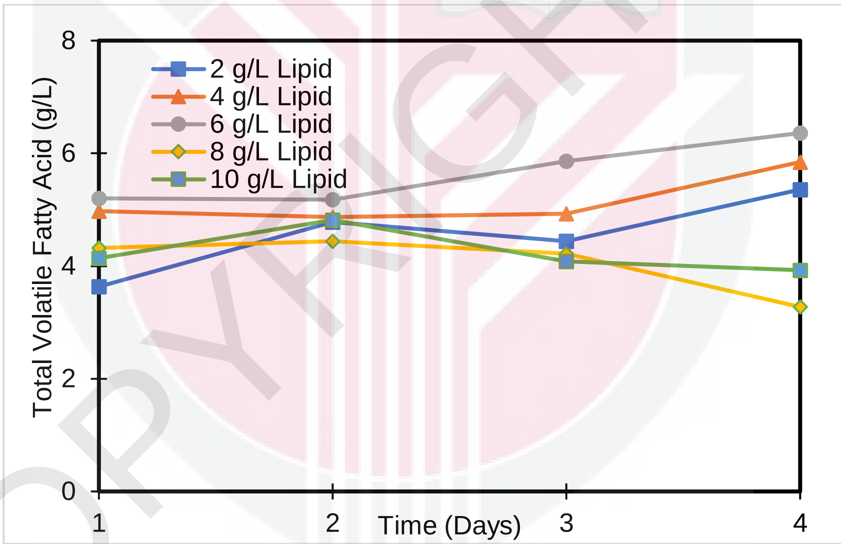

Figure 4.36: Total VFA Concentration for two-stage AD in Tank 1

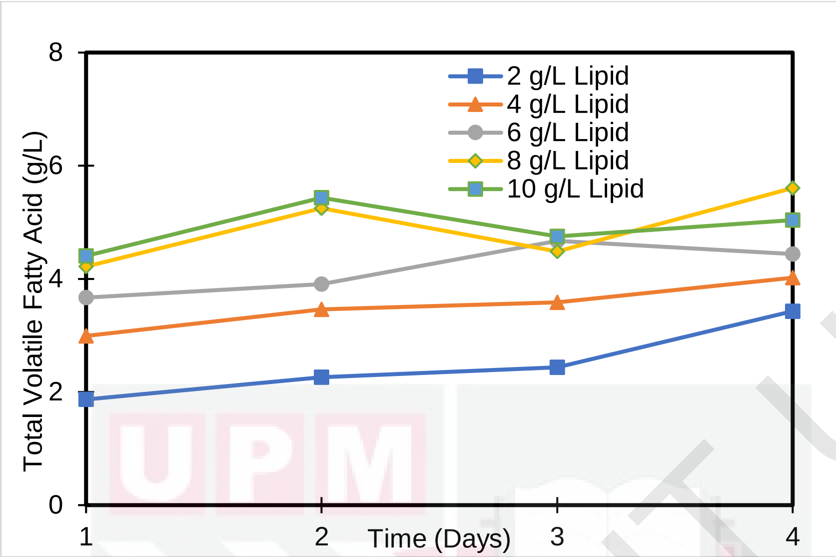

Figure 4.37: Total VFA concentration for two-stage AD in Tank 2

The pH for tank 1 (Figure 4.38) was parallel to the total VFA results. For 2, 4 and 6 g/L lipid concentrations, the total VFA increased over time; thus, the system's pH reduced. Meanwhile, for 8 and 10 g/L lipid concentrations, the total VFA decreased from day 2; thus, this also reduced the pH. Interestingly, the increase in total VFA in Tank 2 did not reduce the pH on day 3 of digestion (Figure 4.39), which may be influenced by AN buffering capacity, as explained in Subsection 4.3.2.

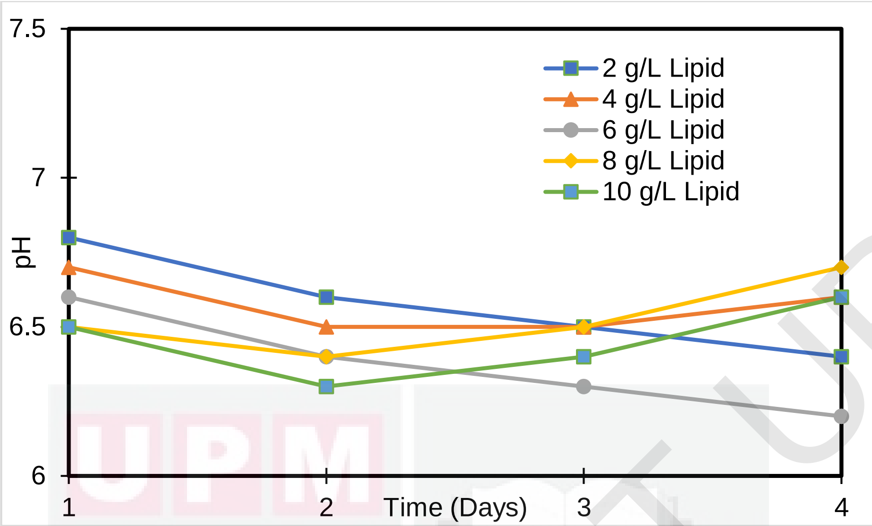

Figure 4.38: pH for two-stage AD in Tank 1.

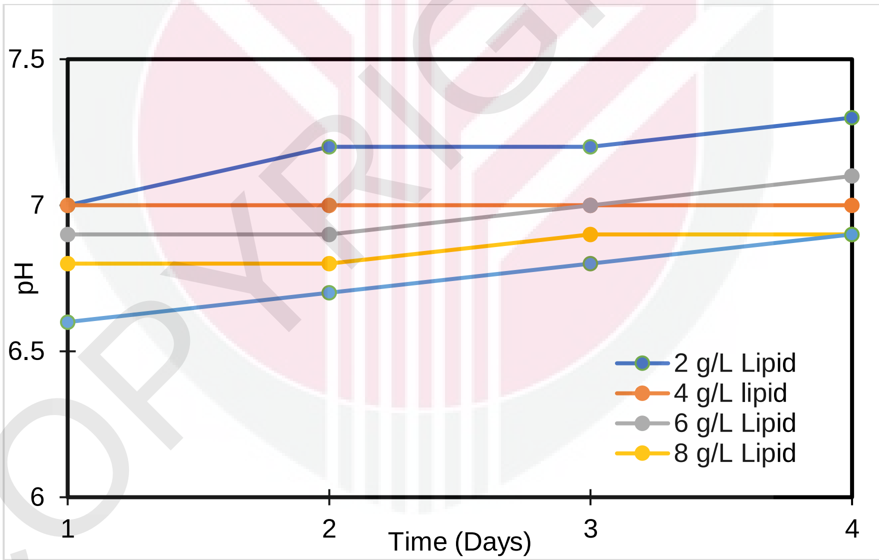

Figure 4.39: pH for two-stage AD in Tank 2

In one-stage AD when fed with high lipid concentration substrate, it was observed that the total VFA increased over time except for 10 g/L on days 3-4 (Figure 4.40). The reduction of total VFA at 10 g/L lipid concentration could be by the reduction of microbial activity due to sludge floatation, as shown in Figure 4.41. Lipids and LCFAs can be adsorbed on the surface of sludge granules to form a stable oily film. This oily film may obstruct mass transfer between granules and bulk liquid. As a result of hunger or the accumulation of extracellular enzymes that cannot diffuse quickly enough to the exterior of the granules, cell lysis may occur inside the sludge granules. Concurrently, gaseous product export can become less efficient, accumulating gas bubbles within sludge granules, thus triggering sludge flotation (Leitão et al., 2006). From Figure 4.42, the pH of the one-stage digester reduced with higher lipid concentration. The reduction of pH is consistent with the total VFA production in the digester.

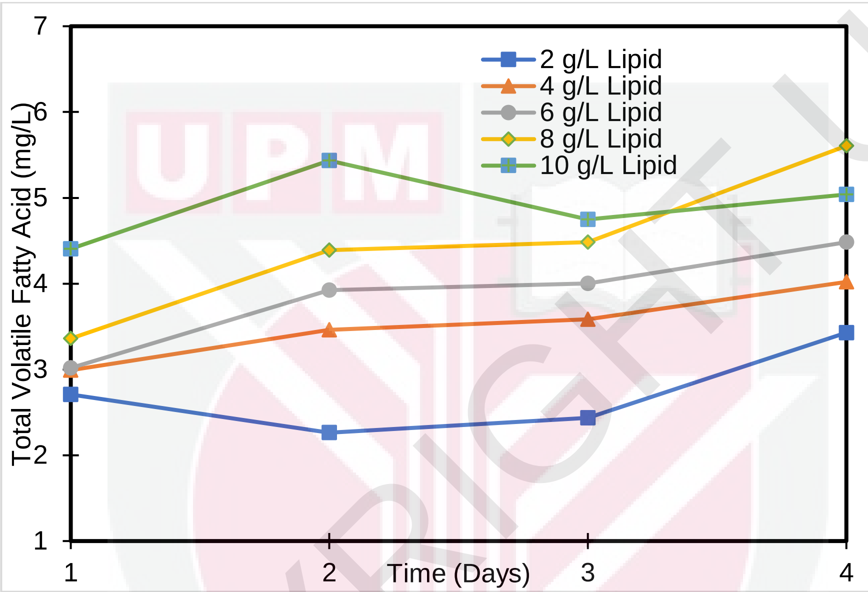

Figure 4.40: Total VFA Concentration for one stage AD

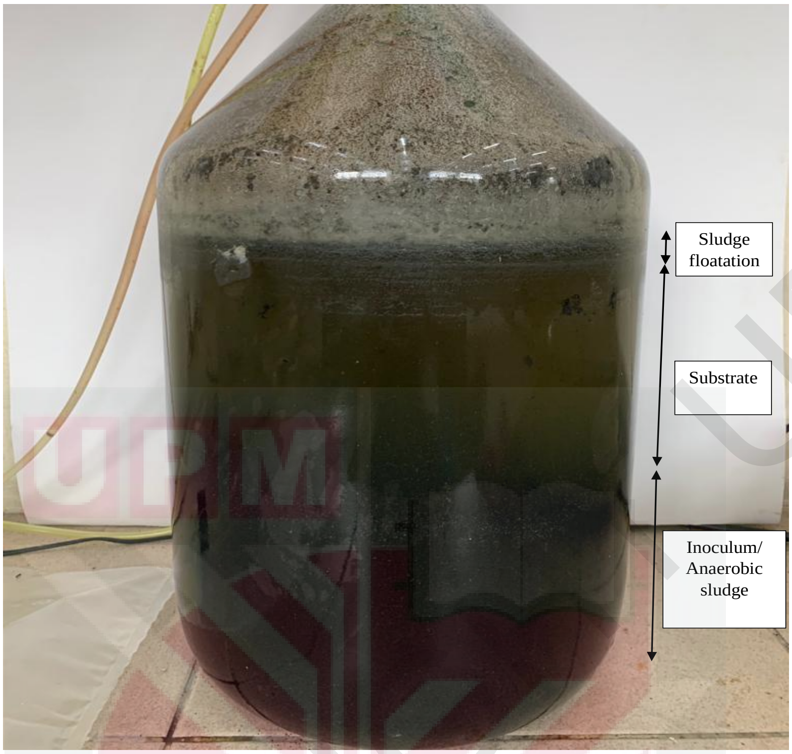

Figure 4.41: Sludge floatation in one-stage AD fed with 10 g/L Lipid wastewater.

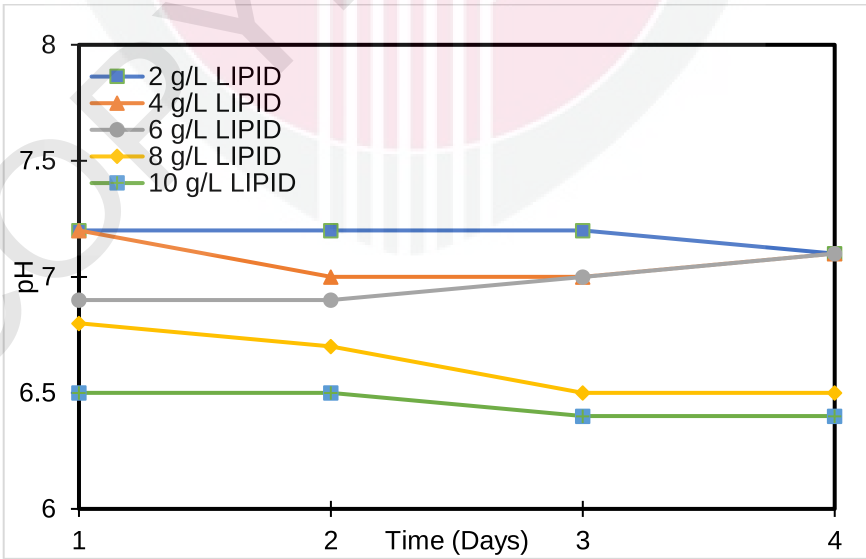

Figure 4.42: pH of one-stage AD

4.5.6 FOG Removal

The hydrolysis of lipids in AD is a fast reaction, which converts lipids into fatty acids and glycerol; thus, the FOG in the sample is lower over time. The β-oxidation pathway is a rate-limiting step that causes the accumulation of intermediates on the surface of the digester (Affes et al., 2017), which was validated through the fatty acid analysis (Subsection 4.5.1.1). From Figure 4.43, the FOG contents in the Tank 1 digester were low for all substrate lipid concentrations. For Tank 2, the initial FOG concentration ranges from 0.5-2 g/L for every feeding, which is significantly low; thus, 100% of FOG content was removed in all lipid concentrations. Whereas for the one-stage AD (Figure 4.44), only a small amount of FOG was analyzed. This is because in one stage, the methanogens consume the LCFA intermediates simultaneously as they are produced (Li et al., 2014) thus reducing the lipid inhibition in the digester. There was no scum observed on the surface of the digester in one-stage AD.

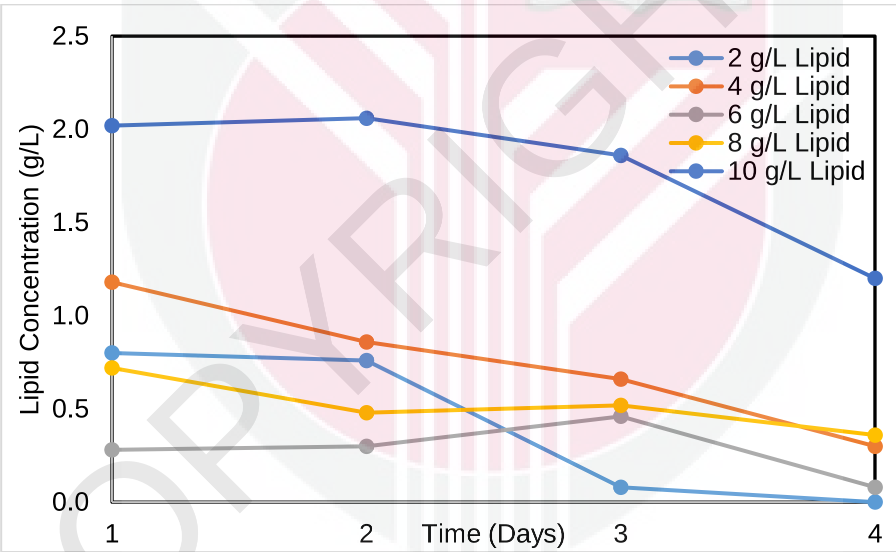

Figure 4.43: FOG content in two-stage AD Tank 1

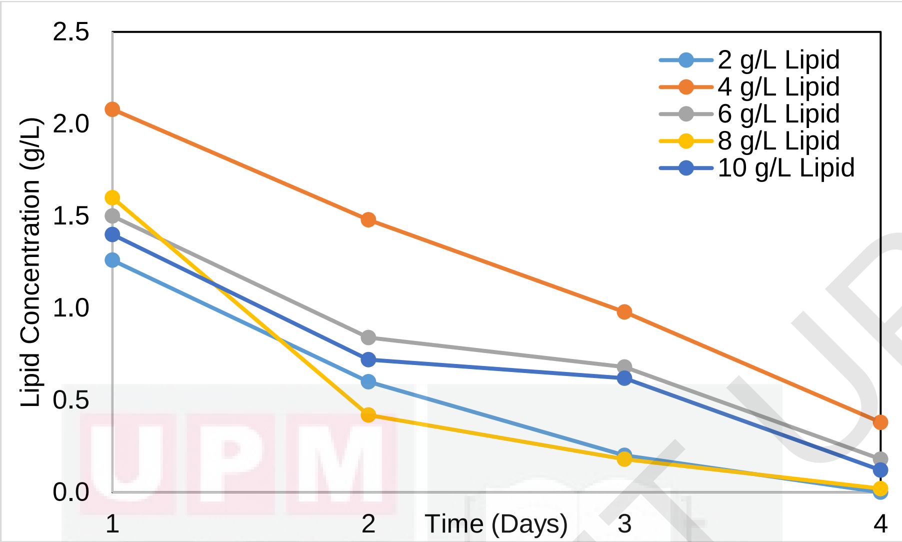

Figure 4.44: FOG content in one-stage AD

4.5.7 Biogas Accumulation

The cumulative biogas accumulation was seemingly higher in one-stage AD than in two-stage AD, as shown in Figures 4.45 and 4.46. The highest biogas accumulation was at a substrate lipid concentration of 10 g/L at 0.615 L/l/d in two-stage AD and 1.835 L/l/d in one-stage AD. Besides that, the increase in biogas production was directly proportioned with substrate lipid concentration for one-stage AD. Whereas in two-stage AD, the continuous digestion process outperformed the batch process when treating animal wastes, increasing biogas production by 34.2% when the two optimum yields were compared. This is because the rate of bacteria growth was faster in continuous feeding (Obiukwu & Nwafor, 2016). However, in this study, the biogas yield was significantly lower due to the lower microbial activity from fatty acid accumulation. It was reported that the accumulation of intermediate products such as propionate, ethanol, or acetate, particularly in the undissociated form, inhibits several microbial species, decreasing methane production. Propionate acid is also harmful to methanogens (Al-Sulaimi et al., 2022; Wang et al., 2009)

The increase in lipid concentration in the system increases the accumulation of LCFA. The ability of LCFA to cause inhibition is related to the adsorption capacity of the microbial cell membrane; forming a barrier that disrupts membrane capabilities and limits the transport of substrates and products is associated with their inhibitory mechanism (Rasit et al., 2015). It was observed that the accumulation of scum on the digester surface occurred quickly, and the characterization of scum revealed a high concentration of protein and lipids (Table 4.2). Wu et al. (2016) revealed that high lipid concentration reduces protein and carbohydrate degradation capability. In this study, the poor degradation rate of protein, lipid, and carbohydrate results in the accumulation of surface-active compounds on the surface of the digester (Tank 1) due to their hydrophobic characteristics (Beneventi et al., 2001). Thus, lesser active compounds are digested and transferred into Tank 2, reducing biogas accumulation.

In one-stage AD, biogas production is significantly higher than in two-stage AD. The high biogas production correlates to efficient mass transfer within the digester. According to the findings of a study, two-stage AD is not necessarily superior to single-stage AD in terms of substrate-specific biogas production yields, but it may offer the possibility of higher loading and production rates (Janesch et al., 2021). All process phases are carried out in parallel in the dominant single-stage AD, benefiting intercellular hydrogen transfer (Moeller & Zehnsdorf, 2016).

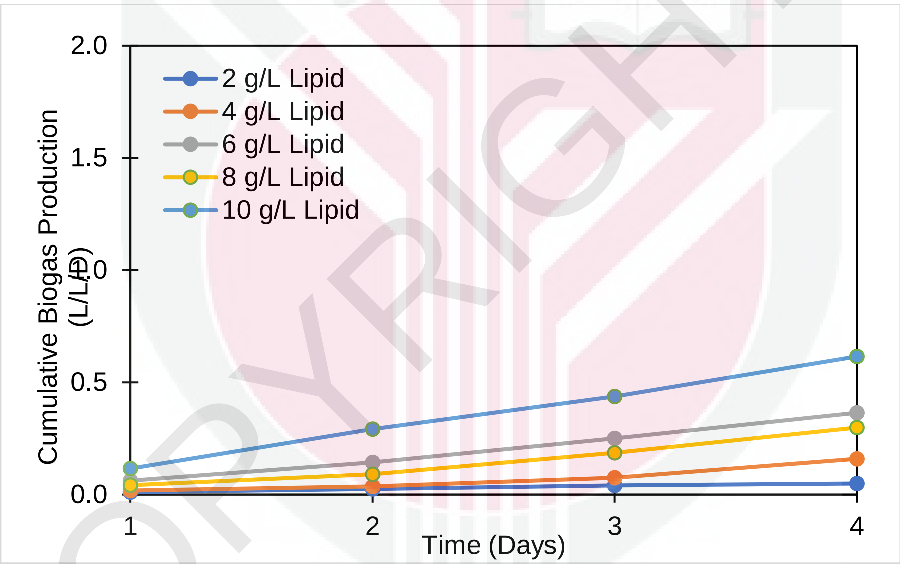

Figure 4.45: Biogas Accumulation in two-stage AD in Tank 2

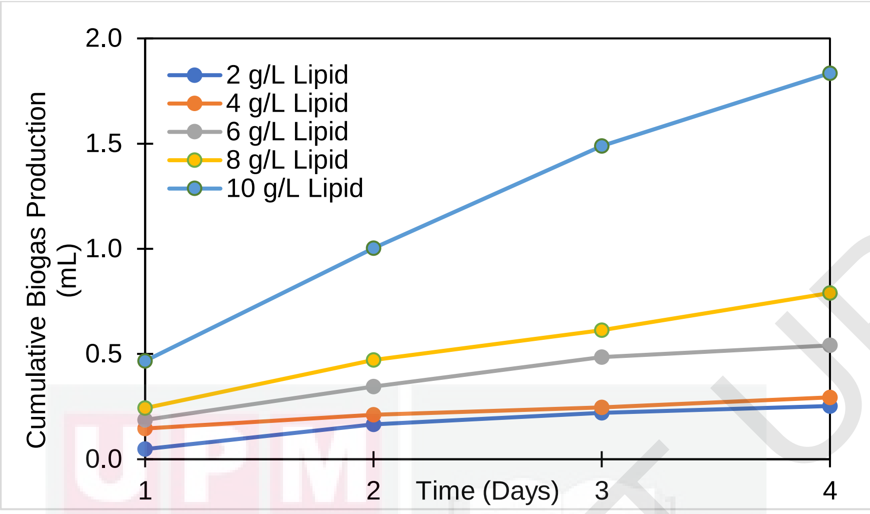

Figure 4.46: Biogas Accumulation in one-stage Anaerobic Digestion

4.6 Effect of antifoams on scum and foam in two-stage AD

This research uses two types of antifoams: natural oils and bio-surfactants. The antifoams were implemented in the two-stage AD because in the one-stage AD scum was not present even at high lipid concentrations. Rapeseed oil (0.25 v/v%, 0.5%v/v) and palm oil (0.5% v/v) were implemented for natural oils. While for biosurfactants, a preliminary study was first conducted to investigate the performance of AS. The preliminary study was conducted through a one-stage AD (Batch test).

4.6.1. Effect of biosurfactants on one-stage AD

The dosage of biosurfactants like rhamnolipids is crucial for their influence on hydrolysis-acidogenesis. As a result, preliminary research is critical (Johnravindar et al., 2022). Rhamnolipid (RL) and Tea Saponin (TS) were used in this study to improve the acidogenesis and hydrolysis process. According to previous studies, each biosurfactant was dosed at optimum dosage (Huang et al., 2015; Li et al., 2019). RL was dosed according to total suspended solid (TSS) content, while TS was dosed according to to dry solid (DS) content.

4.6.1.1 COD removal

Figure 4.47 shows that COD was the highest when one-stage AD was fed with 0.02 g RL g^{-1} TSS indicating that the performance of the digester is enhanced when using RL. The utilization of surfactants to aid in the emulsification and disposal of oils from wastewater has been investigated for the treatment of wool-scouring wastewater, which has a high COD and O&G (Nakhla et al., 2003). After 16 days, the addition of biosurfactant to raw wastewater improved COD removal. This indicates that the addition of RL improved COD removal in wastewater. It was observed that the addition of RL of 0.02 g RL/g TSS had the highest COD removal compared to other dosage, which is consistent with a previous study(Xu et al., 2021a). It has been demonstrated that RL may efficiently improve the solubility of organic substances and solubilize hydrophobic substances, making it easier for the cell to uptake (Al-Tahhan et al., 2000). Additionally, it could make the cell surface more hydrophobic, increasing the cell's interaction with the less soluble substrate as compared to TS (Li et al., 2019). The results are consistent that overall, bio-surfactants can improve hydrolysis and acidogenesis in AD digesters however, rhamnolipid had the best outcomes.

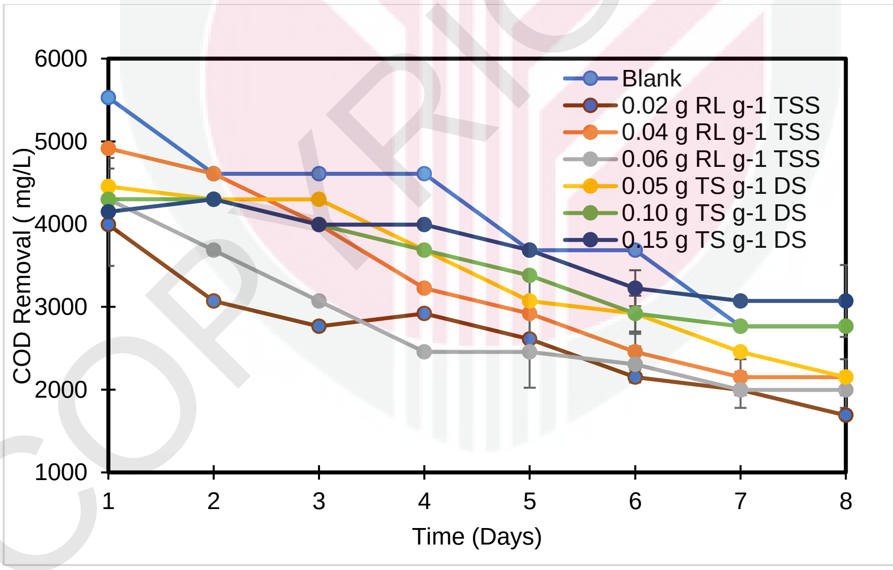

Figure 4.47: COD removal in one stage AD fed with RL and TS

4.6.1.2 Protein removal

The removal efficiency of protein in Figure 4.48 indicates that the addition of biosurfactant improved the digestion process. It is observed that rhamnolipid at a dosage of 0.02 g RL g-1 TSS had the highest protein removal, whereas the highest protein removal when fed with tea saponin was 0.05 g TS g-1 DS, similar to COD removal. Rhamnolipid and saponin can increase the surface activity and solubilization of organic matter, resulting in higher protein removal compared to blank. All samples with biosurfactant had higher protein removal than blank except for 0.10 g TS g-1 DS on day 4, which slightly increased. In AD, protein is hydrolyzed to amino acids, which are then acidified to VFA, and methane is produced. It was explained that certain amino acids would be challenging to biodegrade and acidify during the anaerobic biological treatment process. A study conducted on the effect of RL addition on protein-rich wastewater concluded that RL was able to decrease cell surface hydrophobicity and increase cell membrane permeability of protein (Wang et al., 2021)

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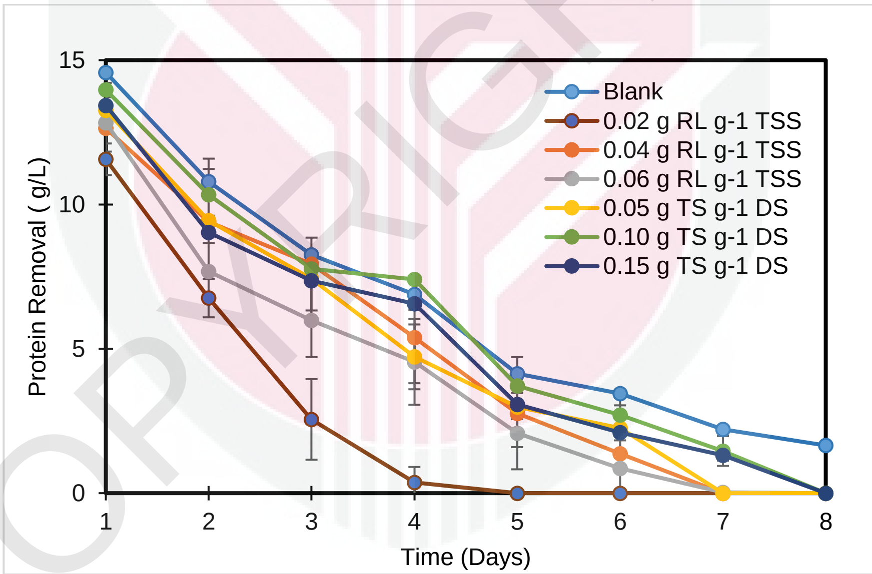

Figure 4.48: Protein removal in one stage AD fed with RL and TS

4.6.1.3 Total VFA Concentration and PH

The total VFA concentration improved drastically when biosurfactants were added to the substrate for one-stage AD. Figure 4.49 indicates that the highest total VFA production was achieved when RL was added at a dosage of 0.02 g

RL g^{-1} TSS, whereas the highest VFA production was achieved at a dosage of 0.05 g TS g^{-1} DS for TS. When compared with blank, TS had a lesser impact on hydrolysis and acidogenesis than RL. Both RL and TS contain glycosyls that can be hydrolyzed into saccharides. The produced saccharides have the potential to be a superior substrate for acid production. The high surfactant surface activity was the primary reason for the improved organic matter solubilization and hydrolysis, which benefited VFA production (X. Huang et al., 2015a). Besides that, bacteria that produce acid were able to function longer, indicating RL promoted the acid-producing ability of those microbes. This could be attributed to RL releasing enough soluble carbon compared to TS. On Day 4, the TVFA was the highest; the peak was due to the maximum food intake by the bacteria and the drop in VFA beyond that the inoculum actively consumed the VFA to produce gas which can be observed in Figure 4.49.

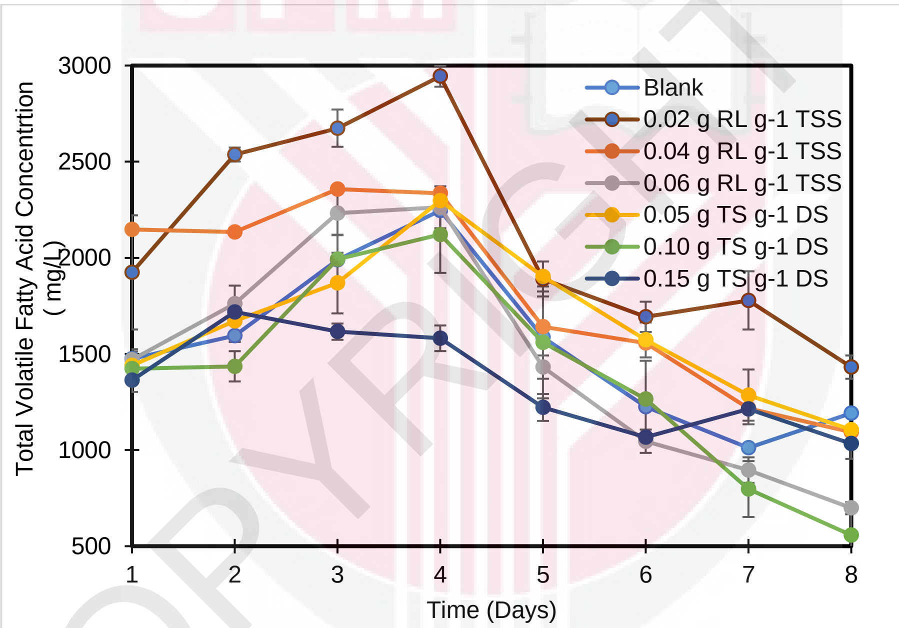

Figure 4.49: Total VFA concentration in one stage AD fed with RL and TS

The pH variations are shown in Figure 4.50, respectively. The pH of each concentration and blank fell from 7 to 6.5-6.8 in the first 4 days, concurring with total VFA production at the start of the experiment. Methanogens then convert the short-chain fatty acids hydrolyzed from protein and lipid, essential intermediates, to produce methane. The pH ranges from 6.7 to 7.4 for both digesters added with RL and TS, which are still within the optimum range. The blank sample had the highest pH compared to the samples added with biosurfactants, indicating that bio-surfactant addition improved the production of organic acids in the digester (Li et al., 2014). The lowest pH was observed for

0.02 g RL g-1 TSS addition as observed for RL and 0.05 g TS g-1 DS for TS. The addition of surfactants can alter the system's pH by enhancing the production of VFA as shown in Figure 4.47.

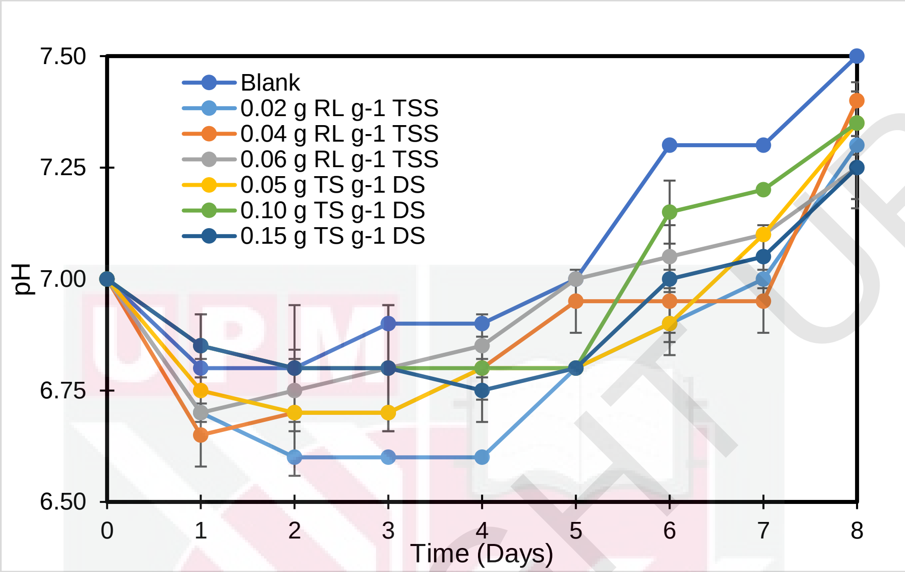

Figure 4.50: pH of one stage AD fed with biosurfactants RL and TS

4.6.1.4 Biogas Accumulation

It was observed that the highest biogas accumulation was at 0.02 g RL g^{-1} TSS as shown in Figure 4.51. As expected, all the digesters fed with bio-surfactants increased biogas production as compared to blank (X. Huang et al., 2015a). The usage of bio-surfactants has improved digestion when used FPW as a substrate. RL had a higher biogas yield compared to TS. The increased acid production might be accompanied by increased protons and high substrate loading, which could boost the growth of a critical methanogenesis community archaeon (Li et al., 2019). It is widely known that rhamnolipids have an excellent impact on accelerating the biodegradation of lipids such as oil because they can reduce interfacial tension and increase solubility significantly (H. Zhang, Long, et al., 2009). Thus, providing the system with a much more cohesive environment and enabling microbial activity to increase, resulting in higher biogas production. The addition of tea-saponin has shown promising results. However, the slight reduction of gas compared to rhamnolipid could be due to the interference of hydrogen transfer between methanogens (Guo et al., 2008).

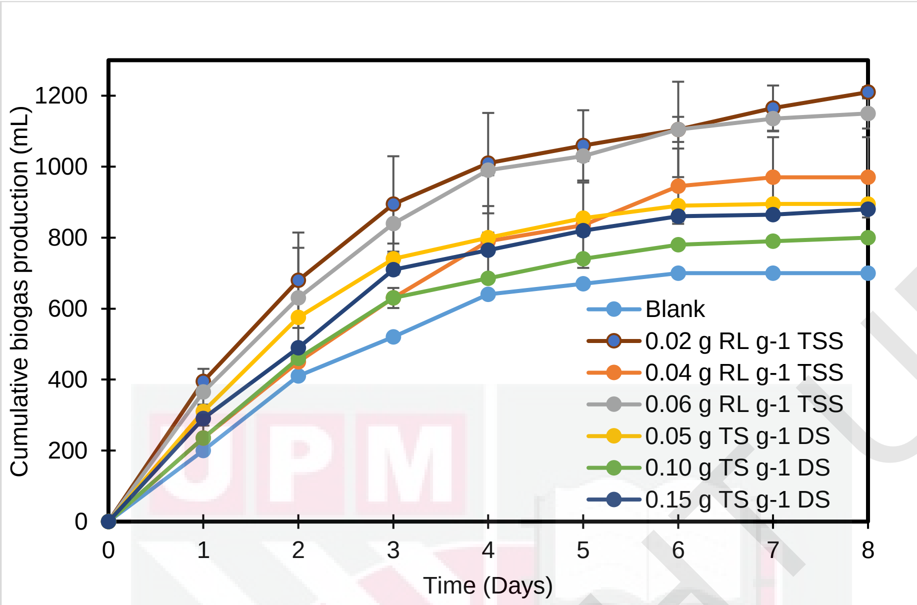

Figure 4.51: Biogas Accumulation of one-stage AD fed with RL and TS

4.6.2 Effect of antifoam on scum in two-stage AD

The two-stage digester was run according to subsection 4.3. 6 g/L Lipid was used as blank since it had high biogas production, total VFA, AN, and COD, and protein removal efficiency. The dosage of rapeseed oil (RO) used in this research was 0.25% and 0.5% v/v, palm oil was 0.5% v/v, and RL at a dosage of 0.02 g RL g-1 TSS. TS was not used in this section as RL performed better in the preliminary study.

4.6.2.1 Scum reduction efficiency

The scum reduction efficiency test revealed that the highest reduction percentage was 32% when FPW was fed with RO 0.5% v/v as an antifoam, as shown in Figure 4.52. The reduction of scum level in this study is correlated to the antifoam properties of RO. Since the foam is present on the surface of the digester below the scum, it is deduced that the presence of RO reduced the scum level by removing the foam. Fatty acids, such as oleic acid, and neutral oils with carboxylic groups bonded to a molecule of glycerol, such as RO, are hydrophobic compounds with low water solubility. As a result of their hydrophobic nature, they accumulate on the foaming surface and rapture the foaming film via bridging mechanisms (Denkov, 2004). When RO dosage was reduced to improve the reactor's performance, the percentage of scum reduction capability decreased significantly to 11% on day 4 of digestion. The decrease in scum reduction efficiency is consistent with previous findings that a higher dosage of RO is needed for more reduction of foam and scum (Kougias et al., 2015; Thakur et al., 2019). Besides that, another type of natural oil that was investigated was PO. PO with a dosage of 0.5% v/v had a scum reduction percentage of only 1.9%, considerably lower than RO. Palmitic acid is known to be degraded five times slower. Thus, it accumulates on the digester surface resulting in lower microbial activity in the digester (Dasa et al., 2016; Silvestre et al., 2014). The resulting scum produced on the digester surface was also clumped together (Figure 4.53) with a pungent smell. Comparatively, the scum produced when fed with RO was loose, as shown in Figure 4.54. The PO and RO residue were also observed in the digester effluent, as shown in Figures 4.55 and 4.56, respectively. RO includes monounsaturated fatty acids such as oleic acid and saturated and polyunsaturated-like linoleic acids. As a result, a mixture of various fatty acids may have improved rapeseed oil's propensity to destabilize foam, leaving an aqueous mixture (Kougias et al., 2015). At the same time, PO has a higher density, which could cause reduced efficacy in reducing foam and scum.

Surfactants are supposed to improve the microbial bioavailability of hydrophobic substrates by increasing their dispersion in aqueous environments or modifying cell surfaces (Zhang et al., 2009). Conversely, RL increased the scum level by 23% compared to blank results, demonstrating that a biosurfactant at optimum concentration positively affects hydrolysis and acidogenesis (Li et al., 2019). It has also been shown that RL surfactant can increase the abundance of acid-producing bacteria (Zhou et al., 2015), resulting in higher production of scum.

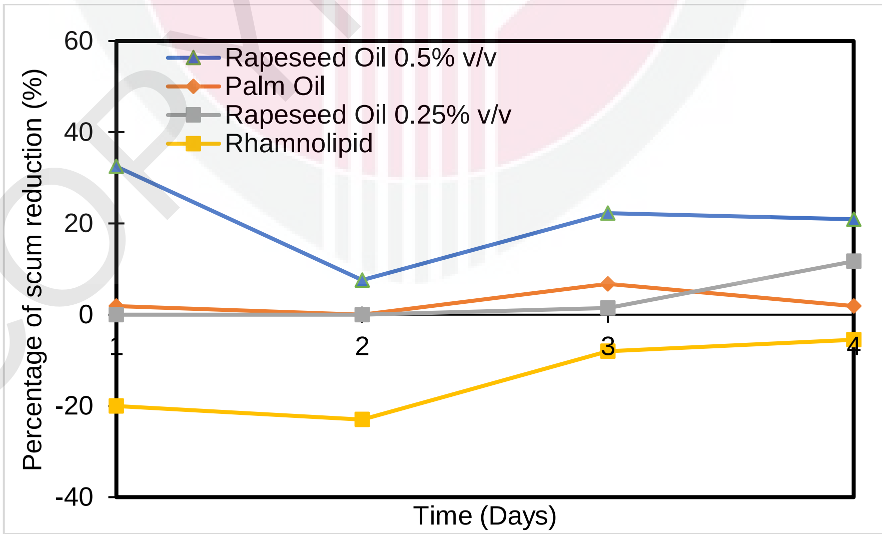

Figure 4.52: Percentage of scum reduction in two-stage AD (Tank 1).

Figure 4.53: Scum formation in Tank 1 two-stage AD treated with palm oil

Figure 4.54: Two-stage AD effluent treated with palm oil

Figure 4.55: Scum formation in tank 1 two-stage AD treated with rapeseed oil (0.5%v/v)

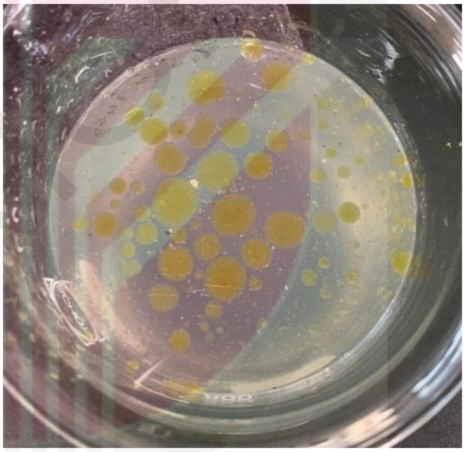

Figure 4.56: Two-stage AD effluent treated with rapeseed oil (0.5% v/v)

4.6.2.2 Percentage of COD removal

From Figure 4.57, the percentage of COD removal in Tank 1 when fed with antifoam was highest with RO at a dosage of 0.5% v/v, followed by PO, Blank, RL, and RO at 0.25% v/v. The initial COD of RO when dosed at 0.5% v/v was 11,239 mg/L. However, the COD was reduced to 1456 mg/L on day 4. For PO, the initial COD was 12,230 mg/L, and it was reduced to 1747.2 mg/L. The high reduction in COD firstly is because when natural oil was fed in the tank, the COD reading was taken after homogenization, but in the digester, the RO accumulates on the surface as soon as the feeding ends. Besides that, the mixture's organic matter (i.e., protein) attracts to the natural oil when introduced since it is a hydrophobic compound, thus reducing the organic content in the digester. This explains the higher COD when treated with 0.25% v/v RO. For Tank 2, the percentage of COD removal was the highest for RO at 0.5% v/v, PO, Blank, RL, and RO at 0.25% v/v (Figure 4.58), similar to Tank 1 results. The accumulation of organic matter in the surface of Tank 1 reduced the transfer of the organic matter to Tank 2. The low COD removal of RL and RO 0.25% as compared to blank is due to the high amount of scum on the digester surface. The presence of RL improved the acidogenesis rate in tank 1, thus resulting in higher scum production. As this is prolonged, the digester experience overload and lower microbial activity. Whereas the low dosage of RO resulted in higher scum levels causing similar conditions as RL.

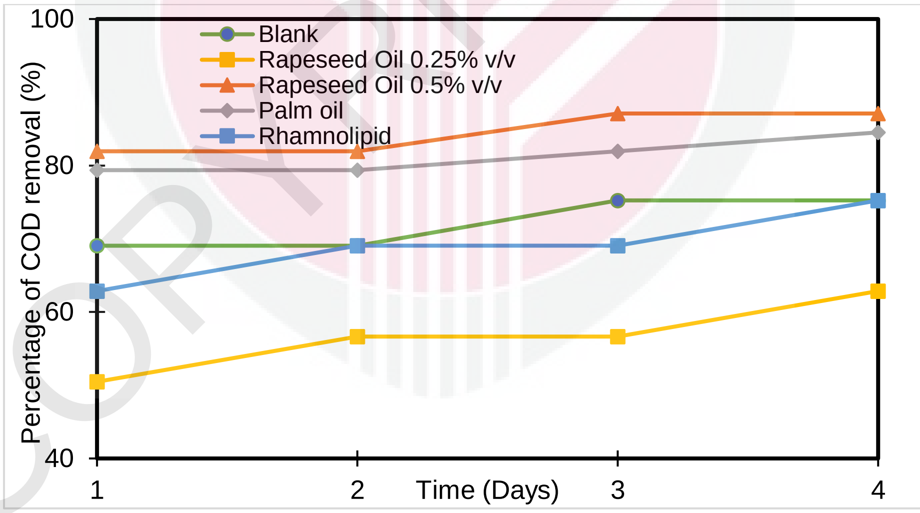

Figure 4.57: Percentage of COD removal in two stage AD in tank 1

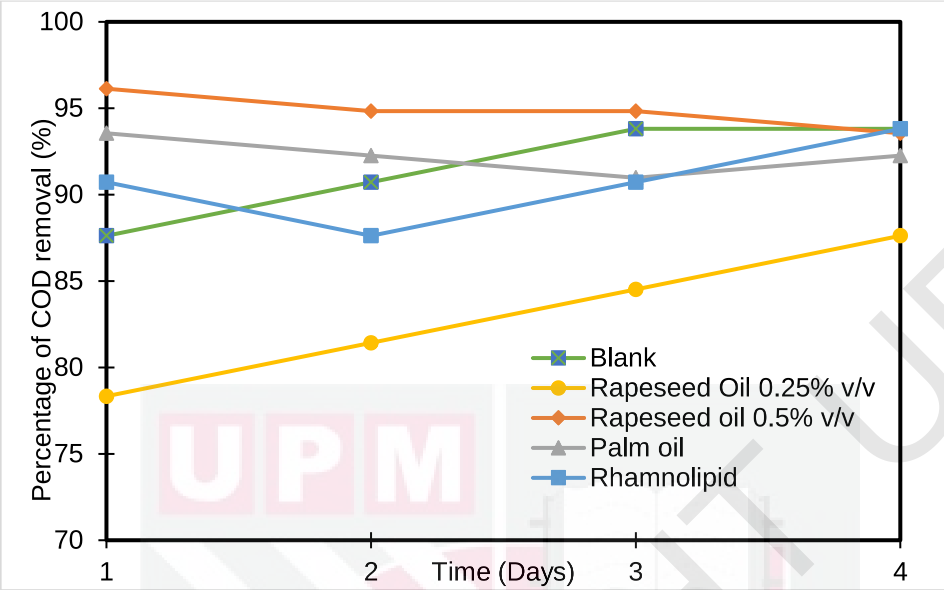

Figure 4.58: Percentage of COD removal in two stage AD in Tank 2

4.6.2.3 Protein removal efficiency

The percentage of protein removal was the highest when RO at 0.25% v/v was used in Tank 1 followed by RL, Blank, RO at a dosage of 0.5% v/v, and PO on day 4 (Figure 4.59). It was observed that the protein removal was good when the natural oils were used as antifoam at a high dosage. PO has some concentration of protein left in both Tanks 1 and 2 (Figure 4.60) of the digesters. Besides that, 0.05% v/v RO also had a lower percentage of removal concentration when compared to 0.25% v/v rapeseed oil dosage and blank on day 4. The lower removal percentage could be due to less microbial activity since higher organic matter ratios are too numerous for the available bacteria to digest (Mahat et al., 2020). Besides that, the lesser protein degradation could also be due to the accumulation of fatty acids in the digester. The digestion with RL had excellent protein removal for both tanks, indicating that the protein was successfully degraded in the digester. RL can improve the hydrolysis stage significantly, thus improving the rate of protein degradation (Lovaglio et al., 2011).

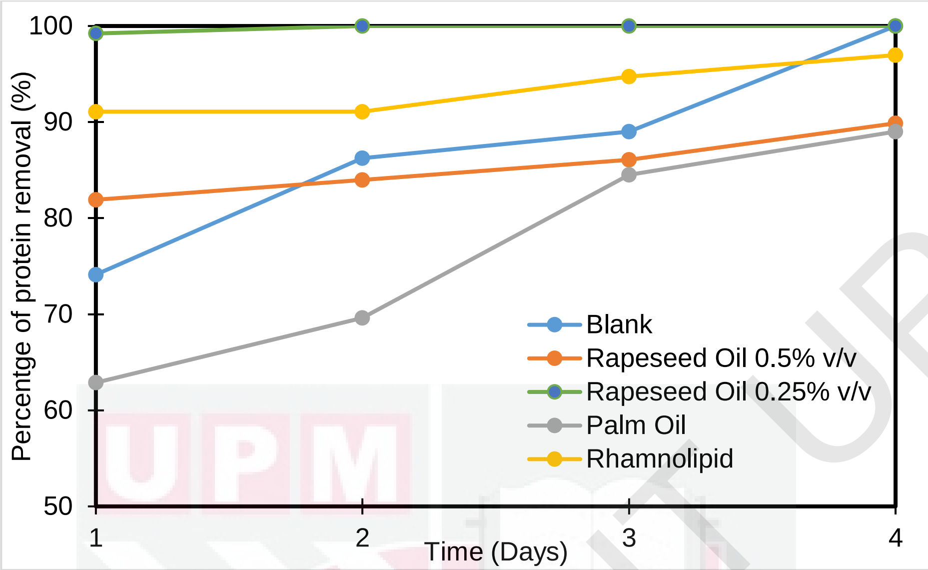

Figure 4.59: Percentage of protein removal in two-stage AD in Tank 1.

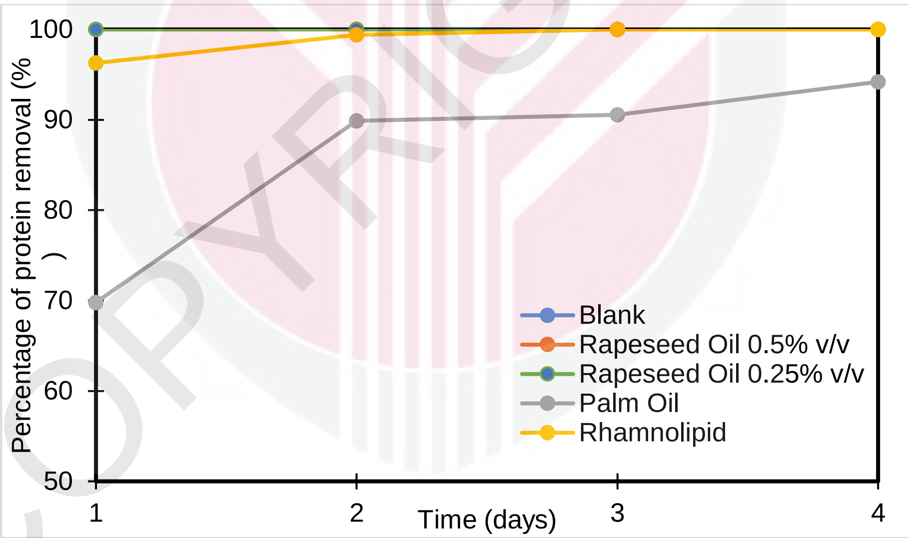

Figure 4.60: Percentage of protein removal in two stage AD in Tank 2

4.6.2.4 Percentage of lipid removal

The lipid removal percentage is presented in Figures 4.61 and 4.62 for Tank 1 and 2, respectively. The highest lipid removal in Tank 1 is blank at 99.3%, while the lowest was RO at a dosage of 0.5% v/v. This result is consistent with the COD percentage removal. Nevertheless, the lipid removal for all antifoams in Tank 2 is higher than 94%, which indicates good performance. However, the biogas accumulation in Figure 4.66 is low for natural oil study. It could be due to the lower organic content in digester Tank 2.

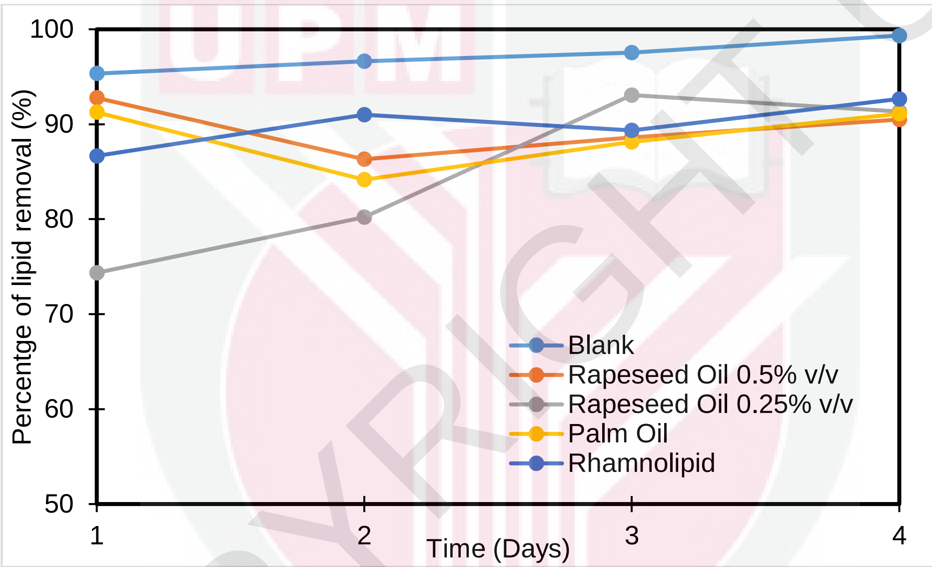

Figure 4.61: Percentage of lipid removal in two-stage AD in Tank 1

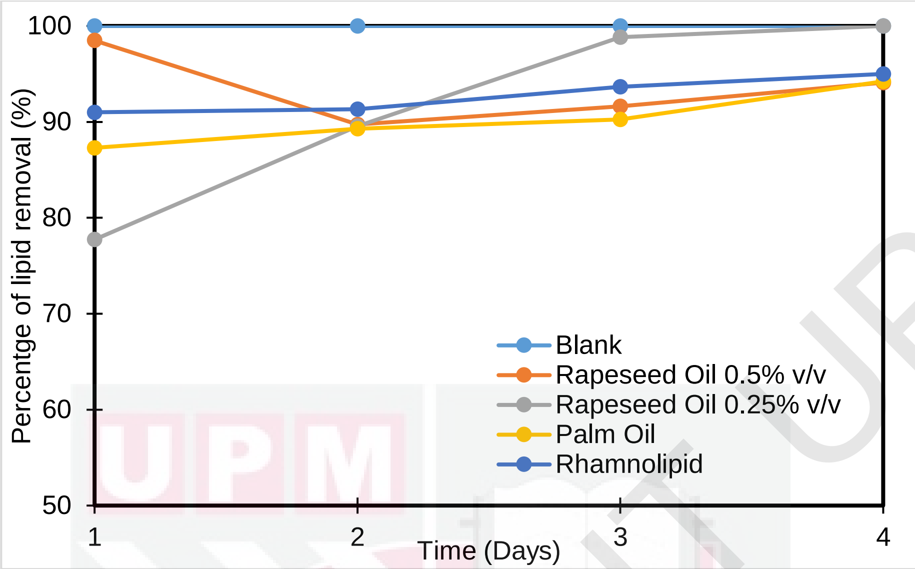

Figure 4.62: Percentage of lipid removal in two-stage AD in Tank 2

4.6.2.5 AN Concentration

The AN concentration observed in this part of the research is relatively low when fed with 0.5% v/v of rapeseed and palm oil in Tank 1 (Figure 4.63) and Tank 2 (Figure 4.64). There are several reasons for the reduction of AN concentration in the digester. Firstly, is the accumulation of total VFA (Figures 4.65 and 4.66) in the tanks due to excessive production of FA causes a restriction in the bacterial activity in the digester resulting in lower ammonia concentration in the digestate (Yenigün & Demirel, 2013). Besides that, since previous analysis indicated a low COD and protein removal, the AN concentration could also be affected due to the lack of intermediate compounds. Whereas for RL, the experimental results are consistent with COD removal, confirming that RL can promote the release of dissolved organic matter in the co-digestion system, providing adequate material assurance for succeeding the methanogenesis process. The percentage of protein removal in Tank 1 (Figure 4.59) and 2 (Figure 4.60) was high, corresponding to the AN concentration. Over time, there is a slight increase in AN concentration for all antifoams except RL, which is more stagnant and indicates an increasing amount of AN in the digester; however, it is very slow.

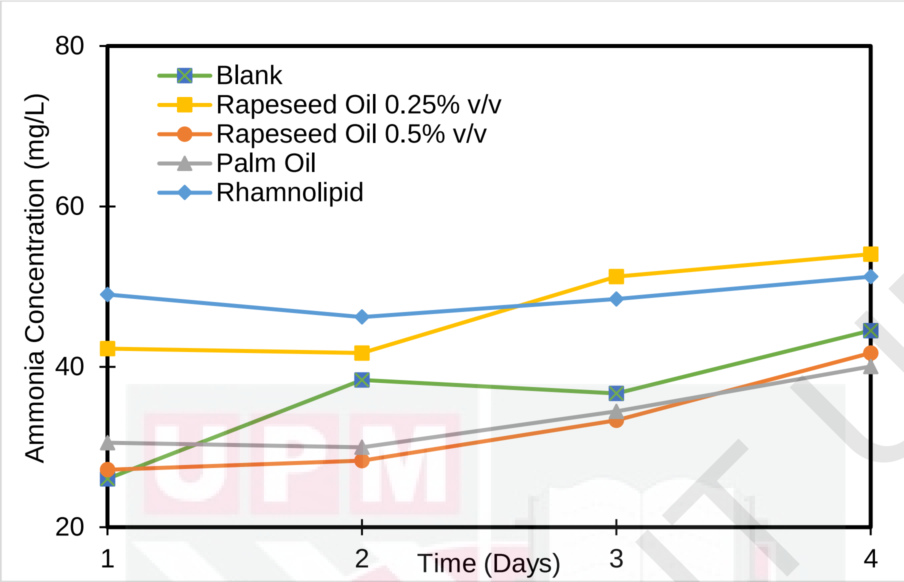

Figure 4.63: AN Concentration for two-stage AD in Tank 1.

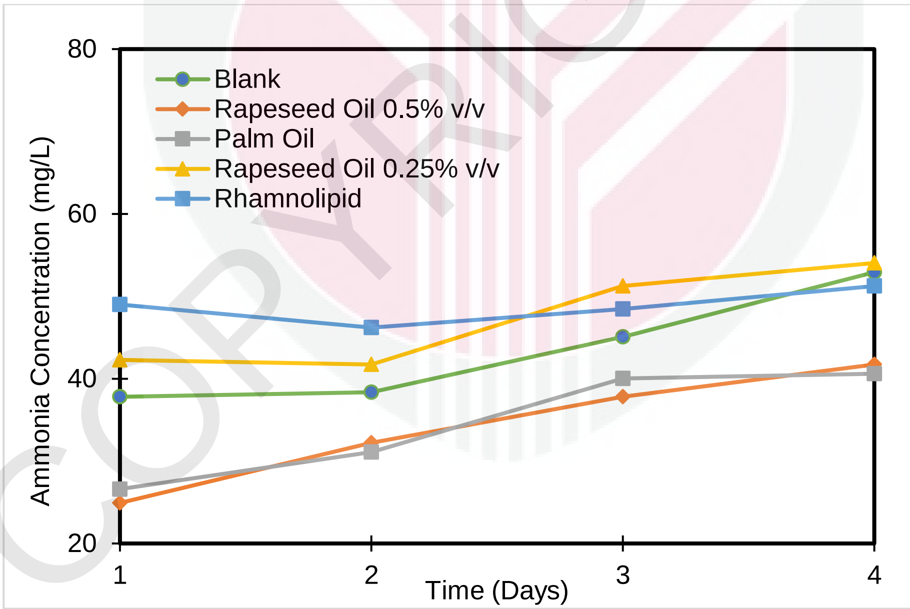

Figure 4.64: AN Concentration for two-stage AD in Tank 2

4.6.2.6 Total VFA Concentration and pH

The total VFA concentration was reduced in the digesters when the feed was added with natural oils as antifoam. From Figure 4.65 and Figure 4.66, the total VFA of the blank was higher in both tanks 1 and 2 when compared to natural oils dosage (RO and PO). In PO, there are high concentrations of palmitic acid; thus at, concentrations greater than 1.1 g/L inhibited anaerobic digestion by about 50% under mesophilic conditions. Whereas, for RO, the most abundant products were butyric, acetic, and propionic acids. High ammonium, sodium, and VFA concentrations hampered VFA production during the acidogenic stage (Magdalena et al., 2019). For Rhamnolipid, the total VFA was the highest on day 1 of digestion at 7868.57 mg/L in Tank 1, but the total VFA decreased over time. The reduction of VFA in the system could be due to increased scum level because it hinders the activity of acidogenic bacteria. The increase in scum level is correlated to the improvement of hydrolysis when rhamnolipid is added in the digester resulting in higher LCFA concentrations. However, in Tank 2, the total VFA increased. The accumulation of VFAs in Tank 2 caused a reduction in methanogenesis activity, thus resulting in a slightly lower biogas yield than blank, which was explained in Subsection 4.4.2.7.

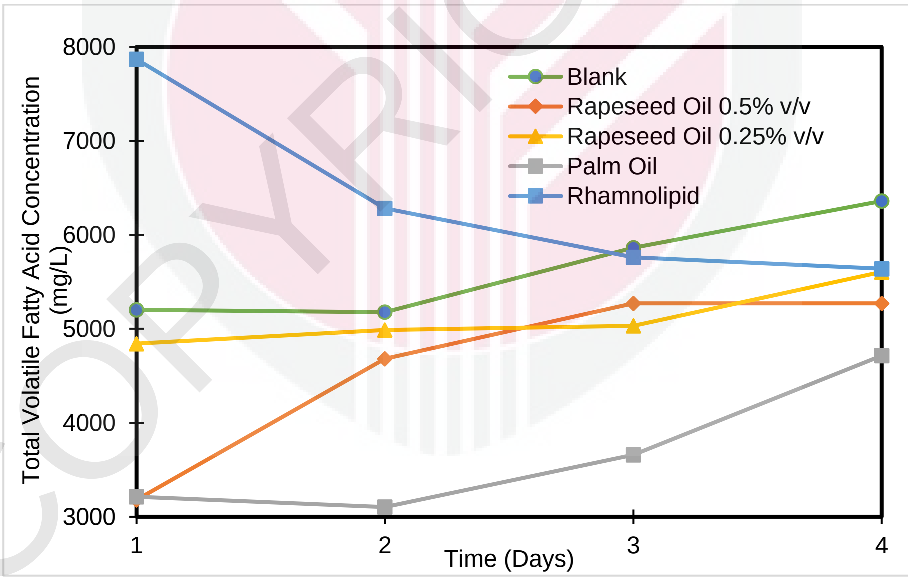

Figure 4.65: Total VFA Concentration for two-stage AD in Tank 1

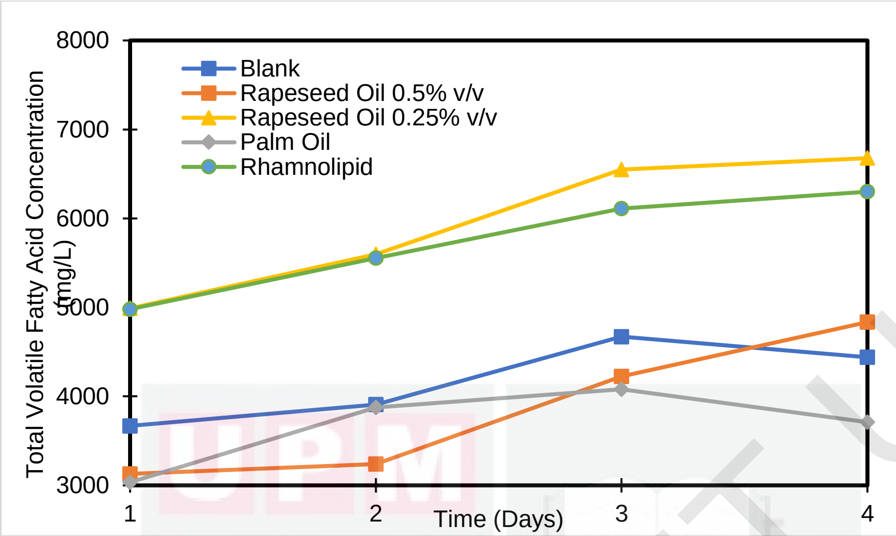

Figure 4.66: Total VFA Concentration for two-stage AD in Tank 2

Corresponding to the total VFA, the pH of Tank 1 (Figure 4.67) and Tank 2 (Figure 4.68) shows that the addition of antifoams reduced the pH of the digester. RL-fed digestion has a high pH in Tank 1 even though there was high production of organic acids. The reduction of pH over time for RL could be associated with the accumulation of LCFA in the digester as shown in Figure 4.52, the scum level was higher as compared to other digesters. AN that acts as a buffering medium increased in the system leading to this. In Tank 2, palm oil had the lowest pH, which could be due to the accumulation of palmitic acid (Pereira et al., 2005)

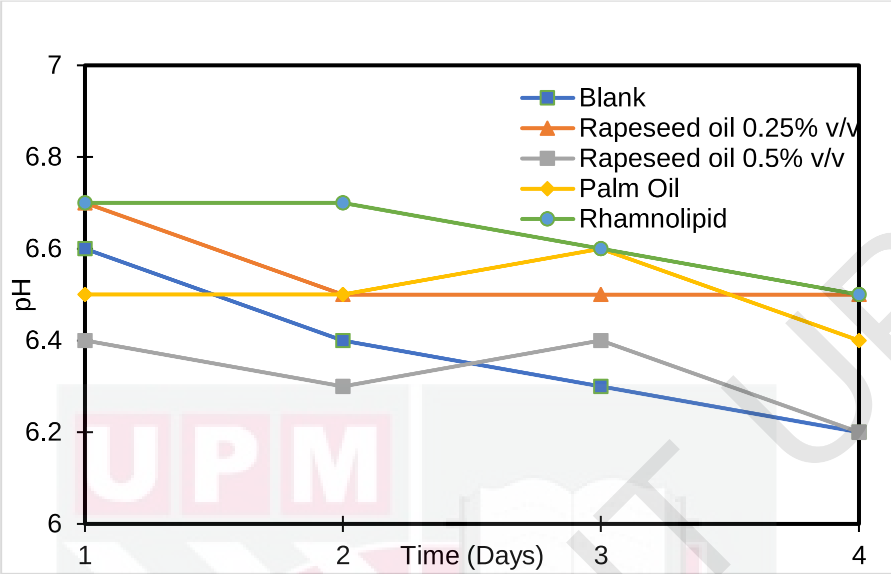

Figure 4.67: pH for two-stage AD in Tank 1.

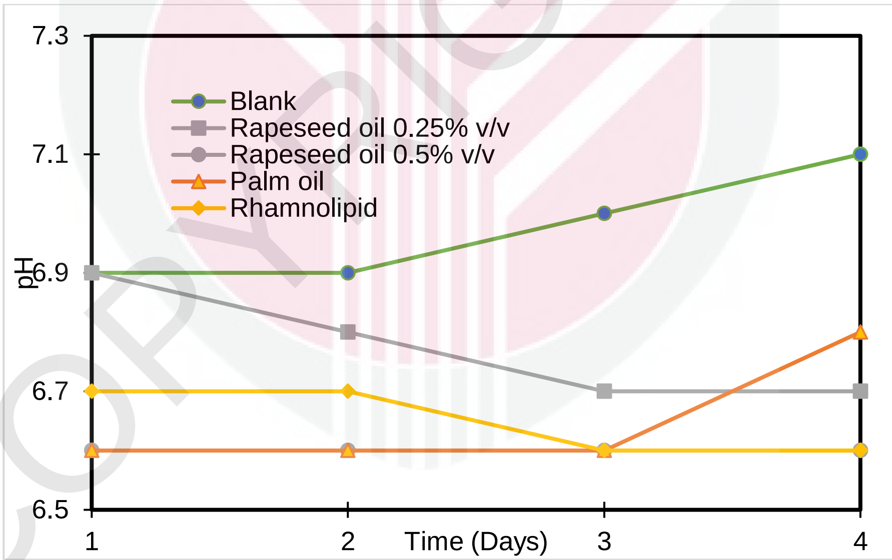

Figure 4.68: pH for two-stage AD in Tank 2

4.6.2.7 Biogas Accumulation

The biogas accumulation of two-stage AD, when added with different types of antifoams, is shown in Figure 4.69. It was observed that the blank run that did not have any antifoam had the highest biogas volume at 0.364 L/L/d, followed by the feed added with RL at 0.312 L/L/d. Meanwhile, the addition of 0.25% v/v RO as antifoam produced biogas at 0.271 L/L/d, palm oil at 0.220 L/L/d, and the lowest was for rapeseed oil of 0.5% v/v (0.201 L/L/d). This observation states that the added antifoam did not increase biogas production, even though some antifoam helps reduce scum production. Aside from the possible harmful impact, LCFA build-up on sludge might create a physical barrier and impede the transfer of substrates and products (e.g., biogas release), causing the initial methane generation to be delayed (Pereira et al., 2005).

The slight reduction of biogas when the feed was added with RL as the antifoam might be due to it being unsuitable for methanogenesis. Zhou et al. (2013) discovered that the sludge fermentation liquid of WAS treated with RL significantly improves solubilization and acidification. Extracellular polymeric substances (EPS) and bacterial aggregations produced by RL depart the connected cell surface. Although biosurfactants can reduce surface tension and increase surface activity by forming micelle aggregates (Jahan et al., 2020), the acidification products VFAs, are substrates for methanogenesis and can be quickly metabolized to methane by methanogen. Another study revealed that the presence of RL increased waste-activated sludge hydrolysis and acidogenesis in the acidogenic phase, resulting in more substrates for methane production in the methanogenic phase. The addition of Rhamnolipid during the acidogenic phase improved methane production during the methanogenic phase. The cumulative methane yield increased as the RL dosage was increased from 0 to 0.04 g/g TSS (Xu et al., 2021a. In this study, the biogas yield was slightly lower than the blank, but no inhibition was noted.

Natural oils such as rapeseed oil are good antifoams to reduce foam and scum levels (Kougias et al., 2015; Thaku & Barjibhe, 2019. However, there was a significant reduction in biogas production when natural oils were added as an antifoam. This could correlate to the increased stress on the microorganism when natural oils are added to the digester. High rapeseed oil addition to the feed at 0.5% v/v and palm oil at 0.5% v/v increased the organic matter noted by initial COD of 11,289 mg/L and 12,320 mg/L, which is very high for AD. The digester COD increased significantly, thus resulting in a higher requirement for organic matter removal (Mahat et al., 2021).

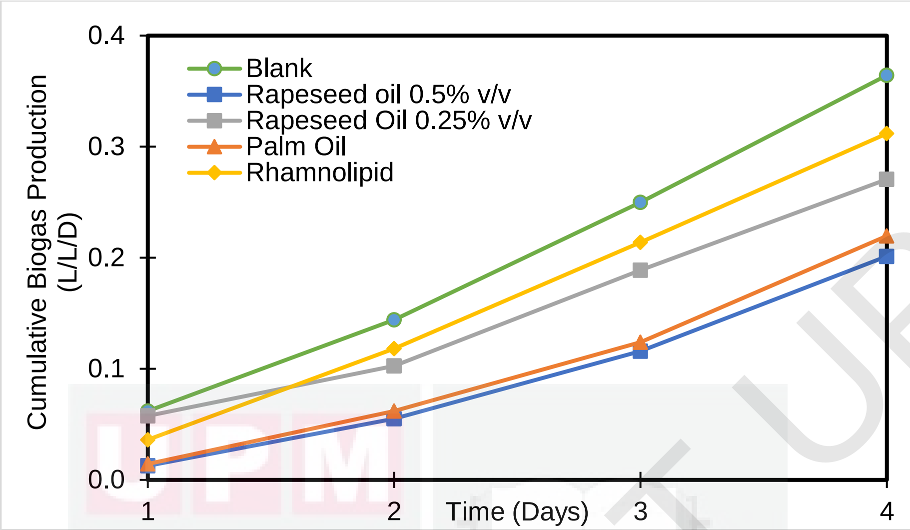

Figure 4.69: Biogas Accumulation in two stage AD with antifoam

4.6.2.8 Foaming Tendency

The foaming tendency of tank 1 and tank 2 was not observed with the foaming test. However, the foaming was present in the digester when fed with 0.25% v/v rapeseed oil (Figure 4.70) and blank, as shown in Figure 4.71. At a higher dosage of natural oils (0.5% v/v), the digester foaming was absent. A study conducted by Kougias et al. 2015 revealed that rapeseed oil was found to be a better antifoam because it did not disrupt the overall bio methanation process and contributed positively to biogas productivity. Furthermore, the foam suppression efficiency of RO increased as the dosage was increased. A higher dosage of natural oil can reduce the digester's foaming (Kougias et al., 2014). The foaming test did not reveal any foaming tendency for the sample in both tanks.

Figure 4.70: foam formation in Tank 1 two-stage AD treated with rapeseed oil (0.25% v/v)

Figure 4.71: Foam formation in tank 1 for blank

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

5.1 Conclusions

This research aimed to investigate the effect of proteins and lipids on foam and scum in one-stage and two-stage anaerobic digestion (AD). The substrate used was synthetic wastewater (SW) as a protein-based substrate and FPW, a high-strength food processing wastewater for the inoculum; one stage was inoculated with cow manure (CM) and activated sludge (AS), while the two-stage was inoculated with AS.

From observation, it was observed that the foam formation was significantly high in the one-stage AD inoculated with CM fed with SW on the first day of digestion (21 g/L protein) at 1.16 mL foam/ mL air-min. AS had a lower foaming tendency at 1.00 mL foam/ mL air-min on day 3. Surprisingly, the two-stage digester did not have foaming when fed with high protein concentration using SW. For both digesters, scum was not present even at high protein concentrations. Next, the effects of lipids were investigated by introducing FPW with AS. The scum production was not present in one-stage AD. However, sludge floatation was observed at 10 g/L lipids.

In contrast, the scum production increased with lipid concentration in two-stage AD (Tank 1) over time. The best performance obtained was at 6 g/L with a biogas accumulation of 0.364 L/L/d and COD removal of 93.8 %, whereas one-stage AD had a biogas accumulation of 0.531 L/L/d with a COD removal of 72.5%. The fatty acid analysis of scum in the two-stage digester revealed that FPW had a high percentage of palmitic acid at 655.34 mg/100 g while scum had a composition of 1028 mg/100 g while the oleic and α-linoleic acid was 327.12 mg/100 g and 88.21 mg/100 g respectively for FPW and 214.56 mg/100 g and 1636.50 mg/100 g for scum. The high percentage of fatty acids in the scum sample validates that scum is produced from the accumulation of fatty acids.

This research also aimed to reduce scum formation in the two-stage digester (Tank 1) by using natural oils – rapeseed oil (RO) and palm oil (PO) and biosurfactants – rhamnolipids (RL) and tea saponin (TS). The results showed that RO at a dosage of 0.5% v/v reduced scum by 32% and PO at 7%. However, biogas reduced significantly to 0.201 L/L/d for RO and 0.220 L/L/d for PO compared to blank (0.364 L/L/d). The preliminary study was first conducted using biosurfactants to obtain the optimum dosage of 0.02 g RL g^{-1} TSS in one-stage

AD. The optimum dosage was deployed into the two-stage AD; however, it did not reduce scum. In fact, there was an increase in scum production by 23%. There was also slightly lower biogas production at 0.312 L/L/d compared to blank. Natural oils have a good chance of reducing scum in AD, but further research is required to optimize the dosage. Bio-surfactants increased the acidogenesis and hydrolysis rate in AD but did not reduce scum formation in this study.

5.2 Recommendations for future research

Natural oils have an excellent potential to reduce scum; however, a significant reduction in biogas produced was noted. Thus, the optimization of dosage could be applied for better performance. Besides that, enzyme pre-treatment of lipase has shown promising advancements for reducing lipid inhibition in AD. FOG treatment with lipases may boost hydrolysis and increase biomethane generation. Besides that, this study concluded that foam and scum are different. Thus, a detailed mechanism could be explored and researched to understand the problem better.

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LIST OF PUBLICATIONS

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