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Analysis of semi-continuous biogas generation using optimal combination of Duckweed and Cow dung from batch-based production

by

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A THESIS

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The undersigned certify that they have read, and recommended to the Institute of Engineering for acceptance, a thesis entitled **"Analysis of semi-continuous biogas generation using optimal combination of Duckweed and Cow dung from batch-based Production"** submitted by Pradeep Kumar Yadav, in partial fulfillment of the requirements for the degree of Master of Science in Renewable Energy Engineering.

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ABSTRACT

The utilization of renewable energy sources has become imperative in the face of increasing energy demands and environmental concerns. Biogas, a versatile and sustainable energy carrier, holds significant potential as an alternative to conventional fossil fuels. This study delves into the production of biogas through the anaerobic digestion process, focusing on the synergistic interaction between duckweed and cow dung as substrates. The research aims to optimize the composition of these substrates in a batch-based production system to enhance biogas yield through continuous process.

In this investigation, various proportions of duckweed (DW) and cow dung (CD) were combined to create different substrate mixtures. The batch-based production method facilitated controlled experimentation to evaluate the influence of substrate ratios on biogas generation. Parameters such as temperature, pH, total solids (TS), volatile solids (VS), and gas composition were monitored to assess the effectiveness of each mixture.

The results revealed that the optimal blend of duckweed and cow dung significantly influenced biogas production. The highest biogas yield was observed in 50% cow dung and 50% duckweed combinations and highest composition of methane in mix of 40% cow dung and 60% duckweed highlighting the importance of substrate composition in enhancing the anaerobic digestion process. In addition to this, the research findings indicated that the NPK (nitrogen, phosphorus, and potassium) value reached maximum in the slurry composed of 60% cow dung and 40% duckweed, highlighting its optimal suitability as a fertilizer. The reduction in total solids and volatile solids after anaerobic digestion indicated effective conversion of organic matter to biogas.

This study contributes valuable insights into the potential of utilizing duckweed and cow dung as co-substrates for biogas production. The findings underscore the significance of optimal substrate composition in achieving maximum biogas yield.

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LIST OF ACRONYMS AND ABBREVIATIONS

AD	Anaerobic Digestion
BOD	Biological Oxygen Demand
COD	Chemical oxygen demand
CD	Cow Dung
C/N Ratio	Carbon Nitrogen Ratio
CM	Cow Manure
CSTR	Continuous Stirred Tank Reactor
DW	Duckweed
HTR	Hydraulic Retention Time
MSW	Municipal Solid Waste
OLR	Organic Loading Rate
SMY	Specific Methane Yield
VMP	Volumetric Methane Production
VFA	Volatile Fatty Acid
TS	Total Solid
VS	Volatile Solid
GHG	Green House Gases
SWAT Lab	Soil, Water and Air Testing Laboratories

CHAPTER ONE: INTRODUCTION

1.1 Background of the Study

As the world grapples with the challenges of climate change, escalating energy demands, scare and expensive fuel resources and the need for more sustainable practices, researchers and environmentalists are continually seeking innovative solutions to mitigate these issues. One such promising avenue is the utilization of duckweed as a renewable resource for biogas production.

In Nepal, the installation of biogas for household purpose had been in practice for 28 years. More than 300000 household plants have been built on 2008 and the biogas model which has been promoted in Nepal is mostly GGC-2047. Different national and international organizations such Alternative energy promotion center, Nepal biogas promotion association and other international organization such giz (german support) have played important role promoting biogas in Nepal.("Nepal Improved Biogas Plant- overview report of Research and deveopment phase," 2015)

Duckweed, a tiny aquatic plant that floats on the surface of still or slow-moving water bodies, has recently gained significant attention due to its extraordinary growth rates and its ability to efficiently capture nutrients from water. Hence, duckweed can be used for two purpose; waste water treatment and biogas production.(Rashmi Verma, 2015) It belongs to the family Lemnaceae and encompasses several genera, the most common of which include Lemna, Spirodela, Wolffia, and Landoltia.(Peigao Duan, 2012)

The key factor that makes duckweed an attractive candidate for biogas production is its high nutritional content and the potential for fast biomass generation.(Yadav et al., 2017) It exhibits remarkable growth rates, doubling its biomass in a matter of days under favorable conditions. Additionally, duckweed thrives in various water sources, such as ponds, lakes, and wastewater treatment plants, and it can even grow on nutrient-rich agricultural runoff.

Biogas production from duckweed involves a biological process called anaerobic digestion. During anaerobic digestion, microorganisms break down organic matter in the absence of oxygen, resulting in the production of biogas. The primary components of biogas are methane (CH_4) and carbon dioxide (CO_2), with trace amounts of other gases. Methane, the main constituent of biogas, is a valuable renewable energy source that can be used for heating, electricity generation, and as an alternative to fossil fuels. This paper aims to explore the potential of duckweed as a promising feedstock for biogas production, focusing on its unique characteristics, cultivation methods, and the anaerobic digestion process. Additionally, it will delve into the environmental benefits of using duckweed as a biogas source, including wastewater treatment and nutrient recycling, while also considering the challenges and opportunities associated with this eco-friendly approach.

In the following sections, the study delves into the intricate details of duckweed cultivation, the biogas production process, and the various benefits this sustainable approach can offer. By harnessing the potential of duckweed for biogas production, we can move one step closer to a greener and more sustainable future, reducing our carbon footprint and advancing towards a circular economy model.

Anaerobic digestion (AD) is one of the highly accepted technologies for biogas production from organic matter obtained from municipal solid waste (MSW). Methane gas can be used as alternative to liquid petroleum gas (LPG) and natural gas. The residue after the digestion can be used as bio-fertilizer for high crop yield in the field which can replace artificial/mineral fertilizer. Biogas from organic waste helps to producing energy with environmental benefits (Mohamad Y. Mustafa, 2015).

This study is the further investigation of the work carried out by Mr. Bibek Shakya who uses batch type digester model for the production of biogas from duckweed and cow dung at different proportion. Six different ratios of cow dung and duckweed was used in batch model where two high yielding mix was found to be 60% CD and 40% DW followed by 40%CD and 60%DW. This study focuses on quality and quantity of biogas production from semi-continuous type biogas at different mixture ratio of cow dung and duckweed. This research also lays light on utility of the fertility of slurry from semi-continuous type biogas model.

1.2 Statement of the Problem

The growing demand for sustainable and renewable energy sources, coupled with the need for effective waste management solutions, presents a unique challenge that requires innovative approaches. The combination of duckweed and cow dung as a feedstock for biogas production holds great promise in addressing these concerns. However, several critical issues and uncertainties surround this approach, necessitating further investigation and research.

- **Biogas production from biomass keeps importance in the context of Nepal**
  Biogas holds paramount importance in Nepal as a transformative solution to address multiple pressing challenges. In a country where energy scarcity, indoor air pollution, deforestation, and waste management are persistent issues, biogas emerges as a beacon of sustainable change. By harnessing organic waste such as animal dung and agricultural residues, biogas provides clean and renewable energy for cooking and lighting, alleviating the burden on households while curbing deforestation. Moreover, its role in waste management not only mitigates environmental concerns but also generates nutrient-rich fertilizers, bolstering agricultural productivity and rural livelihoods. As Nepal strives for economic growth, improved health, and environmental conservation, biogas stands as a versatile tool that empowers communities, mitigates climate impact, and catalyzes a sustainable future for the nation.
- **Biogas production from in blending of cow dung and duckweed is maximum in certain ratio in batch production**
  Biogas production achieved through the blending of cow dung and duckweed represents a significant breakthrough, with the attainment of optimal results observed at specific ratios during batch production. This innovative approach capitalizes on the synergistic interaction between these two organic materials, where their combined composition contributes to a remarkable increase in biogas generation.

1.3 Objectives

Main objective of this study is to analyze the potential of biogas generation through Semi-continuous anaerobic digestion, using high biogas yielding proportion of duckweed and cow dung as co-substrates in batch model.

Specific objectives of this study are

- To blend cow dung and duckweed in varying proportions and assess the initial parameters.
- To analyze the biogas yield resulting from different combinations of cow dung and duckweed.
- To analyze the composition of the generated biogas and determine the NPK value of the slurry extracted from the digesters.

1.4 Limitations

While using a Semi-continuous feed system for duckweed and cow dung mix to biogas production offers several advantages, it also comes with certain limitations that need to be considered. Some of the limitations include:

1. Substrate Variability: The continuous feed system is more susceptible to fluctuations in feedstock characteristics, including the nutrient content and moisture levels of the duckweed and cow dung mix. These variations may lead to inconsistent biogas production and difficulties in maintaining optimal conditions within the digester.
2. Leakage of gas: Biogas formed inside the digester is likely to leak form the gaps made for feeding substrate despite of m-seal sealing. Also the ring lock used to tighten the jar might leak gas due to inside pressure.
3. Mixing Challenges: In a semi-continuous feed system, achieving uniform mixing of the duckweed and cow dung mixture can be challenging, especially if the feedstock has varying densities or particle sizes. The liquid solution of cow dung and water get settle down while solid substrate of duckweed floats up. This lead to the difficulty of not proper draining of slurry mixture forms the slurry pipe.

CHAPTER TWO: LITERATURE REVIEW

While co-digestion enhances anaerobic digestion efficiency, leading to increased biogas production and improved biodegradability of organic matter, practical applications in household and commercial biogas plants in Nepal still predominantly rely on the mono-digestion of cow dung. Co-digestion involves the combined treatment of multiple biomasses possessing complementary characteristics that enhances biogas production. This happens due to favorable synergies effect within the essential nutrients contained in co-substrates.(Maria Rosaria Provenzano, 2011). This study aims to emphasize the significance of co-digestion, specifically focusing on the potential benefits of utilizing high-nutrient-containing, easily cultivable duckweed plants as a biodegradable substrate.

2.1 Biogas from Cow Dung, Duckweed and their co-digestion

As a component of biogas production and waste management, cow dung is commonly employed in these plants. The resulting residues serve as organic fertilizer, while the biogas is utilized as a fuel source. A study conducted (Molla Rahman Shaibur, 2021) based on interview with 12 random dairy farmers in ziala village of Bangladesh found biogas from cow dung provided high user satisfaction and biogas saved use of conventional fuels finally uplifting the socio-economic condition of farmers. Cow dung stands out as a substrate extensively employed in biogas production due to its significant potential for gas generation. In a study conducted by (Harilal S. Soathia, 2012), three distinct substrates were employed to assess biogas yield. The findings revealed that the gas production from cattle dung per kilogram is 0.0376 m3, surpassing that from other sources such as pig dung and droppings. The table below provides an overview of gas production from various organic materials during both winter and summer seasons.

Table 1.1: Biogas productions from different organic matter in winter and summer

Material	Amount of gas (m^3/kg of fresh material)	
	Winter	Summer
Cattle dung	0.036	0.092
Pig dung	0.07	0.10
Dropping	0.07	0.16

Co-digestion enhances the efficiency of biogas production. An experiment (Hongyan Ren, 2018) was conducted to produce biogas using duckweed alone, excess sludge alone, and a combination of duckweed with excess sludge. The findings showed that the mixture of these two substrates yielded significantly higher cumulative gas, 11% more than duckweed alone. Additionally, the methane content was observed to be 13% higher compared to the duckweed-only group and 9% higher than the excess sludge group.

The co-digestion of organic matter can significantly enhance the efficiency of the technology, resulting in optimal biogas production. In a study conducted (Archana Kasinath, 2021), it was discovered that treating mechanically and fermenting chicken manure with maize silage resulted in a 27% increase in methane production compared to using chicken manure alone. Additionally, the same study observed a 26.5% increase in methane production through the co-digestion of horse manure waste with corn Stover. In a separate study conducted by (Nyirenda Austin Kawelamzenje, 2021) human waste (HW) and canteen food waste (CFW) were employed as individual substrates. The results showed that the total volume of biogas produced was 185 m^3 per kg of substrate for HW and 58.9 m^3 for CFW when used independently. However, when HW and CFW were co-digested together, the total yield increased to 265 m^3 per kg of substrate. This research (Emmanuel Pax, Muzenda, & Lekgoba, 2020) highlights the significance of co-digestion in optimizing biogas production. The ideal substrate mixture for biogas production was determined to be a mixing ratio of cow dung to food waste at 1:2, resulting in an optimum gas volume of 25,595.7 Nml. Different gas volumes were recorded for various cow dung to food waste ratios: 18,756.6 Nml for 2:1, 14,042.5 Nml for 1:1, 13,940.8 Nml for 1:3, and 13,839.1 Nml for 3:1. Notably, when the co-digestion contained a higher proportion of food waste compared to cow dung, a greater volume of biogas was produced.

The co-digestions involving fruit and vegetable waste, fish offal, and dissolved air flotation sludge exhibited greater effectiveness compared to the digestion with cattle slurry alone. Specifically, concerning the specific methane yield (m^3 CH_4 kg^{-1} VS removed), co-digestions containing fish offal and brewery sludge showed higher values than the control digestion relying solely on cattle slurry. Additionally, both co-digestions with poultry manure (at 7.5% and 15% total solids) outperformed their respective control (cattle slurry alone) in terms of cumulative methane production, with the system having a lower concentration of poultry manure yielding a higher specific methane yield(Callaghan, Wase, Thayanithy, & Forster, 1999).

Besides this, a study by (Fikadu, 2021) points the fact that co-digestion not only enhances the performance of biogas production, it also adjusts the pH value of the substrate to optimum required.

2.2 Biodegradability of duckweed

The quantity of biogas generated from 100% duckweed was greater than that produced from 100% cattle manure (CM) and reached maximum in the mixture containing 25% DW and 75% CM. These results could be attributed to the greater abundance of biodegradable material within duckweed compared to cattle manure, providing a richer source of energy for microbial activity throughout the entire digestion process (Fikadu, 2021). This observation is consistent with the findings(Charles J Banks, 2013).

2.3 Factor affecting biogas production

Several factors can significantly influence biogas production in anaerobic digestion systems. Some key factors are Feedstock Composition, Carbon-to-Nitrogen Ratio (C/N Ratio, Temperature, Retention Time, Organic loading rate, pH Level, Mixing and Agitation

A study conducted by (Yadav et al., 2017) used duckweed and cow dung in four different proportions 0:100, 90:10, 75:25 and 50:50 with cumulative gas production 11620, 305, 11695 and 12070 ml respectively on 35th day showing 50:50 to be highest potential for gas production, here the increment ratio of duckweed (DW) in the mixture from 25% to 75%, found to be declining the cumulative biogas yield. Interestingly, their study revealed that a lower concentration of water hyacinth led to higher cumulative biogas production within the system. In contrary to this another research carried out by (Fikadu, 2021) where duckweed and cow dung was used in five different proportions 100:0, 75:25, 50:50, 25:75, 0:100 and found to the cumulative bio gas production to be 1015.5, 1040, 1159, 1206, and 862, respectively which revealed attractive potential of duckweed as feedstock when mixed in 25% of duckweed and 75% of cow manure. This is the optimal blend for the digestion of duckweed with cattle manure. The biogas yield from duckweed exceeded that from cattle manure by 15.17%.

In a same study conducted by (Yadav et al., 2017) distinct results were observed in biogas production through anaerobic digestion of duckweed (DW) and cattle manure (CM) on the 35th day. The biogas production was measured at 20 ml, 580 ml, 610 ml, and 560 ml for the mixtures of DW and CM in ratios of 90:10, 50:50, 75:25, and 100:0, respectively. Notably, the highest production was achieved with the ratio of 75:25, amounting to 610 ml. This finding underscores the dynamic nature of gas production over time, influenced by the varying combinations of duckweed and cow manure.

The carbon-to-nitrogen (C/N) ratio is a crucial factor influencing bacterial growth and methane production. The ideal C/N ratio falls within the scientifically recommended range of 20-30:1. Inadequate nitrogen can hinder bacterial growth, while excessive nitrogen, surpassing 40, tends to be insufficient, limiting the growth of microbial communities and diminishing their overall capacity (Charles J Banks, 2013).

Production of biogas also depends on dilution ratio of substrate with water content as well. Substrate was diluted in different ratios ranging from 1:1 to 1:4 of mass of cow dung and mass of water. The experiment showed that as the dilution increased the biogas production per kg of cow dung increased. The biogas yield for 1:3 dilution increases by 30% than those of 1:1. The biogas volume per gram of Vs was 227,265,273,270 and 277 ml for 1:1, 1:2.5, 1:3, 1:3.5 and 1:4 FD (Gautham P. Jeppu, 2022).

There is a correlation between temperature and both biogas and methane production, with elevated temperatures resulting in increased yields. Notably, the digester operated at 35 °C exhibited the highest biogas production rate and methane content. Throughout the study, fomenters maintained at 35 °C showcased the most substantial biogas production (10377 ml), achieving the highest methane yield of 64.47%. In contrast, thermophilic reactors at 50 °C exhibited lower biogas and methane generation, followed by room temperature reactors that demonstrated even lesser production levels (Rameshrabu Ramaraj, 2016).

Amount of biogas produced from the organic substrate has influence of OLR (Organic loading rate). Specifically, it is observed that the biogas yield increased as the OLR was raised up to a certain point and then began to decrease. Thus the biogas yield with increasing OLR produced after that then shows inverse result. The OLR value with 1.5 kg/m^3, 2 kg/m^3, 2.5kg/m^3, 3kg/m^3 showed cumulative biogas yield of 0.255 kg, 0.200 kg, 0.150 kg and 0.100 kg at the end of 14^{th} , 30^{th} , 45^{th} and 60^{th} day which indicated increase in gas yield up to 1.5 kg/m^3 and then decline above this (Ejiroghene kelly orhorhoro, 2018). Similarly another study conducted by (Tabassum, Wall, & Murphy, 2016) shows biogas production generated through continuous digestion and cultivated seaweeds with dairy slurry. The OLR was varied from 4 kg/VS/m3/d to 5 kg/VS/m3/d for two different species of Seaweed and found optimum conditions of mono-digestion both species as 4 kg/VS/m3/d.

Biogas produced for continuous model in a portable stainless digester of capacity 500 liter where cow dung was used as substrate. The slurry was fed in two variations for the investigation as 5 liter and 10 liter per day. The biogas production for 5 liter per day found to be 51.7 liter biogas/day in 30 days and found 82 liter per day for the feeding of 10 liter per day keeping the hydraulic same retention time. The quality of biogas i.e. the average methane content was found to be 58.75 % in 5 liter/day where it was found 56.40% methane for 10 liter/day. This showed better loading for 5 liter per day (Ketut Adi Atmika, 2019).

pH is one of important factor for biogas production from the co digestion of cow dung and duckweed. It offers a transparent assessment of system performance, revealing potential issues such as system failure or inadequate buffering capacity, which can impede digestion. Conversely, elevated pH levels can also restrict the methanogenesis process. The pH value is contingent upon several factors, including VFA (volatile fatty acid) concentration, bicarbonate concentration, system alkalinity, and the fraction of CO2 in the digester gas (Nathaniel sawyerr, 2019).

A research conducted by (Md. Tahmid Fahan Himel, 2018) on production of biogas form using ingredients cow dung, poultry wastes, kitchen wastes, municipal solid wastes. The findings indicated that a pH range of 7.2 to 7.4 is optimal for both the combined mixture and individual components to facilitate biogas production in Bangladesh. Another research by (Mohamad Y. Mustafa, 2015) found To optimize the digestion rate, the pH level should be maintained between 5.5 and 8.5, while the temperature remains within the range of 30 to 60 degrees Celsius.

Substrate-to-inoculum ratio is a crucial parameter influencing the efficiency and stability of biogas production in anaerobic digestion systems.
In a study conducted by (Emmanuel Pax Makhura, 2020) on biogas production, the substrate-to-inoculum ratio was varied at 1:1, 2:1, and 3:1 for both cow dung and food waste. The research reported cumulative biogas yields for reactors with cow dung at 12847 Nml, 3598.3 Nml, and 4199.4 Nml for the ratios of 2:1, 3:1, and 1:1,

respectively. An increase in the Substrate/Inoculum ratio from 1:1 to 2:1 resulted in a notable 67% rise in the accumulated biogas volume. Conversely, reactors with food waste and inoculum exhibited cumulative biogas yields of 110.2 Nml, 70.1 Nml, and 46.7 Nml for the ratios of 2:1, 3:1, and 1:1, respectively. When compared to cow dung, a 58% increase in cumulative biogas yield was observed with an elevated Substrate/Inoculum ratio from 1:1 to 2:1 for food waste. This underscores that the 2:1 ratio produced the highest volume of biogas, and an increase from 2:1 to 3:1 resulted in a decrease in biogas yield. Similarly in contrast to this, For the Biochemical Methane Potential (BMP) of maize, the authors observed only minor changes in methane yield at various inoculum-to-substrate ratio (based on VS) values (1:1, 1.5:1, 2:1, and 3:1). The optimal ratio was found to be 1:1 (Raposo et al., 2009).

2.4 Types of Digesters

The selection of an appropriate bio digester configuration is critical in optimizing biogas production from organic materials. This literature review explores the characteristics of batch, continuous and semi-continuous bio digesters, aiming to provide insights into their advantages and limitations. Furthermore, the review addresses the rationale behind choosing a semi-continuous bio digester over batch and continuous types, highlighting its potential benefits for specific applications.

Batch, continuous, and semi-continuous bio-digesters are different operational configurations used in anaerobic digestion systems for the production of biogas from organic materials.

2.4.1 Batch Bio digester:

Batch Bio digester is simple in design and operation. It operates in discrete cycles with a predetermined amount of organic material. It is Suitable for small-scale applications and research purposes. It applicability is in scenarios with intermittent feedstock availability.

2.4.2 Continuous Bio digester:

Continuous Bio digester is more complex design and control mechanisms. It allows for the continuous feeding of organic material and suitable for large-scale biogas production in industrial settings. It has continuous and uninterrupted gas production. It is well-suited for consistent feedstock supply.

2.4.3 Semi-Continuous Bio digester:

Semi-Continuous Bio digester is periodic loading of substrate batches with simultaneous gas production. It has intermediate complexity in design and operation and combines features of both batch and continuous systems. It is flexible in adapting to variations in feedstock availability and enhanced control over digestion conditions.
It has potential for more stable gas production compared to batch systems and is applicable for medium medium-scale applications.

2.5 Choosing Semi-Continuous over Batch Bio digester:

Semi-continuous bio digesters offer a middle ground, allowing periodic substrate loading while maintaining continuous and more stable gas production compared to batch reactors. This is beneficial for applications where a consistent biogas output is desired gas production. Semi-continuous reactors involve periodic substrate loading, reducing the need for frequent shutdowns and restarts. This can lead to less downtime for maintenance and operational adjustments, contributing to overall system efficiency. Additionally, semi-continuous design allows for a more consistent gas production compared to batch systems, potentially leading to optimized biogas yields. This reliability is essential for applications where a steady supply of biogas is crucial.

A report by (Farghali et al., 2020) observed anaerobic digestion for batch and semi-continuous method and reported semi-continuous model performance 25 to 42 % more methane generation than batch one. Along with this, it also revealed 45 to 66 % more gas yield in semi-continuous than batch production. Another study conducted by (Mahnert, Heiermann, & Linke, 2005) indicated that in the production of biogas through the co-digestion of grass and cattle slurry, batch digestion resulted in 0.36 m3/(kg VS) of gas production, whereas continuous digestion exhibited a higher rate of 0.5 m3/(kg VS) leading to 39% increase in gas yield.

CHAPTER THREE: METHODOLOGY

This research builds upon previous investigations into biogas production from a combination of duckweed and cow dung, initially conducted using a batch model in six jars with different compositions of cow dung (CD) and duckweed (DW): 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100. Notably, the digesters with cow dung and duckweed ratios of 60:40 and 40:60 exhibited the highest biogas yields. These two optimal models were subsequently chosen for further exploration using a semi-continuous model. Additionally, a new model was introduced with an equal 50:50 ratio of cow dung and duckweed to assess gas production. The subsequent phase involved the selection of three distinct substrates, featuring cow dung and duckweed in ratios of 60:40, 40:60, and 50:50, for a semi-continuous process. The methodology adopted to carry out this research is outlined in the flow chart shown below.

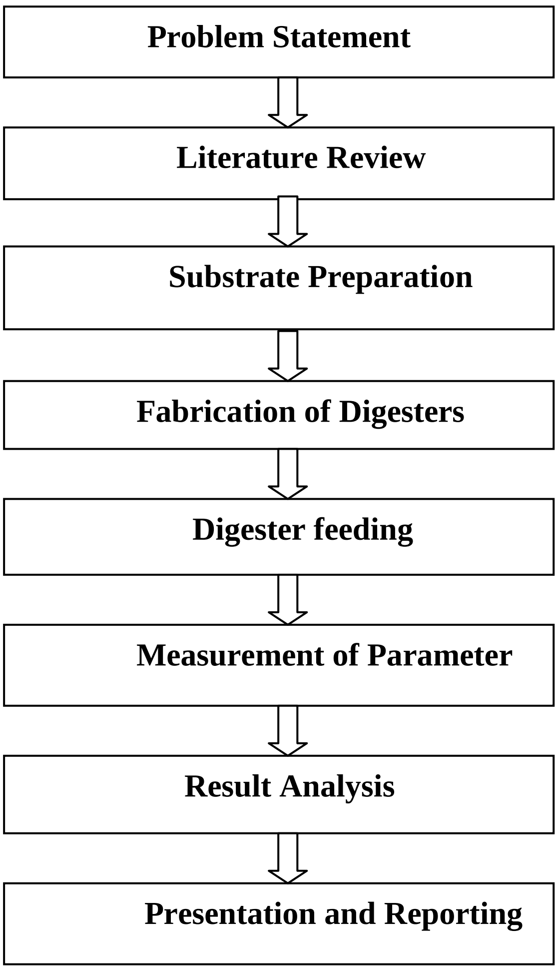

Figure 3.1: Flowchart for experimental setup

3.1 Experimental setup

The aim of this experimental setup is to investigate the biogas production potential from semi-continuous biogas reactor using a 20-litre water jar as the main reactor vessel. The reactor was fed with a mixture of cow dung and duckweed in different ratio, with the addition of inoculums to establish the desired microbial community inside the digester. The setup includes an inlet pipe for substrate addition, a slurry outlet pipe, a plastic for digester insulation, weighing machine etc. The set up was made in the open place with sunny exposure for sufficient heat absorption for microbial activity of digester.

3.2 Materials and Equipments:

The components utilized in the construction of the experimental setup, including the fabrication materials and the process of measuring biogas volume and composition for biogas production, are outlined below.

i. 20-litre water jar (main reactor vessel) (3 no.s)
ii. Cow dung (substrate material)
iii. Duckweed (co-substrate material)
iv. 1.5-inch diameter PVC pipe (inlet pipe)
v. 1-inch diameter PVC pipe (slurry outlet pipe) and elbows
vi. Inoculum (methanogenic microorganisms)
vii. Drill or hole-cutting tool
viii. Cork to seal tight the inlet pipe
ix. Gas collection system (e.g., gas storage container, gas meter)
x. Temperature and pH sensors
xi. Gas analyzer or recording system

Below is the outline of the experimental arrangement for the continuous biogas system. It involves a biogas reactor developed from a water jar, complete with an inlet feed pipe, an outlet slurry pipe, and a gas pipe fitted with a valve. The reactor is completely sealed to prevent any leaks and ensure precise monitoring of biogas production. Three similar reactors were constructed where different ratios of duckweed and cow dung were added into it.

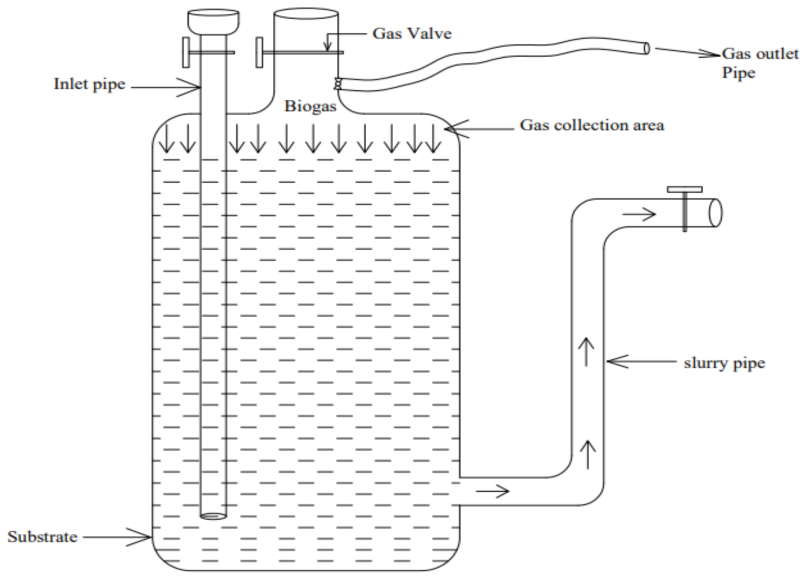

Figure 3.2: Experimental set up for semi-continuous biogas reactor

3.3 Methods

The detailed description of the sequential method employed in this experiment is outlined below. The method includes all the steps from the cultivation of duckweed to the gas production and its analysis.

3.3.1 Preparation of Substrate

Going through different studies conducted in production of biogas form various organic matter, duckweed seemed to be one of the high potential aquatic plant for biogas production. Cultivation of duckweed was done beside the boy's hotel of Pulchowk Engineering Campus. It took two month for proper of growth for ready for harvesting. Thus cultivated Duckweed was collected and cow dung was collected a cow farm near Nakkhu Located at Lalitpur district of Nepal. The inoculum was collected from existing biogas plant situated at Quarter building of Pulchowk Campus. Thus collected substrate was mixed in the ratio of 3 different ratio as 60:40, 40; 60, and 50:50 ratio of cow dung and duckweed. Inoculum was also added in the given amount as the catalyst to initiate the microbial reaction. Inoculum helps in initiating and enhancing biogas production. The amount of substrate was added in the amount as stated in the table below.

3.3.2 Reactor Fabrication

A 20-litre water jar, cleaned with distilled water to remove potential contaminants, was selected to create a biogas reactor. Using a red hot GI pipe of diameter 1.5 inch, a hole was made adjacent to the jar's mouth. A CPVC pipe of dia. 1.5 inch fitted into it, extending just slightly above the jar's bottom level.

Additionally, another hole with a 1-inch diameter was created on bottom side wall to facilitate the arrangement of the slurry pipe. A 1-inch slurry outlet pipe was attached to the bottom of the water jar. Care was taken to ensure that this pipe extended almost to the substrate level within the reactor, allowing the digested substrate to exit through the slurry pipe.

3.3.3 Loading the Reactor

Set of three drinking water jar each capacity 20 liters were taken for fabrication of digester. The digester was fed to two-third volume with the substrate with regular feeding. The hydraulic retention time was taken for 45 days. Substrate was prepared taking cow dung and duckweed in three different proportions along with addition of inoculums as shown in the table below. The substrate prepared was mixed toughly in round plastic tough and fed into the digester.

Table 3.1: Mass of Substrate feed at the beginning

<table>
<tr><th rowspan="2">Batch
(CD:DW)</th><th colspan="4">Mass (in kg) of</th><th rowspan="2">Total mass (m)=a+b +c</th><th rowspan="5">Total volume to be filled (liter)
=0.7×20
= 14</th></tr>
<tr><th>Cow Dung (a)</th><th>Duckweed (b)</th><th>Water (c)</th><th>Inoculums</th></tr>
<tr><td>B1 (60:40)</td><td>3.78</td><td>2.52</td><td>7</td><td>0.7</td><td>14</td></tr>
<tr><td>B2 (40:60)</td><td>2.52</td><td>3.78</td><td>7</td><td>0.7</td><td>14</td></tr>
<tr><td>B3 (50:50)</td><td>3.15</td><td>3.15</td><td>7</td><td>0.7</td><td>14</td></tr>
<tr><td>Total</td><td>9.45</td><td>9.45</td><td>21</td><td>2.1</td><td>42</td><td></td></tr>
</table>

The OLR (Organic Loading Rate) was calculated using formula below. The total capacity of substrate in a digester is 14 kg and retention days were taken as 45 days. The formula to calculate the organic loading rate is given as

$$Organic\ Loding\ Rate(OLR) = \frac{Total\ capacity\ \ of\ the\ digestor}{No.of\ retention\ \ days}$$

$$= \frac{14}{45} \qquad = 0.31 \text{ kg/day}$$

Loading rate of 0.31 kg/day was distributed differently as stated in the table below

Digester volume = Volume of digester/day × Retention period (day)

= 0.31×45 = 13.95 ~14 kg

Also the volume of gas holder is left in the above of digester jar Three-tenth of total volume which equivalent to 6 L space.

Volume of gas holding space inside digester = $\frac{3}{10}$×20 liter = 6 liters

Total digester volume = volume of digester + volume of gas holding space

= 14 liters + 6 liters = 20 liters

The inoculum was maintained at 10% of the total substrates, resulting in a substrate and inoculum ratio of 9:1.

3.3.4 Initial parameter for continuous type biogas

Parameters affecting the gas were recorded in regular basis using different equipments. Initial parameter of the bathes B1 (60%CD+40%DW), B2 (40%CD+60%DW) and B3 (50%CD+50%DW) was measured. Temperature was measured using Thermocouple thermometer and pH was measured using pH meter. Likewise, TS, VS and C/N Ratio were measured at were measured at Soil, Water and Air Testing Laboratories Pvt. Ltd. (SWAT Lab) situated at Baluwatar, Kathmandu. The values are of initial parameter taken at the time of first fed is illustrated below.

Table 3.2: Initial parameters of different batches recorded

Parameters	Batches		
	B1 (60%CD+ 40%DW)	B2 (40%CD+ 60%DW)	B3 (50%CD+ 50%DW)
Initial Temperature (°C)	26	26	25
Initial pH	6.9	7.0	6.8
TS (% Dry Basis)	14.61	6.51	10.06
VS (% of TS)	76.43	73.09	79.12
C/N Ratio	30:1	32:1	31:1

Examining the parameters from the table above, the total solids (TS) reached its highest value for the ratio of cow dung to duckweed (CD/WDW) at 60:40 and its lowest for 40:60. This leads to the assumption that the 40:60 ratios are likely to have the highest gas yield. It is based on the findings of (I. N. Itodo, 1999), which suggest that gas yield tends to increase as the total solids value decreases.

Similarly another forecasting can be made based on the volatile solid where VS is maximum for 50:50 ratios and minimum for 40:60 ratio of cow dung and duckweed. From conclusion drawn by (Negassa & Fikadu, 2021) which says higher the VS value, larger fraction of organic mass is bio degradable resulting in highest gas production. This brings to fact that 50:50 model is going to be the highest gas yielding digester.

After introducing three pre-defined ratios of cow dung and duckweed into the digester, airtight condition was maintained securely fastening the jar's mouth using a tie screw and a new lid. To raise the temperature required for anaerobic digestion, the digester was placed within artificial greenhouse made of transparent plastic. The table below summarizes the computation of the daily feed, specifically the Organic Loading Rate (OLR), for various digesters, incorporating both cow dung and duckweed.

Table 3.3: OLR of Cow dung and Duckweed in kg

Cow dung and duckweed ratio inside the digester	Cow dung	Duckweed	Water	Total
60:40	0.062	0.093	0.155	0.31
40:60	0.093	0.062	0.155	0.31
50:50	0.0775	0.0775	0.155	0.31

3.3.5 Gas measurement System

Formation of gas at the top portion of the water jar start just after the mixture is introduced inside the reactor. Gas was measured in the regular interval of 3 days in each of three digesters. Volume of gas was measured using water displacement method. In this method, a tough full of water is taken and measuring cylinder is slanted gently to fill it with water ensuring no air into it and made the position upright. The gas pipe from the digester was inserted into measuring cylinder from below ensuring the gas valve is open. As soon as the gas pipe is inserted into the measuring cylinder inside the water and gas valve is opened the gas begin to displace water showing a measurement reading for gas volume in the cylinder. At times, enough gas accumulates to fill the measuring cylinder. In such instances, a second round of gas measurement is conducted by expelling the previous gas. This process is repeated for subsequent measurements until gas inside the cylinders was depleted.

3.3.6 Monitoring of Data

Different parameters such as temperature, pH, and gas yield and gas composition was measured at a regular interval. Temperature was measured with the help of Thermocouple K type Fluke 53 ii B Thermometer. Temperature was measured for both outside surrounding and inside the green house which is attached in Appendix. The pH was measurement with the help of digital pH meter (HANNA). pH was also measured in the regular interval of 3 days. PH value of cow dung and duckweed was recorded as

Table 3.4: pH value recorded during the feeding of substrate at room temperature

Substrates	pH value
Cow dung	6.7
Duckweed	4.3
Cow dung and Duckweed mix(60:40)	6.9
Cow dung and Duckweed mix(40:60)	6.9
Cow dung and Duckweed mix(50:50)	6.9
Inoculums culture	6.7

3.3.7 Calculation of Gas yield per kg of substrate

For 60:40 cow dung and duckweed

Total substrate at the beginning = 14 kg

Hydraulic Retention Time = 45 days

Daily feed = 0.31 kg

Total feed amount fed daily = 45×0.31 = 13.95 kg

Total fed = 14 +13.95 = 27.95

Gas Yield = $\frac{\text{Total gas yield}}{\text{Total substrates fed into digester}} = \frac{8470\ (\text{ml})}{27.95(\text{kg})}$ = 0.31 liter/kg = 0.00031 m^3/kg

Similarly for 40:60 mix of cow dung and duckweed

Gas Yield = 0.00045 m^3/kg

Finally, for 50:50 ratio is

Gas Yield = 0.00056 m^3/kg

3.3.8 Calculation of TS and Vs Removal

The Volatile Solids (VS) removal in a biogas model is a measure of the reduction in the organic content of the feedstock during the anaerobic digestion process. VS removal is often expressed as a percentage, indicating how much of the volatile solids have been converted to biogas, microbial biomass, and other byproducts. The calculation was made after the AD when the slurry comes out. The slurry was taken to lab and its TS and VS was measured. The removal of TS and VS was calculated using for below mentioned formula

$$TS\ Removal\ \% = \frac{Initial\ TS\ content - Final\ TS\ content}{Initial\ TS\ content} * 100\%$$

Similarly VS Removal can be calculated as

$$VS\ Removal\ \% = \frac{Initial\ VS\ content - Final\ VS\ content}{Initial\ VS\ content} * 100\%$$

3.3.9 Biogas and digestate Analysis

Biogas and digestate was analyzed by evaluating the composition and properties of these two outputs from anaerobic digestion processes. Here's a brief overview of what each analysis entails:

Biogas Composition using gas analyzer:

Methane (CH_4) Content: Determining the percentage of methane in biogas is crucial since methane is the primary component responsible for its energy value. Higher methane content indicates a more energy-rich biogas.

Carbon Dioxide (CO_2) Content: CO_2 is another significant component of biogas. The proportion of CO_2 affects the calorific value of biogas, and lower CO_2 levels are generally desirable for energy purposes.

Trace Gases: Analyzing for trace gases, such as hydrogen sulfide (H_2S), ammonia (NH_3), and siloxanes, is important for both environmental and operational reasons. These gases can have corrosive or harmful effects and may need to be removed or controlled.

Digestate Analysis:

Nutrient Content: Assessing the levels of nutrients like nitrogen, phosphorus, and potassium in the digestate is essential for understanding its value as a fertilizer.

CHAPTER FOUR: RESULTS AND DISCUSSION

This semi-continuous biogas setup serves as a continuation of a previous batch production experiment conducted with six different models of bio digesters. In the batch experiment, various ratios of cow dung (CD) and duckweed (DW) were tested, including 100% CD, 80% CD + 20% DW, 60% CD + 40% DW, 40% CD + 60% DW, 20% CD + 80% DW, and 100% DW. Among these, the combinations of 60% CD + 40% DW and 40% CD + 60% DW demonstrated the highest biogas yields.

In addition to these two successful ratios, a new mixture of 50% CD + 50% DW was introduced in the semi-continuous setup. The table below illustrates the volume of gas produced in the three semi-continuous type digesters.

4.1 Gas volume for different digesters

The study spanned 45-days, involving regular gas measurements. Commencing on August 12th and extending until 25th September, a consistent gas flow persisted due to the continual provision of feed substrate. Referring to the range of biogas retention times spanning 40 to 100 days, as outlined by (Amrit Nakarmi, 2015) , this experiment specifically adopted a 45-day retention time. The figure below shows the volume of gas yield for semi-continuous feeding of substrate with varying mix.

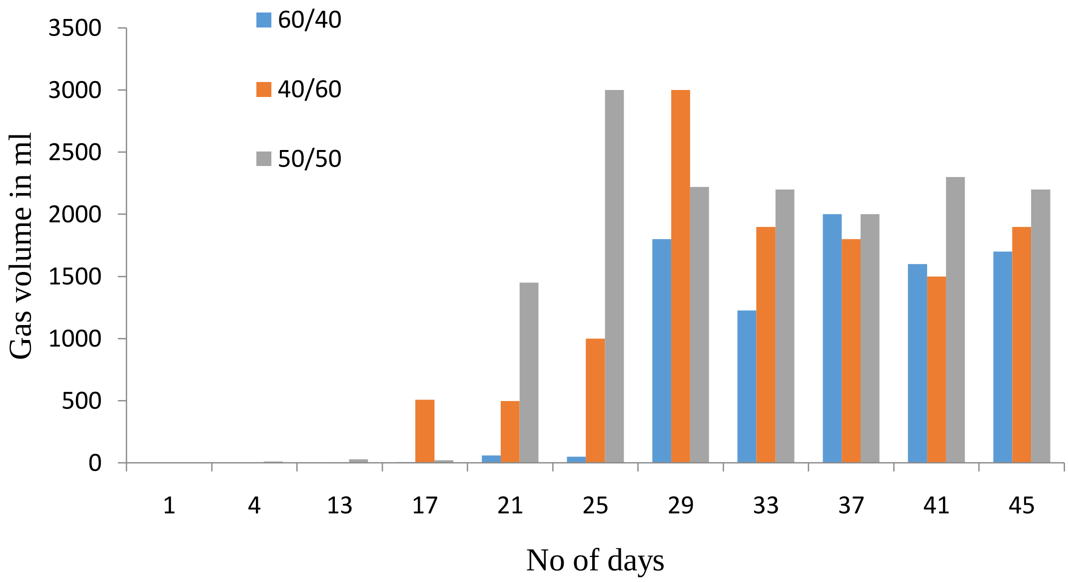

Figure 4.1: Gas production in different digesters with days

From the figure above, it is observed that the volume of gas produced in each digester is increasing with time up to 29th day after which the gas production stabilizes for the rest of the time. This happened due to the reason that gas production may start early but the efficiency and completeness of digestion takes time equal to hydraulic retention time. In this case, the substrate seems to be fully digested till 29th day and stabilizes thereafter. It is also being observed that the gas production for the mix of 50:50 is higher in every phase of experiment, while the digesters with having 40:60 and 60:40 of cow dung and duckweed shows competitive dynamics i.e. seems dominating one another with time. This might be due to impact of some other factors like temperature in biodegradation of cow dung and duckweed. Thus, overall production of gas was found to be highest for 50:50 ratios. This shows that equal proportion of cow dung and duckweed when mixed makes the optimal blend for highest microbial activity.

4.2 Cumulative gas production vs. daily gas production

The cumulative and daily gas yield of the different digester fed with varying ratio of cow dung and duckweed has been measured and analyzed with the help of figure illustrated below. The cumulative gas production curve seems to be increasing with time where as daily curve are fluctuating up to certain time and then get stabilizes indicating relative constant gas production.

4.2.1 Gas production in CD: DW =60:40

The figure 4.2 shows the cumulative and daily gas yields of the digester where cow dung and duckweed are mixed in 60:40 proportions. The begins to form from the day seventeen and volume of gas start to increase rapidly from 25th day. After that, the gas yield shows rapid fluctuatation up to 40th day and then stabilizes thereafter. This may be due to complete digestion of the substrate inside the digester fed initially. Total volume of gas produced from 60:40 was found to be 46 % lower than highest producing digester i.e. 50:50. This shows the 60:40 ratio of cow dung and duckweed has lesser microbial activity than 50:50 and thus producing less gas. The possible reason for this can be high percentage of cow dung in mix where a portion of undigested organic matter i.e partial fermentation in the intestinal tract of cow which later could not from uniform mix with duckweed leading to lesser co-digestion between the substrate reported by (Negassa & Fikadu, 2021).

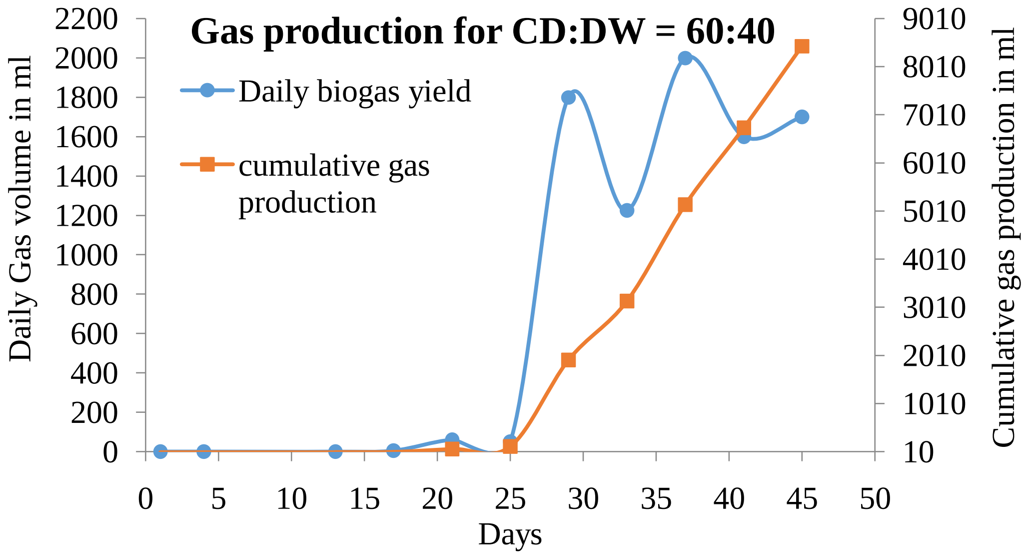

Figure 4.2: Volume of gas production in 60:40 Duckweed and Cow dung mix

4.2.2 Gas production in CD: DW =40:60

The figure 4.3 shows the cumulative and daily gas yields of the digester where cow dung and duckweed are mixed in 40:60 proportions. The gas begins to form from the day second but in negligible quantity and volume of gas start to increase noticeably from the day 19. After day 19, the gas yield continues to fluctuate with continuously feed of substrate into the digester till 35th day and then moving towards stabilization. This indicates the complete digestion of substrates is ongoing till 35th day and then gas production becomes slight lower and continued till the Hydraulic retention time. The volume of the gas production is 20% lesser than 50:50 ratio of cow dung and duckweed. This may be due the high percentage of duckweed in the mixture which contains lignin, cellulose and hemi cellulose that takes comparatively more time to break down and get converted into methane reported by (Facchin et al., 2013). This forms a relatively weak blend for co-digestion of cow dung and duckweed leading to less gas production.

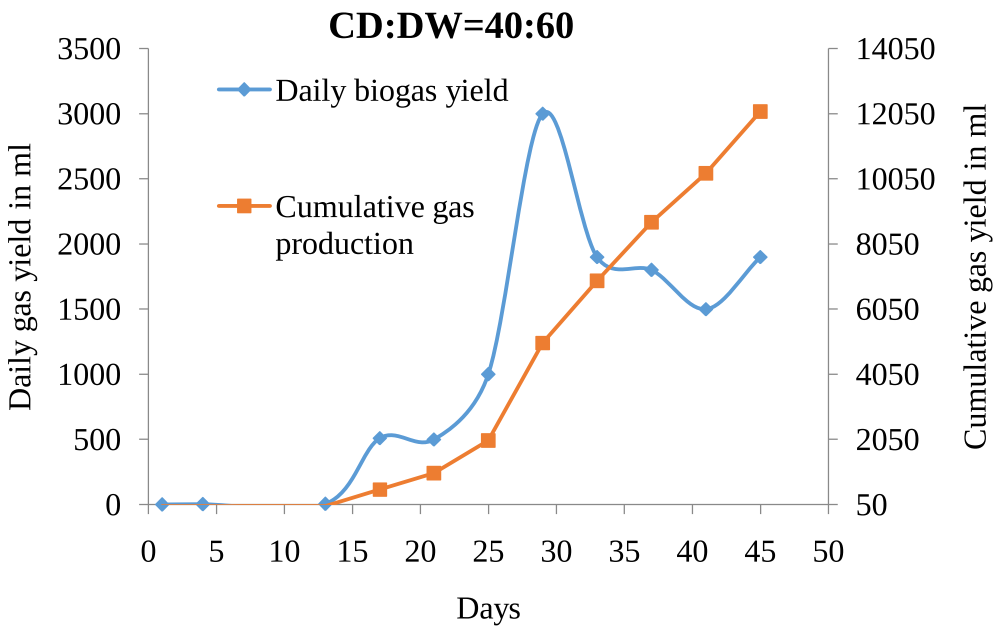

Figure 4.3: Volume of gas production in 60:40 Duckweed and Cow dung mix

4.2.3 Gas production in CD: DW =50:50

The figure 4.4 shows the cumulative and daily gas yields of the digester where cow dung and duckweed are mixed in 50:50 proportions. The gas begins to form from the day 2 but in negligible quantity and volume of gas start to increase noticeably from the day 20. After day 20, the gas yield reaches maximum to 2700 ml on 26th day and then fluctuate till 33rd day and stabilized thereafter. The stabilization of gas for this ratio seems earlier than other two blends of cow dung and duckweed. This shows the 50;50 blend has quicker response for microbial activity between substrates.

The volume of gas obtained from 50:50 digester found to be highest than other two digesters i.e. 40:60 and 60:40 digesters. This result is supported by the research conducted by (Yadav et al., 2017) It shows the optimal blend of cow dung and duckweed when mixed in equal proportion. The probable reason for high gas yield can be high volatile solid when measured at initial parameter i.e. 79.12%. Higher the volatile solid higher is the organic matter present to be converted into biogas is suggested by a conducted by (Fikadu, 2021).

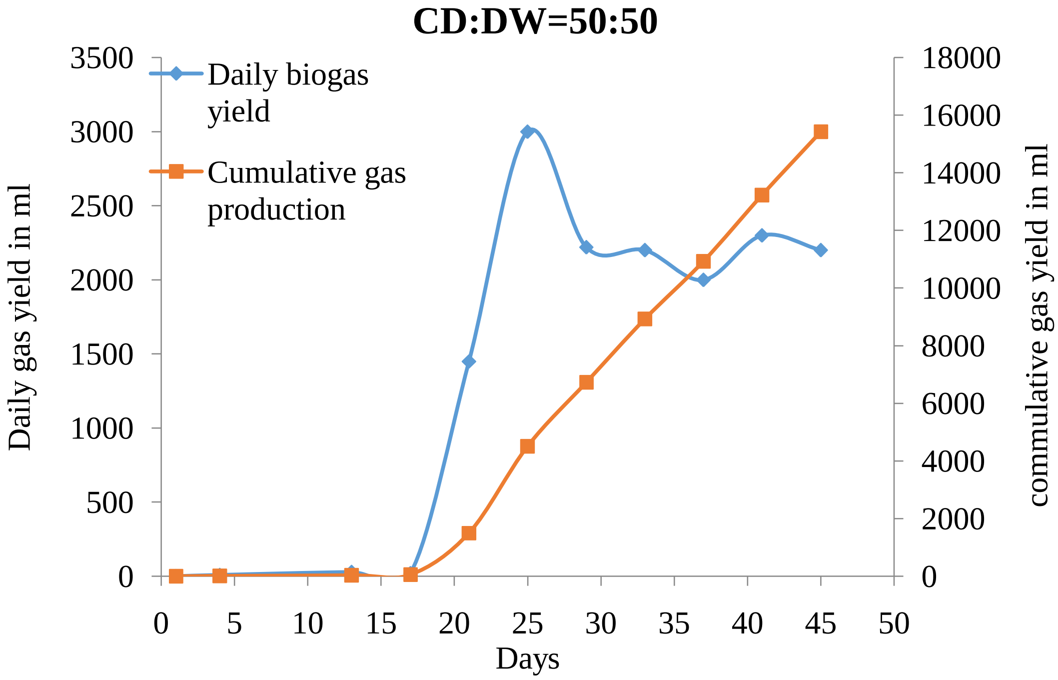

Figure 4.4: Volume of gas production in 50:50 Duckweed and Cow dung mix

4.2.4 Cumulative Gas Production

The figure 4.5 shows the cumulative production of biogas from different digesters. The first one i.e. cow dung and duckweed ration of 60:40 has least gas production i.e. 8470 ml in 45 days where as 50:50 has highest gas production i.e. 15715 ml. This shows the co-digestion of duckweed with cow dung in equal is optimal blend for microbial activity and biogas production. This outcome aligns with a study conducted by (Yadav et al., 2017), which emphasized that an equal proportion of duckweed demonstrates a greater potential for biogas generation. In contrary, (Fikadu, 2021) finds highest yield of biogas was obtained from cow dung and duckweed of 75:25 when experimented for different mix of 0:100, 75:25, 50:50, 25:75, 0:100. i.e. the highest gas yield was observed in the mixture with a higher proportion of cow dung. This might have happened due to the duration of hydraulic retention time. HRT was taken for 30 days where as this study has taken 45 days. This is attributed to the enriched nutrient content of duckweed, particularly nitrogen and phosphorus, which, when combined with cow dung for co-digestion, significantly enhances the efficiency of anaerobic digestion.

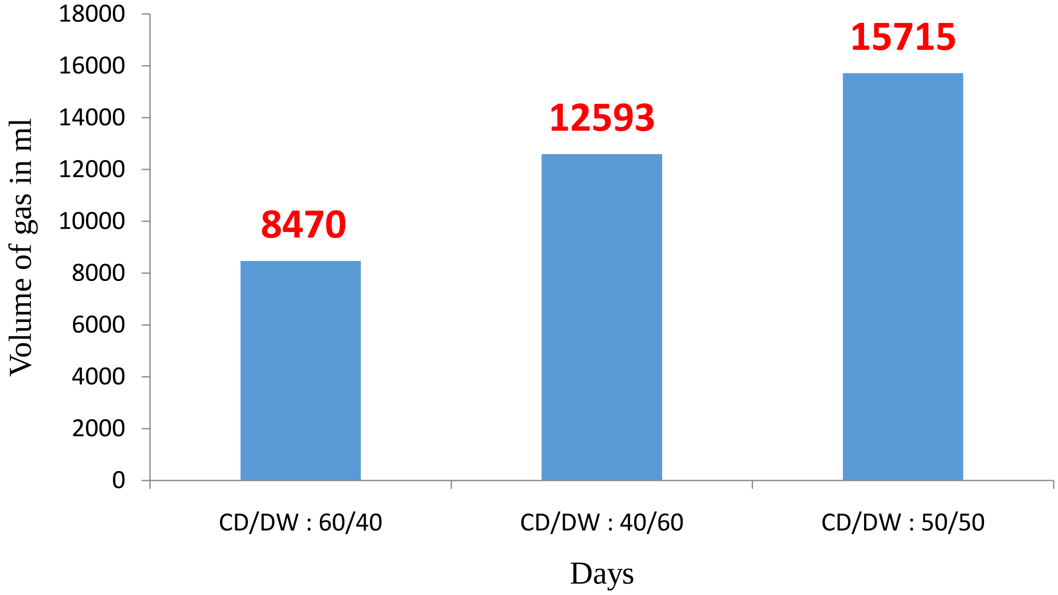

Figure 4.5: Cumulative gas production with HRT 45 days

4.3 Effects of Total Solid and Volatile Solid in biogas production

The initial total solid content for all three samples before anaerobic digestion (AD) varied from 6.51% to 41.61%, based on 10 grams of each sample. After AD, the total solid content ranged from 2.23% to 2.26%. The 60:40 mix of cow dung and duckweed exhibited the highest total solid content before AD, while the 40:60 mix recorded the lowest. A study conducted by (I. N. Itodo, 1999) to see the effect of solid concentration of poultry, cattle and piggery waste slurries on biogas production. Three replicates of 5%, 10%, 15% and 20% of TS concentration of these three substrates digested for 30 day HRT. The result showed increase in biogas production with decrease in TS concentration of the slurries which is not satisfied by this research.
Following the anaerobic digestion (AD) process, there was a notable reduction in total solids (TS) values across all substrate samples in comparison to their pre-AD levels. Notably, the decline was more marked in the mixed substrate samples compared to their individual counterparts. The decrease in TS and VS content implies a conversion into biogas, with the most significant removal was observed in 60% cattle manure (CM) and 40% duckweed (DW) in mixture.

Table 4.1: Experimental results for TS and VS of different substrate in percentage

CD:DW	Initial TS	Final TS	TS removal	Initial VS	Final VS	VS removal
60:40	14.61	2.43	83	76.43	73.09	9
40:60	6.51	2.23	66	70.19	69.36	3
50:50	10.06	2.26	78	79.12	70.19	6

Though highest removal of TS and VS removal after AD indicates highest gas formation, but it was not observed in this experiment, it showed highest gas production in 50:50. This is due to the fact that 60:40 ratio has higher 60 % cow dung and 40% duckweed and finding of (Fikadu, 2021) reveals cattle dung contains more simple degradable monomers fermentable bacteria that degrades these molecules to Volatile fatty acids and then to ammonia and finally those ammonia into methane gas. So the Total solid removal found to highest in 60:40 blends of cow dung and duckweed.

The same study (Fikadu, 2021) which was carried out for different percentage of cow dung and duckweed found highest removal of TS in the mixture where cow dung percent was dominant i.e. 75% cattle manure and 25% duckweed and mixture. These findings indicate a positive correlation between total solids removal and content of cow dung in the mixture. i.e. the TS and VS removal for 60:40 cow dung and duckweed ratio is 83% and 9 % where as 78% and 6% for 40:60 mix.

4.4 Effect of pH

The pH level is a crucial marker for evaluating methanogenic activity in biogas formation. In the absence of alternative indicators, pH values are commonly utilized to gauge the conditions within the digester. A study (Simon Jayaraj, 2014) investigates the impact of pH on biogas yield using five laboratory-scale batch reactors, each maintained at pH levels of 5, 6, 7, 8, and 9. The reactors operated under mesophilic temperature conditions with a hydraulic retention time of 30 days. The findings reveal a significant increase in biogas yield and degradation efficiency for the substrate at pH 7, in comparison to other pH values. The variation in pH was observed to be varying between 6.7 to 7.3 which lie in suitable range of biogas production backed by a study (Nathaniel sawyerr, 2019).

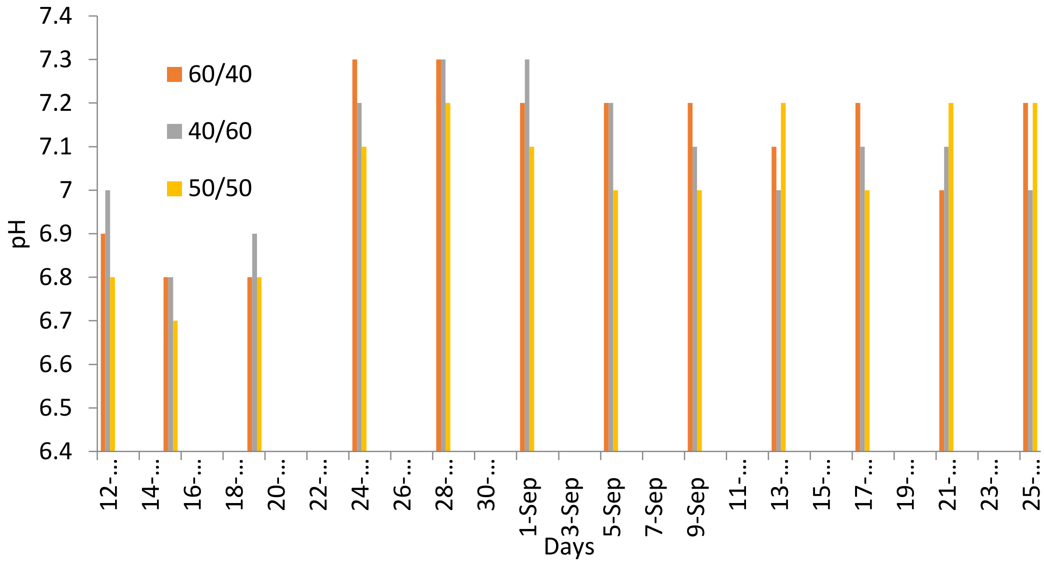

Figure 4.6: pH variations with time

Above figure shows the pH fluctuations for three different semi-continuous digesters with corresponding days. It shows the gas production first decline with the decrease in pH around 18th August i.e. 7th day. This is the phase where acidogenesis occur where gas production is comparatively less after which it enters into methogenesis phase where gradually gas production increases takes place. From the graph it can be concluded that pH fluctuations vary from 7.0 to 7.3 which is highest gas yielding zone backed by a research (Md. Tahmid Fahan Himel, 2018) that reveals the suitable pH 7.2 to 7.4 for maximum biogas production. The study also says the production becomes maximum at 7.3 pH. Thus the pH measured during this experiment was within the optimal range appropriate for maximum gas production.

Different stages of the anaerobic digestion (AD) process achieve varying optimal pH values. These pH fluctuations occur during the biological transformations observed at different phases of the AD process. Specifically, a pH level below 5 is encountered during the production of organic acids, a process occurring at the acetogenesis stage (L.Arsova, 2010).

4.5 Hydraulic retention time

Hydraulic retention time (HRT) is the average time spent inside the bio-digester by the substrate before it discharges out as slurry. It's an important parameter because it influences the efficiency of organic matter degradation and biogas production. The reactors made for batch production was recorded as 40-50 days. According to study (Md. Tahmid Fahan Himel, 2018), the hydraulic retention time is 30 to 55 days. The HRT for this experiment was taken for 45 days for 3 model of continuous type biogas with different blend ratio into the reactor. The digesters were feed with the constant OLR up to 45 days. Despite of HRT for 45 days, gas production pattern shows that it was 30 days a newly fed remained inside the digesters because gas production up to 30 days seems increasing and stabilizes after 30^{th} day.

In batch production, the digester is loaded with a specific amount of substrate, the digestion process occurs, and then the digester is emptied before starting a new batch. In contrast, semi-continuous production involves a constant inflow of substrate and outflow of digested material, allowing for a continuous process. For continuous-type digesters, the average retention time is a crucial parameter to maintain efficient biogas production.

As per the investigation conducted (R. Singh, 1985), the retention time is influenced by a range of factors including Total Solids, Volatile Solids, Cellulose, Lignin, Nitrogen percentage, Carbon nitrogen ratio, pH, and Total volatile fatty acids. Their findings also suggest that there exists an inverse relationship between the rate of gas production and the duration of retention time, meaning that higher gas production rates tend to correspond with shorter retention times.

The study on semi-continuous type biogas predicted that different blend ratios and retention times affect biogas production. It was seen nearly constant amount of gas being produced after the HRT which was slight lower than earlier.

The digester was maintained an average temperature of approximately 30 to 40ºC where the hydraulic retention time for cow dung and duckweed blended substrate last to 30 days, with gas production commencing on the 5^{th} day. Furthermore, the gas production is delineated in terms of volume percentage across four distinct intervals of the hydraulic retention time. According to (Mohamad Y. Mustafa, 2015), in the initial slot spanning 0-10 days, approximately 10% of the total gas was generated. This percentage substantially increased to around 35% during the subsequent period of 11-20 days. The third interval, covering 21-30 days, recorded the highest gas production, accounting for approximately 46% of the total volume. Finally, the gas production diminished to approximately 9% within the fourth slot of 31-40 days, and no further gas production was observed beyond the 40^{th} day.

4.6 Organic loading rate

Organic loading rate is the mass of feedstock added per day per digester for growth rate of methanogenesis bacteria during AD It is measured in kg/L/day. The OLR for this experiment was kept 0.31 kg per day. It was calculated dividing the volume of digesters by hydraulic retention time. This amount was partitioned in different parts according to the blend proportion of cow dung and duckweed in the digesters.

The specific methane rate (SMR) remains constant with increase in loading rate but it rise the volumetric methane production (VMP) linearly. During this time, HRT get reduced due to the reason of addition of material to satisfy the organic load. According to the experiment carried out by (Charles J Banks, 2013), HRT would reach the safe limit value of 12 days with an OLR of 5 kg VS/m^3. If this value limit is crossed, it would start to wash out the methogens resulting in fall in the specific yield due to reason of less contact period and reduced mass quantity.

Organic Loading Rate (OLR) is a crucial parameter in the context of anaerobic digestion (AD) processes, which are used for the conversion of organic materials into biogas. In this process, microorganisms break down organic matter in the absence of oxygen, producing biogas as a byproduct.

A study was conducted by (Ejiroghene Kelley Orhorhoro, 2018), the amount of biogas produced from the organic substrate, was influenced by the OLR. Specifically, they observed that the biogas yield increased as the OLR was raised up to a certain point and then began to decrease. Thus the biogas yield with increasing OLR produced inverse result. The OLR value with 1.5 kg/m^3, 2 kg/m^3, 2.5kg/m^3, 3kg/m^3 showed cumulative biogas yield of 0.255 kg, 0.200 kg, 0.150 kg and 0.100 kg at the end of 14th , 30th , 45th and 60th day.

In a single-stage AD reactor, the biogas yield increased with increasing OLR up to 1.5 kg/m^3 of reactor volume. Beyond this point, when the OLR was further increased, the biogas yield started to decline. This suggests that there is an optimal range of OLR that leads to the highest biogas production in single-stage reactors.

In the case of a three-stage continuous AD reactor, the optimal OLR range for maximizing biogas yield was found to be between 1.5 and 3.0 kg/m^3. This indicates that the three-stage reactor had a broader range of OLR within which biogas production was optimized.

Similarly a study conducted (Muhammad Rizwan Tabassum, 2016) on biogas production generated through continuous digestion and cultivated seaweeds with dairy slurry. The OLR was varied from 4 kg/VS/m^3/d to 5 kg/VS/m^3/d for two different species of Seaweed and found optimum conditions of mono-digestion both species as 4 kg/VS/m^3/d.

A study was conducted by (Ejiroghene Kelley Orhorhoro, 2018) infers the amount of biogas produced from the organic substrate, was influenced by the OLR. Specifically, they observed that the biogas yield increased as the OLR was raised up to a certain point and then began to decrease.

In a single-stage AD reactor, the biogas yield increased with increasing OLR up to 1.5 kg/m^3 of reactor volume. Beyond this point, when the OLR was further increased, the biogas yield started to decline. This suggests that there is an optimal range of OLR that leads to the highest biogas production in single-stage reactors is 1.5 kg/m^3.

In the case of a three-stage continuous AD reactor, the optimal OLR range for maximizing biogas yield was found to be between 1.5 and 3.0 kg/m^3. This indicates that the three-stage reactor had a broader range of OLR within which biogas production was optimized.

4.7 Effect of temperature

Temperature plays a pivotal role in biogas production as it profoundly influences the activity of the microorganisms responsible for anaerobic digestion, the process through which organic materials are broken down to produce biogas. Different temperature ranges have varying effects on biogas yield and methane content, which are crucial parameters in assessing the efficiency and potential of biogas generation from the given feedstock mixture.

The experiment was carried out at Pulchowk campus Msc Hostel (27.6801123361518, 85.31917764075332) in the month of august and September where temperature of surrounding varied between 25^0C to 31^0C in the day time which is slight low temperature for efficient biogas production. A study (Leenawat Artsupho, 2016) identified the ideal temperature for maximizing biogas yield to be between 29°C and 40°C. This range is known to foster the most favorable conditions for the microbial community involved in the anaerobic digestion process. Another study (Rameshprabhu Ramaraj, 2016) findings indicate a correlation between temperature and both biogas and methane production, with elevated temperatures resulting in increased gas yields. Notably, the digester operated at 35 °C exhibited the highest biogas production rate and methane content.

Biogas production below 30°C declines as low temperature slow down the growth of methanogenic bacteria which is responsible for production of CH_4(Nyirenda Austin Kawelamzenje, 2021). So, to elevate the temperature, the digesters were positioned outdoors in an open area and covered with a greenhouse, contributing to a temperature increase of 4-6°C. The variation of temperature obtained using plastic covered green house is illustrated below. The maximum temperature obtained was 38.2 degree Celsius inside the green house where as minimum temperature obtained was 25.2 degree Celsius.

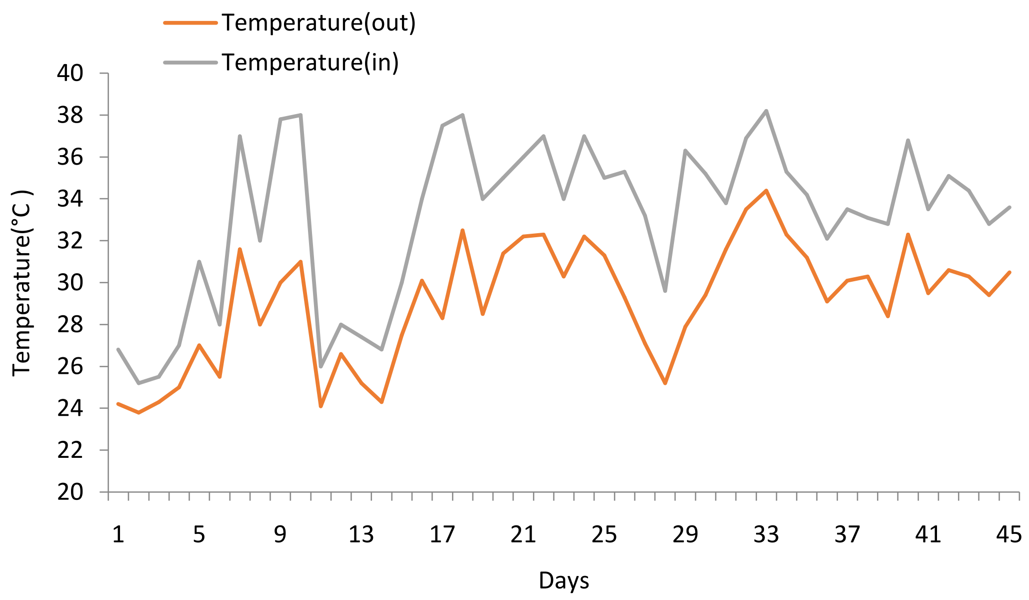

Figure 4.7: Recorded temperature at experimental site

4.8 C:N Ratio

It is vital element of anaerobic digestion which shows the carbon to nitrogen proportion in the substrate. The process of anaerobic digestion operates with a carbon-to-nitrogen (C:N) ratio that utilizes carbon around 25-30 times more than nitrogen. The ideal balance falls within the range of 20-30:1 for C:N ratio This ratio's manipulation is achievable through co-digestion, a method of combining different waste types to enhance biogas production (Fikadu, 2021). By blending low-carbon content waste with high-nitrogen content waste, a balanced C:N ratio can be attained. This synergic effect of C:N ratio helps to obtain optimum value that supports in maximum gas production. Occasionally, temperature variations can also impact the C:N ratio.

In the context of semi-continuous type anaerobic digestion experiments, the effects of different mixtures of cow dung and duckweed on C:N ration was analyzed. These mixtures were formulated in ratios of 60%CD & 40% DW, 40% CD & 60% DW, and 50%&50%, representing the proportion of cow dung to duckweed. The initial C:N ratios of the mixtures were determined to be 30:1 for the 60%CD & 40% DW ratio, 32:1 for the 40% CD & 60% DW ratio, and 31:1 for the 50%&50% ratio.

4.9 Effect of dilution

Traditionally, biogas plant is run at a dilution ratio of cow dung and water of 1:1. Dilution in the ration of 1:1 indicates the Total solid of 10%. In this experiment the dilution was made after the cow dung and duckweed was mixed in three different ratio. i.e. 60:40, 40:60 and 50:50. Water was mixed in the 1:1 ratio as the Total solid measured was 14.61%, 6.51% and 10.06% for 60:40.40:60 and 50:50 respectively. The dilution was made 1:1 due to the fact for comparison of different substrates at similar dilution. In contrary to this, study conducted by (Gautham P. Jeppu, 2021) used the substrate dilution in different ratios ranging from 1:1 to 1:4 of mass of cow dung and mass of water. The experiment showed that as the dilution increased the biogas production per kg of cow dung increased. The biogas yield for 1:3 dilution increases by 30% than those of 1:1. The same study showed there is no increase of gas beyond dilution of 1:3 and the methane content was also recorded maximum at the dilution of 1:2. These finding was based on biogas yield from cow dung only which is different with this research.

4.10 Gas Composition evaluation

The content of biogas produced in three digester was measured using gas analyzer (GASBOARD 3200 Plus) and composition found in each type of digester was illustrated below. The gas analyzer measured concentration of methane, carbon dioxide and oxygen among which methane is the main constituent required for combustion of gas. It is found that highest average methane content found in (CD: DW=40:60) i.e. 37.79% proportion of cow dung and duckweed followed by CD: DW=50:50 (29.90%) and DW=60:40 (19.45%). The probable reason for high methane content found in (CD: DW=40:60) may be the C/N ratio of the substrate. The C/N ratio of CD: DW=40:60 was maximum among three digesters i.e. 32.1 followed by CD: DW=50:50 (31.1) and CD: DW=60:40 (30:1).

The biogas produced did not meet the expected methane content, as indicated by a study conducted by (yadav, 2019), which reported methane production ranging from 63.5% to 64.3% during the co-digestion of cow dung and duckweed. The likely cause for this discrepancy could be attributed to the use of a semi-continuous digester model over batch one. The highest methane content semi-continuous model was found to lesser than batch one. The possible reason for this might be incomplete digestion. The semi-continuous model resulted in a slurry output without digested fully, possibly due to the introduction of a new feed mix filling the digester.

Tables 4.2 show the content of methane, carbon dioxide and oxygen in three distinct digesters. Gas composition was measured on 25th, 35th and 45th day of experiment which was found almost identical and the gas content obtained as shown below table.

Table 4.2: Gas composition on 25th, 35th and 45th day

CD:DW	CH_4 (%)	$C0_2$ (%)	O_2(%)	H_2S (ppm)
Day 25				
60:40	22.81	25.68	1.83	0
40:60	37.90	34.45	0	750
50:50	30.47	30.38	0.67	0
Day 35				
60:40	16.53	17.76	5.18	0
40:60	37.78	34.33	0	1036
50:50	30.35	30.24	0	0
Day 45				
60:40	19.02	20.54	3.75	0
40:60	37.69	34.17	0	960
50:50	28.89	29.08	0.29	0

The percentage of average methane was obtained to be highest in 40:60 proportion of cow dung and duckweed ratio. The average methane, carbon dioxide and oxygen were found as shown by figure 4.8.

The average methane (CH_4) was content was found to be in the range 19.45 to 37.79 % which is lower than the mark. The methane content in biogas from semi-continuous biogas production should be between 50 to 60% (Nasir, Ghazi, Omar, & Idris, 2013). The decrease could be because of how the digester is designed. The pipes for the slurry and the substrate feed are too close inside the digester. This causes a short circuit between the substrate feed and the slurry ready to come out. Because of this, the substrate feed doesn't get digested well before it comes out through the slurry pipe.

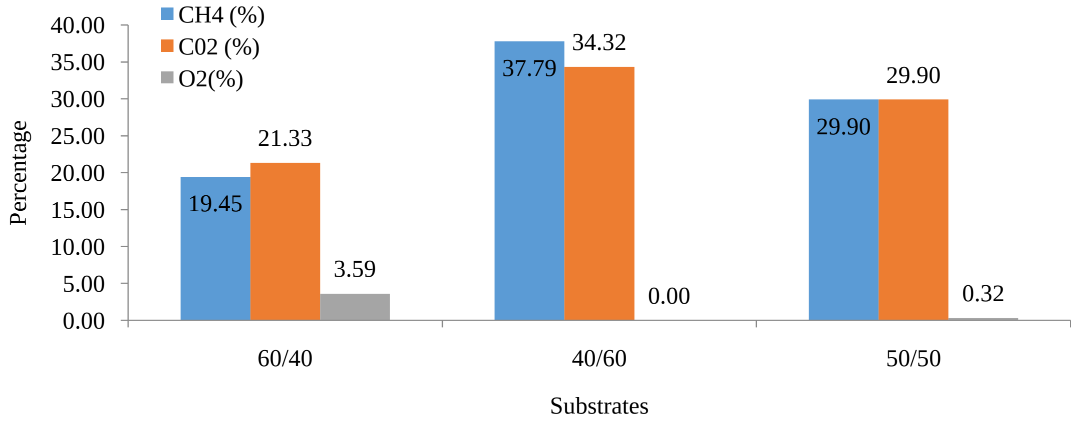

Figure 4.8: Gas compositions

4.11 NPK

NPK content in the slurry was measured to ensure its usefulness to use it as fertilizer. Presence of nitrogen, phosphorus and potassium compounds in the fertilizer helps to promote the growth of plants. The table below shows results was found from the lab test regarding nitrogen, phosphorus and potassium content. Nitrogen (N) content was found to be highest for 60:40 blends of Cow Dung and Duckweed where as lowest for 40:60 i.e. 0.10. Phosphorus (P) was found to be highest for 60:40 and lowest for 40:60. However the value is almost similar for all three blends. Similarly, the value for Potassium (K) was found to be highest and lowest for 60:40 and 40:60. The highest value of nitrogen, phosphorus and potassium in 60:40 ratios of cow dung and duckweed shows its usefulness for fertilizer for the growth of plants. The reason for NPK being highest in the 60:40 may be due to its highest content of cow dung in feed.

Table 4.3: NPK value of slurry obtained

CD : DW	60:40	40:60	50:50
Nitrogen (N), %	0.15	0.10	0.12
Phosphorus (P),mg/kg	49.81	47.97	48.16
Potassium(K), mg/kg	220.99	159.16	164.88

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Following conclusions can be drawn from this study

Biogas production was examined across six different ratios of cow dung and duckweed (100:0, 80:20, 60:40, 40:60, 20:80, 0:100) for batch production. Notably, the ratios of 60:40 and 40:60 exhibited the highest gas production. Along with these highest two, 50:50 mix of cow dung and duckweed was added and analyzed for semi-continuous biogas production. Maintaining a Hydraulic Retention Time (HRT) of 45 days and an Organic Loading Rate (OLR) of 0.31 kg/day, the 50:50 ratios revealed the maximum gas production. Also the methane content was found to be highest in 40:60 blends of cow dung and duckweed. Moreover, pH fluctuations were observed to influence gas yield at various stages of biogas production. During the acidogenesis phase, gas yield was lower as pH declined, while it increased during the methanogenesis phase as pH began to rise. Initial TS for measured for all three ratios of mixture where 50/50 was found to be 10.06 which were found to one of the factor the highest gas production from this mix. Also the NPK of slurry of measured was measured for its usefulness to be used as bio fertilizer and it was found to highest where cow dung was dominant. i.e. 60:40 mix.

5.2 Recommendations

The recommendations of this study are

- The experiment can be extended towards variation on dilution ratio to find the optimum water balance in the digester. i.e 1:3 ratio of substrate to waste dilution ratio is optimum.
- The jar selected for digester can be equipped with stirrer mechanism at the fabrication phase.
- The volatile fatty acid level can be monitored because control of VFA levels is crucial for optimizing biogas yield and maintaining the efficiency of the anaerobic digestion process.
- Digester should be designed to avoid short-circuit and produce high gas yield due to complete digestion.
- Substrate to inoculum ratio can be varied for gas yield i.e 2:1 is the optimum value.

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APPENDIX B: INSIDE AND OUTSIDE TEMPERATURE

Days	Temperature(out)	Temperature(in)
1	24.2	26.8
2	23.8	25.2
3	24.3	25.5
4	25	27
5	27	31
6	25.5	28
7	31.6	37
8	28	32
9	30	37.8
10	31	38
11	24.1	26
12	26.6	28
13	25.2	27.4
14	24.3	26.8
15	27.5	30
16	30.1	34
17	28.3	37.5
18	32.5	38
19	28.5	34
20	31.4	35
21	32.2	36
22	32.3	37
23	30.3	34
24	32.2	37
25	31.3	35
26	29.3	35.3
27	27.1	33.2
28	25.2	29.6
29	27.9	36.3
30	29.4	35.2
31	31.6	33.8

Days	Temperature(out)	Temperature(in)
32	33.5	36.9
33	34.4	38.2
34	32.3	35.3
35	31.2	34.2
36	29.1	32.1
37	30.1	33.5
38	30.3	33.1
39	28.4	32.8
40	32.3	36.8
41	29.5	33.5
42	30.6	35.1
43	30.3	34.4
44	29.4	32.8
45	30.5	33.6

APPENDIX C: EQUIPMENT AND METHOD USED LABORATORY

S.N.	Material of Test	Test Method Specification against which tests are performed	Used Equipment
1.	Solid TS (%)	Laboratory Testing Procedure for soil and water sample analysis, 2009 (Through Moisture Content)	Oven
2.	Solid VS (%)	Guide to Laboratory Establishment for Plant Nutrient Analysis, FAO, 2008	Oven plus Muffle (CE-Optics Technology, Delhi- 110034)
3.	Carbon (%)	Guide to Laboratory Establishment for Plant Nutrient Analysis, FAO, 2008 Chapter 3, 38-39	Oven plus Muffle
4.	Potassium	Laboratory Testing Procedure for soil and water sample analysis, Irrigation Research and Development, Government of Maharashtra, India, 2009 Chapter B II-7, 61-65	Flame Photometer(ESICO-Model-1382)
5.	Phosphorus	Laboratory Testing Procedure for soil and water sample analysis, Irrigation Research and Development, Government of Maharashtra, India, 2009 Chapter B II-6, 56-60	Spectrophotometer (CT-2400)
6.	Total Kjeldahl Nitrogen	4500-N_{org} C. Semi Micro Kjeldahl Method 23rd edition	Kjeldahl (Digestion and Distillation) (KELDLUS-KELVAC-VA)

For sample weighing 4 digit digital balance- (RADWAG- As 202.R2)

APPENDIX D: EQUIPMENTS USED DURING EXPERIMENT

Thermometer

pH meter

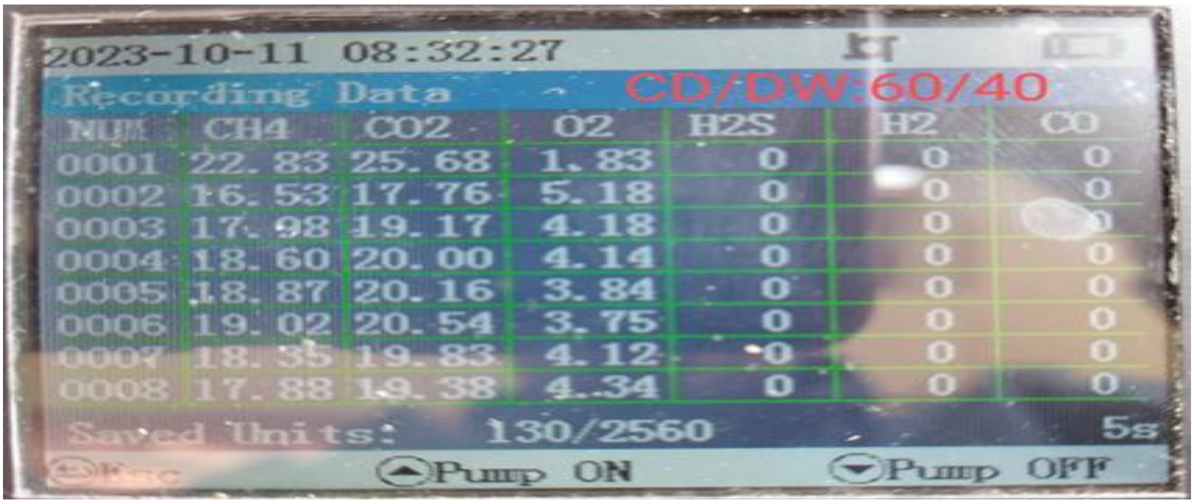
Digital weighing Scale

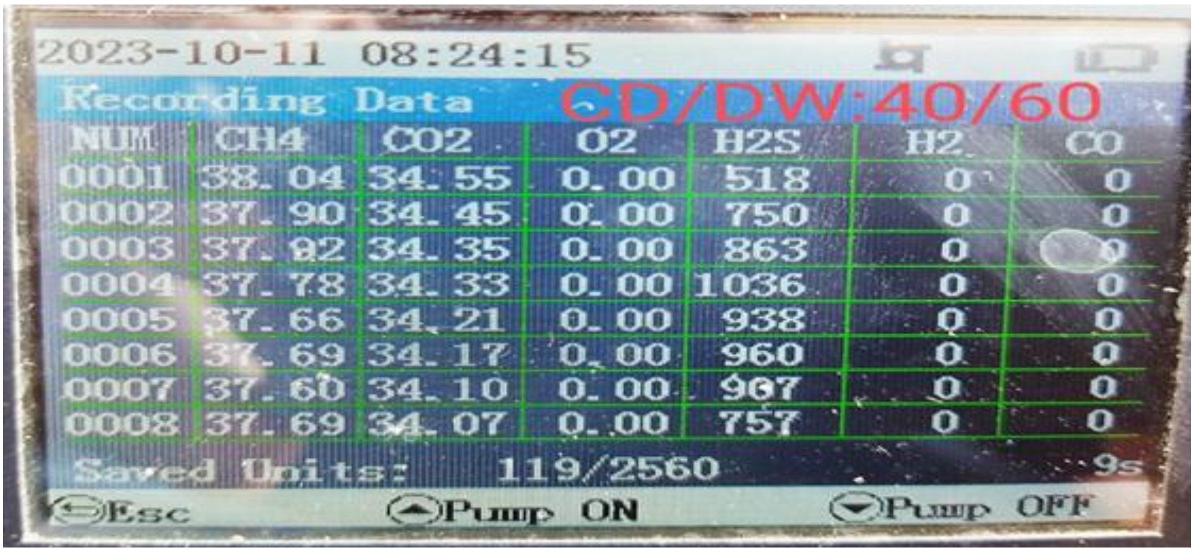
Gas Analyzer

Measuring Cylinder

Iron Stand with Clamp

APPENDIX E: GAS COMPOSITON MEASURED BY GAS ANALYSER





Gas composition for cow dung and duckweed in 60:40, 40:60 and 50:50 mix

APPENDIX F: PHOTOS

Figure: Fabrication of digester

Figure: Collection of duckweed

Figure: Digester Feeding

Figure: pH measurement

Figure: Temperature measurement

Figure: Digesters inside green house

Figure: Weighing of substrate

Figure: Biogas volume measurement

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Date: November 26, 2023

To Whom It May Concern:

This is to certify that the paper titled "***Analysis of Semi-continuous Biogas Generation using Optimal Combination of Duckweed and Cow Dung from Batch-based Production***" (Submission# **609**) submitted by **Pradeep Kumar Yadav** as the first author has been accepted after the peer-review process for presentation in the 14^{th} IOE Graduate Conference being held during Nov 29 to Dec 1, 2023. Kindly note that the publication of the conference proceedings is still underway and hence inclusion of the accepted manuscript in the conference proceedings is contingent upon the author's presence for presentation during the conference and timely response to further edits during the publication process.

Bhim Kumar Dahal, PhD
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Analysis of Semi-continuous Biogas Generation using Optimal Combination of Duckweed and Cow Dung from Batch-based Production

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Abstract
With the ongoing expansion of the global population, the demand for energy continues to rise steadily. Consequently, ensuring energy security has become an imperative concern. Traditionally, biogas production relied solely on cow dung as its primary substrate. However, contemporary research endeavors are now concentrating on the use of cow dung in conjunction with other organic materials. The co-digestion of organic matter can greatly improve the technology's efficiency, leading to optimal biogas production. The growing demand of energy has made the necessity of third generation source of energy. This study is specifically focused on exploring the potential of duckweed as an additive to cow dung for enhancing biogas production. The initial phase of this research involved batch-type biogas experiments where six digesters with cow dung and duckweed in the ratio of 100:null, 80:20, 60:40, 40:60, 20:80 and 0:100. Among these, top two high yielding ratios (60:40 and 40:60) was chosen for semi-continuous production by adding one more blend of 50:50 for comparative purposes. Throughout the study, various parameters influencing biogas production; pH, temperature, gas yield, TS, VS was consistently monitored across the three biogas digesters. Notably, the cumulative gas production recorded on 45^{th} day for 60:40, 40:60 and 50:50 was 8470, 12593 and 15712 ml. Similarly pH was measured in optimum range between 6.9 to 7.3 thought the experiment. The TS removal for 60:40, 40:60 and 50:50 was found to be 83%, 66% and 78% respectively where as VS removal was found to be 9%, 3% and 6% consecutively. The methane content was found to be maximum for the 40:60 mix i.e 38.04 %. The NPK measurement of slurry obtained from different blend was conducted for its usefulness as organic fertilizer. Nitrogen, Phosphorus and Potassium content was found be maximum in 60:40 ratio i.e. 0.15%, 49.81% and 220.99 mg/kg and lowest in 40:60 mix i.e 0.12%, 47.97 mg/kg and 159 mg/kg respectively. This indicates that slurry from a higher proportion of cow dung in co-digestion is a more suitable fertilizer for plant growth.

Keywords
co-digestion; duckweed; semi-continuous biogas generation; cowdung

1. Introduction

Biogas production using anaerobic digestion (AD) has become a preferred technology for renewable energy generation. AD enables the production of biogas, a sustainable fuel source [1]. Duckweed grow naturally in freshwater surfaces and can serve as a biomass source for biogas generation. Duckweed, a rapidly proliferating aquatic plant, poses no competition with humans for food and land [2]. Duckweed is appropriate organic matter to be blended with cow dung to produce biogas. There is greater abundance of biodegradable material within duckweed compared to cattle manure, providing a richer source of energy for microbial activity throughout the entire digestion process [3]. The organic matter goes through four stages for biogas production: Hydrolysis, Acidogenesis, Acetogenesis and Methnogenesis [4]. Overreliance on fossil fuels has caused climate change, environmental harm, and health issues. About 80% of global energy comes from fossil fuels.In places like Ethiopia, rural communities rely on harmful traditional fuels like firewood. Population growth and climate change have intensified the search for renewable energy sources by researchers and organizations. Duckweed, a tiny aquatic plant, has vast biogas potential and can thrive in nutrient-rich waters, including wastewater, at a low cost, providing an affordable feedstock [3]. Duckweed exhibits exceptional growth rates, often doubling in size within 2 to 3 days under optimal conditions. Duckweed biomass is ideal for biogas production due to its low lignin content, which makes microbial degradation easier. The leftover liquid waste retains valuable nutrients and can be used as fertilizer for growing more duckweed. Methane production from duckweed is higher that of grass and water spinach [5]. Biogas was generated through both continuous and batch processes at temperatures of 30°C, 35°C, and 37°C. It was determined that the highest biogas yield and the greatest methane composition were attained at 37°C [6]. To optimize the digestion rate, the pH level should be maintained between 5.5 and 8.5, while the temperature remains within the range of 30 to 60 degrees Celsius [7].

2. Literature Review

While co-digestion enhances anaerobic digestion efficiency, leading to increased biogas production and improved biodegradability of organic matter, practical applications in household and commercial biogas plants in Nepal still predominantly rely on the mono-digestion of cow dung. This study aims to emphasize the significance of co-digestion, specifically focusing on the potential benefits of utilizing high-nutrient-containing, easily cultivable duckweed plants as a biodegradable substrate.

The co-digestion of organic matter can significantly enhance the efficiency of the technology, resulting in optimal biogas production. In a study conducted by [8], it was discovered that treating mechanically and fermenting chicken manure with maize silage resulted in a 27% increase in methane production compared to using chicken manure alone. Additionally, the same study observed a 26.5% increase in methane production through the co-digestion of horse manure waste with corn stover [8]. In a separate study conducted by [9] Human Waste (HW) and Canteen Food Waste (CFW) were employed as individual substrates.The results showed that the volume of biogas produced was 58,900 L for HW and 36,300 L for CFW when used independently. However, when HW and CFW were co-digested together, the yield increased to 22,600 L.This research highlights the significance of co-digestion in optimizing biogas production [9].

A study conducted by [5] used duckweed and cow dung in four different proportions 0:100, 90:10, 75:25 and 50:50 with cumulative gas production 11620, 305, 11695 and 12070 ml respectively on 35th day showing 50:50 to be highest potential for gas production, here the increment ratio of duckweed (DW) in the mixture from 25% to 75%, found to be declining the cumulative biogas yield. Another research carried out by [3] where duckweed and cow dung was used in five different proportions 100:0, 75:25, 50:50, 25:75, 0:100 and found to the cumulative bio gas production to be 1015.5, 1040, 1159, 1206, and 862, respectively which revealed attractive potential of duckweed as feedstock when mixed in 25% of duckweed and 75% of cow manure.

The carbon-to-nitrogen (C/N) ratio is a crucial factor influencing bacterial growth and methane production. The ideal C/N ratio falls within the scientifically recommended range of 20-30:1 [3]. Inadequate nitrogen can hinder bacterial growth, while excessive nitrogen, surpassing 40, tends to be insufficient, limiting the growth of microbial communities and diminishing their overall capacity [10].

There is a correlation between temperature and both biogas and methane production, with elevated temperatures resulting in increased yields. Notably, the digester operated at 35 °C exhibited the highest biogas production rate and methane content. Throughout the study, fermenters maintained at 35 °C showcased the most substantial biogas production (10377 ml), achieving the highest methane yield of 64.47%. In contrast, thermophilic reactors at 50 °C exhibited lower biogas and methane generation, followed by room temperature reactors that demonstrated even lesser production levels [1].

Production of biogas also depends on dilution ratio. Substrate was diluted in different ratios ranging from 1:1 to 1:4 of mass of cow dung and mass of water. The experiment showed that as the dilution increased the biogas production per kg of cow dung increased. The biogas yield for 1:3 dilution increases by 30% than those of 1:1 dilution ratio. The biogas volume per gram of Vs was 227,265,273,270 and 277 ml for 1:1, 1:2.5, 1:3, 1:3.5 and 1:4 FD (Feed Dilution) [11].

Amount of biogas produced from the organic substrate has influence of OLR (Organic Loading Rate). Specifically, it is observed that the biogas yield increased as the OLR was raised up to a certain point and then began to decrease. Thus the biogas yield with increasing OLR produced after that then shows inverse result. The OLR value with 1.5 kg/m^3, 2 kg/m^3, 2.5 kg/m^3, 3 kg/m^3 showed cumulative biogas yield of 0.255 kg, 0.200 kg, 0.150 kg and 0.100 kg at the end of 14^{th} , 30^{th}, 45^{th} and 60^{th} day which indicated increase in gas yield up to 1.5 kg/m^3 and then decline above this ([12]. Similarly another study conducted by [13] shows biogas production generated through continuous digestion and cultivated seaweeds with dairy slurry. The OLR was varied from 4 $kg.VS^{-1}.m^{-3}.d^{-1}$ to 5 $kg.VS^{-1}.m^{-3}.d^{-1}$ for two different species of Seaweed and found optimum conditions of mono-digestion both species as 4 $kg.VS^{-1}.m^{-3}.d^{-1}$. Similarly, another research showed production of biogas also depends on dilution ratio. Substrate was diluted in different ratios ranging from 1:1 to 1:4 of mass of cow dung and mass of water. The experiment showed that as the dilution increased the biogas production per kg of cow dung increased. The biogas yield for 1:3 dilution increases by 30% than those of 1:1 dilution ratio. The biogas volume per gram of Vs was 227, 265, 273, 270 and 277 ml for 1:1, 1:2.5, 1:3, 1:3.5 and 1:4 FD (Feed Dilution) [11].

3. Materials and Methods:

3.1 Methodology

This research continues the study of biogas production from duckweed and cow dung which was carried out batch model. Six different jars were used as biogas digesters, each containing varying proportions of duckweed (DW) and cow dung (CD) including 100% CD, 80% CD + 20% DW, 60% CD + 40% DW, 40% CD + 60% DW, 20% CD + 80% DW, and 100% DW These digesters yielded different results. Among them, the two digesters i.e 60% CD + 40% DW, 40% CD + 60% DW showed the highest biogas yield in batch model were selected for further experiment in semi-continuous model. Additionally, another model was introduced with a 50:50 ratio of duckweed and cow dung. Finally three different substrate i.e. 60% CD + 40% DW, 40% CD + 60% DW and 50% CD + 50% DW was carried out using semi-continuous process. Further design of digesters and equipment used are discussed below.

3.2 Materials

In this study, plastic drinking water jar is used as bio-digester for the production of biogas which has a capacity of 20 liters, 1 inch CPVC pipe, 1.5 inches CPVC pipe, 1 inch pipe, angle elbows, hose, CPVC solvents, cutting tool, stand holder and measuring cylinder Various materials were utilized in both the construction and biogas production phases. These included steel pipes of different sizes (1.5 inches and 1 inch), a weighing machine, thermometer, pH meter, clamps, gum, paper tape, screwdrivers, hand gloves, distilled water, and jar covers. The primary raw materials employed for biogas production in the bio-digester were cow dung, duckweed, inoculum, and water. The nitrogen content of the slurry was calculated using Kjeldahl (Digestion and Distillation) (KELDLUS-KELVAC-VA similarly, The phosphorus was measured by Spectrophotometer (CT-2400) and Potassium was measured using Flame Photometer (ESICO-Model-1382). These measurements were carried out on soil and water testing lab.

3.3 Design Consideration

The requirement of designing of a Bio-digester is volume of digester, arrangement for slurry feed and digestate outlet, retention period and amount of organic matter (substrate) to feed on daily basis. Size of biogas is calculated by the relation: Capacity of digester (kg) = Daily feed-in (kg/day) * Retention time (day)

Figure 1: Three digesters setup with different substrate mix

3.4 Volume design of the Bio-digester

Total volume of digester's feed per day is given as Total daily feeding volume = 0.31 kg/day From the above equation Digester volume = Volume of digester/day × Retention period (day) = 0.31×45 = 13.95 ∼ 14 kg. Also the volume inside the jar for holding gas is left, i.e, Three-tenth of total volume which is equivalent to 6 L space. Volume of gas holding space inside digester = 3/10×20 liter = 6 liters
Total volume of digester = digester's volume occupied by substrate + volume of gas holding space = 14 liters + 6 liters = 20 liters.

3.5 Substrate Collection

Duckweed required for this study was cultivated inside the Pulchowk Campus making a pond beside football playground and cow dung was collected from a cow farm nearby village of Nakhhu, Lalitpur. The duckweed was washed with clean water and dried for few hours to ensure removal of extra water content. Other important component inoculums were collected from the existing biogas plant situated in the Pulchowk Campus. In the course of collection of cow dung, duckweed and inoculums, necessary health precaution was taken was taken by using hand gloves and mask.

3.6 Feeding of Digester

As the experimental set up was done using semi-continuous model, feeding mode was made at regular interval of time. Loading the digester once was done to two-third i.e. 14 liters and one-third were left empty for gas accumulation.The digester was then fed with calculated amount of co-digestate and regular expel of slurry outlet. The daily feed rate is determined using the equation used above.Three jars were taken as digester for the co-digestion of duckweed and cow dung in the ratios 60:40, 40:60 and 50:50.The initial feeding was done as per the following figure: Initially, digester was fed by the fermentable

Batch	Cow Dung (a)	Duckweed (b)	Water (c)	Inoculums	Total mass a+b+c
B1 (60% CD + 40% DW)	3.78	2.52	7	0.7	14
B2 (40% CD + 60% DW)	2.52	3.78	7	0.7	14
B3 (50% CD +50% DW)	3.15	3.15	7	0.7	14
Total	9.45	9.45	21	2.1	42

Figure 2: Initial feed quantity in kg

material (Cow dung, duckweed, water and inoculum) as per Figure 3. The initial parameters of fed substrate were recorded as shown in Figure 4. Daily feeding was considered with the design criteria in accordance of retention period of 45 days. It is calculated by diving the initial feeding by the hydraulic retention time.The daily feeding has been calculated using the equation below Organic Loading Rate(OLR)= (Total capacity of the digester)/(No.of hydraulic retention days)
= 14/45 = 0.31 kg/day

Organic loading rate is the daily feed of substrate fed into the digester in regular basis.It was calculated as per the mix ration where water was made 50% of the remaining mix i.e. mixture of cow dung and duckweed to maintain the proper TS.The figure stated 2 shows the amount of substrate used to feed daily into the digester. The initial parameters which are crucial for the biogas

Cow dung and duckweed ratio	Cow dung	Duckweed	Water	Total
60:40	0.062	0.093	0.155	0.31
40:60	0.093	0.062	0.155	0.31
50:50	0.0775	0.0775	0.155	0.31

Figure 3: Daily feeding rate of substrate in kg

production and its evaluation were recorded as figure 4. The value of temperature, pH, total solid, volatile solid and carbon to nitrogen ration was measured below.

3.7 Gas Measurement

The water displacement method, employed for collecting gas, operates by enabling gas to displace an equivalent volume of water The water filled in a rectangular tough and measuring cylinder was filled with water was made upright mouth down into tough. The gas pipe from the digester was inserted into the measuring cylinder from downward position of measuring cylinder. Gas valve fitted at the mouth of digester was loosen to let the gas into the measuring cylinder there by replacing the water and gas volume. Gas measured with the help of granulated scale on cylinder.This method was utilized to measure the

Initial parameters	CD:DW =60:40	CD:DW =40:60	CD:DW =50:50
Initial temperature	26	26	26
Initial pH	6.9	7.0	6.8
TS(% dry basis)	14.61	6.51	10.06
VS (% of TS)	76.43	73.09	79.12
C/N Ratio	30:1	32:1	31:3

Figure 4: Initial parameters of the substrate

quantity of gas generated on every four-day.

4. Results and Discussion

The experimental setup was prepared for the co-digestion of duckweed and cow dung in three different jars with varying proportions and different parameters such as pH change, temperature, gas yield was monitored regularly. Data was collected using appropriate instruments and interpretation and analysis of obtained data has been done with the help of charts, graphs and tables. The table below presents the volume of biogas production in ml using varying proportions of cow dung and duckweed mix, specifically 60:40, 40:60 and 50:50 over consecutive days.

4.1 Experimental results

The gas production in the different digesters having varying ratio of cow dung and duckweed was measured in every 4 days using water displacement method and result was obtained in stated below in figure 5. From the figure 5, it is seen that the gas

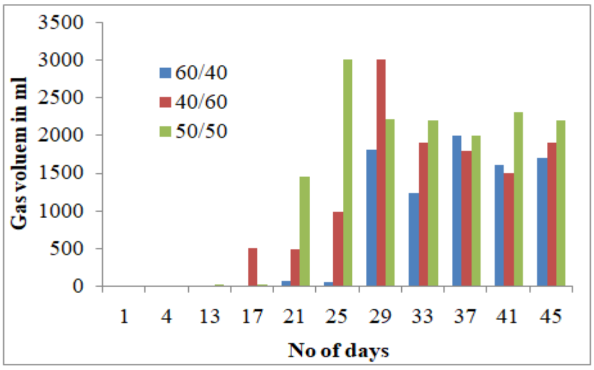

Figure 5: volume of gas yield and temperature in a regular interval (ml)

formation start begins from the thirteenth day of feeding and daily fed was continued up to forty five days and gas volume was also measured accordingly which is shown in the figure. Based on these findings, it is evident that the digester employing a 50:50 ratio of cow dung and duckweed yields the highest gas production, while the digester with having 40:60 ratio produces the lowest gas volume. This outcome aligns with a study conducted by[5], which emphasized that an equal proportion of duckweed and cow manure mix as substrate demonstrates a greater potential for biogas generation. This is attributed to the enriched nutrient content of duckweed, particularly nitrogen and phosphorus, which, when combined with cow dung for co-digestion, significantly enhances the efficiency of anaerobic digestion.

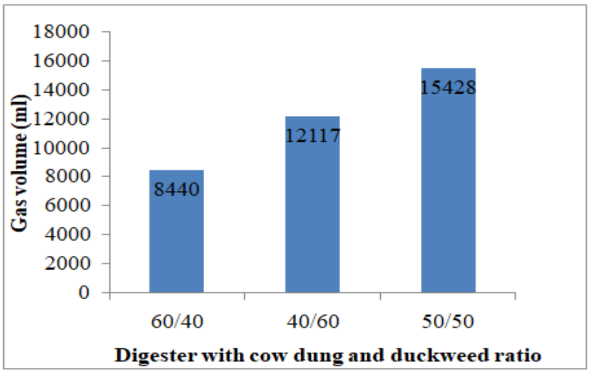

Figure 6: Cumulative gas production

The figure 6 shows the cumulative production of biogas from different digesters. The first one i.e cow dung and duckweed ration of 60:40 has least gas production i.e. 8470 ml in 45 days where as 50:50 has highest gas production i.e. 15715 ml.The result resemble with the result with findings of [5] which shows highest gas production of biogas form the blend of 50:50 duckweed and cow dung. The equal proportion co-digestion of duckweed with cow dung emerged as the optimal blend, fostering enhanced microbial activity and maximizing biogas production.

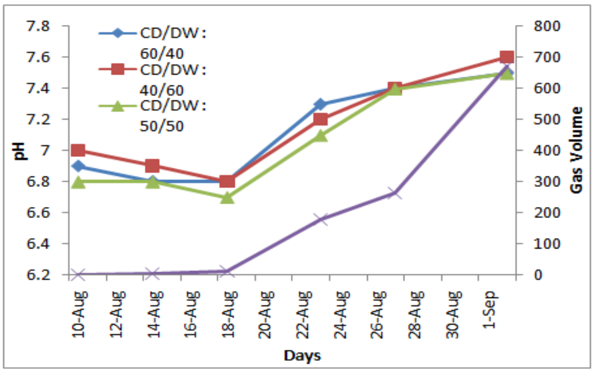

Figure 7: pH fluctuations of substrate with time

The figure 7 illustrates a declining pH during 18th August, indicating the progression into the biochemical stages of acidogenesis. During this phase, amino acids, sugars, and fatty acids undergo transformations into intermediate products like Propionate, Butyrate, Lactate, Ethanol, and more. As the pH subsequently increases, it signifies the entry into methanogenesis phase, during which these intermediate products are converted into methane and carbon dioxide. The pH remained in the range of 6.7 to 7.3 during the experiments which are appropriate for biogas production. A study conducted by [4] found the suitable pH range for production of biogas to be 7.2-7.4. However, as pH of 7.3 is the highest gas producing potential. C/N ratio is one of important that affects production of biogas during anaerobic digestion. The initial C/N ratio was found to be in 30:1, 32:1 and 31:3 for three different substrates.

Very high C/N may cause low gas production due to nitrogen degradation by microbes.A study conducted by [14] for biogas production from corn stalks and chicken manure which concluded C/N below 18 is favorable for best gas production from stated organic matter [14]. The temperature in which bio digester is maintained has the significant impact on biogas production.The suitable temperature for highest biogas production is said to be 35^0C [1]. During the experiment carried out for 45 days, the temperature was between 28^0C to 39^0C. In cold climate and night the temperature was maintained using green house from white transparent plastic of thickness 90 GSM.

4.2 TS and VS

Total solid and volatile solid content determines the behavior of the microbial activities involved in anaerobic digestion of organic matter. Total solid was measured using oven and Muffle (CE-Optics Technology, Delhi- 110034). Biogas yield for the substrate having 50:50 biogas productions is highest having total solid 10.06 where as lowest in 60:40 (CD:DW) ratio with TS 14.61 with agree with the research carried out by [15]. Total solid removal is highest in 60:40 i.e.83% where as lowest in 40:60 i.e 66%. Also a study by [12] shows the total solid of 10.16 % produced highest gas production which agrees with this experimental i.e. Substrate of 50:50 having TS of 10.06 highest gas yields. "It has been noted that the 50:50 mixture, specifically the initial volatile solids (VS) is 79.12% of total solids (TS) which is the highest among the three different substrates, produces the highest gas yield. These findings align with the research conducted by [12], indicating that gas production increases as the percentage of volatile solids rises." Removal of

CD:DW	Initial TS	Final TS	TS removal	Initial VS	Final VS	VS removal
60:40	14.61%	2.43%	83%	76.43%	73.09%	9%
40:60	6.51%	2.23%	66%	70.19%	69.36%	3%
50:50	10.06%	2.26%	78%	79.12%	70.19%	6%

Figure 8: Experimental results for TS and VS of different substrate

total solid denotes the conversion of organic matter into biogas during the anaerobic digestion process.The experiment showed the highest TS removal in case of 60:40 blend of cow dung and duckweed and lowest in 40:60 which indicates the highest gas production should in 60:40 Similarly the volatile solid removal was also found to be highest in 60:40 blends and lowest in 40:60 which again shows there should be highest gas production in 60:40 rather than 50:50 blends. There is also other factor for gas such as pH, temperature,C/N ratio.

4.3 Gas Composition Evaluation

The content of biogas produced in three digester was measured using gas analyzer (GASBOARD 3200 Plus) and composition found in each type of digester was illustrated below. The gas analyzer measured concentration of methane, carbon dioxide and oxygen among which methane is the main constituent required for combustion of gas. It is found that highest methane content is present in the mix (CD: DW) of 40:60 proportion of cow dung and duckweed where as lowest was found in 60/40 mix model. The probable reason for high methane content may be the CN ratio of the substrate i.e the CN ratio of 40:60 was maximum among three digesters i.e. 32.1. The figure 9 shows the content of methane, carbon dioxide and oxygen in the digesters.

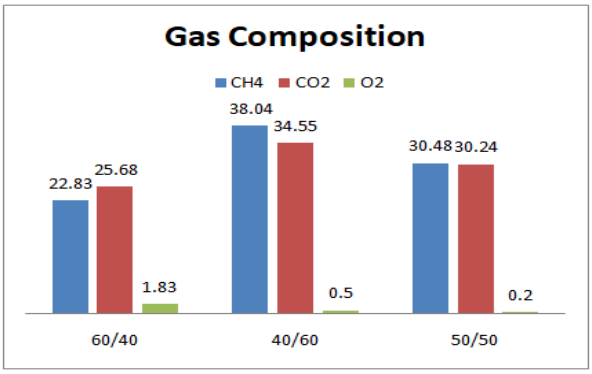

Figure 9: Gas Composition in different digesters

4.4 NPK

NPK content in the slurry was measured to ensure its usefulness to use it as fertilizer. Presence of nitrogen, phosphorus and potassium compounds in the fertilizer helps to promote the growth of plants. Fertilizer with higher NPK content increases the concentrations of essential nutrients, supporting robust and healthy plant growth. The NPK measurement of slurry obtained from different blend was conducted for its usefulness as organic fertilizer in lab. Nitrogen, Phosphorus and Potassium content was found be maximum in 60:40 ratio i.e. 0.15%, 49.81% and 220.99 mg/kg respectively and lowest in 40:60 mix i.e 0.12%, 47.97 mg/kg and 159 mg/kg. Similarly, the value for Potassium (K) was found to be highest and lowest for 60:40 and 40:60 which concludes highest potential of slurry out of 60:40 mixes to use as fertilizer. It has also provided an insight that high value of NPK is found in the mix with dominant cow dung. Figure 10 shows values of NPK on slurry of each mix of cow dung and duckweed.

5. Conclusion

Biogas production was examined across six different ratios of cow dung and duckweed (100:null, 80:20, 60:40, 40:60, 20:80, 0:100) for batch production. Notably, the ratios of 60:40 and 40:60 exhibited the highest gas production. These two ratios were chosen, alongside a 50:50 ratio of cow dung and duckweed, for semi-continuous biogas production. Maintaining a Hydraulic Retention Time (HRT) of 45 days and an Organic Loading Rate (OLR) of 0.31 kg/day, the 50:50 ratios revealed the maximum gas production. Also the methane content was found to be highest in 40:60 blend of cow dung and duckweed. Moreover,

CD: DW	Nitrogen (%)	Phosphorus (mg/kg)	Potassium (mg/kg)
60:40	0.15	49.81	220.99
40:60	0.10	47.97	159.16
50:50	0.12	48.16	164.88

Figure 10: NPK value of slurry obtained

pH fluctuations were observed to influence gas yield at various stages of biogas production. During the acidogenesis phase, gas yield was lower as pH declined, while it increased during the methanogenesis phase as pH began to rise. Initial TS for measured for all three ratios of mixture where 50/50 was found to be 10.06 which were found to one of the factor the highest gas production from this mix. Also the NPK of slurry of measured was measured for its usefulness to be used as bio fertilizer and it was found to highest where cow dung was dominant in the mix.

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