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Co-digestion of Duckweed with Cow dung for Biogas 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 **"Co-digestion of Duckweed with Cow Dung for Biogas Production"** submitted by Bibek Shakya (078MSREE006), in partial fulfillment of the requirements for the degree of Master of Science in Renewable Energy Engineering.

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ABSTRACT

This thesis explores the synergistic potential of co-digesting cow dung and duckweed as a novel approach for biogas production. The increasing demand for renewable energy sources necessitates innovative solutions to enhance biogas yield and sustainability. The study investigates the combined digestion of cow dung and duckweed, evaluating their biogas production capabilities. Through a comprehensive analysis of process parameters such as feedstock ratios, hydraulic retention times, temperature, pH levels, total solids, volatile solids and removal efficiencies, this research aims to elucidate the optimal conditions for maximizing biogas production from the co-digestion process.

The experiment conducted in this thesis concluded that after 60 days of observation involving batch production from four different mixture ratios and two controls it was found that the highest daily average and cumulative biogas yield were recorded for the mixture of 60% cow dung and 40% duckweed. These values were respectively 1.94 L/day and 116.64 L. This was followed up by the mixture of 40% cow dung and 60% duckweed whose respective values for daily average and cumulative biogas yield for the same time period were 0.95 L/day and 56.79 L. Also, the highest methane composition was observed for 100% duckweed batch which was 45.36%.

The findings of this study contribute to the understanding of co-digestion dynamics and provide valuable insights for the development of efficient and environmentally friendly biogas production systems. As biogas continues to gain prominence as a clean energy source, the outcomes of this research have implications for sustainable waste management and energy generation practices, fostering a more resilient and eco-conscious future.

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LIST OF ABBREVIATIONS

AD	Anaerobic Digestion
AEPC	Alternative Energy Promotion Centre
BOD	Biological Oxygen Demand
BSP	Biogas Support Program
CBS	Central Bureau of Statistics
CD	Cow Dung
C/N	Carbon/Nitrogen
COD	Chemical Oxygen Demand
CAGR	Cumulative Annual Growth Rate
DW	Duckweed
F/I	Feed-to-inoculum
FY	Fiscal Year
GHG	Green House Gas
GoN	Government of Nepal
HRT	Hydraulic Retention Time
KfW	German Development Bank
LPG	Liquified Petroleum Gas
MTOE	Million Tons of Oil Equivalent
MoF	Ministry of Finance
NPK	Nitrogen Phosphorous Pottasium
NREP	National Rural Energy Programme
OC	Organic Carbon
RETs	Renewable Energy Technologies

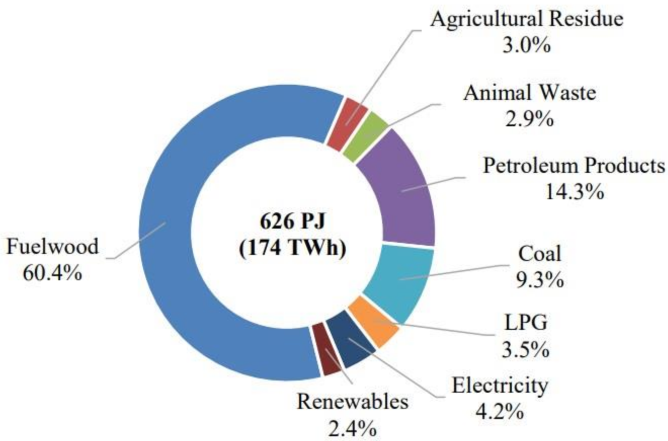
SWAT	Soil, Water and Air Testing
TOD	Time of Day
TKN	Total Kjeldahl Nitrogen
TS	Total Solids
VFAs	Volatile Fatty Acids
VS	Volatile Solids
WB	World Bank
WECS	Water and Energy Commission Secretariat

CHAPTER ONE: INTRODUCTION

1.1 Background of the Study

1.1.1 Energy scenario of Nepal

Nepal's primary energy consumption is predominantly driven by biomass, a non-commercial form of energy. Traditional sources such as fuelwood, agricultural residues, and animal waste continue to dominate the energy landscape. Nevertheless, there is a noticeable transition towards commercial energy sources like coal, petroleum products, and electricity, with a growing emphasis on renewable energy sources. The recent surge in electricity consumption is also noteworthy. The growth rate of energy consumption over the last decade is 4% (MoF, 2021). In 2018, the country's energy consumption pattern indicated a primary energy consumption of nearly 14 Million Tons of Oil Equivalent (MTOE). Projections anticipate a steady growth with a Cumulative Annual Growth Rate (CAGR) of 3.8%, leading to an estimated 22 MTOE by the year 2030 (WECS, 2022).



(WECS, 2022)

Figure 1.1 Energy Consumption by Fuel Types in 2021

In Nepal's residential sector, primary energy sources include fuelwood, agricultural residue, animal waste, biogas, and various forms of biomass. Urban residential areas, primarily for cooking and lighting, see a shift towards hydro and solar energy as alternatives to traditional sources. In the industrial sector, major energy sources comprise coal, fuelwood, diesel, and electricity.

As per the 2013 National Survey of Energy Consumption and Supply Situation in Nepal, the commercial sector relies on fuelwood, LPG, coal, and grid electricity as significant fuel sources. Non-renewable energy consumption in the commercial sector constitutes 34% of the overall energy consumption (WECS, 2013). The National Rural Energy Programme (NREP) has established a comprehensive framework for implementation within local communities throughout the country. Aligned with this policy, Nepal aims to augment its energy mix by emphasizing renewable sources, with a targeted increase of 20%. This involves diversifying the energy consumption pattern, extending its reach to encompass various industrial and commercial sectors (WECS, 2022).

1.1.2 Biogas in Nepal

According to the Waste Management baseline study conducted by the Central Bureau of Statistics (CBS) in 2020, each municipality, on average, generated approximately 1.2 kilo tons of organic waste. If the organic fraction of municipal solid waste (OFMSW) in Kathmandu is utilized, it can generate nearly 140,000 m^3 of biogas. This biogas production has the potential to fill up around 21,000 Liquefied Petroleum Gas (LPG) cylinders daily, resulting in significant savings amounting to hundreds of millions rupees (WECS, 2022). Being introduced for the first time in 1955 in Nepal, the Government of Nepal (GoN) officially launched the biogas program in 1975. Following the launch of the Biogas Support Program (BSP) with help from the Dutch government in 1992, this program gained additional traction in the nation. With the major aim of promoting renewable energy technologies (RETs), lifting the rural residents' living standards by providing them with clean, sustainable energy, and contributing to eradication of human activities leading to environmental deterioration, a wholesome & dedicated organization called Alternative Energy Promotion Center (AEPC) was established in the year 1966. With the assistance of the GoN, the German Development Bank (KfW), and the World Bank (WB), AEPC is implementing BSP.

Despite being a well-established technology, the full potential of biogas utilization has yet to be realized. The limited capacity of biogas to meet household energy demands, especially in cold climates, poses a significant barrier to widespread adoption. Referred to colloquially as "Gobar gas," indicating "gas from cattle dung," the majority of household biogas systems in Nepal rely on cattle dung. The primary socioeconomic factors influencing biogas usage in Nepal are income levels and the extent of landholdings. In the hilly regions of the country, households encounter challenges in implementing biogas technology, highlighting the complexities associated with installing and operating such systems in mountainous terrain. The scarcity of biogas installation and maintenance service providers, coupled with a lack of accessible banking facilities, serves as a substantial hindrance to the widespread adoption of biogas in Nepal.

1.1.3 Biogas production process

Biogas production from renewable sources is a promising approach when it comes to providing clean energy and reducing GHG emissions. The use of organic waste materials for biogas production has gained significant attention due to their potential as renewable energy sources. Anaerobic digestion of organic waste materials is one of the most widely used methods for biogas production. Anaerobic digestion (AD) is a biological process that is widely used for the treatment of organic wastes to produce biogas, which is a valuable source of renewable energy. Biogas typically contains methane (CH_4) and carbon dioxide (CO_2), and its composition and yield depend on the feedstock used in the AD process. AD is completed in three steps, each of which is described briefly as follows: -

1. Hydrolysis:

This is the stage where slurry is made by dilution of the organic material in water. The intricate polymers present in these types of organic materials, such as cellulose and hemicellulose need to be broken down into simpler compounds to be accessible to the microorganisms. This is where the water and enzymes produced by the microbes play a crucial role. In the presence of hydrolytic fermentative bacteria (mostly anaerobes), these complex polymers are hydrolysed into simpler compounds like organic acids (usually highly volatile) and alcohols.

As an example, cellulose which is basically a polymer, undergoes degradation, first in to dimers & then into monomers where, the monomer here is glucose ($C_6H_{12}O_6$). The enzyme ‘cellulase’, plays a crucial role in this process. Among the most prevalent anaerobic cellulose fermenters in natural environments belong to the Clostridium genus & these micro-organisms are frequently found in a variety of environments, such as manure, compost, and soil.

2. Acidogenesis:

Here, first of all acetic acid (CH_3COOH) is obtained from the previously formed volatile acids. Another category of bacteria exist which aid in transforming uncomplicated organic materials through redox reactions. This process results in the production of acetate ($C_2H_3O_2^-$), Hydrogen (H_2), and CO_2 all of which collectively act as nutrients for the methanogens. There are two processes that occur, namely acetogenic dehydrogenation and acetogenic hydrogenation. Both of these processes involve different groups of acetogenic bacteria. In the former, obligate hydrogen-producing acetogenic bacteria convert fatty acids into $C_2H_3O_2^-$, H_2, and CO_2 while in the latter, $C_2H_3O_2^-$ and other acids are produced (Karki, 2005).

3. Methanogenesis or Methanisation:

Finally, $C_2H_3O_2^-$, H_2 & CO_2 are converted by methanogens into CH_4, CO_2, H_2O. The primary acids produced in the previous stage are processed by methanogens to produce CH_4. Majority of the methane production comes from acetic acid. The reaction that leads to the formation of methane is called methanisation, which occurs as represented by the following equations:

$$\underset{\text{(Acetic acid)}}{CH_3COOH} \longrightarrow \underset{\text{(Methane)}}{CH_4} + \underset{\text{(Carbon dioxide)}}{CO_2}$$

$$2CH_3CH_2OH + CO_2 \longrightarrow CH_4 + CH_3COOH$$

(Ethanol) (Carbon dioxide) (Methane) (Acetic acid) *

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$$

(Carbon dioxide) (Hydrogen) (Methane) (Water)

1.2 Statement of the problem

Among the various types of organic wastes, cow dung is the most commonly used feedstock for biogas production due to its high organic content and availability. Duckweed, on the other hand, is a rapidly growing aquatic plant that has a high protein and carbohydrate content coupled with a low lignin content, which makes it a suitable substrate for AD. Additionally, various studies have shown that the use of duckweed as a co-substrate can enhance the overall biogas yield, improve the nutrient balance, and reduce the hydraulic retention time (HRT) of the process, thus further improving the efficiency and sustainability of the biogas production process. Furthermore, duckweed is abundant and can be grown easily, even in wastewater or nutrient-rich environments, which makes it a sustainable feedstock for biogas production. Duckweed biomass is easy to harvest and handle too.

Thus one of the major problems that this thesis aims to address is the problem of feedstock scarcity dilemma. This is more relevant in the present context of Nepal as people are leaving abroad in huge numbers for employment or other purposes leaving behind a dearth of cattle and consequently cattle dung. Approximately 500,000-600,000 Nepalis migrate annually to join the international labor market (Ministry of Labour, 2022). The rural household have been the frontrunners when it comes to rearing cattle and since the rural population has been largely affected by the current brain-drain scenario, the overall population of cattle has been greatly affected too. The total number of cattle in the country for the FY 2020/21 was 7,466,841 and it was 7,413,197 for FY 2021/22 which indicates a drop by 0.72% (MOALD, 2023). If this trend continues, there is a high chance of an impending cow dung deficiency in the near future. Thus the overdependence on cow dung as feedstock for biogas production in the communities and the decreasing availability of cow dung due to rapid urbanization and brain-drain in the country is one issue that calls for the need of co-digestion and/or the use of an alternative feedstock altogether. This is where duckweed comes in, as it is not only an ideal alternative to cow dung but also proves to be an ideal co-substrate for cow dung.

The problem addressed in this research is also the lack of information on the optimization of biogas production using a mixture of cow dung and duckweed as a feedstock. Previous studies have shown that the ratio of feedstock used in AD has a significant impact on the biogas yield and composition. The collaboration in co-digestion boosts methane yield by fostering positive synergies within the digestion medium, leveraging bacterial diversities across various wastes, and supplying essential nutrients through the co-substrates. The co-digestion process offers a solution to the challenge of feedstock scarcity. Moreover, incorporating supplementary biomass not only increases biogas yield but also makes biogas plants economically feasible (Li, Jha, & Bajracharya, 2014). Although biogas production from cow dung and other substrates has been extensively studied, there is limited information on the use of duckweed as a co-substrate in the AD process. Furthermore, there is no consensus on the optimal mixing ratio of cow dung and duckweed for biogas production. Therefore, a comparative analysis of the biogas yield and composition from varying ratios of duckweed and cow dung in AD is necessary to optimize the process and evaluate the potential of using duckweed as a co-substrate.

This problem is significant as the demand for renewable energy is increasing due to the depletion of fossil fuels and the need to reduce GHG emissions. Biogas production from cow dung and duckweed has the potential to not only address the energy crisis but also reduce the environmental impact of waste disposal. The findings of this study will provide valuable information for researchers, policymakers, and practitioners in the field of biogas production and waste management. Therefore, this study will contribute to the development of a sustainable and efficient biogas production process using duckweed and cow dung mixture as a feedstock that can address both energy and environmental challenges.

1.3 Objective of the study

The main objective of this study is to determine the optimum mixing ratio of cow dung & duckweed that gives maximum biogas yield.

The specific objectives of this study are

- To mix duckweed and cow dung in six different proportions and test their parameters
- To analyze the biogas yield & composition from these six mixtures

1.4 Limitations

- The availability of duckweed and cow dung may vary depending on the season and location, which may affect the continuity of the experiment.
- The study will not consider the environmental impacts of using cow dung and duckweed as feedstock for biogas production, such as GHG emissions, water use, and nutrient runoff.
- The study will be conducted in a small-scale anaerobic digestion system (20 L water jars), which may not fully represent the conditions of large-scale biogas production systems.

CHAPTER TWO: LITERATURE REVIEW

2.1 Duckweed & its various uses

Lemna minor, commonly known as lesser duckweed or common duckweed, belongs to the subfamily Lemnoideae within the arum family Araceae (Klaus, Nokolai, & Eric, 2013). *Lemna minor*, a freshwater floating aquatic plant, features one to four leaves per individual, each accompanied by a single root extending into the water. Through the growth of additional leaves, the plants undergo division, emerging as distinct entities. The roots measure 1–2 cm in length, while the leaves are oval, ranging from 1 to 8 mm in length and 0.6 to 5 mm in width. They exhibit a light green hue, possess three veins (occasionally five), and incorporate small air spaces that aid in floatation.

Widely distributed across the globe, *Lemna minor* is native to the majority of Africa, Asia, Europe, and North America. It demonstrates a sub cosmopolitan presence and thrives in freshwater ponds and sluggish streams, excluding regions with arctic and subarctic climates.

Duckweed exhibits rapid growth, characterized by a reproduction rate approaching exponential expansion at low plant density. Its growth rate is approximately 64 times greater than that of corn (Ziegler et al., 2014). Duckweed possesses high photosynthetic efficiency and higher biomass than other plants and under favourable conditions are found to double its biomass within 24 hours. Duckweed can be found in diverse ecosystems ranging from the alkaline water lakes, eutrophic water to even industrial wastewaters (Borisjuk et al., 2015). The optimal growth conditions of *L. minor* have been found at pH values of 6.5 – 8 and 6 – 33 °C (Leng, 1995).

Cultivating duckweeds typically requires proactive management efforts. These petite, free-floating plants are vulnerable to aggregation, forming accumulations that create open water surfaces conducive to algal growth. To mitigate this, it is recommended to establish long, narrow ponds oriented perpendicular to the prevailing wind. Achieving an even distribution of added nutrients within the ponds can be realized through multiple inlets. Sustaining a dense plant cover on the water surface and preventing excessive thickness necessitate coordinated practices of harvesting and nutrient replenishment (Hasan, 2009).

The fertilization needs for cultivating duckweed are contingent upon two factors: the water source and the geographical origin of the selected plant isolate. When cultivating *L. minor* in ponds filled with rainwater, an additional application of nitrogen, phosphorus, and potassium is required (Bergmann, 2000). To sustain high growth rates and crude protein contents, the Total Kjeldahl Nitrogen (TKN) level should not fall below 20 mg/L. Optimal growth has been observed with phosphorus concentrations in the range of 6-154 mg/L (Hasan, 2009). Due to the elevated concentrations of ammonium and other minerals in effluents from domestic animal production, there is often a necessity to dilute them to achieve a balanced nutrient concentration. Studies suggest that sewage water, being rich in potassium and phosphorus, can serve as a viable medium for duckweed cultivation. However, adjustments are required to optimize nitrogen concentrations.

Duckweed is also gaining attention as a valuable animal feed due to its high nutritional content and potential to serve as a cost-effective and sustainable alternative to traditional feed sources. Duckweed biomass also has a high potential for ethanol production (Cheng & Stomp, 2009). The duckweed starch content can be manipulated by adjusting growth conditions (e.g., pH, nutrient content, etc.) that affect proliferation. The aforementioned researchers argue that *Spirodela Polyrrhiza* has shown a great potential for starch production.

Duckweed has also been recognized as an effective and environmentally friendly tool for wastewater treatment due to its ability to remove nutrients and contaminants from water bodies. Duckweed-based wastewater treatment systems have been implemented on a full scale in various locations, including the USA, Bangladesh, and China (R. A. Leng, 1999). When grown in nutrient-rich wastewater, duckweed can act as a natural water purification system, providing several benefits. Duckweed is particularly efficient at removing excess nutrients like nitrogen and phosphorus from wastewater. These nutrients are major contributors to water pollution, and their removal helps to prevent eutrophication and algal blooms in water bodies. Biological Oxygen Demand (BOD) is a measure of the organic material present in water that consumes oxygen during decomposition. Duckweed can help reduce BOD levels in wastewater by consuming organic matter as a food source. Some species of duckweed have the ability to accumulate heavy metals such as cadmium, lead, and mercury. By absorbing these pollutants, duckweed can help in the remediation of water contaminated with heavy metals. Duckweed has the potential to remove certain pathogens from wastewater, acting as a natural bio filter that can reduce the presence of bacteria and other harmful microorganisms. Duckweed-based wastewater treatment systems can be relatively low-cost compared to traditional treatment methods, making them particularly suitable for small-scale or decentralized wastewater treatment applications. However, it's important to note that the success of using duckweed for wastewater treatment depends on various factors, including the type of wastewater, environmental conditions, and the species of duckweed used.

2.2 Biogas production from cow dung

Cow dung is a widely available and inexpensive organic material that has been used for biogas production for many years. Numerous studies have explored the use of cow dung for biogas production and found that it can produce a high quantity of biogas with a high methane content. Biogas production efficiency was highest with cow dung, followed by ship manure demonstrating moderate effectiveness, and pig manure exhibiting comparatively lower efficiency (Fikadu, Kumsa, & Gemechu, 2020).

A study on the generation of biogas from cow dung in Nigeria concluded that cow dung has the potential to serve as a viable substrate for biogas production. Implementing such production on a commercial scale could not only offer an alternative energy source but also serve as a method of waste disposal (Onwuliri, Onyimba, & Nwaukwu, 2013).

Research has shown that incorporating both cow dung and food waste can enhance the efficiency of biogas production. The findings of this study indicate that the combined waste slurry yields a greater volume of gas compared to cow dung slurry alone, attributing this difference to the higher nutrient content present in food wastes (Chibueze, Okorie, Oriaku, Isu, & Peters, 2017).

Among various mixtures and controls, the dry co-digestion of cow dung and pig manure in the ratio of 60%:40% demonstrated superior methane yield and exhibited the highest efficiency (Li et al., 2014). The experimental results showed that this co-digestion resulted in higher methane yields (as high as 18%), higher VS removals (as high as 13%) & greater Chemical Oxygen Demand (COD) degradation (as high as 13%).

Hence, co-digestion of the said substrates in the said ratio resulted in positive synergism.

2.3 Biogas production from duckweed

Duckweed possesses a high energy content, which contributes to its potential as a bioenergy source. Owing to its ultra-high biomass accumulation rate, short reproduction cycle, growth in floating water, and ease of harvesting, duckweed is a high-quality raw material for developing bioenergy, such as ethanol, butanol, and biogas. Thus, it is a promising raw material for bioenergy production.

Several studies have shown that duckweed can produce biogas with a high methane content. For example, duckweed can be used to produce methane, with a yield of nearly 400 mL of CH_4 g^{-1} (Calicioglu & Brennan, 2018). Abundant in protein and starch while possessing a low lignin content, duckweed emerges as an excellent biomass feedstock for energy production (Chen et al., 2022). One research investigation (Chusov et al., 2022) presents findings on anaerobic digestion processes within bioreactors utilizing composite mixtures composed of initial and residual biomass from *Lemna minor* duckweed, along with additives such as inoculum in the form of manure, food waste, and spent sorbents. The objective was to determine the biogas potential, including biogas volume and methane content. According to the study, loading the bioreactors with equal amounts of primary and residual biomass from *Lemna minor* duckweed revealed that the specific biogas yield from the residual duckweed biomass was slightly lower compared to the primary biomass. Notably, the methane content in the biogas from the primary biomass was high by almost 1.5 times.

The study (Clark & Hillman, 1996) investigated the improvement of biogas production in laboratory-scale anaerobic digesters by using duckweed & poultry manure as co-substrates. The authors emphasize iron's significance as the primary micronutrient for anaerobic bacteria. Duckweed, with a substantial iron content was found to significantly impact the rate of decomposition without affecting the overall biogas production volume, as per the authors. This effect was specifically linked to duckweed's elevated iron content, a conclusion corroborated by subsequent semi-continuous experiments.

Similarly in a batch test to determine the biogas production of lesser Duckweed (*Lemna minor*) and Duckweed fern under mesophilic conditions (Banning, 2011), the anaerobic digestibility of both duckweeds was found to be comparable. After shredding, the biogas production of both duckweeds was the same whereas the biogas production of non-shredded duckweed was less.

A study into the effects of the use of duckweed as a coproduct in anaerobic digestion of dairy manure (Henderson, S.L., Triscari, & Reinhold, 2012) concluded that a substantial increase of nearly 1.5 to 2 times greater methane production was observed from co-digestion of 2% dry mass duckweed with dairy manure at 35°C.

A team of researchers from Thailand had conducted a study of co-digestion of bloomed water fern and duckweed biomass with swine manure, which may prove relevant here. As per this study (Souvannasouk, Unpaprom, & Ramaraj, 2021), the biogas output from the co-substrates resulted in a generation of approximately 70% CH_4 and 34% CO_2 which is a great quality of biogas. This study suggested that the said co-substrates could serve as a reliable biomass energy source to produce biogas.

2.4 Biogas production from co-digestion of cow dung and duckweed

Cow dung and duckweed are two feedstock that have been studied for biogas production, and their mixture has been reported to enhance biogas yield and quality. However, there is a gap in knowledge regarding the optimal ratio of duckweed and cow dung for biogas production, as well as the effect of varying ratios on biogas composition. This literature review aims to provide a comprehensive overview of the previous studies that have investigated the comparative analysis of biogas yield and composition from varying ratios of duckweed and cow dung in anaerobic digestion.

Conducting batch-type anaerobic digestion for 55 days at 37°C, the co-digestion of duckweed (DW) with cow dung (CD) in different ratios (DW:CD = 90:10, 75:25, and 50:50) revealed cumulative biogas production was highest for the batch with mixture ratio of 50:50. This was approximately 12L. Furthermore, the methane content in the biogas generated from co-digested feedstock was comparable to that from cow dung alone (Yadav et al., 2017).

In a separate study, the cumulative biogas production was investigated for different mixtures, similar to the above study (DW: CD = 100:0, 75:25, 50:50, 25:75 & 0:100). The findings underscore the promising potential of duckweed as a valuable feedstock for biogas production, with optimal results observed when co-digesting the plant with cattle manure at a ratio of 25% to 75% (Negassa & Fikadu, 2021).

The literature review thus shows that both duckweed and cow dung have the potential to produce biogas through anaerobic digestion but it also indicates that there is a research gap in the study of biogas production from a mixture of cow dung and duckweed. Although several studies have explored the use of cow dung and other co-substrates, such as agricultural waste and food waste, there is limited information on the use of duckweed as a co-substrate in the AD process. The optimal ratio of duckweed and cow dung for biogas production and the effect of varying ratios on biogas yield & composition remain unclear. Therefore, this research will aim to fill this research gap by conducting a comparative analysis of biogas yield and composition from varying ratios of duckweed and cow dung in AD.

CHAPTER THREE: RESEARCH METHODOLOGY

The research methodology can be summarized in the following figure which shows the step-wise procedures employed during the research work

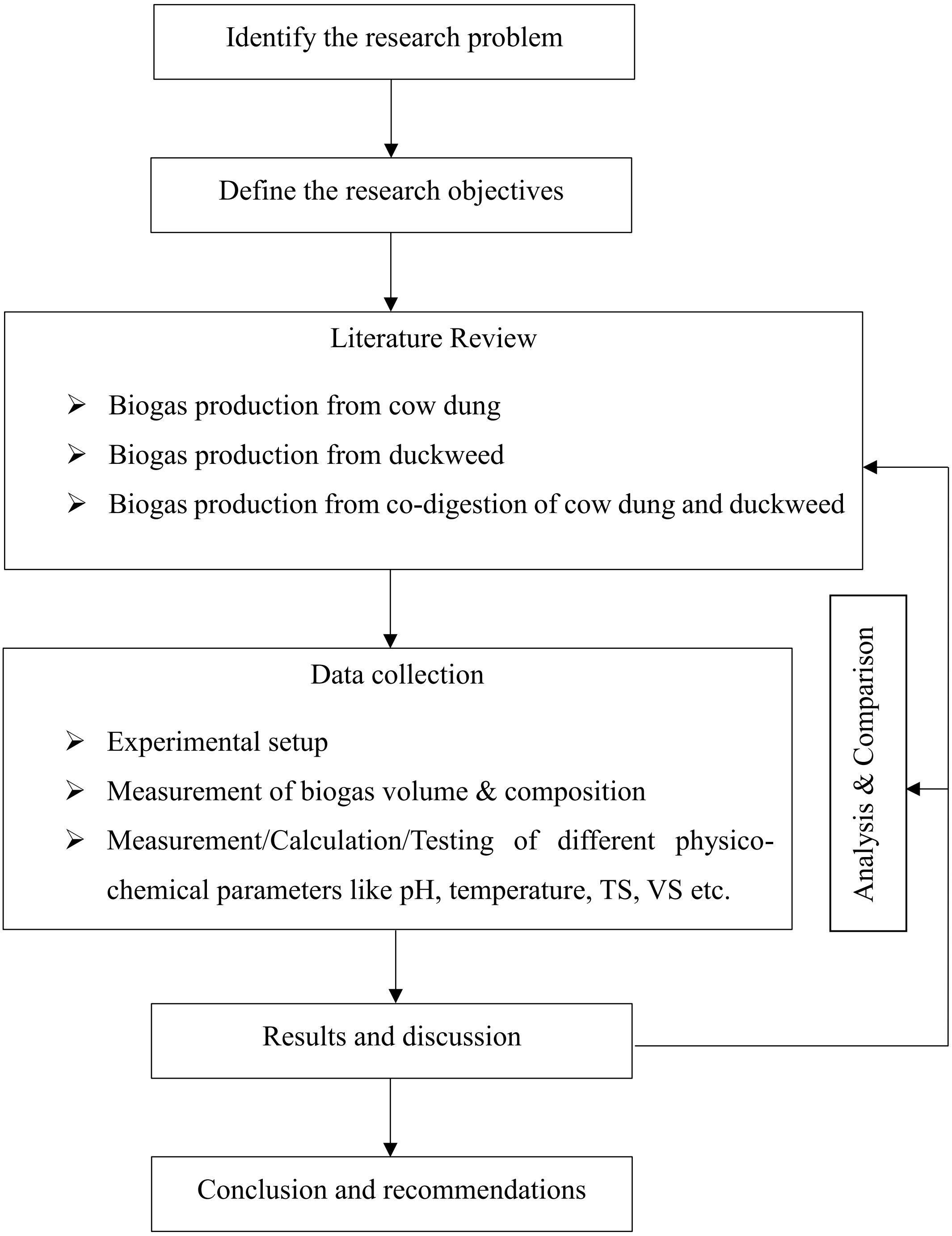


Figure 3.1 Research Methodolgy

2.1 Feedstock collection

Cultivated duckweed was collected from the man-made ponds constructed inside the premises of Institute of Engineering, Pulchowk Campus, Lalitpur whereas cow dung was purchased from "K.C. Cow Firm" situated at Bagdol, Lalitpur, Nepal.

2.2 Substrate preparation

Cow dung (CD) and Duckweed (DW) were then thoroughly mixed in varying ratios viz. 100% CD, 80% CD + 20% DW, 60% CD + 40% DW, 40% CD + 60% DW, 20% CD + 80% DW & 100% DW. Digested slurry/effluent was collected from a household biogas plant that used only cow dung as the substrate & this slurry was used as fresh inoculum. The plant was present in the living quarter premises of Pulchowk Campus. According to (Karki, 2005), to ensure effective solubilization of organic content, maintaining a solid-to-water ratio of 1:1 on a unit volume basis (i.e., equivalent volumes of water and solid) is recommended when utilizing domestic wastes. However, in the case of dry dung, which requires crushing before being introduced into the digester, the water quantity must be adjusted accordingly to achieve the desired input consistency. For instance, the ratio may vary from 1:1.25 to even 1:2 depending on the specific requirements.

The mixing ratios of CD, DW, water and inoculum for these six mixtures was done as in Table 3-1. Here, the mass of inoculum used was kept as 10% of the total mass of the substrate. The mass of each component was measured using a digital weighing scale (DT 510). These mixtures were kept in six different batch reactors (20L water jars) which were named B1, B2, B3, B4, B5 and B6 respectively. The lids of these water jars were sealed tight using a stainless-steel hose clamp. A hole was drilled at the neck of the jars & a pneumatic flow control valve was fixed on the hole. A clear transparent gas pipe was connected to the valve for gas outlet. The valve was further sealed using M-seal. These mixtures were then stored at room temperature and biogas yield were kept under observation for the following days. The jars were shaken manually each day after taking the measurement so as to ensure proper mixing of substrates.

Table 3.1 Mixing ratios of cow dung, duckweed, water and inoculum in different batches

Batch	Mass (in kg) of				Total mass (m)=a +b+c	Total volume to be filled (litre) =0.7*20
	Cow dung (a)	Duck weed (b)	Water (c)	Inoculum (d) = 0.1 × (a+d)		
B1 (100% CD)	6.3	0	7	0.7	14	14
B2 (80% CD + 20%DW)	5.04	1.26	7	0.7	14	
B3 (60% CD + 40% DW)	3.78	2.52	7	0.7	14	
B4 (40% CD + 60% DW)	2.52	3.78	7	0.7	14	
B5 (20% CD +80% DW)	1.26	5.04	7	0.7	14	
B6 (100% DW)	0	6.3	7	0.7	14	
Total	18.9	18.9	42	4.2	84	

3.3 Measurement of biogas volume

The daily biogas volume obtained from each reactor was measured using the downward water displacement method. A 1000ml measuring cylinder was used and as such the volume measurement was done in multiple steps by controlling the flow of gas with the help of the pneumatic control valve.

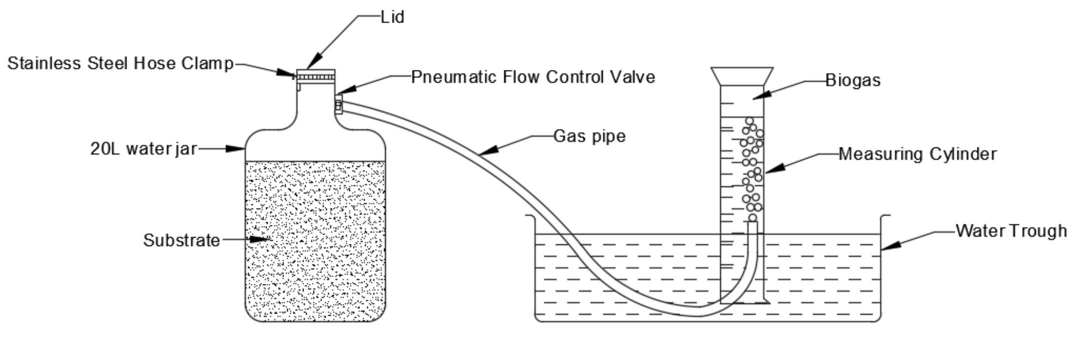


Figure 3.2 Schematic Diagram of the Experimental Setup

3.4 Measurement of biogas composition

The biogas composition which includes CH_4 %, CO_2 %, O_2%, H_2S in ppm & CO % as obtained from all six reactors were measured using Biogas Analyser (GASBOARD 3200 Plus). The readings were taken in an interval of not more than 10 days starting from Day 20. The results are tabulated in the Appendix B: Biogas Composition. It is important to note here that only CH_4 % & CO_2 % have been shown in the appendix as the content of the remaining constituents was very less.

3.5 Measurement of temperature

The initial temperatures of the feedstock were measured using a thermocouple thermometer (FLUKE 53II B). The daily ambient temperature was measured using a wall mounted thermometer.

3.6 Measurement of pH

The initial & final pH of the substrates as well as the pH of inoculum & mixing water was measured using a pH meter (pHep Pocket-Sized pH Meter-HANNA Instruments).

3.7 Measurement of total solids, volatile solids & carbon/nitrogen ratio

Other parameters which include Total Solids (TS), Volatile Solids (VS), Carbon/Nitrogen Ratio (C/N Ratio) & Nitrogen-Phosphorous-Potassium (N-P-K) value were measured at Soil, Water and Air Testing Laboratories Pvt. Ltd. (SWAT Lab) situated at Baluwatar, Kathmandu using standard methods.

3.8 Calculation of organic carbon

The feedstock's organic carbon (OC) content was calculated from the data of volatile solids using an empirical equation given by (Badger, C.M, Bogue, & Stewart, 1979) as:

$$\% \text{ Carbon} = \frac{\% \text{ VS}}{1.8}$$

Where, VS = Volatile Solids

Table 3.2 Summary of all parameters and measurement methods

S.N.	Parameter	Instrument/Equipment Used	Procedure/Test Method
1.	Ambient Temperature	Wall mounted thermometer	Direct measurement
2.	Substrate Temperature	Thermocouple Thermometer (FLUKE 53II B)	Direct measurement
3.	pH	pH meter (pHep Pocket-Sized pH Meter-HANNA Instruments)	Direct measurement
4.	Total Solids (TS) (%)	Oven	Laboratory Testing Procedure for soil and water sample analysis, 2009 (Through Moisture Content)
5.	Volatile Solids (VS) (% of TS)	Oven plus Muffle (CE-Optics Technology, Delhi- 110034)	Guide to Laboratory Establishment for Plant Nutrient Analysis, FAO, 2008
6.	Organic Carbon (OC)	N/A	Calculated using empirical equation $\% \text{ Carbon} = \frac{\% \text{ VS}}{1.8}$ developed by (Badger, C.M, Bogue, & Stewart, 1979)

7.	Carbon/Nitrogen Ratio		
	Carbon (%)	Oven plus Muffle	Guide to Laboratory Establishment for Plant Nutrient Analysis, FAO, 2008, Chapter 3, 38-39
	Nitrogen (Total Kjeldahl Nitrogen-TKN)	Kjeldahl (Digestion and Distillation) (KELDLUS-KELVAC-VA)	4500-N_{org} C. Semi Micro Kjeldahl Method 23rd edition
8.	Biogas Volume	Measuring Cylinder (1000ml)	Downward displacement of water
9.	Biogas Composition	Biogas Analyser (GASBOARD 3200 Plus)	Direct Measurement

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Evaluation of daily and cumulative biogas yield

The daily biogas yield and cumulative biogas yield from all the six batch reactors were plotted against the corresponding days and the results were obtained as seen in the following curves:

4.1.1 Control batch B1 (100% CD)

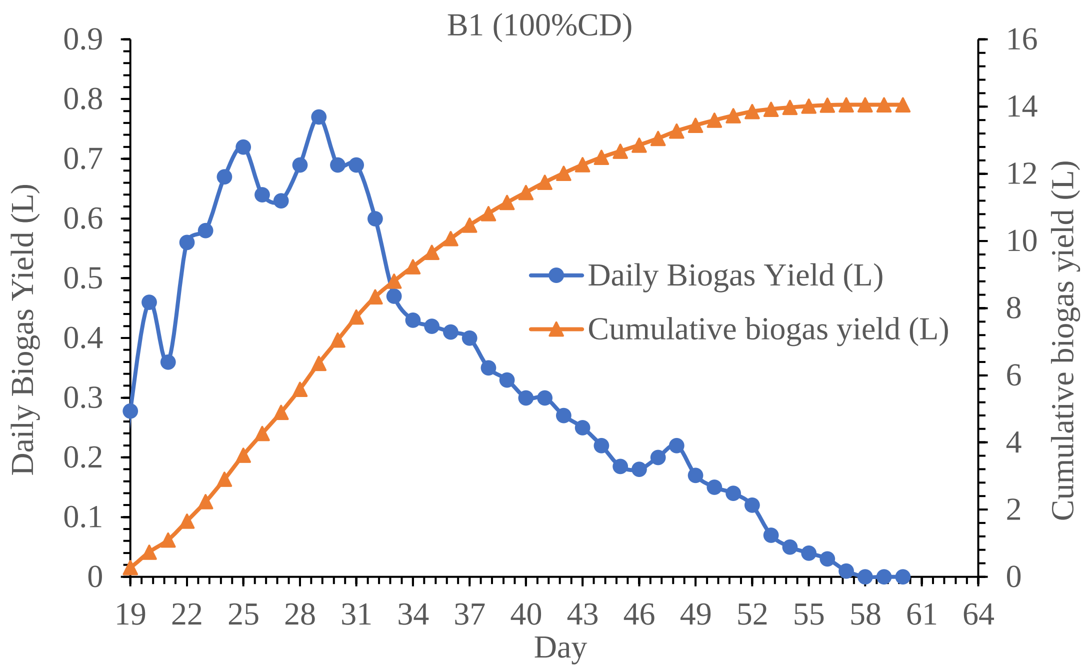


Figure 4.1 Daily Biogas Yield (L) & Cumulative Biogas Yield (L) vs Day for B1

From Figure 4. 1, we can infer that, the maximum biogas yield was 0.77L on the 29th day whereas the minimum biogas yield was 0.01L on the 57th day. The daily average biogas yield was 0.234L/day and the cumulative biogas yield for 60 days was 14.053L. Thus, the biogas production per kg of cow dung per day can be calculated as:

$$Biogas\ production\ per\ kg\ of\ cow\ dung\ per\ day = \frac{Cumulative\ \ production}{Amount\ of\ cow\ dung\ used \times HRT}$$

Where, HRT= Hydraulic Retention Time

$$= \frac{14.053}{7 \times 60} = 0.034 \frac{L}{kg} / day$$

First and foremost, the amount of biogas produced from 100% CD in this experiment is far below what is generally obtained. According to (Werner, Stöhr, & Hees, 1989) biogas production per kg of cow dung ranges from 23L/kg to 40L/kg for a retention time of 40-100 days. i.e., standard biogas production per kg of cow dung per day (assuming an HRT of 70 days) ranges from 0.328 L/kg per day to 0.571 L/kg per day. So, the obtained figure is one tenth of the minimum standard value.

It is important to analyse the possible reasons for the low production obtained from 100% CD in this experiment. Since the ambient temperature for all the mixtures were same throughout the experiment, we can rule out the effect of temperature. As suggested by (Werner et al., 1989) design parameters for sizing if a biogas plant involves pH value within a range of 6 to 7. According to (Irshad, Eneji, Hussain, & Ashraf, 2013), pH of fresh cow manure was 8.5 and that of manure compost was 7.4. Similarly, according to (Belyeu, 2017), cattle manure tends toward a neutral pH. Studies by Penn State University showed dairy manure at pH of 7.0, while manure mixtures from the University of Ngaoundere ranged from approximately 5.9 to 6.9. So, the pH clearly seems to be the major factor behind the substantial drop in biogas production from 100% CD as it was recorded at a value of just 5.5 at the beginning of the experiment.

The digester's internal pH usually drops to acidic levels owing to the initial acidogenesis process of biogas formation. Then a hinderance or cessation of the AD process follows because methanogens are highly senstitive to pH and struggle to thrive when the pH falls (specifically when below 6) (Karki, 2005). This can be an explanation for why biogas production fell to such low value. The quality of water used for mixing might also have a role to play for this as it was collected from the nearby local source and its pH was found to be 6.5 which is again in the acidic range. As reported by (Deublin & Steinhauser, 2008) the lower production from 100% CD may be attributed, in part, to the partial fermentation that commonly occurs in the animal's intestinal tract. Conversely, the increased production from the mixtures might be a result of a balanced nutrient composition, enhanced buffer capacity, and a diminished impact of toxic compounds, stemming from the amalgamation of substrates (Fulford, 1988), (Macias-Corral et al., 2008).

Another factor which might have contributed to the low yield from 100% CD in this experiment is the C/N ratio of the substrate. Theories on biogas suggest that the optimal C/N ratio of feedstock is 30:1. This ratio can only be kept up in cases of co-digestion of cattle dung with other substrates (Karki, 2005). This ratio has been precisely achieved for the mixture batch B3 thus validating the high yield obtained from this mixture batch. The fermentative bacterium utilizes carbon at a rate 25 to 30 times faster than nitrogen, emphasizing the need for an optimal C/N ratio of 25–30:1. Any deviation from this ratio has the potential to impede the process. A higher C/N ratio implies nitrogen depletion before carbon digestion, while a lower C/N ratio results in excess nitrogen relative to carbon, leading to elevated ammonium concentrations that are toxic to anaerobic bacteria (Mittal, 1996). Although the C/N ratio of the substrate here was 27:1 which falls within the optimal range, it still is the lowest when compared with the other substrates used in the experiment. This might be a reason as to why this particular batch gave little yield as compared to the other batches.

There might be also be a possibility of the overall quality of cow dung used in the experiment being poor. Since there was a prevalence of lumpy-skin disease in cattle (especially cows) during the time of cow dung purchase, the quality of the dung might have suffered. Hence through this study it has been found that it is imperative to check beforehand the quality of cow dung while performing these kinds of experiments or study through various chemical composition analysis available.

4.1.2 Mixture batches B2 (80% CD+20% DW) to B5 (20% CD+80% DW)

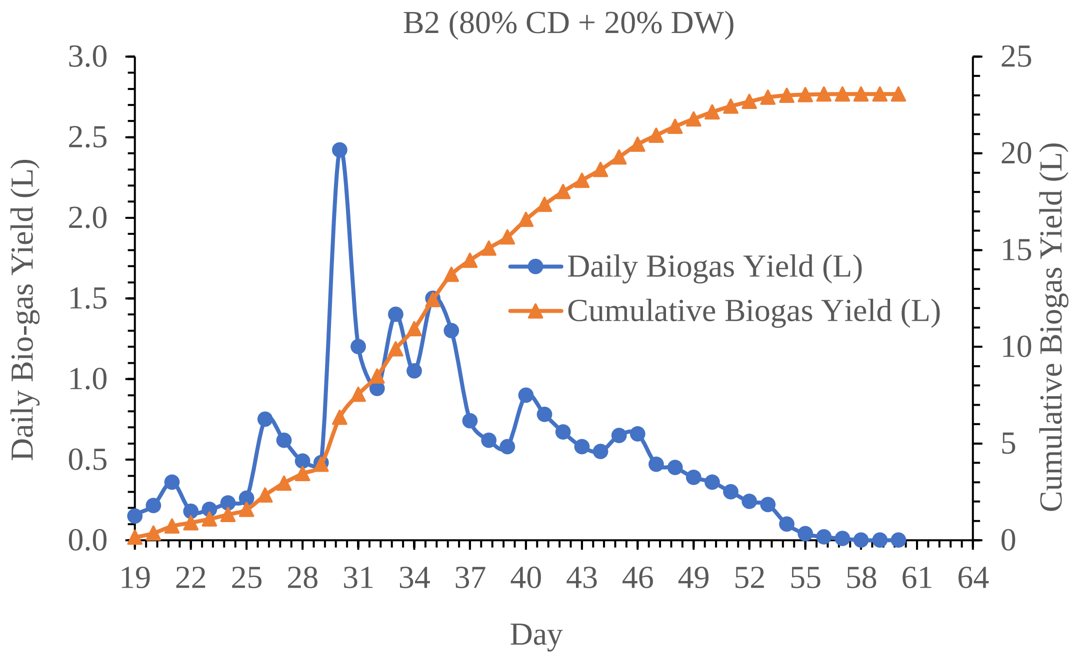


Figure 4.2 Daily Biogas Yield (L) & Cumulative Biogas Yield (L) vs Day for B2

From the Figure 4.2, we can infer that, the maximum biogas yield was 2.42L on the 30th day whereas the minimum biogas yield was 0.01L on the 57th day. The daily average biogas yield was 0.38L/day and the cumulative biogas yield for 60 days was 23.07L.

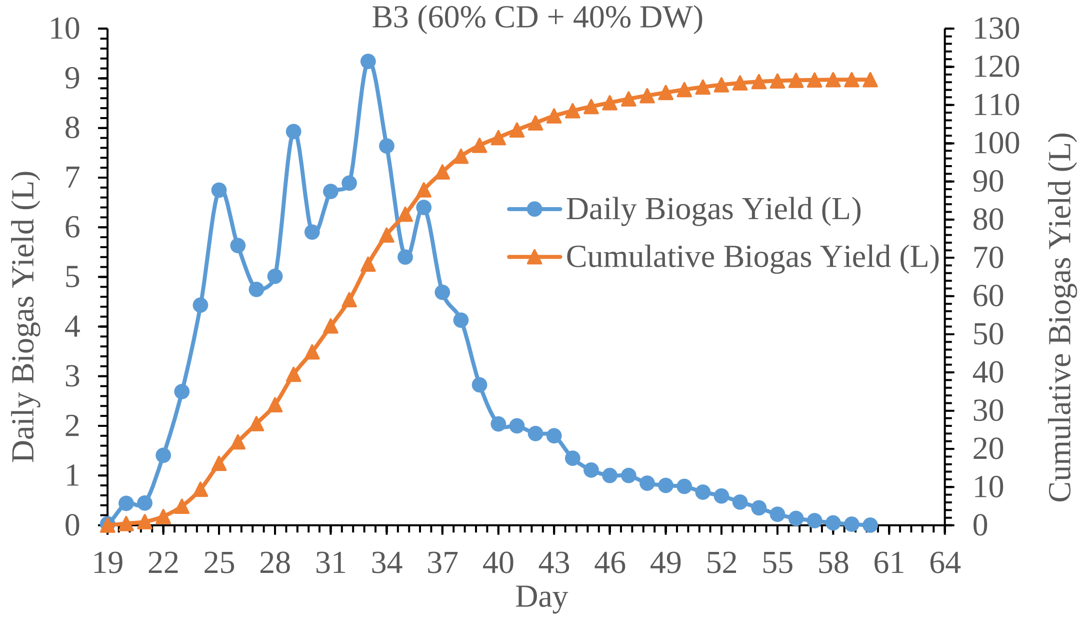


Figure 4.3 Daily Biogas Yield (L) & Cumulative Biogas Yield (L) vs Day for B3

From the Figure 4. 3, we can infer that, the maximum biogas yield was 9.34L on the 33rd day whereas the minimum biogas yield was 0.02L on the 59th day. The daily average biogas yield was 1.94L/day and the cumulative biogas yield for 60 days was 116.64L.

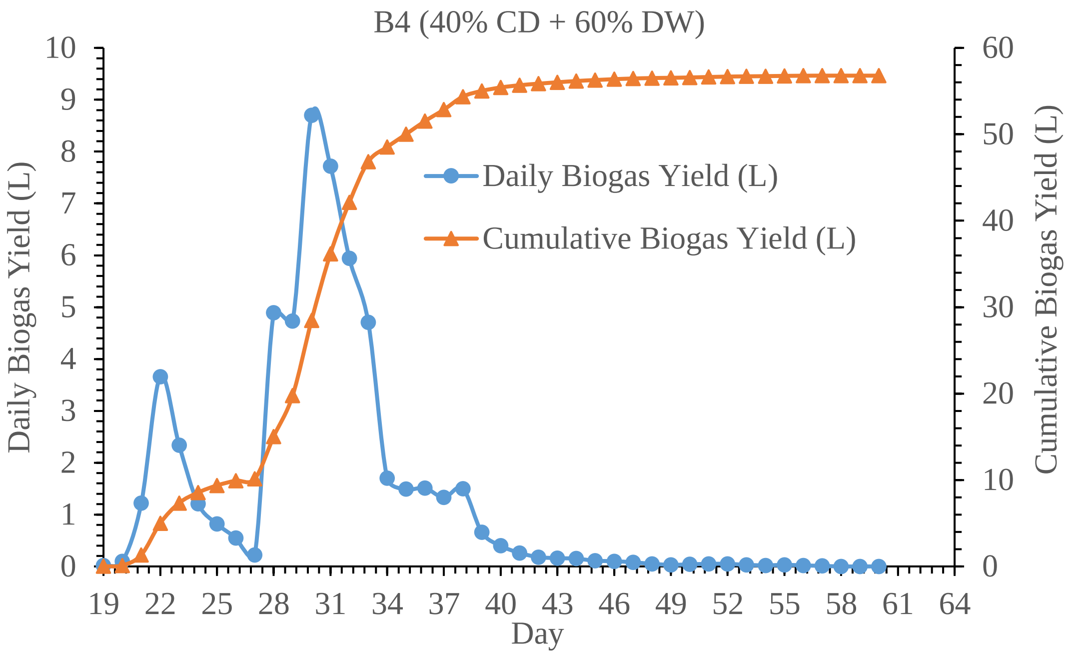


Figure 4.4 Daily Biogas Yield (L) & Cumulative Biogas Yield (L) vs Day for B4

From the Figure 4.4, we can infer that, the maximum biogas yield was 8.7L on the 30th day whereas the minimum biogas yield was 0.01L on the 57th day. The daily average biogas yield was 0.95L/day and cumulative biogas yield was 56.79L.

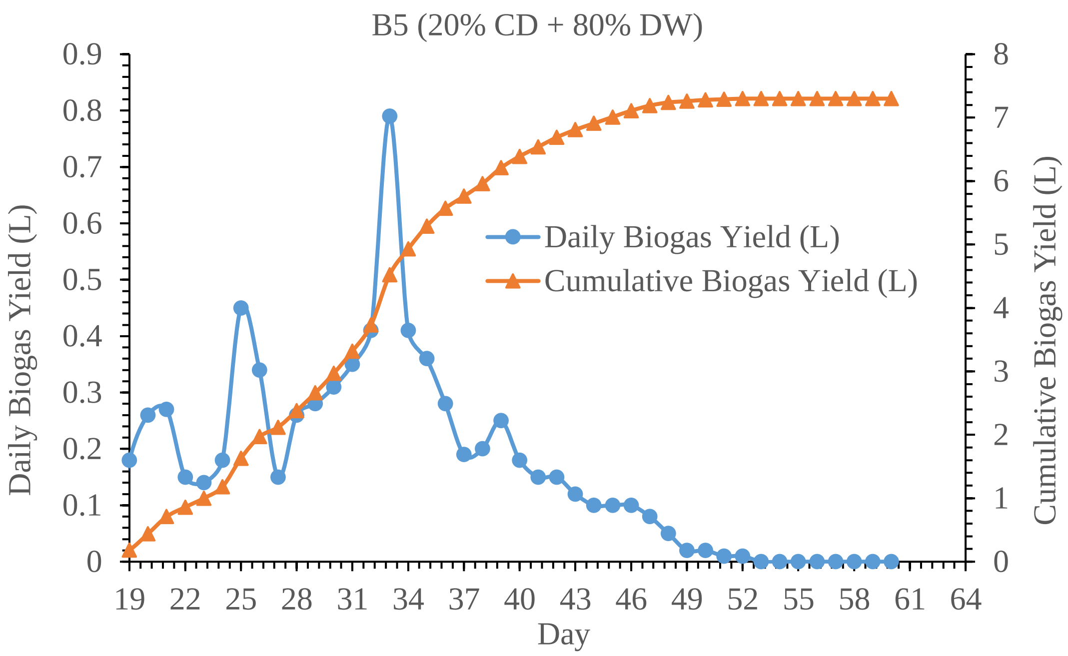


Figure 4.5 Daily Biogas Yield (L) & Cumulative Biogas Yield (L) vs Day for B5

From the curve obtained for B5, we can infer that, the maximum biogas yield was 0.79L on the 33rd day whereas the minimum biogas yield was 0.01L on the 51st & 52nd day. The daily average biogas yield was 0.12L/day and the cumulative biogas yield for 30 days was 7.3L.

For the batches B2 to B5, where cow dung and duckweed were mixed in varying ratios, the graphs of daily biogas yield vs day are patterned quite similarly. Biogas production follows a kinetic pattern where it starts slowly, increases to a peak, and then gradually tapers off as the digestion process completes. The biogas production increases very slowly and gradually during the initial phase (first 3 to 4 days); with the exception of B3 where this is rather swift and continues up to the 7th day. Then the daily biogas yield shows a zig-zag pattern with multiple peaks and troughs.

The data obtained also indicates that the maximum daily yield from all four of these batches occurred during the period from 30th to 33rd day. This suggests probable increase in overall microbial community & its activity during this period. This result is also supported by the results of (Yadav et al., 2017) where peak yield values for samples from mixing of CD & DW in 10%:90%, 25%:75% and 50%:50% ratios were obtained on the 35th day. The researchers of this experiment had performed similar batch production like the one done in the present experiment. They had used 5kg cow dung as the base feedstock and had concluded that considering an HRT of 55 days the optimum mixing ratio for co-digestion of DW and CD has been found to be at 1:1.

Another similar research by (Negassa & Fikadu, 2021) involved using five 0.6 L plastic bottles as batch digesters for co-digestion of CD and DW. This team used DW: CD in 75:25, 50:50 & 25:75 ratios along with 100% CD and 100% DW as controls. Daily biogas produced from the mixtures of varying ratios peaked during a period of 14-22 days which is not in accordance with the present experiment's peak days. However, the end results of the two experiments do tend to concur as (Negassa & Fikadu, 2021) concluded that the ratio of CD & DW at 75%:25% gave the highest cumulative biogas yield.

The minimum yield so far has been obtained on the 57th day for batches B1 to B2 (towards the end), 59th day for batch B3 (towards the very end) and 52nd day for batch B5(quite early). This can be attributed to the fact that biogas volume is obviously very low towards the end of the AD process. This result is supported by the aforementioned literatures (Yadav et al., 2017) & (Negassa & Fikadu, 2021) .

4.1.3 Control batch B6 (100% DW)

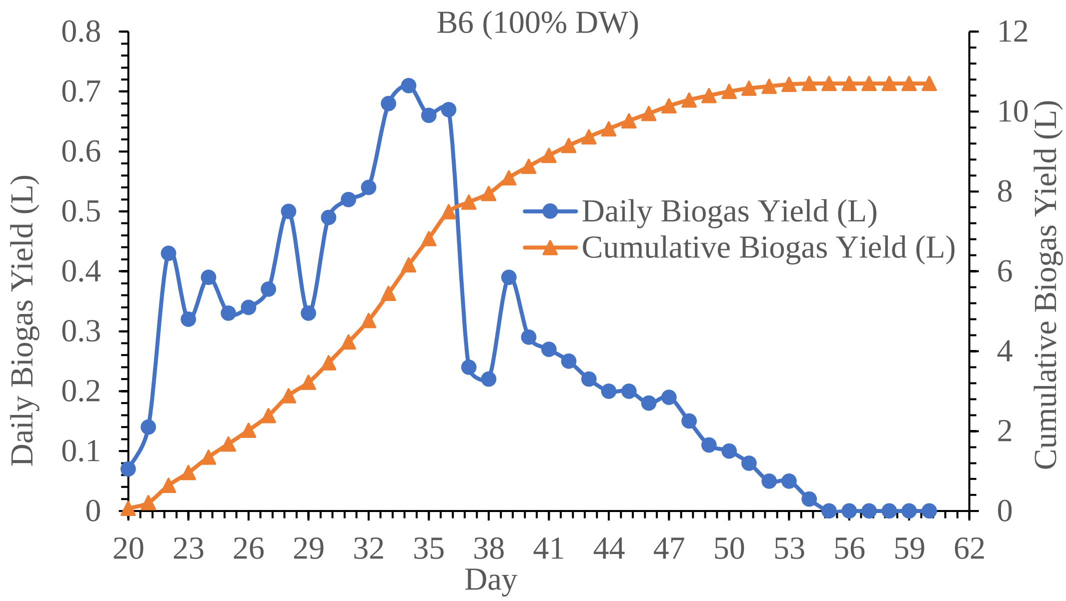


Figure 4.6 Daily Biogas Yield (L) & Cumulative Biogas Yield (L) vs Day for B6

From the Figure 4.6, we can infer that, the maximum biogas yield was 0.71L on the 34th day whereas the minimum biogas yield was 0.02L on the 54th day. The daily average biogas yield was 0.18L/day and the cumulative biogas yield for 60 days was 10.7L.

First of all, the biogas production for B6 started a day later than that for the rest of the batches which is an interesting observation. This follows the finding from (Facchin et al., 2013) which states that duckweed being a lignocellulosic biomass needs more time to breakdown into more labile carbon moieties which then subsequently gets converted into precursor for methanogenesis. Like for the mixture batches, the daily biogas yield from B6 also follows a similar pattern of initial rise for the first 3 days followed by zigzag pattern in between (which spans from the 3rd to the 39th day) and eventually a phase of gradual decline. However, it is important to note here that the first two phases are somewhat different from the rest of the mixture batches- B2 to B5. The initial rise here is rather swift than gradual and it is steep too (0.07 L on the first day followed by

0.14 L on the second day which peaked swiftly to a value of 0.43L on the third day). This might be because once the lignocellulosic content of duckweed biomass is broken down, the biogas production shows a rapid increment but this obviously lasts only for a short span of time. The trend is then followed by a zig zag pattern with numerous peaks and troughs along the way. It can be predicted that the biogas production from duckweed is perhaps more sensitive to temperature fluctuations than the mixture combinations. The results of (Ramaraj & Unpaprom, 2016) may be relevant here. In their research investigating the impact of temperature on biogas production from duckweed, the following conclusion was drawn: The experimental findings reveal that, within typical temperature ranges, faster decomposition occurs at higher temperatures. From a technical standpoint, only the mesophilic range (35℃) and the thermophilic range (50℃) are of interest, as anaerobic degradation at room temperature (23-28℃) is markedly slow. Since, the present experiment had temperature fluctuations which fall within both the room and mesophilic ranges (thus a wide range of temperature) the biogas production might have followed such a random zigzag pattern as seen on the curve.

4.1.4 Summary of biogas yield data

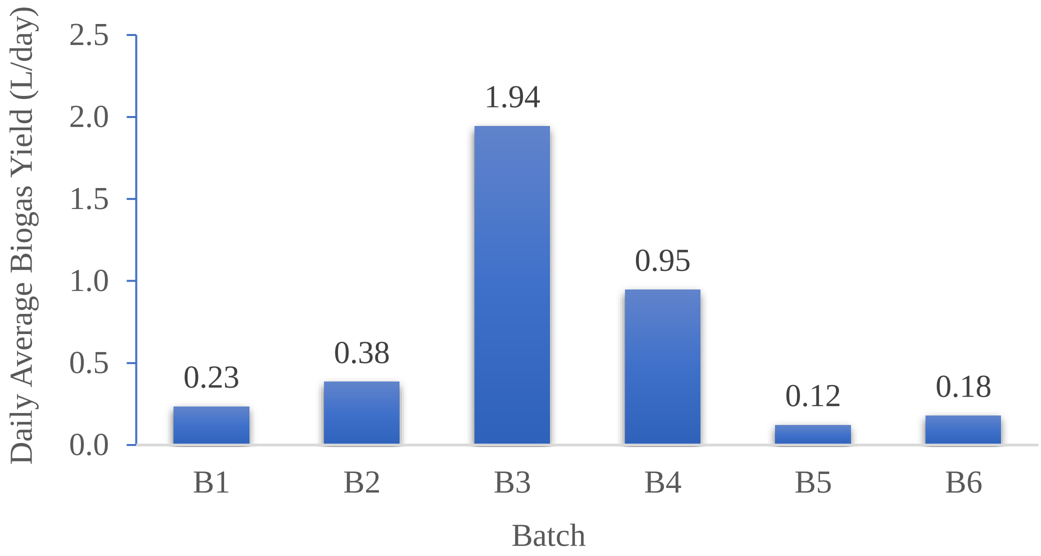


Figure 4.7 Daily Average Biogas Yield (L/day) of All Batches

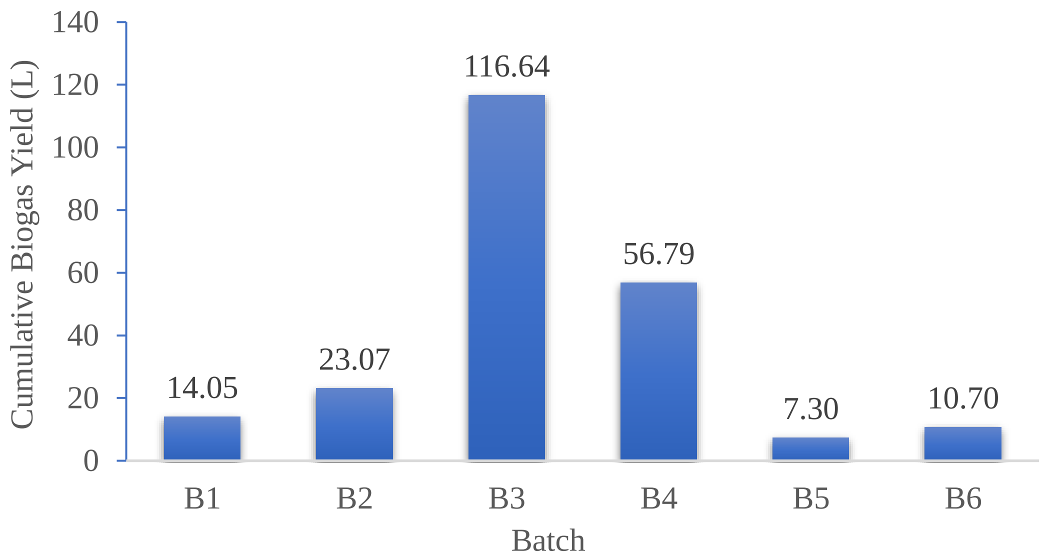


Figure 4.8 Cumulative Biogas Yield (L) of All Batches

The daily average biogas yield obtained from each reactor namely B1, B2, B3, B4, B5 & B6 were found to be 0.23L/day, 0.38L/day, 1.94L/day, 0.95L/day, 0.12L/day and 0.18L/day respectively. This is also shown in the chart above Figure 4.7 Daily Average Biogas Yield (L/day) of All Batches. Thus, the descending order of daily average biogas yield is B3 > B4 > B2 > B1 > B6 > B5 i.e., the maximum daily average biogas yield was obtained from batch B3 (mixture of 60% CD & 40% DW) followed by batch B4 (the mixture of 40% CD & 60% DW) whereas the minimum daily average biogas yield was obtained from batch B5 (the mixture of 20% CD & 80% DW).

Similarly, the cumulative biogas yield for 60 continuous days of biogas production as obtained from each reactor namely B1, B2, B3, B4, B5 & B6 were found to be 14.05L, 23.07L, 116.64L, 56.79L, 7.3L & 10.7L respectively. This is also shown in the chart above Figure 4.8 Cumulative Biogas Yield (L) of All Batches. Thus, the descending order of cumulative biogas yield is also B3 > B4 > B2 > B1 > B6 > B5 i.e., the maximum cumulative biogas yield was obtained from the batch B3 (mixture of 60% CD and 40% DW) followed by batch B4 (mixture of 40% CD and 60% DW) whereas the minimum cumulative biogas yield was obtained from batch B5 (the mixture of 20% CD & 80% DW).

4.2 Process stability and consistency

For batches B1 to B5, the biogas production began only from the 19th day of the completion of setup whereas for batch B6 it began even later; from the 20th day of the completion of setup. Thus, the initial 18-19 days showed no significant biogas production. This suggests that the microbial community needed more time to acclimatize or adapt and establish itself in the digester. The reason behind this might be the initial pH of the substrates as all of them were either below the favourable pH value (for B1) or towards the lower end (for B2, B4 & B5) as a pH value between 6 & 7 is considered ideal for maximum methane production. The pH level within a biogas digester is influenced by the retention time. As the AD progresses, the proteins release ammonia through hydrolysis, which subsequently generates ammonium (NH_4) ions in water. As the NH_4 level rises, the pH elevates to reach alkaline values (Karki, 2005).

The maximum yield, both daily average and cumulative, obtained from B3 also conforms to the pH criterion as its pH was 6.5, which is the most favourable among all the batches in this experiment. On the other hand, duckweed being a lignocellulosic biomass contains lignin and cellulose, which needs more time to break down. Hence, biogas production in all the digesters containing a mixture of duckweed and cow dung might have started so late.

According to (Hobson, Bousfield, & Summers, 1981) the availability of biodegradable material is more in DW than in CD. Biogas production is a directly dependent on the amount of organic content of the feedstock and its biodegradability (Macias-Corral et al., 2008). However, the biogas yield from 100% CD in this experiment still exceeded the yield from 100% DW. This was most probably due to the fact that the percentage of VS from TS content of DW slurry was 71.61% whereas that of CD slurry was 79.88%. This implies that in comparison to cow dung used, the duckweed used in the experiment actually had lesser fraction of biodegradable mass. In addition, the organic content or carbon content of cow dung used exceeds that of duckweed used in this case as OC of CD = 44.38 & OC of DW = 39.78.

As the percentage of duckweed (DW) in the mixing ratio increased beyond 40%, there was a decrease in the cumulative biogas yield, aligning with findings from (Yadav et al., 2017). This observation is consistent with the results of an experiment conducted by (Callaghan, F.J., Wase, Thayanithy, & Forster, 1999) using water hyacinth, poultry manure and cow dung, where higher cumulative biogas production was produced in the system with the lower concentration of water hyacinth. Consequently, the co-digestion of cow manure (CM) and duckweed (DW) was found to be more effective and/or efficient when the proportion of DW did not exceed 40%.

Conversely, the notably low biogas production from batch B5 (80% CD and 20% DW) could be attributed to the presence of lignin and intricate molecules. These components require an extended duration for hydrolysis, thereby failing to offer adequate precursors to methanogenic bacteria consequently hindering microbial growth and impeding the process of methanogenesis (Manyi-Loh et al., 2013).

4.3 pH and organic carbon

The initial pH values of all six slurry samples fell within the range of 5.5 to 6.5. The pH value of 100% CD slurry was 5.5 which is well below the optimal pH value of 6 to 7 as suggested by (Werner et al., 1989) & is hence highly unfavourable for biogas production. Owing to this, the biogas yield might have been very low from this batch (B1). According to (Negassa & Fikadu, 2021) the pH value of 100% DW was 6.03 which is comparatively less optimal and in line with (Thy, S., Preston, & Ly., 2003) & (Yadvika, Santosh, Sreekrishnan, Kohli, & Rana, 2004). However, the initial pH value of DW used in the present experiment was 6.6 (within optimal range) yet was not able to produce much biogas as compared to other substrates. Slurry samples from the mixture batches showed a rise in pH value when compared to 100% CD but less than that of 100% DW. This suggests that co-digestion serves as an effective method for adjusting the pH value to the optimal level (Negassa & Fikadu, 2021).

The OC values before & after AD are as shown in the Table 4.2 Values of different physico-chemical parameters

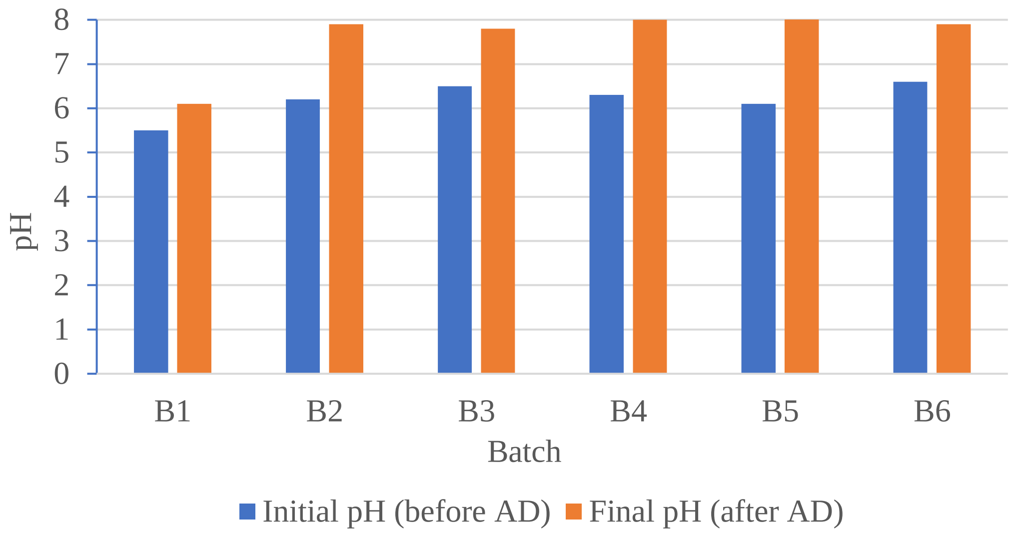


Figure 4.9 Chart showing initial and final pH of all six slurries

4.4 Temperature

Temperature is a crucial factor that significantly affects biogas production in AD processes. The enzymatic activity of bacteria is heavily contingent on temperature, with methanogens displaying inactivity at both extremely high and low temperatures. Optimal gas production takes place at mesophilic temperatures (30-40°C). Significant enhancement in biogas yield can be achieved at temperatures as high as 55°C, but the destruction of bacterial enzymes at elevated temperatures causes decline in the biogas yield. Consequently, for thermophilic digestion, the temperature range should be within 45°C and 55°C.

Conversely, in colder ambient temperatures around 10°C, gas production virtually ceases. Increasing the temperature allows for a considerable reduction in the digestion time. This implies that a shorter retention time is required when elevating the digester temperature to the desired level (Karki, 2005).

The experiment was performed in a lab situated inside the Pulchowk Campus premises (27.6811° N, 85.3185° E). It was carried out during the summer/monsoon season, precisely from the month of June to August. The ambient temperature condition was thus on the favourable range for biogas production as mesophilic temperature range was achieved during this season. As this season was accompanied by frequent rainy days the outside ambient temperature would drop to a value as low as 21°C; however, the inside ambient temperature of the lab was mostly on the warmer side. The roof structure of the lab where the batch reactors were kept were made of tin thus absorbing much heat from the sun and this too might have helped the case. The minimum ambient temperature recorded during gas measurement was 21°C & the maximum temperature recorded was 33°C. Refer Appendix A: Temperature and Time of Day (TOD) Log

4.5 Total solids, volatile solids and percentage removal

The initial TS of all dry samples (10gm) fell within the range from 5.3% to 18.52%. The maximum TS (18.52%) before AD was measured in the dry sample of the mixture of 80% CD and 20% DW whereas the minimum TS (5.3%) was documented in the dry sample of 100% DW. According to (Karki, 2005), the dilution should be adjusted to keep the TS within the range of 5 to 10%. Excessive dilution may cause solid particles to settle at the digester's bottom, while overly concentrated slurry can hinder the flow of gas. Going by this rule, the slurry prepared for batches B5 (80% CD + 20% DW) & B6 (100% DW) apparently seem to be a case of over dilution keeping in mind TS values of dry samples of B5 and B6 substrates were already 10.06% and 5.3% respectively. If it is the case, then it stands out as the major reason for low biogas yield from these batches.

The VS before AD ranged from 71.61% to 79.88%. The VS determined for dry duckweed and cow dung samples were 71.61% and 79.88% respectively. This result confers with (Fulford, 1988) whose report mentions that VS in animal and human wastes lies precisely in the range from 77% to 90%.

After completion of AD, the final TS and VS of all six substrates were determined and then the percentage of TS removal & the percentage of VS removal was calculated using the following formulas:

TS removal (%) = $\frac{TS_i - TS_f}{TS_i} \times 100$

Where, TS_i=Initial Total Solid (TS before AD) &

TS_f= Final Total Solid (TS after AD)

VS removal (%) = $\frac{VS_i - VS_f}{VS_i} \times 100$

Where, VS_i= Initial Volatile Solid (VS before AD) &

VS_f= Final Volatile Solid (VS after AD)

Both TS and VS reduction are good parameters for evaluating the efficiency of anaerobic digestion (Abubaker & Ismail, 2012) & it is a good indicator of biogas production (Anonymous, 1981).

Table 4.1 TS Removal (%) and VS Removal (%) of all six batches

Batch	Initial TS (%)	Final TS (%)	TS Removal (%)	Initial VS (% of TS)	Final VS (% of TS)	VS Removal (%)
B1	17.72	6.06	65.80	79.88	82.9	9.13
B2	18.52	3.5	81.10	77.78	78.62	11.65
B3	14.61	3.09	78.85	76.43	72.52	8.93
B4	6.51	1.77	72.81	73.09	64.56	3.62
B5	10.06	1.24	87.67	79.12	58.88	7.23
B6	5.3	0.9	83.02	71.61	62.73	3.23

4.6 Evaluation of biogas composition

The Figure 4.10 CH_4 Content (%) Vs Time (Days) below represents the methane concentration profile of the biogas from different ratios of feedstock in all the six batch reactors. The methane content was measured using a biogas analyser (GASBOARD 3200 Plus). The highest methane content of 45.36 % was observed for the batch B6 i.e., 100% DW followed by B5, B4, B3, B2 and B1. This is in accordance with the C/N ratio values of the substrates as duckweed had the highest C/N ratio followed by the mixtures with decreasing content of duckweed.

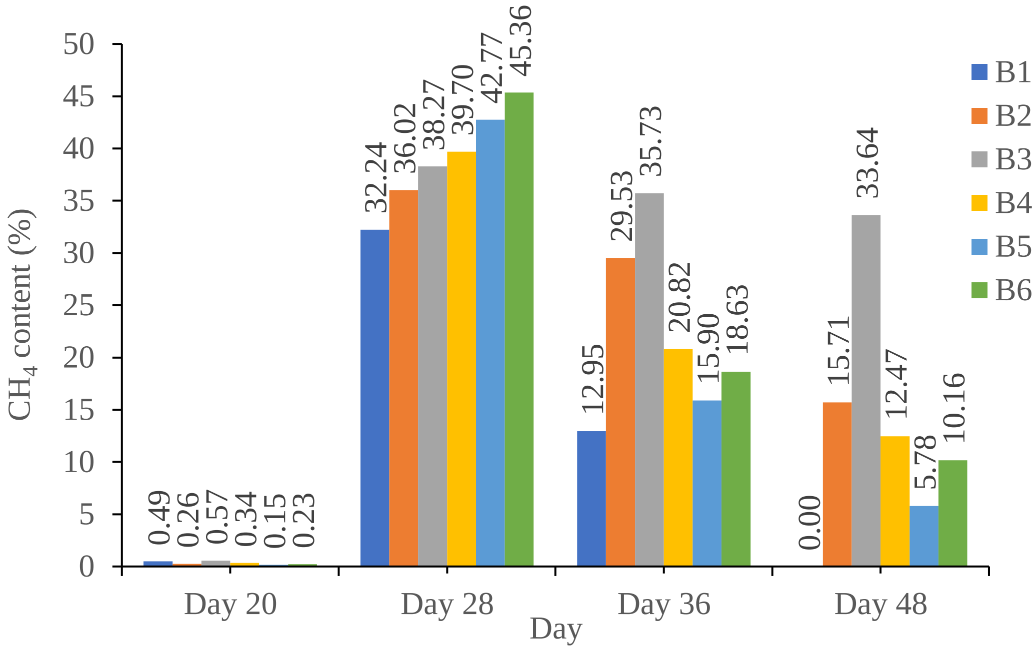


Figure 4.10 CH_4 Content (%) Vs Time (Days)

The inference "Higher the C/N ratio, higher the methane yield" is further conformed by (Yadav et al., 2017) where CD had higher C/N ratio (22.7) than DW (5) and consequently gave higher methane yield. The composition of biogas obtained from all the six batches are tabulated in Appendix B: Biogas Composition

If we look at the ideal average composition of biogas, it consists of 50-70% CH_4, 30-40% CO_2, 5-10% H_2, 1-2% N_2, about 0.3 % water vapour and trace amounts of H_2S. However, it is important to note that the kind of input material (feedstock or substrate) has a major influence on the percentage composition of biogas, and it may change depending on the experimental setup (Karki, 2005).

The CH_4 content of biogas obtained from the six batches in this experiment is comparatively lower when compared with the theoretical value. This might be due to the acidic pH of mixtures as the production of methane is greatly influenced by the pH of the input mixture. The methanogenic process is not supported by an acidic environment. Similarly, temperature has a major impact on the enzymatic activity of bacteria, which is essential for the synthesis of methane. Extreme heat or cold renders the methanogens dormant (Karki, 2005). In the present experiment temperature was in the favourable range so it might certainly not be among the reasons for the lower methane content of biogas.

4.7 Hydraulic retention time

Retention time, also referred to as Hydraulic Retention Time (HRT), is the average duration that a specific quantity of input resides in the digester. This duration is computed by dividing the total volume of the digester by the volume of feedstock added per day.

Moreover, the retention time is influenced by the nature of the feedstock & surrounding temperature (as discussed before). Recommended retention times for cattle dung vary based on climatic conditions. In hilly (colder/temperate) regions, previous research suggests an HRT of 60-70 days, while in the Terai region (warmer/tropical climatic condition), recommended HRT is 45-55 days (Karki, 2005). While HRT is often associated with continuous or semi-continuous digestion processes, it still plays a role in batch production systems as well. In a batch anaerobic digestion system, the retention time is the duration for which a specific batch of feedstock remains within the digester before the digestion process is completed. In the present experiment, the AD has been studied so far for a period of 60 days from the time of completion of setup meaning an HRT of 60 days. The longer the retention time, the more time the microorganisms have to degrade complex organic materials into simpler compounds. Longer retention times allow for more complete breakdown of organic matter, resulting in increased biogas production. Longer retention times can extend the period during which biogas production is at a significant level, resulting in higher cumulative biogas production for a given batch. Some complex compounds, like lignocellulosic materials, require more time to break down fully. A longer retention time provides the necessary opportunity for these compounds to be converted into simpler compounds that can be more readily fermented into biogas.

In this experiment, the overall retention time has been found to be more than 50 days. Duckweed being a lignocellulosic feedstock seems to have a lengthy HRT as compared to cow dung.

Table 4.2 Values of different physico-chemical parameters

Parameters	Batch					
	B1	B2	B3	B4	B5	B6
Initial Temperature of Substrate/Slurry (°C)	30	29	31	30	29	29
Initial pH of slurry (Before AD)	5.5	6.2	6.5	6.3	6.1	6.6
Final pH of slurry (After AD)	6.1	7.9	7.8	8.0	8.1	7.9
TS (% Dry Basis) of dry mixture sample (Before AD)	17.72	18.52	14.61	6.51	10.06	5.30
VS (% of TS) of dry mixture sample (Before AD)	79.88	77.78	76.43	73.09	79.12	71.61
TS (% Dry Basis) of dry mixture sample (After AD)	6.06	3.5	3.09	1.77	1.24	0.90
VS (% of TS) of dry mixture sample (After AD)	82.90	78.62	72.52	64.56	58.88	62.73
C/N Ratio of dry mixture sample	27:1	28:1	30:1	32:1	33:1	34:1
Organic Carbon (Before AD)	44.38	43.21	42.46	40.61	43.96	39.78
Organic Carbon (After AD)	46.06	43.68	40.29	35.87	32.71	34.85
Also, pH of inoculum used = 5.9 & pH of water used for mixing = 6.5						

4.8 Feed-to-inoculum ratio

The feed-to-inoculum (F/I) ratio refers to the relationship between the quantity of organic feedstock material and the amount of inoculum or microbial culture added to initiate the anaerobic digestion process & it is a crucial parameter in the AD process.

The inoculum, often referred to as digestate or sludge from a well-established biogas digester, contains the necessary consortium of microorganisms (bacteria, archaea, etc.) responsible for breaking down complex organic compounds into methane and carbon dioxide.

The optimal F/I ratio is essential for efficient biogas production. Generally, a balanced ratio ensures effective microbial activity, rapid degradation of organic matter, and optimal biogas yield. The ratio can vary depending on the type of feedstock, the composition of the inoculum, and the specific conditions of the digester.

There are many research involving experiments to determine the ideal F/I ratio. In a study by (Jha, 2018) the impact of increasing the inoculum amount on the semi-dry anaerobic digestion process of cow dung with excess sludge over an 84-day period at 15±1ºC, or psychrophilic temperature was evaluated. It was determined that the psychrophilic semi-dry anaerobic digestion process produced the best specific methane yield when it was inoculated with a mass of 45% of the substrate, out of the 15%, 30%, 45%, and 60% inoculum used.

Similarly, another study by (Liu, Zhang, El-Mashad, & Dong, 2009) involved using four distinct F/I ratios viz.1.6, 3.1, 4.0, and 5.0 for the thermophilic digestion tests, while only one ratio (3.1) was used for the mesophilic digestion tests. The experiment was carried out for a period of 25 days & the substrate used was a mixture of 50% food waste and 50% green waste. Here, the maximum biogas yield was obtained for the F/I ratio 4.0 which was 784 mL/g VS for food waste. The biogas and methane yields from the mesophilic digestion of food waste, green waste, and their combination were less than those at thermophilic temperature at the ratio of 3.1.

In another experiment, to find the potential biogas yield from each proportion, the anaerobic co-digestion process was developed and optimized at different inoculum to feedstock ratios of 1:0, 0:1, 1:3, 3:1, and 1:1. The bio-digester recorded the lowest biogas potential at a ratio of 0:1, while the biogas potential at an inoculum to feedstock ratio of 3:1 was the highest (Asante Sackey et al., 2018).

Thus, the amount of inoculum used in the present experiment proves to be inadequate when compared with the aforementioned literature. This might be a reason why there was a prolonged delay in biogas production from all batches and also why the overall biogas yield obtained from most batches apart from the mixture batch B3 was low.

4.9 Comparative analysis of results

As a comparative analysis is necessary for the understanding of the nature of results obtained from the experiment, the Table 4.3 Comparative analysis of results summarizes the observations made from the experiment, the inferences drawn based on the observation, the possible reasons and the literature in support or in contradiction to the reasoning.

Table 4.3 Comparative analysis of results

S.N.	Observation	Inference	Possible Reasons	Support / Contradiction
1.	Max. biogas yield from mixture batch B3 (CD:DW=60:40)	Optimal mixing ratio of CD:DW = 60:40	Good pH (6.5)	Optimum pH = 6 to 7 (Karki, 2005) & (Werner et al., 1989)
			Good VS removal (8.93%)	Removal of VS suggests its conversion to biogas (Negassa & Fikadu, 2021) & (Yadav et al., 2017)
			Ideal C/N ratio of substrate (30:1)	Optimum C/N ratio =25-30:1 (Yadav et al., 2017)

S.N.	Observation	Inference	Possible Reasons	Support / Contradiction
2.	Initial 18-19 days showed no significant biogas production	Microbial community might have needed more time to acclimatize or adapt to and establish itself in the digester.	Initial pH of substrates which was largely in the acidic range B1=5.5 B2=6.2 B3=6.5 B4=6.3 B5=6.1 B6=6.6	It is advisable to keep the digester's pH level within the range of 6 to 7 for optimal performance (Karki, 2005).
				As the concentration of ammonium (NH_4) ions rises, the pH value elevates beyond 8 (Karki, 2005).
				Maximum yield is obtained from B3 (pH= 6.5), **however**, B6 (pH=6.6) gave minimum yield.

S.N.	Observation	Inference	Possible Reasons	Support / Contradiction
3.	Biogas Production from B1(100% CD) >B6(100% DW)	CD is more productive than DW	Availability of biodegradable material (% of VS from TS content)	More in DW than in CD (Hobson et al., 1981) **however**, % of VS from TS content of DW was 71.61% whereas that of CD was 79.88%
				The organic carbon content of the feedstock directly influences the production of biogas(Macias-Corral et al., 2008). Here, OC of CD = 44.38 & OC of DW = 39.78

S.N.	Observation	Inference	Possible Reasons	Support / Contradiction
4.	Low biogas production with increment of DW content beyond 40%	DW proportion in the mix is optimal at 40%.	Availability of biodegradable material (%VS)	Similar results by (Yadav et al., 2017)
				Similar results by (Callaghan et al., 1999)
5.	Low production from B6 (100% DW) in spite of favourable pH (6.6)	Other factors at play	Over dilution	Dilution should be performed to uphold total solids (TS) within the range of 5 to 10% (Karki, 2005) Here, TS of B5 =10.06% & B6 = 5.3%.
6.	Very low biogas yield from B1 (100% CD)	Some critical factors at play	Acidic pH of substrate = 5.5	For cow manure, pH was 8.5 in fresh and 7.4 in manure compost (Irshad et al., 2013)
				Acidic condition lowers down methane formation (Karki, 2005).

				Acidic condition arises partly due to the partial fermentation that usually takes place in the intestinal tract of the animal (Deublin & Steinhauser, 2008)
			Low C/N ratio (27:1)	Low C/N ratio ⇒ high ammonium concentration which is toxic to anaerobic bacteria (Yadav et al., 2017)
			Overall quality of cow dung being poor	Prevalence of lumpy skin disease in cows during the time of study might be a cause of poor quality cow dung

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In the pursuit of enhancing biogas production through co-digestion of Duckweed (DW) with Cow dung (CD), this study conducted experiments using six anaerobic batch reactors with varying proportions of the two substrates. The reactor configurations included 100% CD, 80% CD: 20% DW, 60% CD: 40% DW, and their respective vice versa arrangements, along with 100% DW.

The findings of this research reveal that the highest biogas yield was achieved in the batch with a mixture of 60% CD and 40% DW, followed by the batch with 40% CD and 60% DW. This indicates that an optimal balance between cow dung and duckweed proportions significantly influences the efficiency of biogas production. The synergistic effects of these substrates in the 60% CD: 40% DW mixture contributed to a higher biogas yield, showcasing the potential for enhanced methane generation through co-digestion.

Moreover, not only did the 60% CD: 40% DW batch exhibit superior biogas yield, but it also demonstrated the highest average methane content among all configurations. This underscores the importance of carefully determining substrate proportions for maximizing both quantity and quality of biogas output.

5.2 Recommendations

Based on the research carried out following recommendations have been drawn out

1. **Quality assessment of substrates**: The quality assessment of the substrates for co-digestion i.e., both cow dung and duckweed should be done properly beforehand. This would involve prior testing of all the required parameters including chemical composition of the substrates.
2. **pH monitoring & optimization**: The pH of the mixtures need to be regularly monitored in order to keep track of pH as it is one of the most critical factors that affect the biogas production. Batch reactors in future studies should be designed in such a way so as to incorporate a pH measuring mechanism for this

purpose. A pH controlling addition could also be made in the form of some chemical compound as and when needed.

3. **Surplus analysis**: Various other analytical methods can be added during experiment to measure parameters such as Volatile Fatty Acids (VFAs), COD, TKN etc. Microbial community analysis can be one such ambitious addition too.
4. **Repetition of experiment in triplicates**: To further validate the results, it is advisable to carry out the batch experiments in triplicates.
5. **F/I ratio**: The F/I ratio should be reviewed in the future studies to obtain better biogas yield and fasten the start-up process.
6. **Process Scaling:** Consideration should be given to scaling up the process from batch reactors to continuous flow systems or larger-scale digesters. This would provide insights into the scalability and practical application of the co-digestion approach in real-world scenarios.
7. **Nutrient Supplementation:** Investigation of the potential benefits of nutrient supplementation to enhance microbial activity during co-digestion could be done such as addition of trace elements or other nutrients that may positively impact the anaerobic digestion process.
8. **Economic Viability:** Evaluation of the economic feasibility of implementing the co-digestion process on a larger scale can be done which includes assessing the cost-effectiveness of acquiring and processing duckweed in conjunction with cow dung.
9. **Environmental Impact:** Assessment of the environmental implications of co-digestion, including any potential reduction in greenhouse gas emissions and the overall sustainability of the process can be explored.

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APPENDIX

Appendix A: Temperature and Time of Day (TOD) Log

S.N.	Date	Time of gas measurement	Ambient Temperature at the time of gas measurement	Max. Temp.	Min. Temp.
1.	10-Jul-23	1:00 PM	30℃	31 ℃	22 ℃
2.	11-Jul-23	12:10 PM	31℃	31℃	21℃
3.	12-Jul-23	1:10 AM	29℃	30 ℃	20 ℃
4.	13-Jul-23	12:15 PM	28℃	28 ℃	21 ℃
5.	14-Jul-23	12:45 PM	26℃	27 ℃	21 ℃
6.	15-Jul-23	1:10 AM	28℃	29 ℃	21 ℃
7.	16-Jul-23	1:10 AM	29℃	30 ℃	21 ℃
8.	17-Jul-23	5:05 PM	23℃	29 ℃	21 ℃
9.	18-Jul-23	5:05 PM	23℃	29 ℃	20 ℃
10.	19-Jul-23	4:40 PM	25℃	31 ℃	21 ℃
11.	20-Jul-23	12:10 PM	30℃	32 ℃	21 ℃
12.	21-Jul-23	5:10 PM	25℃	31 ℃	21 ℃
13.	22-Jul-23	5:05 PM	26℃	32 ℃	21 ℃
14.	23-Jul-23	4:30 PM	25℃	31 ℃	21 ℃
15.	24-Jul-23	1:10 PM	30℃	30 ℃	20 ℃
16.	25-Jul-23	3:10 PM	26℃	27 ℃	20 ℃
17.	26-Jul-23	5:55 PM	24℃	30 ℃	21 ℃
18.	27-Jul-23	1:00 PM	27℃	29 ℃	20 ℃
19.	28-Jul-23	1:00 PM	28℃	29 ℃	20 ℃
20.	29-Jul-23	12:50 PM	26℃	26 ℃	21 ℃
21.	30-Jul-23	2:10 PM	27℃	28 ℃	21 ℃
22.	31-Jul-23	2:00 PM	29℃	31 ℃	20 ℃
23.	1-Aug-23	5:30 PM	23 ℃	32 ℃	21 ℃
24.	2-Aug-23	5:30 PM	23 ℃	31 ℃	21 ℃
25.	3-Aug-23	5:30 PM	23 ℃	30 ℃	22 ℃
26.	4-Aug-23	5:30 PM	23 ℃	30 ℃	21 ℃
27.	5-Aug-23	5:30 PM	23 ℃	30 ℃	20 ℃

S.N.	Date	Time of gas measurement	Ambient Temperature at the time of gas measurement	Max. Temp.	Min. Temp.
28.	6-Aug-23	5:30 PM	21 ℃	25 ℃	20 ℃
29.	7-Aug-23	5:30 PM	22 ℃	28 ℃	20 ℃
30.	8-Aug-23	5:30 PM	22 ℃	26 ℃	20 ℃
31.	9-Aug-23	5:30 PM	23 ℃	22 ℃	29 ℃
32.	10-Aug-23	5:30 PM	23 ℃	21 ℃	30 ℃
33.	11-Aug-23	5:30 PM	23 ℃	22 ℃	31 ℃
34.	12-Aug-23	5:30 PM	22 ℃	21 ℃	27 ℃
35.	13-Aug-23	5:30 PM	20 ℃	19 ℃	23 ℃
36.	14-Aug-23	5:30 PM	21 ℃	19 ℃	25 ℃
37.	15-Aug-23	5:30 PM	22 ℃	19 ℃	31 ℃
38.	16-Aug-23	5:30 PM	22 ℃	20 ℃	29 ℃
39.	17-Aug-23	5:30 PM	23 ℃	21 ℃	32 ℃
40.	18-Aug-23	5:30 PM	23 ℃	21 ℃	32 ℃
41.	19-Aug-23	5:30 PM	24 ℃	22 ℃	32 ℃
42.	20-Aug-23	5:30 PM	24 ℃	21 ℃	32 ℃

Appendix B: Biogas Composition

Day 20	Content	
	%CH4	%CO2
B1	0.49	0.20
B2	0.26	0.21
B3	0.57	0.48
B4	0.34	0.40
B5	0.15	0.33
B6	0.23	0.39

Day 28	Content	
	%CH4	%CO2
B1	32.24	21.61
B2	36.02	16.75
B3	38.27	23.50
B4	39.70	17.63
B5	42.77	10.24
B6	45.36	7.67

Day 36	Content	
	%CH4	%CO2
B1	12.95	34.13
B2	29.53	29.17
B3	35.73	30.87
B4	20.82	7.75
B5	15.90	4.11
B6	18.63	3.41

Day 48	Content	
	%CH4	%CO2
B1	0.00	21.69
B2	15.71	31.26
B3	33.64	6.12
B4	12.47	3.94
B5	5.78	0.00
B6	10.16	2.95

Appendix C: Instruments Used

Fig: Thermocouple thermometer

Fig: Biogas Analyser

Fig: pH meter

Fig: Digital Weighing Scale

Fig: Wall Mounted thermometer

Fig: 1000ml Measuring Cylinder

Fig: Iron Stand with Clamp

Fig: Pneumatic Flow Control Valve

Fig: Water Trough

Appendix D: Lab Equipment

Fig: Muffle Furnace

Fig: KELDLUS-KELVAC-VA

Fig: Flame Photometer (ESICO-Model-1382)

Fig: Oven

Fig: Dessicator

Fig: Spectrophotometer (CT2400)

Appendix E: Photos

Co-digestion of Duckweed with Cow dung for Biogas Production

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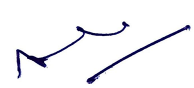

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Date: November 26, 2023

To Whom It May Concern:

This is to certify that the paper titled "***Co-digestion of Duckweed with Cow dung for biogas production***" (Submission# **546**) submitted by **Bibek Shakya** as the first author has been accepted after the peer-review process for presentation in the 14th 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.

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Co-digestion of Duckweed with Cow Dung for Biogas Production

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Abstract

The increasing demand for renewable energy sources necessitates innovative solutions to enhance biogas yields and sustainability. The study investigates the combined digestion of Cow Dung (CD) and Duckweed (DW), evaluating their biogas production capabilities. Through a comprehensive analysis of process parameters such as feedstock ratios, hydraulic retention times, temperature, pH levels, total solids, volatile solids and removal efficiencies this research aims to elucidate the optimal conditions for maximizing biogas production from the co-digestion process. The experiment conducted in this thesis concluded that after 60 days of observation involving batch production from four different mixture ratios and two controls it was found that the highest daily average and cumulative biogas yield were recorded for the mixture of 60% cow dung and 40% duckweed. These values were respectively 1.94 L/day and 116.64 L. This was followed up by the mixture of 40% cow dung and 60% duckweed whose respective values for daily average and cumulative biogas yield for a period of 60 days were 0.95 L/day and 56.79 L. The highest methane content was observed for 100% duckweeed batch which was 45.36%. The findings of this study contribute to the understanding of co-digestion dynamics and provide valuable insights for the development of efficient and environmentally friendly biogas production systems. As biogas continues to gain prominence as a clean energy source, the outcomes of this research have implications for sustainable waste management and energy generation practices, fostering a more resilient and eco-conscious future.

Keywords

anaerobic digestion, bio energy,downward water displacement method

1. Introduction

Nepal's primary energy consumption is predominantly driven by biomass, a non-commercial form of energy. Traditional sources such as fuelwood, agricultural residues, and animal waste continue to dominate the energy landscape. Nevertheless, there is a noticeable transition towards commercial energy sources like coal, petroleum products, and electricity, with a growing emphasis on renewable energy sources. The recent surge in electricity consumption is also noteworthy. The growth rate of energy consumption over the last decade is 4% [1]. In 2018, the country's energy consumption pattern indicated a primary energy consumption of nearly 14 Million Tons of Oil Equivalent (MTOE). Projections anticipate a steady growth with a Cumulative Annual Growth Rate (CAGR) of 3.8%, leading to an estimated 22 MTOE by the year 2030. [2]

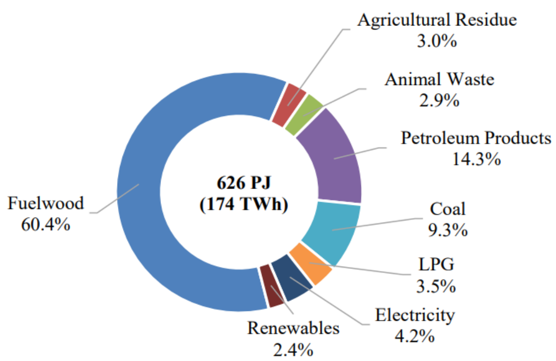


Figure 1: Energy Consumption by Fuel Types in 2021 [2]

According to the Waste Management baseline study conducted by the Central Bureau of Statistics (CBS) in 2020, each municipality, on average, generated approximately 1.2 kilo tons of organic waste. If the organic fraction of municipal solid waste (OFMSW) in Kathmandu is utilized, it can generate nearly 140,000 m^3 of biogas. This biogas production has the potential to fill up around 21,000 Liquefied Petroleum Gas (LPG) cylinders daily, resulting in significant savings amounting to hundreds of millions of rupees[2].

Being introduced for the first time in 1955 in Nepal, the Government of Nepal (GoN) officially launched the biogas programme in 1975. Following the launch of the Biogas Support Programme (BSP) with help from the Dutch government in 1992, this program gained additional traction in the nation. The Alternative Energy Promotion Center (AEPC) was founded in 1996 with the primary goal of informing the public and promoting the use of renewable energy technologies (RETs) in order to lift the rural residents' living standards, provide them with clean, sustainable energy, and stop environmental deterioration. With the assistance of the GoN, the German Development Bank (KfW), and the World Bank (WB), AEPC is implementing BSP. Despite being a well-established technology, the full potential of biogas utilization has yet to be realized. The limited capacity of biogas to meet household energy demands, especially in cold climates, poses a significant barrier to widespread adoption. Referred to colloquially as "Gobar gas," indicating "gas from cattle dung," the majority of household biogas systems in Nepal rely on cattle dung. The primary socioeconomic factors influencing biogas usage in Nepal are income levels and the extent of landholdings. In the hilly regions of the country, households encounter challenges in implementing biogas technology, highlighting the complexities associated with installing and operating such systems in mountainous terrain. The scarcity of biogas installation and maintenance service providers, coupled with a lack of accessible banking facilities, serves as a substantial hindrance to the widespread adoption of biogas in Nepal.

Lemna minor, commonly known as lesser duckweed or common duckweed, belongs to the subfamily Lemnoideae within the arum family Araceae [3].Duckweed exhibits rapid growth, characterized by a reproduction rate approaching exponential expansion at low plant density. Its growth rate is approximately 64 times greater than that of corn [4]. Duckweed can be used to produce methane, with a yield of nearly 400 mL of CH_4 gm^{-1} [5]. The problem addressed in this research is the lack of information on the optimization of biogas production using a mixture of cow dung and duckweed as a feedstock. Although biogas production from cow dung and other substrates has been extensively studied, there is limited information on the use of duckweed as a co-substrate in the AD process. Furthermore, there is no consensus on the optimal mixture ratio of cow dung and duckweed for biogas production.

2. Literature Review

2.1 Biogas production from cow dung

Cow dung is a widely available and inexpensive organic material that has been used for biogas production for many years. Numerous studies have explored the use of cow dung for biogas production and found that it can produce a high quantity of biogas with a high methane content. Biogas production efficiency was highest with cow dung, followed by ship manure demonstrating moderate effectiveness, and pig manure exhibiting comparatively lower efficiency. [6]. A study on the generation of biogas from cow dung in Nigeria concluded that cow dung has the potential to serve as a viable substrate for biogas production. Implementing such production on a commercial scale could not only offer an alternative energy source but also serve as a method of waste disposal[7].

Research has shown that incorporating both cow dung and food waste can enhance the efficiency of biogas production. The findings of this study indicate that the combined waste slurry yields a greater volume of gas compared to cow dung slurry alone, attributing this difference to the higher nutrient content present in food wastes [8].

Among various mixtures and controls, the dry co-digestion of cow dung and pig manure in the ratio of 60%:40% demonstrated superior methane yield and exhibited the highest efficiency[9]. The experimental results showed that this co-digestion resulted in higher methane yields (as high as 18%), higher VS removals (as high as 13%) & greater Chemical Oxygen Demand (COD) degradation (as high as 13%). Hence, co-digestion of the said substrates in the said ratio resulted in positive synergism.

2.2 Biogas production from duckweed

Due to its rapid growth rate and nutrient richness, duckweed proves to be an excellent substrate for biogas production. Several studies have shown that duckweed can produce biogas with a high methane content. Abundant in protein and starch while possessing a low lignin content, duckweed emerges as an excellent biomass feedstock for energy production [10].

One research investigation [11] presents findings on anaerobic digestion processes within bioreactors utilizing composite mixtures composed of initial and residual biomass from *Lemna minor* duckweed, along with additives such as inoculum in the form of manure, food waste, and spent sorbents. The objective was to determine the biogas potential, including biogas volume and methane content. According to the study, loading the bioreactors with equal amounts of primary and residual biomass from *Lemna minor* duckweed revealed that the specific biogas yield from the residual duckweed biomass was slightly lower compared to the primary biomass. Notably, the methane content in the biogas from the primary biomass was high by almost 1.5 times.

2.3 Biogas production from cow dung and duckweed as co-substrates

Cow dung and duckweed are two feedstock that have been studied for biogas production, and their mixture has been reported to enhance biogas yield and quality. However, there is a gap in knowledge regarding the optimal ratio of duckweed and cow dung for biogas production, as well as the effect of varying ratios on biogas composition. This literature review aims to provide a comprehensive overview of the previous studies that have investigated the comparative analysis of biogas yield and composition from varying ratios of duckweed and cow dung in anaerobic digestion.

Conducting batch-type anaerobic digestion for 55 days at 37°C, the co-digestion of duckweed (DW) with cow dung (CD) in different ratios (DW:CD = 90:10, 75:25, and 50:50) revealed cumulative biogas production was highest for the batch with mixture ratio of 50:50. This was approximately 12L. Furthermore, the methane content in the biogas generated from co-digested feedstock was comparable to that from cow dung alone.[12].

In a separate study, the cumulative biogas production was investigated for different mixtures, similar to the above study (DW:CD = 100:0, 75:25,50:50, 25:75 & 0:100). The findings underscore the promising potential of duckweed as a valuable feedstock for biogas production, with optimal results observed when co-digesting the plant with cattle manure at a ratio of 25% to 75% [13].

The study [14] investigated the improvement of biogas production in laboratory-scale anaerobic digesters by using duckweed & poultry manure as co-substrates. The authors emphasize iron's significance as the primary micronutrient for anaerobic bacteria. Duckweed, with a substantial iron content was found to significantly impact the rate of decomposition without affecting the overall biogas production volume, as per the authors. This effect was specifically linked to duckweed's elevated iron content, a conclusion corroborated by subsequent semi-continuous experiments.

The literature review thus shows that both duckweed and cow dung have the potential to produce biogas through anaerobic digestion but it also indicates that there is a research gap in the study of biogas production from a mixture of cow dung and duckweed. Although several studies have explored the use of cow dung and other co-substrates, such as agricultural waste and food waste, there is limited information on the application of duckweed as a co-substrate in the AD process. The optimal ratio of duckweed and cow dung for biogas production and the effect of varying ratios on biogas yield & composition remain unclear. Therefore, this research will aim to fill this research gap by conducting a comparative analysis of biogas yield and composition from varying ratios of duckweed and cow dung in AD.

3. Methodology

3.1 Experimental Setup and Procedure

Cultivated duckweed was collected from the man-made ponds constructed inside the premises of Institute of Engineering, Pulchowk Campus, Lalitpur, Nepal. Cow dung was purchased from "K.C. Cow Firm" situated at Bagdol, Lalitpur, Nepal. Cow dung (CD) and Duckweed (DW) were then thoroughly mixed in varying ratios viz. 100% CD, 80% CD + 20% DW, 60% CD + 40% DW, 40% CD + 60% DW, 20% CD + 80% DW & 100% DW. Digested slurry/effluent was collected from a household biogas plant that used cow dung as substrate & this slurrywas used as fresh inoculum. The plant was present in the living quarter premises of Pulchowk Campus.

Batch	Mass (in kg) of				Total mass (m)=a +b+c	Total volume to be filled (litre) =0.7*20
	Cow dung (a)	Duck weed (b)	Water (c)	Inoculum (d) = 0.1 × (a+d)		
B1 (100% CD)	6.3	0	7	0.7	14	14
B2 (80% CD + 20%DW)	5.04	1.26	7	0.7	14	
B3 (60% CD + 40% DW)	3.78	2.52	7	0.7	14	
B4 (40% CD + 60% DW)	2.52	3.78	7	0.7	14	
B5 (20% CD +80% DW)	1.26	5.04	7	0.7	14	
B6 (100% DW)	0	6.3	7	0.7	14	
Total	18.9	18.9	42	4.2	84	

Figure 2: Mixing ratios of cow dung, duckweed, water and inoculum in different batches

According to [15],to ensure effective solubilization of organic content, maintaining a solid-to-water ratio of 1:1 on a unit volume basis (i.e., equivalent volumes of water and solid) is recommended when utilizing domestic wastes. However, in the case of dry dung, which requires crushing before being introduced into the digester, the water quantity must be adjusted accordingly to achieve the desired input consistency. For instance, the ratio may vary from 1:1.25 to even 1:2 depending on the specific requirements.

The mixing ratios of CD, DW, water and inoculum for these six mixtures was done as shown in the Figure2. Here, the mass of inoculum used was kept as 10% of the total mass of the substrate. The mass of each component was measured using a digital weighing scale (DT 510). These mixtures were kept in six different batch reactors (20L water jars) which were named B1, B2, B3, B4, B5 and B6 respectively. The lids of these water jars were sealed tight using a stainless-steel hose clamp. A hole was drilled at the neck of the jars & a pneumatic flow control valve was fixed on the hole. A clear transparent gas pipe was connected to the valve for gas outlet. The valve was further sealed using M-seal. These mixtures were then stored at room temperature and biogas yield were kept under observation for the following days. The jars were shaken manually each day after taking the measurement so as to ensure proper mixing of substrates.

Parameters	Batch					
	B1	B2	B3	B4	B5	B6
Initial Temperature of Substrate/Slurry (°C)	30	29	31	30	29	29
Initial pH of slurry (Before AD)	5.5	6.2	6.5	6.3	6.1	6.6
Final pH of slurry (After AD)	6.1	7.9	7.8	8.0	8.1	7.9
TS (% Dry Basis) of dry mixture sample (Before AD)	17.72	18.52	14.61	6.51	10.06	5.30
VS (% of TS) of dry mixture sample (Before AD)	79.88	77.78	76.43	73.09	79.12	71.61
TS (% Dry Basis) of dry mixture sample (After AD)	6.06	3.5	3.09	1.77	1.24	0.90
VS (% of TS) of dry mixture sample (After AD)	82.90	78.62	72.52	64.56	58.88	62.73
C/N Ratio of dry mixture sample	27:1	28:1	30:1	32:1	33:1	34:1
Organic Carbon (Before AD)	44.38	43.21	42.46	40.61	43.96	39.78
Organic Carbon (After AD)	46.06	43.68	40.29	35.87	32.71	34.85
Also, pH of inoculum used = 5.9 & pH of water used for mixing = 6.5						

Figure 3: Values of different parameters as measured, tested and/or calculated

3.2 Measurement of Biogas Volume

The daily biogas volume obtained from each reactor was measured using the downward water displacement method. A 1000ml measuring cylinder was used and as such the volume measurement was done in multiple steps by controlling the flow of gas with the help of the pneumatic control valve.

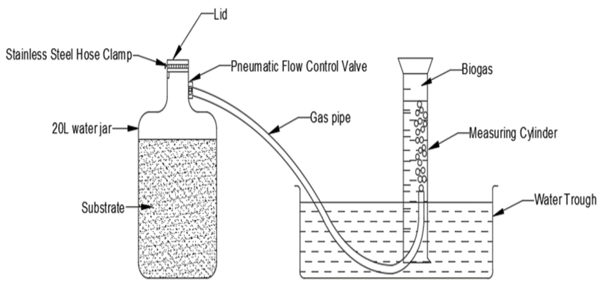


Figure 4: Schematic Diagram of the Experimental Setup

3.3 Measurement of Biogas Composition

The biogas composition which includes CH_4 %, CO_2 %, O_2%, H_2S in ppm & CO % as obtained from all six reactors were measured using Biogas Analyser (GASBOARD 3200 Plus). The readings were taken in an interval of not more than 10 days starting from Day 20. (Day 20 refers to the 20^{th} day from the start of experiment)

3.4 Measurement of Temperature

The initial temperatures of the feedstocks were measured using a thermocouple thermometer (FLUKE 53II B). The daily ambient temperature was measured using a wall mounted thermometer.

3.5 Measurement of pH

The initial & final pH of the substrate as well as the pH of inoculum & mixing water were measured using a pH meter (pHep Pocket-Sized pH Meter-HANNA Instruments).

3.6 Measurement of Total Solids, Volatile Solids and Carbon-Nitrogen Ratio

Other parameters which include Total Solids (TS), Volatile Solids (VS) and Carbon/Nitrogen Ratio (C/N Ratio) were measured at Soil, Water and Air Testing Laboratories Pvt. Ltd. (SWAT lab) situated at Baluwatar, Kathmandu using standard methods.

3.7 Calculation of Carbon Content

The feedstock's organic carbon (OC) content was calculated from the data of volatile solids using an empirical equation given by [16] as:

$$\% \text{ Carbon } = \frac{\%\text{VS}}{1.8} \tag{1}$$

Where, VS = Volatile Solids

4. Results and Discussion

4.1 Evaluation of Daily and Cumulative Biogas Yield

The daily biogas yield and cumulative biogas yield from all the six batch reactors were plotted against the corresponding days and the results were obtained as seen in the following curves:

4.1.1 Control Batch B1

The biogas production per kg of cow dung per day can be calculated as:

$$\text{Biogas production per kg of cow dung per day} = \frac{\text{Cumulative production}}{\text{Amount of cow dung used } \times HRT} \tag{2}$$

Where, HRT= Hydraulic Retention Time

$$= \frac{14.053}{7 \times 60} = 0.034 \frac{\text{L}}{\text{kg}} / \text{ day} \tag{3}$$

First and foremost, the amount of biogas produced from 100% CD in this experiment is far below what is generally obtained. According to [9] biogas production per kg of cow dung ranges from 0.023 to 0.04 m^3/kg i.e., 23L/kg to 40L/kg for a retention time of 40-100 days. i.e., standard biogas production per kg of cow dung per day (assuming an HRT of 70 days) ranges from 0.328 L/kg per day to 0.571 L/kg per day. So, the obtained figure is one tenth of the minimum standard value.

It is important to analyse the possible reasons for the low production obtained from 100% CD in this experiment. Since the ambient temperature for all the mixtures were same throughout the experiment, we can rule out the effect of

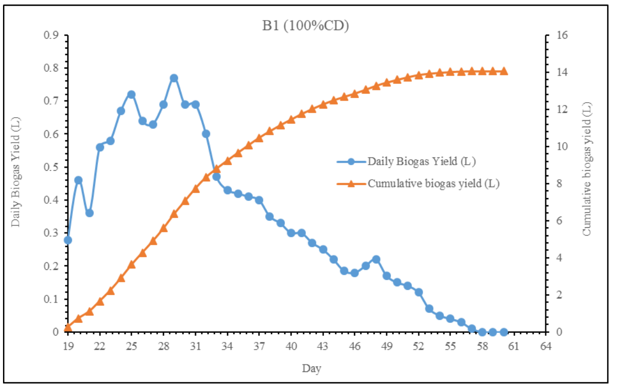


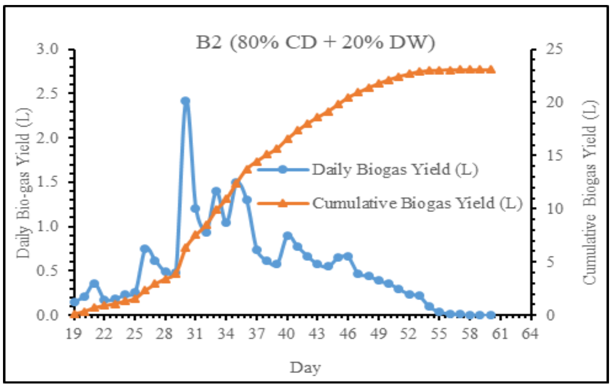
Figure 5: Daily Biogas Yield (L) & Cumulative Biogas Yield (L) vs Day for B1

temperature. As suggested by [17] design parameters for sizing if a biogas plant involves pH value within a range of 6 to 7. According to [18],pH of fresh cow manure was 8.5 and that of manure compost was 7.4. So, the pH clearly seems to be the major factor behind the substantial drop in biogas production from 100% CD as it was recorded at a value of just 5.5 at the beginning of the experiment. The acidic condition lowers down methane formation.

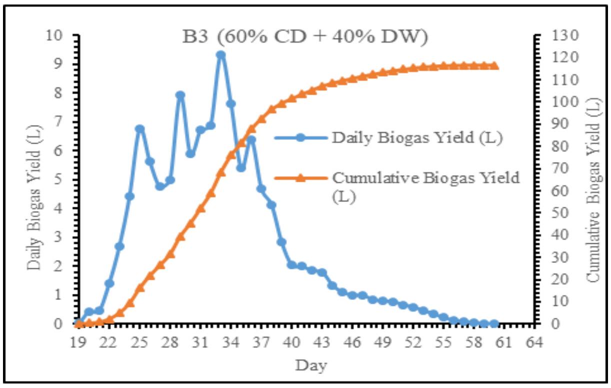
Additionally, substantial quantities of organic acids are generated by acid-forming bacteria in the initial fermentation phase, leading to a decline in the digester's internal pH. This hinderance or cessation of the digestion/fermentation process occurs because methanogenic bacteria are highly pH-sensitive and struggle to thrive when the pH falls,(specifically when below 6).[15].

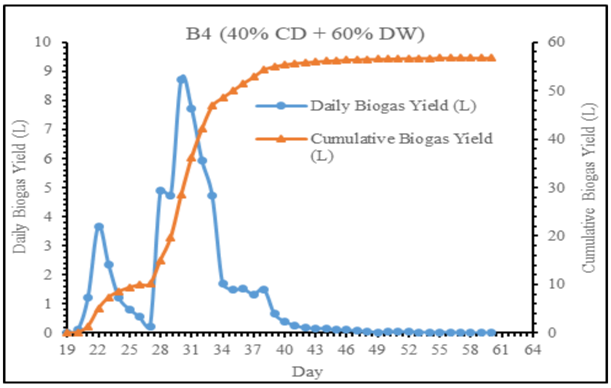
The quality of water used for mixing might also have a role to play for this as it was collected from the nearby local source and its pH was found to be 6.5 which is again in the acidic range. According to [19], the lower production from 100% CD may be attributed, in part, to the partial fermentation that commonly occurs in the animal's intestinal tract. Conversely, the increased production from the mixtures might be a result of a balanced nutrient composition, enhanced buffer capacity, and a diminished impact of toxic compounds, stemming from the amalgamation of substrates[20],[21].

4.1.2 Mixture Batches B2 to B5



For the batches B2 to B5, where cow dung and duckweed were mixed in varying ratios, the graphs of daily biogas yield vs day are patterned quite similarly. Biogas production follows a kinetic pattern where it starts slowly, increases to a peak, and





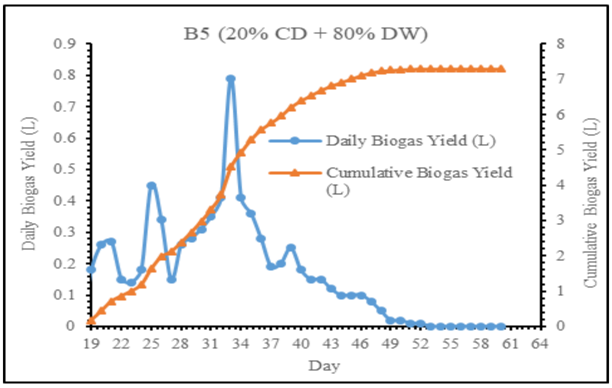


Figure 6: Daily Biogas Yield (L) and Cumulative Biogas Yield (L) for mixture batches B2 to B5

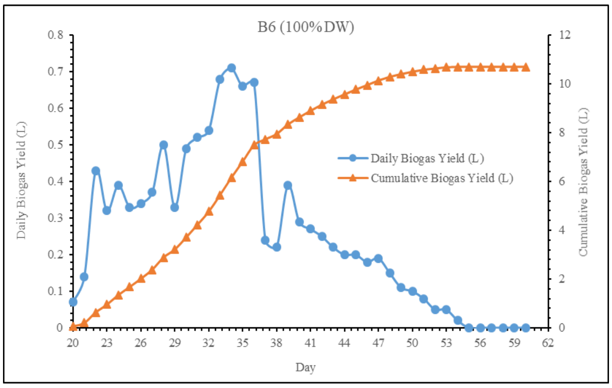


Figure 7: Daily Biogas Yield (L) & Cumulative Biogas Yield (L) for control batch B6

then gradually tapers off as the digestion process completes. The biogas production increases very slowly and gradually during the initial phase (first 3 to 4 days); with the exception of B3 where this is rather swift and continues up to the 7th day. Then the daily biogas yield shows a zig-zag pattern with multiple peaks and troughs.

The data obtained also indicates that the maximum daily yield from all four of these batches occurred during the period from 30^{th} to 33^{rd} day. This suggests probable increase in overall microbial community & its activity during this period. This result is also supported by the results of[12] where peak yield values for samples from mixing of CD & DW in 10%:90%, 25%:75% and 50%:50% ratios were obtained on the 35^{th} day.

Another similar research by [13] involved using five 0.6 L plastic bottles as batch digesters for co-digestion of CD and DW. This team used DW: CD in 75:25, 50:50 & 25:75 ratios along with 100% CD and 100% DW as controls.Daily biogas produced from the mixtures of varying ratios peaked during a period of 14-22 days which is not in accordance with the present experiment's peak days. However, the end results of the two experiments do tend to concur as [13] concluded that the ratio of CD & DW at 75% :25% gave the highest cumulative biogas yield.

The minimum yield so far has been obtained on the 57^{th} day batches B1 to B2 (towards the end), 59^{th} day for batch B3 (towards the very end) and 52^{nd}day for batch B5(quite early). This can be attributed to the fact that biogas volume is obviously very low towards the end of the AD process. This result is supported by the aforementioned literatures [12][13] .

4.1.3 Control Batch B6

The biogas production for B6 started a day later than that for the rest of the batches which is an interesting observation. This follows the finding from [22] which states that duckweed being a lignocellulosic biomass needs more time to breakdown into more labile carbon moieties which then subsequently gets converted into precursor for methanogenesis. Like for the mixture batches, the daily biogas yield from B6 also follows a similar pattern of initial rise for the first 3 days followed by zigzag pattern in between (which spans from the 3^{rd} to the 39^{th} day) and eventually a phase of gradual decline.

4.2 Process Stability and Consistency

Thus, the initial 18-19 days showed no significant biogas production. This suggests that the microbial community needed more time to acclimatize or adapt and establish itself in the digester. The reason behind this might be the initial pH of the substrates as all of them were either below the favourable pH value (for B1) or towards the lower end (for B2, B4 & B5) as a pH value between 6 & 7 is considered ideal for maximum methane production. The maximum yield, both daily average and cumulative, obtained from B3 also conforms with the pH criterion as its pH was 6.5, which is the most favourable among all the batches in this experiment.

According to [23] the availability of biodegradable material is more in DW than in CD. [21] also states that biogas production is a directly dependent on the amount of organic content of the feedstock and its biodegradability. However, the biogas yield from 100% CD in this experiment still exceeded the yield from 100% DW. This was most probably due to the fact that the percentage of VS from TS content of DW slurry was 71.61% whereas that of CD slurry was 79.88%. This implies that in comparison to cow dung used, the duckweed used in the experiment actually had lesser fraction of biodegradable mass. In addition, the organic content or carbon content of cow dung used exceeds that of duckweed used in this case as OC of CD = 44.38 & OC of DW = 39.78.

As the percentage of duckweed (DW) in the mixing ratio increased beyond 40%, there was a decrease in the cumulative biogas yield, aligning with findings from [12]. This observation is consistent with the results of an experiment conducted by [24]. This trend could be attributed to the elevated concentration of total nitrogen (ammonia) resulting from the anaerobic breakdown of proteins, which has the potential to inhibit anaerobic digestion [25]. Consequently, the co-digestion of cow manure (CM) and duckweed (DW) was found to be more effective and/or efficient when the proportion of DW did not exceed 40%.

Conversely, the notably low biogas production in batch B5 (80% cow dung and 20% duckweed) could be attributed to the presence of lignin and intricate molecules. These components require an extended duration for hydrolysis, thereby failing to offer adequate precursors to methanogenic bacteria consequently hindering microbial growth and impeding the process of methanogenesis.[26].

4.3 pH and Organic Carbon

The initial pH values of all six slurry samples fell within the range of 5.5 to 6.5. The pH value of 100% CD slurry was 5.5 which is well below the optimal pH value of 6 to 7 as suggested by [17] & is hence highly unfavourable for biogas production. Owing to this, the biogas yield might have been very low from this batch (B1). [13] stated that the pH value of 100% DW was 6.03 which is comparatively less optimal and in line with [27] & [28]. However, the initial pH value of DW used in the present experiment was 6.6 (within optimal range) yet was not able to produce much biogas as compared to other substrates. Slurry samples from the mixture batches showed a rise in pH value when compared to 100% CD but less than that of 100% DW. This suggests that co-digestion serves as an effective method for adjusting the pH value to the optimal level[13].

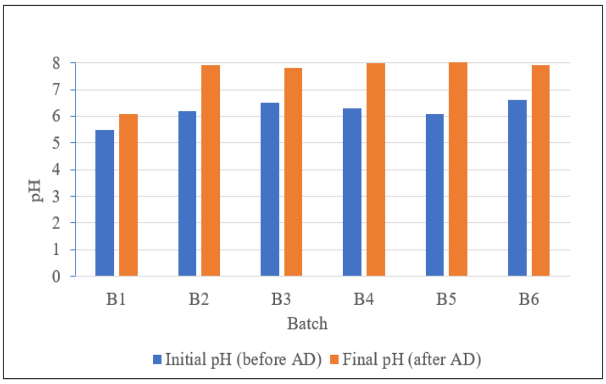


Figure 8: Initial & Final pH of Slurries

4.4 Temperature

The experiment was performed in a lab situated inside the Pulchowk Campus premises (27.6811° N, 85.3185° E). It was carried out during the summer/monsoon season, precisely from the month of June to August. The ambient temperature condition was thus on the favourable range for biogas production as mesophilic temperature range was achieved during this season. As this season was accompanied by frequent rainy days the outside ambient temperature would drop to a value as low as 21°C; however, the inside ambient temperature of the lab was mostly on the warmer side. The roof structure of the lab where the batch reactors were kept were made of tin thus absorbing much heat from the sun and this too might have helped the case. The minimum ambient temperature recorded during gas measurement was 21°C & the maximum temperature recorded was 33°C.

4.5 Total Solids, Volatile Solids and Percentage Removal

The initial TS of all dry samples (10gm) fell within the range of 5.3% to 18.52%. As per [15], the dilution should be adjusted to keep the TS within the range of 5 to 10%. Excessive dilution may cause solid particles to settle at the digester's bottom, while overly concentrated slurry can hinder the flow of gas. Going by this rule, the slurry prepared for batches B5 (80% CD + 20% DW) & B6 (100% DW) apparently seem to be a case of over dilution keeping in mind TS values of dry samples of B5 and B6 substrates are already 10.06% and 5.3% respectively. If it is the case, then it stands out as the major reason for low biogas yield from these batches.

The VS before AD ranged from 71.61% to 79.88%. The VS determined for dry duckweed and cow dung samples were 71.61% and 79.88% respectively. This result confers with [20] whose report mentions that VS in animal and human wastes lies precisely in the range from 77% to 90%. After completion of AD, the final TS and VS of all six substrates were determined and then the percentage of TS removal & the percentage of VS removal was determined using the following formulas:

$$\text{TS removal } (\%) = \frac{TS_i - TS_f}{TS_i} \times 100 \tag{4}$$

Where, TS_i=Initial Total Solid (TS before AD) &
TS_f= Final Total Solid (TS after AD)

$$\text{VS removal } (\%) = \frac{VS_i - VS_f}{VS_i} \times 100 \tag{5}$$

Where, VS_i= Initial Volatile Solid (VS before AD) &
VS_f= Final Volatile Solid (VS after AD)

TS and VS reduction are effective indicators for assessing the efficiency of AD, as highlighted by [29]. Furthermore, these parameters serve as reliable indicators of biogas production, as emphasized by [30].

4.6 Evaluation of Biogas Composition

The figure 10 as shown below, represents the methane concentration profile of the biogas from different ratios of feedstock in all the six batch reactors. The methane content was measured using a biogas analyser (GASBOARD 3200 Plus). The highest methane content of 45.36 % was observed for the

Batch	Initial TS (%)	Final TS (%)	TS Removal (%)	Initial VS (% of TS)	Final VS (% of TS)	VS Removal (%)
B1	17.72	6.06	65.80	79.88	82.9	9.13
B2	18.52	3.5	81.10	77.78	78.62	11.65
B3	14.61	3.09	78.85	76.43	72.52	8.93
B4	6.51	1.77	72.81	73.09	64.56	3.62
B5	10.06	1.24	87.67	79.12	58.88	7.23
B6	5.3	0.9	83.02	71.61	62.73	3.23

Figure 9: TS Removal (%) and VS Removal of all six batches

batch B6 i.e., 100% DW followed by B5, B4, B3, B2 and B1. This is in alignment with the C/N ratio values of the substrates as duckweed had the highest C/N ratio followed by the mixtures with decreasing content of duckweed.

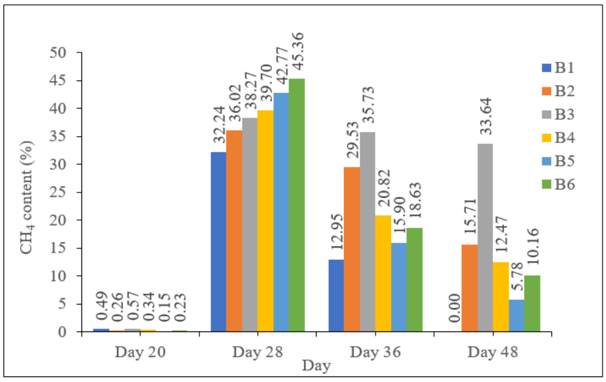


Figure 10: Graph of CH4 content % vs Time (days) for all batches

The inference "Higher the C/N ratio, higher the methane yield" is further conformed by [12]where CD had higher C/N ratio (22.7) than DW (5) and consequently gave higher methane yield.

5. Conclusion

The experiment performed during this research demonstrated the huge potential that duckweed has in terms of biogas production especially when used as a co-substrate with cow dung. Based on the daily average & cumulative biogas production for a period of 60 days, the optimal mixing ratio for cow dung to duckweed has been found to be at ratio of 60%:40%. Similarly, the maximum average methane yield was also obtained from this batch.

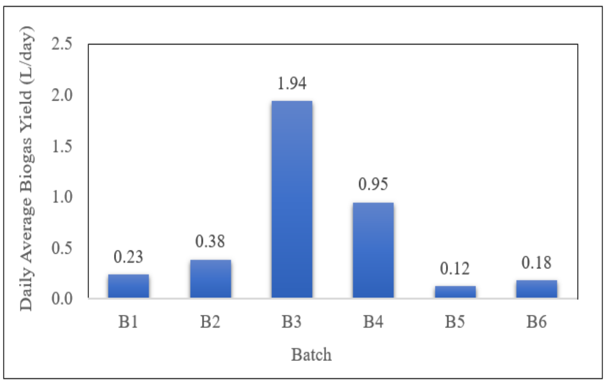


Figure 11: Daily Average Biogas Yield (L/day) of All Batches

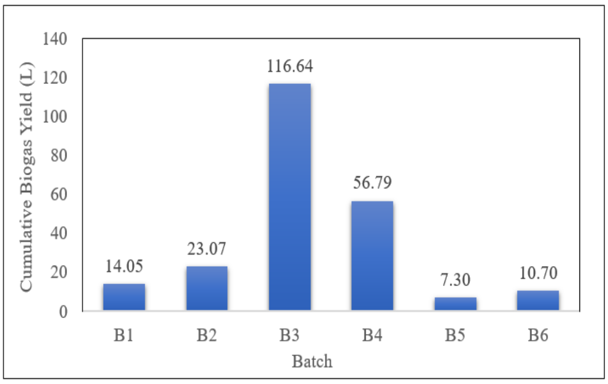


Figure 12: Cumulative Biogas Yield (L) of All Batches

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