

## **INFLUENCE OF MESOPHILIC BACTERIA INOCULATION WITH CHICKEN MANURE FOR BIOGAS PRODUCTION ENHANCEMENT IN ANAEROBIC DIGESTION (AD) PROCESS**

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### **Abstract**

The objective of this study is to investigate biogas production by anaerobic digestion using mesophilic bacteria mixed with Palm Oil Mill Effluent (POME). This project aims to determine the volume of biogas generation and volatile fatty acid (VFA) production from chicken manure via the anaerobic digestion process. Anaerobic digestion (AD) of chicken manure (CM) often faces obstacles, including high total ammonia nitrogen (TAN) concentration, inorganic soil particles, and wood chips. The digestion process was carried under batch mode conditions in Scott bottles of 1.0 L active volume. The bottles were immersed in a water bath to control their temperature at 37°C. The characteristics of total solid, volatile solid of mass fraction, pH, and temperature on the amount of biogas produced were studied. The investigation showed that biogas production can be enhanced by inoculation of another material. The optimum biogas composition in the AD system was recorded by Inoculum I, which was achieved on Day 2 at 560 mL/L. The highest cumulative methane yield was observed in the leachate with Inoculum (I), which was 8976 mL/gVS, while the CML produced 4 mL/g VS. The anaerobic digestion (AD) process augmented with inoculum demonstrated heightened efficacy in biogas generation and VFA concentration reduction during the acidogenic phase, surpassing the observed performance in chicken manure leachate.

Keywords: anaerobic digestion, POME, chicken manure, methane production, bacteria

### **1. INTRODUCTION**

There has been an increase in demand for bioenergy solutions as it has become necessary to increase the energy production of economies. The massive energy consumption in various industries is necessary for the equipment and plants required to sustain their development. In this regard, biogas is a viable option for clean and sustainable energy Zakaria et al. [1]. According to Malaysia Energy Information Hub (MEIH), final energy demand of natural gas in 2016 grew by over 20%, and, by 2020, the energy demand

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represented over 63% of total energy demand from 2012. Most solid organic wastes can be employed as substrates in their manufacture. In contrast to simply covering manure tanks to avoid gaseous emissions, the concept of converting manure storage tanks into inexpensive and primitive anaerobic digestion (AD) plants is gaining popularity.

The AD is a prominent treatment approach and engineering practise that may be used to treat solid waste. It is a biological phenomenon in which microbes' breakdown biodegradable materials in the absence of oxygen. These characteristics are addressed by anaerobic co-digestion (AcoD), which increases biogas generation from low-yielding or difficult-to-digest materials. In AD, the inoculation is the feedstock of the bacteria that initiate the substrate breakdown process. The microbial population, which is originally present in the inoculum, is a significant role in the generation of biogas from different substrates.

The two primary gaseous fuels produced from waste biomass are bio-methane and bio- hydrogen Poudel et al. [2]. Methane ( $\text{CH}_4$ ) and carbon dioxide ( $\text{CO}_2$ ) make up the majority of biogas, but it can also contain portions of other gases such ammonia ( $\text{NH}_3$ ), hydrogen sulphide ( $\text{H}_2\text{S}$ ), hydrogen, oxygen ( $\text{O}_2$ ), nitrogen ( $\text{N}_2$ ), and carbon monoxide ( $\text{CO}$ ). Biogas may be utilized to cook in the home and power 13 microturbines, fuel cells, and other domestic appliances. Furthermore, biohydrogen is a clean fuel with a high energy yield that has the potential to become the dominant fuel source in the future Pavičić et al. [3].

In order to provide sustainable energy, this project focuses on producing biogas from mesophilic bacteria. This is due to mesophilic temperatures supporting a greater diversity of bacteria, and these bacteria are typically more resilient and adaptive to changing environmental conditions. The synthesis of methane in the AD system also depends on complex bacterial and methanogen populations. The sequencing-based technique and data analysis can provide an in-depth understanding of the microbial compositions, inclusion, and relationships between digester performance, biodiversity, and environmental parameters at the plant level, which could help enhance microbial productivity and maximize the methane yields in the AD process of chicken manure. However, with a single substrate, which includes manure, AD produces very little biogas via mono-digestion. Anaerobic co-digestion increases biogas generation since it allows for the simultaneous digestion of two or more substrates. As a catalyst, the inoculum increases the overall production of biogas. The inoculum's microbes typically consume substrate organic materials effectively. Thus, the major goal of this study is to evaluate the effectiveness of the additive's inoculum which is two distinct types of microorganisms in the AD process as well as their effectiveness in creating biogas and methane.

## **2. METHODOLOGY**

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### **2.1. Feedstock and Inoculum Collection**

The chicken excrement was obtained from a client's poultry farm in Tasek Gelugor, Pulau Pinang. The research's starters, known as inoculums, were acquired from Biology Genesis- AquaTM, and the anaerobic sludge from United Palm Oil Industries Sdn. Bhd., which was located in Nibong Tebal. Biology Genesis-AquaTM is an environmentally friendly water treatment based on the innovative Biology GenesisTM combination of human-friendly active microorganisms, helpful enzymes, super catalyzing co-enzymes, and co-factors.

Biology-Genesis is a recently developed mix of human-friendly active microorganisms, helpful enzymes, super catalyzing co-enzymes, and co-factors. It is a natural, organic crop free of harsh chemicals. Biology-Genesis is a source of reliable probiotic bacteria that promotes their growth. All animals, including humans, contain various helpful bacteria in their digestive systems. Animals offer food and a warm environment for these bacteria in exchange for the bacteria's assistance in digesting food, absorbing nutrients, and eliminating hazardous chemicals. Biology-Genesis has been evaluated and found to be non-toxic, non-irritating, non-flammable, non-pathogenic, and non-hazardous by independent laboratories. The feedstock from the sampling procedure was kept in a laboratory cold room at 4 °C until the samples were utilised.

There is a specific method for the inoculum preparation as stated in the application for Biology-Genesis Pro-biotic. The raw sample needs to go through the acclimatization process first before mixing them together into digestate. The process was done by applying 3 to 5 grams of raw sample into 90 ml Palm Oil Mill Effluent (POME) and the solution was kept in room temperature for 3 to 7 days for the bacteria to become actively provident for the AD process. The sampling location and inoculum sampling for both inoculums are shown in Figure 1 and 2.



Fig. 1. Inoculum Sampling (POME)



Fig. 2. Inoculum Sampling (Bacteria)

## 2.2. Chicken Manure Leachate Preparation

AD of chicken manure (CM) typically confronts various problems, including high total ammonia nitrogen (TAN) concentrations, the presence of inorganic soil particles, and the presence of several difficult-to-decompose litters such as wood chips. To address these issues, degradable fractions of CM were removed by leaching, and the resultant chicken manure leachate (CML) was used in the AD batch system. The CML method is illustrated in Figures 3.



Fig. 3. CML Method

## 2.3. Setup and Handling of Batch Reactor

After acclimatization, the inoculums were mixed with chicken manure leachate. For the experiment, a standard mixing ratio of 10% inoculums to substrate was utilised Molaey at el. [4]. The acid and methane stages of the AD process for chicken manure and microorganisms were carried out in the controlled temperature batch reactor based on the ideal conditions for each starter. Scott bottle with a 1000 mL active capacity made up the processing unit. The types of equipment for the batch AD process are shown in Figure 4.



Fig. 4. The Whole Setup of Digester

#### 2.4. Liquid and Gas Sample Collection

The AD system produced two distinct products, which were in the gas and liquid states. The liquid and gas sample collection and data collection took 36 days, from March 12th to April 16th, 2023. The first pipe connection was used to collect the gas sample, while another pipe linkage was utilized to extract the liquid sample. The liquid samples were retained in the centrifuged tube for the other laboratory tests, while the VFA testing sample was stored in the freezer. The liquid sample was suctioned using a syringe as illustrated in Figure 5.



Fig. 5. Liquid and Gas Sample Collection

Fig. 6. Water Displacement Method

The volume of the biogas sample was then evaluated using the water displacement method. The accumulated gases were collected using the water displacement method since one of the features of gas is that it is insoluble in water. As two bodies cannot occupy of the same space at the same time, the amount of gases will cause the water level to rise. The equipment used for the water displacement method was 1L of measuring cylinder, a retort stand, plastic tubing, and a basin to fill the water. The retort stand was utilized in holding tightly the measuring cylinder, while the plastic tubing was used to

force the flow of the gas from the gas bag into the measuring cylinder. The gas sample collection and the types of equipment for the water displacement method are presented in Figures 6.

## 2.5. Analytical Method

Chicken manure (CM) samples were characterised in terms of total solid (TS), volatile solid (VS), chemical oxygen demand (COD), pH, and VS/TS ratio prior to the biogas potential analysis testing. Then, macronutrients (K), micronutrients (Al, B, Bi, Ca, Cu, Fe, K, Mg, Mn, Na, and Sr) were analysed by using ICP-OES analyser, percentage of carbon (C), hydrogen (H), nitrogen (N), and sulphur (S) were tested by using CHNS analyser. In addition, the proportion of each biogas component was determined using the Gas Chromatography (GC) technique to meet the study's goals, and the production of volatile fatty acids (VFAs) was examined using the High-Performance Liquid Chromatography (HPLC) method. Table 1 summarized method No USEPA 1648, USEPA 8156, USEPA 8000 and USEPA 8038 of analytical method.

Table 1. Summarization of Method No USEPA of analytical method

Analytical Method	Method No
(a) Total Solid (TS) and Volatile Solid (VS)	1684
(b) pH	8156
(c) Chemical Oxygen Demand (COD)	8000
(d) Total Ammonia Nitrogen (TAN)	8038

### (e) Gas Chromatography

The elements of biogas generation were examined using a gas chromatograph (Agilent 7890A) fitted with a TCD detector and a Carboxen-1010 PLOT column (30m0.53mm ID 25467). The running duration of each sample was achieved in 15.333 minutes using helium as the carrier gas at a flow rate of 3 ml/min. The oven's temperature setting had a holding temperature of 50°C for 1 minute before rising to 250°C at a rate of 15°C/min and maintaining remained. To achieve an accurate reading of the chromatogram, the GC was calibrated by injecting pure (CH<sub>4</sub>, CO<sub>2</sub>, and N<sub>2</sub>) gases. The data collection of GC results was carried out two times a week for 36 days of the AD system operation.

### (f) High-Performance Liquid Chromatography (HPLC)

The HPLC Agilent Technologies system used to assess the VFA concentrations was outfitted with a C18 column and an ultraviolet detector (UV 210 nm wavelengths). Acetic, propionic, isobutyric, butyric, isovaleric, valeric, formic, heptanoic, hexanoic, and methylvaleric acids were produced in standard solution mixes at concentrations of 2000- 4000-6000-8000-10000 mg/L. The calibration curves used for estimating the concentrations of VFA in the AD sample. The sample from the AD system was filtered via a 0.22 m syringe filter before being injected into the HPLC vial. Then, the VFA sample in the HPLC vial was frozen at -20°C until the testing day. The proportion for each type of acid in the solution and their molar masses as it is essential in determining the concentration of 10 M of VFA standard solution.

### 3. RESULTS AND DISCUSSION

#### 3.1. Characteristics of Chicken Manure

The characteristics of the Chicken Manure Leachate (CML) were summarized and compared to the findings of previous researchers in Table 2:

Table 2. Characteristics of CM

Parameters	Experimental		Previous study	
	Chicken Manure Leachate (CML)	MD Kamal et al. [5]	Cai et al. [6]	
TS (%)	2.0583	1.7784	*	
%VS of TS	69.4692	72.904	*	
VS/TS	33.7508	40.994	*	
pH	7.27	6.85	7.79	
COD (mg/L)	40320	16000	*	
TAN (mg/L)	1890.56	134.4	*	

#### 3.2. Heavy Metals Elements of CM and Inoculum

The comparison of the elements (Al, B, Bi, Ca, Cu, Fe, K, Mg, Mn, Na, and Sr) of the experimental samples and some elements (Ca, K, Mg, Na) from the control and previous study were listed in Table 3.

Table 3. Comparison of Heavy Metals

Elements	Experimental		Previous study	
	Chicken Manure Leachate (CML) (ppm)	Chicken Manure Leachate (CML) with Bacteria (ppm)	Singh et al. [7] (ppm)	Dikinya et al. [8] (ppm)
Ca	95.154	177.692	811	1958
K	2274.06	3113.81	574.25	588.9
Na	599.524	852.261	468.59	468.59
Mg	152.458	341.204	206	75.6
Fe	4.54422	3.13425	*	*
Cu	3.34385	1.25907	*	*
Al	2.90319	1.1761	*	*
Mn	1.87154	2.82759	*	*
B	1.48774	2.02134	*	*
Bi	0.390605	1.28863	*	*
Sr	0.376647	1.00903	*	*

### 3.3. TS Content of Leachate

The relationship between TS and methane generation versus digestion time for both inoculums was displayed in Figures 7 (a) and (b). From the result, the optimum methane yields for CML at 1.99% of TS content on Day 16 while for CML with Inoculum (I) at 7.91% of TS content on Day 2. By comparison, anaerobic co-digestion of excess sludge (ES) with chicken manure (CM) can substantially enhance methane production (82.4–123.1 mL g<sup>-1</sup>-VS<sub>added</sub>) when the co-substrate has a high total solids (TS) content [12]. However, this methane yield still falls short of that achieved by mesophilic bacteria in pure culture.

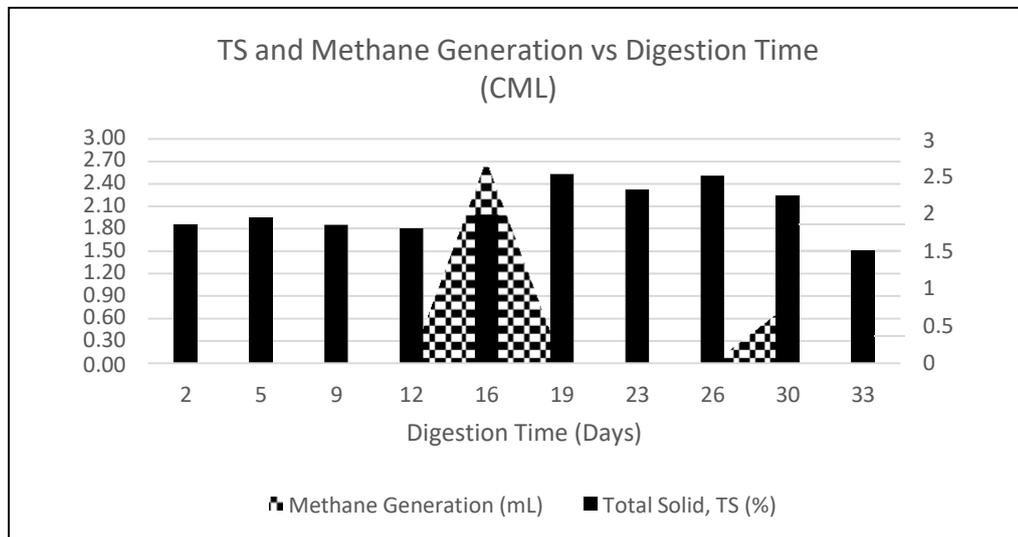


Fig. 7. (a): TS and Methane Generation (CML)

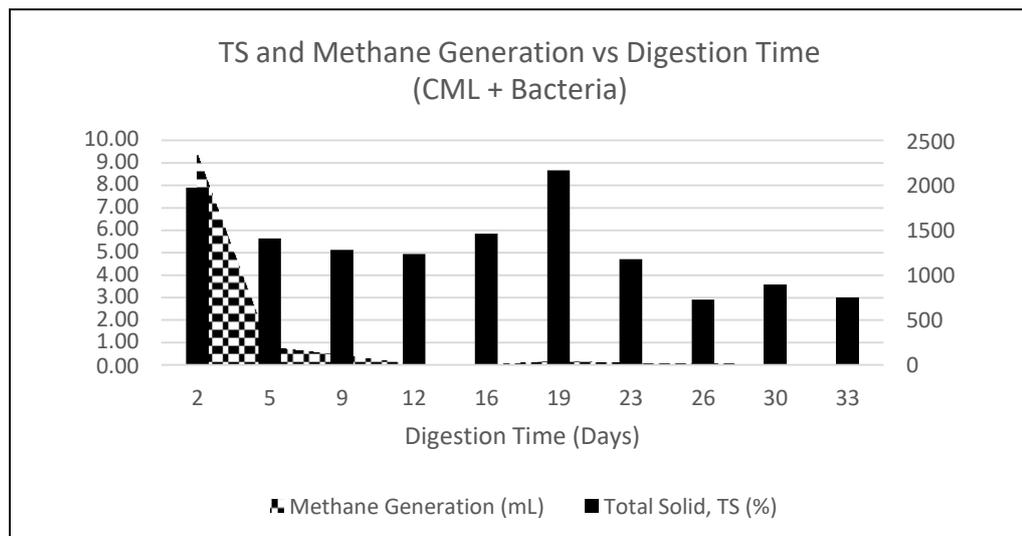


Fig. 7. (b): TS and Methane Generation (CML + Inoculum I)

Figure 7 (a) and (b) show the relationship between TS and methane generation for CML reactor and CML with Inoculum (I) respectively. During the AD process, there was no significant relationship between the TS and the volume of methane generation, but it was important to determine the TS content and the dilution factor at the setup stage of batch digestion Wang et al. [9]. The total solids (TS) content of a substrate exhibits a complex association with methane generation during anaerobic digestion. In general, an increased TS content can result in an increased methane yield, but this association is not always linear. A greater quantity of solids indicates a more substantial amount of organic matter, which is the principal source of nutrition for the microorganisms that generate methane. The microorganisms in an anaerobic digester break down the organic matter in solids through a series of biochemical reactions, releasing methane as a by-product. Hence, the amount of methane produced is directly related to the amount of organic matter available.

Therefore, the relationship between TS content and methane production is often curvilinear. This means that there is an optimal TS content for methane production, and that methane production will decrease at both very low and very high TS contents. The above relationship indicates that the inoculation of bacteria can lead to the establishment of a higher TS content from the start and the production of maximum methane gas at the early stage of the AD process at an optimum TS content.

### 3.4. VS Content of Leachate

The relationship between VS and methane generation versus time for both inoculums was displayed in Figures 8 (a) and (b). From the result, the optimum methane yields for CML at 75.90% of VS content on Day 16 while for CML mixed with Inoculum (I) at 66.93% of VS content on Day 22.

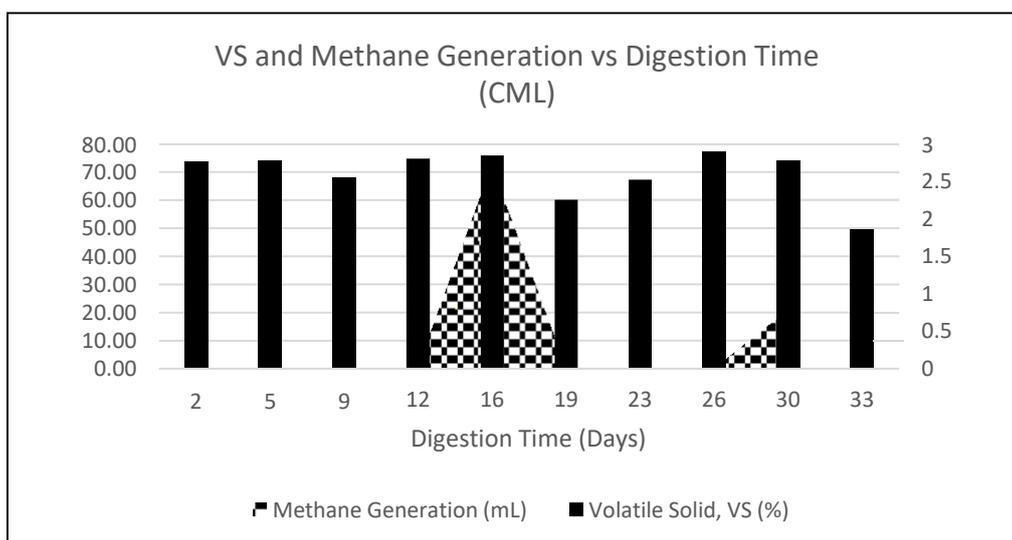


Fig. 8. (a): VS and Methane Generation (CML)

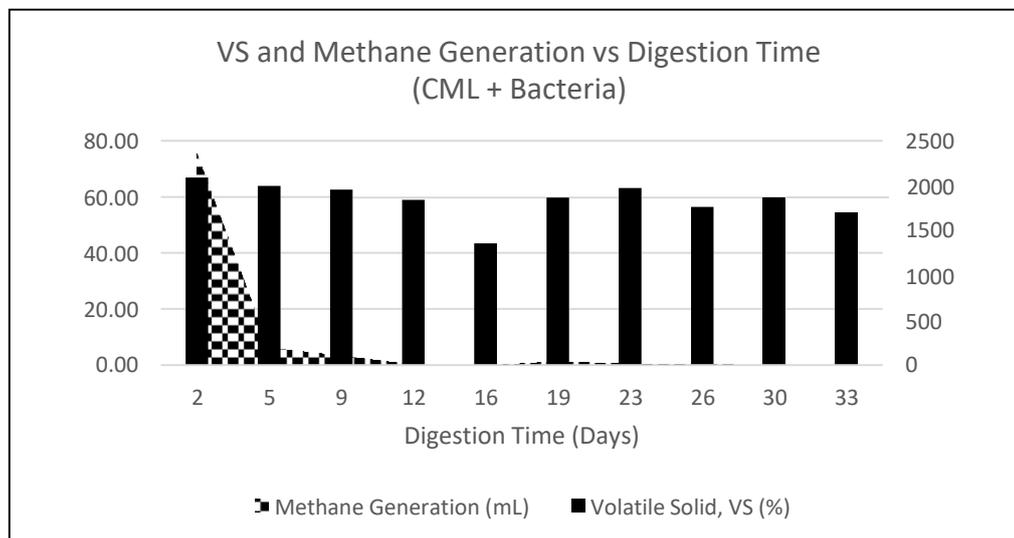


Fig. 8. (b): VS and Methane Generation (CML + Inoculum I)

The volatile solids (VS) content of a material is a significant parameter, as important as the total solids (TS) content when considering biogas production from a slurry. This is because the VS content represents the fraction of the solid material that can be converted into biogas. Although Figure 8 demonstrates that there is no discernible correlation between the volume of methane generated throughout the anaerobic digestion (AD) process and the volatile solids (VS) content, it is nonetheless essential to know the VS content during the AD setup. The CML had a lower VS content of AD setup, producing a lower volume of biogas generation than the Inoculum (I) at the early stage of the digestion process. However, the VS content of CML was higher at Day 16 even it was few days later than Inoculum (I) reactor. In short, the AD setup with a higher VS content contains more organic material to convert into biogas and methane generation. Compared with the inoculum sludge from the wastewater treatment the optimum solid concentration obtained for biogas production is in the range 30 - 32 g L<sup>-1</sup>. It can be concluded that the volatile solid for the inoculum sludge lower than the leachate.

### 3.5. COD of Leachate

In this investigation, both (COD) test samples were collected on the same day as the gas collection sample. As a result, determining the relationship between COD value trends and methane production volume is simplified. On Day 9, the maximum methane generation for CML was 83200 mg/L of COD, while the highest methane generation for Inoculum (I) was 51200 mg/L of COD on Day 2. As demonstrated in Figure 9, a drop in COD concentration followed by a rise in methanogenic activity, which was followed by an increase in methane productivity.

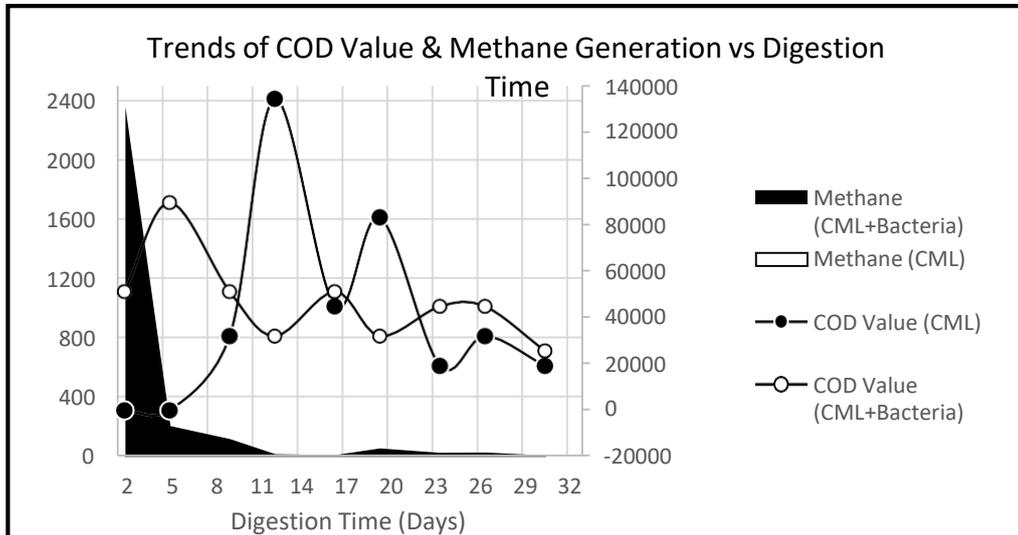


Fig. 9. Trends of COD Value and Methane Generation

### 3.6. TAN of Leachate

The results of the TAN test for the reactors with CML and CML mix with Inoculum (I) were shown in Figure 10.

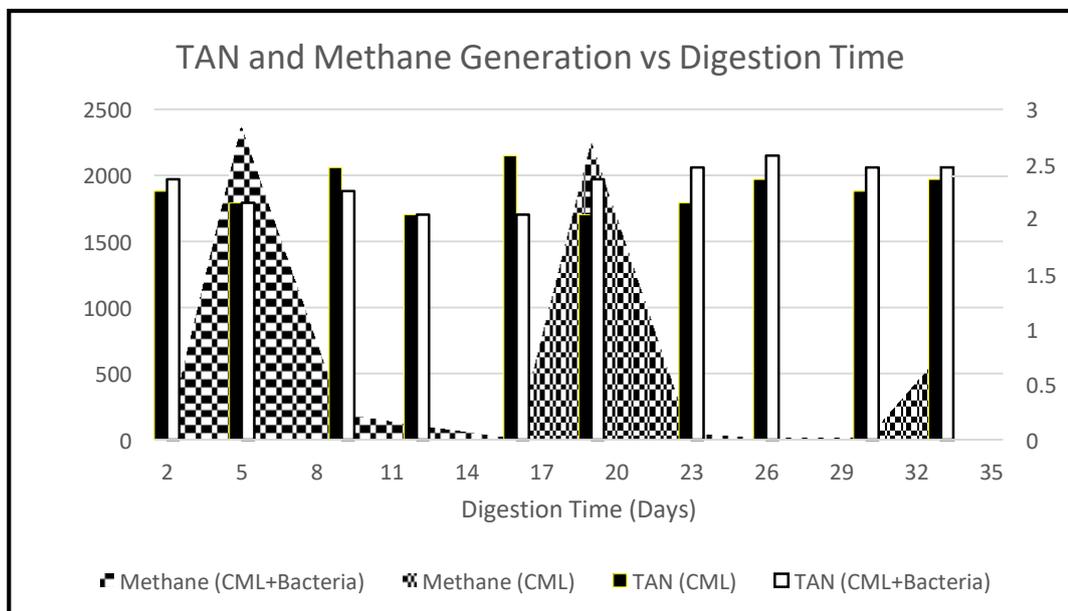


Fig. 10. TAN and Methane Generation

Based on the graph, the increasing trends with a minor fluctuation of the TAN value were identified for both reactors. The highest peak of TAN value for both reactors were at same value which were 2150 mg/L but CML take place on Day 16, while CML+ Inoculum (I) encounter that value on Day 26.

Monitoring the TAN value was important in determining the efficiency of the AD process. TAN is a vital measurement to monitor since it can be hazardous to the methanogenic bacteria which generate methane. At mesophilic temperatures and pH levels of 7, roughly 1.25% of TAN changes to FA, but at the same temperatures and pH levels of 8, approximately 11.25% of TAN transforms to FA, implying that free ammonia (FA) is ten times more hazardous to methanogens at pH 8 than at pH 7 Sarker et al. [10]. On average, the TAN concentrations measured during the co-digestion of sewage sludge (SS) and CM were 2221 gN/m<sup>3</sup> and 2094 gN/m<sup>3</sup>, respectively [13]. Ammonia was produced during digestion and may have a small inhibitory effect on the anaerobic co-digestion of SS and CM. Upon completion of the anaerobic digestion process, a slight decline in biogas production is observed, yet it remains substantial. Concurrently, both pH and TAN levels exhibit a gradual upward trend. In extreme circumstances, excessive TAN concentrations can even kill methanogenic bacteria, effectively stopping the anaerobic digestion process.

### 3.7. Gas Generation

Gas compositions in the reactors were determined using gas chromatography (GC), which revealed the percentage of nitrogen (N<sub>2</sub>), methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>). The percentage of gas for each component versus time for both reactors is presented in Figures 11 (a) and (b).

The acidogenesis phase is the initial stage of the CML reactor, where acidogenic bacteria break down hydrolysis products into simpler compounds. These simpler compounds do not contain enough carbon to form methane gas, so there is no gas present during this phase. The percentage of carbon dioxide in the reactor will increase as the acidogenic bacteria continue to break down the hydrolysis products. This is because carbon dioxide is a by-product of the acidogenesis reaction. As the percentage of CO<sub>2</sub> increases, the acidogenesis phase continues. Graph plotted methane gas yield was lower than expected because the bacteria were not fully acclimated to the conditions in the reactor. This meant that they were not able to carry out methanogenesis, the process of producing methane gas from simpler compounds. As a result, the percentage of nitrogen gas remained high throughout the AD process, with only 0.7736% maximum methane gas present in the maturation phase.

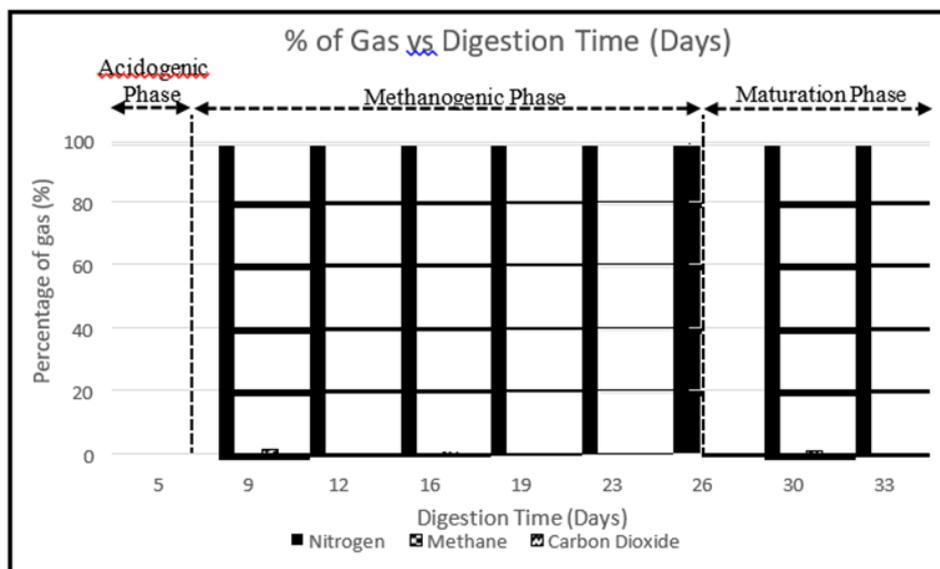


Fig. 11. (a): Percentage of Gas (CML)

In the CML mix with Inoculum (I) reactor by referring to Figure 11 (b), the acidogenesis process began immediately at the start of the retention time. As a result, the percentage of carbon dioxide was the highest at Day 5, followed by nitrogen and methane gas. The production of methane gas then decreased continuously until it reached 0.1367% at Day 19. However, it suddenly peaked at day 23 with a percentage of 11.7619%. The percentage of carbon dioxide gas then steadily decreased until it reached 0% at Day 30. In contrast, the percentage of nitrogen gas gradually increased throughout the AD process. The nitrogen gas produced at high level due to abundance of free ammonia (FA) in the system. FA is a promising methanogen inhibitor. It is membrane permeable, and as it diffuses into the cells, it causes proton imbalance or changes in intracellular pH, which inhibits enzyme processes Sarker et al. [10]. In addition, the excess nitrogen leads to the formation of ammonia, a strong base. This will increase the operating pH above the permissible level of 8.5, which will inhibit the growth of microorganisms and ultimately slow down the rate of gas production Dioha et al. [11].

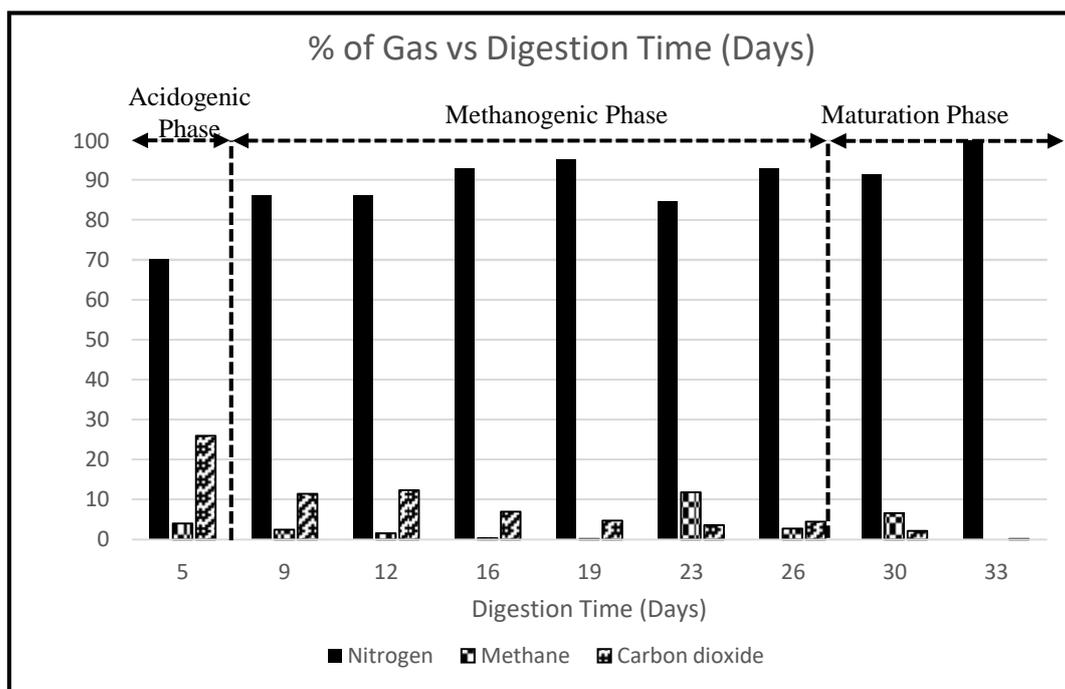


Fig. 11. (b): Percentage of Gas (CML + Inoculum I)

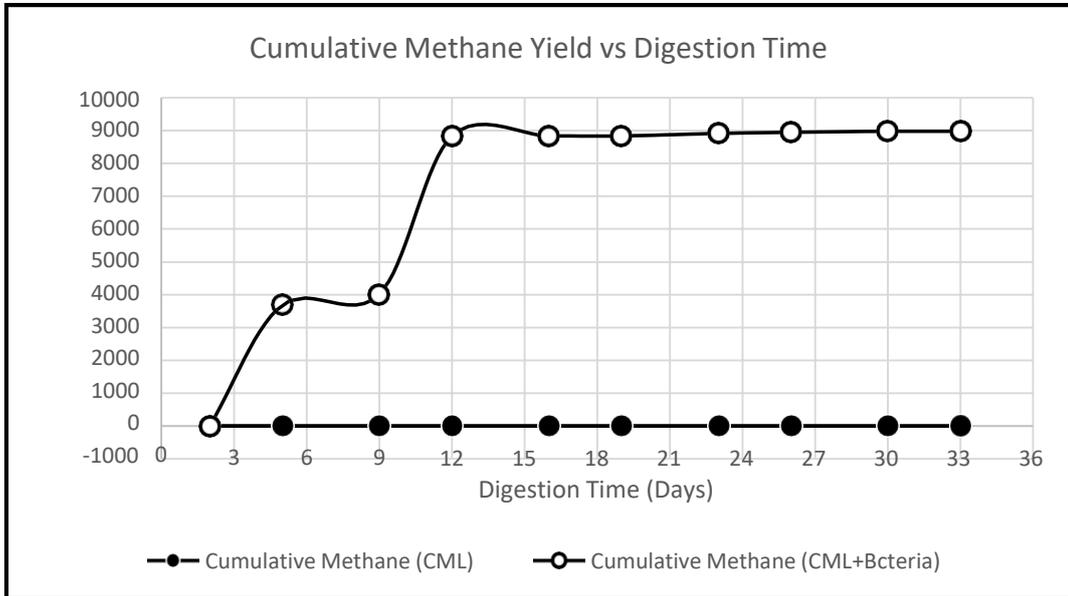


Fig. 12. Cumulative Methane Yield

The highest cumulative methane yield was observed in the leachate with Inoculum (I), which was 8976 mL/gVS, while the CML produced 4 mL/g VS. Inoculum (I) performance in CML was better than CML alone due to the higher cumulative methane yield. The graph of cumulative methane yield versus digestion time for both inoculums was shown in Figure 12.

### 3.8. Volatile Fatty Acid Production

The volume of biogas generated and total VFA production are shown against digestion time in Figure 13. Due to the acidogenic phase, the trends of VFA generation for both reactors ascended from Day 1 to Day 5. An acidic environment may have resulted from a drop in the buffering capacity driven on by an increase in the volatile fatty acid content in the reactors.

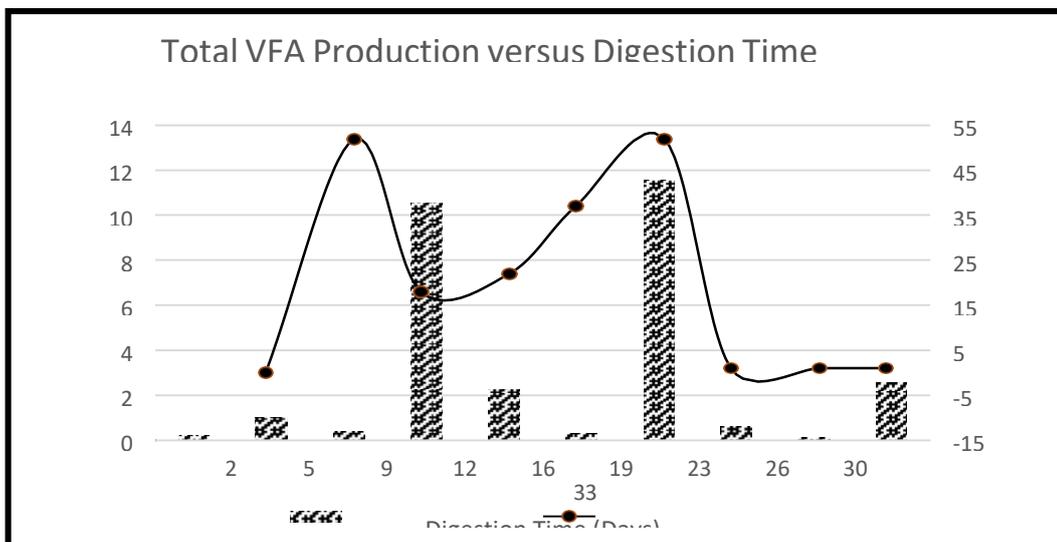


Fig. 13. (a): Total VFA Production (CML)

The leachate of CM solely produced the most biogas when the total VFA production was 11.57 mg/L, whereas the leachate with Inoculum (I) produced the highest biogas when the total VFA production was 22.64 mg/L. The outcome demonstrates that higher biogas generation may be enhanced by lower VFA formation during the methanogenic phase. Additionally, increased VFA generation without any VFA buildup in the acidogenic phase might enhance biogas output. According to the findings, Inoculum (I) was more effective than CML at reducing the VFA concentration in the methanogenic phase from the previous phase.

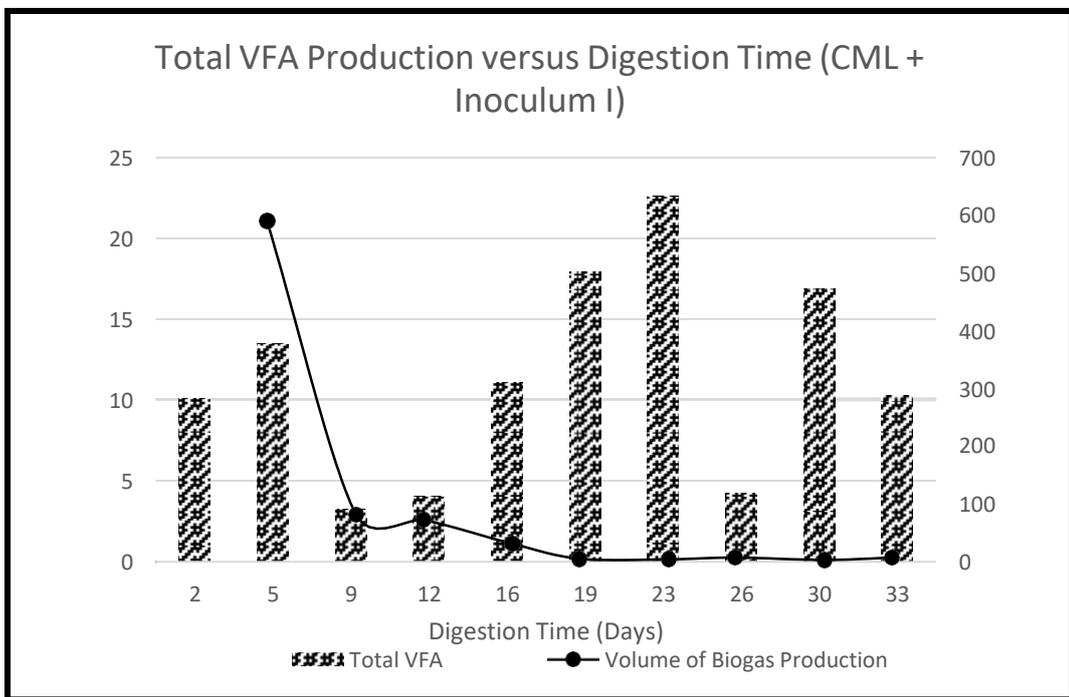


Fig. 13. (b): Total VFA Production (CML + Inoculum I)

#### 4. CONCLUSION

To evaluate biogas production from chicken manure, this study used an anaerobic batch technique. The conclusions reflect the research findings on leachate properties, biogas volume and percentage, and VFA production in the AD system.

In this study, chicken manure leachate (CML) and CML mixed with inoculums were characterized for total solids (TS), volatile solids (VS), total ammonia nitrogen (TAN), chemical oxygen demand (COD), and micronutrient content. The investigation aimed to understand optimal conditions for biogas generation. Results showed that co-digestion of CML and Inoculum I was more efficient in biogas and methane production compared to CML alone. The total biogas outputs were 0.16 mL/L for CML and 20.13 mL/L for CML + Inoculum I. The highest biogas volume occurred on Day 5 for CML + Inoculum I and Day 9 for CML. Inoculum I achieved optimum biogas composition on Day 2. Cumulative methane production was significantly higher in CML + Inoculum I (8976 mL/gVS) compared to CML alone (4 mL/gVS). Both reactors exhibited high N<sub>2</sub> gas levels (70% to 100%), attributed to excess free ammonia causing elevated pH and inhibiting microbial growth.

During the 105-day maturation phase, both reactors experienced declining TS, VS, COD, and methane production after Day 30 due to nutrient limitation and waste stabilization. TAN levels increased during maturation, inversely correlated with methane generation, indicating ammonia inhibition. The study highlighted the importance of acclimatization, with TAN consistently higher than COD readings, indicating incomplete bacterial adjustment to high ammonia levels. The relationship between volatile fatty acid (VFA) production and biogas generation was explored. Reduced VFA production in the methanogenic phase increased biogas generation, while higher VFA production without buildup in the acidogenic phase improved biogas production. The study successfully established relationships between VS, TS, TAN, COD, and methane generation.

Based on the findings, it is recommended to focus on optimizing methane generation in chicken manure leachate (CML) by targeting specific parameters. For CML, the optimal conditions for methane generation were observed at 1.99% of total solids (TS), 75.90% of volatile solids (VS), 83200 mg/L of chemical oxygen demand (COD), and 1792 mg/L of total ammonia nitrogen (TAN). In the case of CML + Inoculum (I), the highest methane generation was achieved at 7.91% of TS, 66.93% of VS, 51200 mg/L of COD, and 1971 mg/L of TAN.

Considering the 105-day maturation phase, it is recommended to closely monitor the declining tendencies observed in both reactors after day 30. This decline in total solids (TS), volatile solids (VS), chemical oxygen demand (COD), and methane production during the maturation phase suggests the need for adjustments to enhance system performance. Strategies to address nutrient restrictions and promote waste stabilization should be explored to maintain optimal biogas production throughout the entire digestion period.

Furthermore, it is advised to investigate and understand the relationships among TS, VS, COD, and methane output during the maturation period. Identifying and addressing any deviations in these relationships could contribute to maintaining consistent and efficient biogas production.

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