



# Biochemical Methane Potential Assessment by Anaerobic Digestion of Locally Available Grasses of Phuentsholing, Bhutan

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## Authors' contributions

This work was carried out in collaboration among all authors. Author YC and SZ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ST, TG, KP and SJ' managed the analyses of the study. Author TCG managed the literature searches. All authors read and approved the final manuscript.

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## ABSTRACT

Renewable energy is not only environmental friendly but also promotes sustainable development. Biogas being one of the abundantly used renewable resource, the enhancement and optimization of the yield of biogas can help in reduction of dependence on imported fuel. Biochemical methane potential (BMP) assessment of grass will determine the production of methane (CH<sub>4</sub>) from this substrate through the process of anaerobic digestion. After determining the parameters such as pH, Biochemical Oxygen Demand (BOD) and Total solids (TS) of three types of local grasses known as Basil, Bermuda and Napier, that affects the production of biogas, Napier grass resulted with the highest potential to produce CH<sub>4</sub> gas. Batch and continuous reactor method under mesophilic condition was adopted. The composition of biogas from continuous reactor was obtained using a biogas analyzer (Biogas 5000 Geotech), from which 30.8% of CH<sub>4</sub>,

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8% of CO<sub>2</sub> and other inert gases were found. Also, methane to carbon dioxide (CH<sub>4</sub>: CO<sub>2</sub>) ratio of 3.81: 1 approximately (80% - 20%) was achieved. Moreover, the batch reactor method showed that 1L Napier grass silage would yield 0.81L of biogas. The concentration of CH<sub>4</sub> gas from Napier grass in hydraulic retention time as short as 20 days was very significant. This study shows that Napier grass can be used as an alternative sustainable source of energy in the country which can improve resource constraints.

*Keywords: Biochemical methane potential; hydrolysis; anaerobic digestion; retention time.*

## 1. INTRODUCTION

With the increase in demand and consumption of energy and increase of environmental issues, renewable energy has played an important role enormously. Conversion of waste into energy has abundant scope as a renewable source in future. Anaerobic digestion is a process which gives biogas as an end product, this process is one of the strategies to manage wastes and also helps in the reduction of production of greenhouse gases such as CO<sub>2</sub>. Most of the countries have adopted anaerobic digestion for the production of biogas mainly for domestic purposes like cooking, heating, etc., as this method is cost-effective and environmental friendly [1]. Even in Bhutan, many people living in rural areas preferred biogas production to fulfill their energy needs due to hardships faced in collecting firewood from the forest. In 2017, 121 LPG cylinders were imported from India, which were distributed to rural households in Nangkor gewog, Bhutan. None of these households had ever used LPG cylinders before. Similarly, more than 125 people from villages in Phuentshopelri (Gomtu) gewog, acquired LPG cylinders for the first time in their lives. Only 63 percent of rural households use imported LPG. The remaining depend on firewood for cooking which affects health and time use, as people have to search for firewood in the forests [2]. Bhutan has over 4000 biogas plants, Trashigang dzongkhag being the most popular in biogas production. However, the biogas was produced by fermenting cattle dung and there was no sign of having an alternative source being used for the production of biogas in Bhutan [3]. The biogas produced contains usually 50-65% methane, 35-50% carbon dioxide [4]. BMP assessment is a common method to determine the potential of CH<sub>4</sub> and biodegradability of wastewater and any waste biomass. Studies have shown that anaerobic digestion of locally available agro-wastes like coconut cake oil, cashew apple waste, and grass from lawn cuttings was done, in which

grass gave the most productive result in terms of yield [5].

In Thailand, a research was conducted on biogas production by using grass silage from different types of available grasses.

Anaerobic digestion of the various grass silage was done and the variation in the biogas yield from different grass substrates was observed. It was concluded that Napier grass had a higher affinity to be used as feedstock and had comparatively higher efficiency to yield biogas. The study showed that Napier grass possessed a higher content of organic substance which enables it to ferment faster and favor the anaerobic digestion [6]. Sawasdee & Pisutpaisal, conducted a study to determine the feasibility of biogas production from Napier grass. The Napier grass was cut and was fermented using mesophilic batch reactors. Three samples of Napier grass with varying solid concentrations (i.e., 5%, 10% and 15%) were prepared and optimum condition was determined. It was found that 5% solid concentration yielded the maximum CH<sub>4</sub> of 54% (i.e., 122.4ml CH<sub>4</sub>/g) and the rate was 4.8ml/hr [7]. Bermuda is also one of the tropical grasses abundantly available in most parts of the world. It holds numerous names like wiregrass, finger grass and Yuma. The grass spreads by its rhizomes and stolons. The seeds are used to produce and reproduce the hybrid Bermuda grass which has better quality in terms of the nutrients [8].

The study area chosen for the current study is located in Rinchending; Phuentsholing, the southern part of Bhutan where local grass such as Basil, Bermuda and Napier grows abundantly. They are most commonly grown and considered as a major fodder crop in Bhutan due to their low water requirement and high nutrient content. It can be cultivated in any kind of land and abundantly found in many parts of the country [9].

Bhutan being a small landlocked country has to heavily depend on the neighboring country

India for the importation of fuel supplies to meet the energy demands [10]. Such research-based exploration of energy potential from locally available grass has not been performed till date. This study aims to assess the Biochemical Methane Potential of three most locally available types of grasses known as Basil, Bermuda and Napier grass, their feasibility of grass as a biogas substrate in Bhutan is determined. In this study the mesophilic temperature for the batch reactor was maintained by water bath and control has been used while conducting BMP analysis which was found limited in the literature. The results from this study intend to provide a baseline for policy implementers and stakeholders to look upon alternative sources of energy in Bhutan.

## 2. MATERIALS AND METHODS

Determination of parameters such BOD, TS and pH were performed experimentally following standard procedure [11,12]. Readings for BOD was taken using BOD sensor-6 and for biogas composition, the daily gas production was measured from the manometer connected to the digester by coke displacement and collected the discharged gas in an air bag and then measured the composition of gas by an Geotech biogas- 5000.

The methodology developed was based on the analysis and assessment of BMP of grass. In this assessment, the substrate used is selected Napier grass silage as the feedstock for the bacteria. For the BMP test, two methods were adopted, a batch reactor and a continuous reactor. During this CH<sub>4</sub> and CO<sub>2</sub> were produced due to anaerobic digestion. The CH<sub>4</sub> produced from the substrate was then measured and the CH<sub>4</sub> potential of the substrate was known. The methodology of this study is mainly divided into four parts which are 1) Pre-field work, 2) Characterization, 3) BMP assessment and 4) Analysis of the result.

### 2.1 Pre-Field Work

Three types of grass namely Basil, Bermuda and Napier were chosen for the characterization. Silage of the grasses was prepared and pulverized about 3mm in size. To select the grass silage that would give the maximum yield of CH<sub>4</sub>, parameters such as pH, total solids and BOD were determined. The fermentation was set up in three containers for three

different grasses, 200g of each of the grasses were taken and 500 ml of water was added in the air-tight containers. It was observed that the grass was fermented in 7 days without any pretreatment. The laboratory scale-anaerobic digesters used in this experiment were operated at room temperature. The pH value for the sample that was collected was mostly acidic i.e., they are below pH 7. Fig. 1 shows a decrease in pH, which concludes that during hydrolysis the solution becomes acidic. For optimizing biogas production and to maintain anaerobic stability, a base was needed to decrease the acidity and make the sample neutral. Substrates in the digester were measured by pH meter every once in two days to know pH profile daily.

Fig. 2 and Table 1 shows the high content of TS & BOD (mg/l) in the sample indicating the presence of a higher number of micro-organisms and organic matter. The organic matter (bacteria) breaks down the organic compounds of the silage (feedstock) and produces CH<sub>4</sub>, also higher BOD results in higher production of biogas [13]. With the characterization and determination of parameters that influence the biogas production [14], it was concluded that Napier grass silage with a pH of 4.59, least Total Solids of 4020 mg/l and the maximum BOD of 342.93 mg/l was expected to give the optimum rate of CH<sub>4</sub> production.

Napier grass was selected as a potential substrate, with which the sludge was used as an inoculum collected from a lagoon in the Phuentsholing city sewage treatment plant. Napier grasses were pulverized into a size of approximately 3 to 5mm and sun-dried, feedstock preparation is also included in the pre-field work and the Napier grass silage was prepared by 1:5 ratio of grass: water. Napier Grasses can undergo pre-treatment with different methods which include alkaline treatment, dilute acid treatment and two-stage treatment. Treatment of substrate resulted in 43.2 % cellulose followed by the 23.2% of hemicellulose. The percentage of the cellulose was increased to 63.2% due to the removal of the hemicellulose after the alkaline treatment [15]. To speed up the hydrolysis process of the substrate, that is to speed up the fermentation and decay of grass, Ca(OH)<sub>2</sub> was used. This pretreatment is done basically to serve two purposes, 1) to ease the hydrolysis process in which the lignocellulose content that is present

in the substrate breaks down into smaller molecules like amino acids and fatty acids and 2) to raise the pH which maintains the anaerobic stability, since the methanogens cannot survive in acidic nature [16].

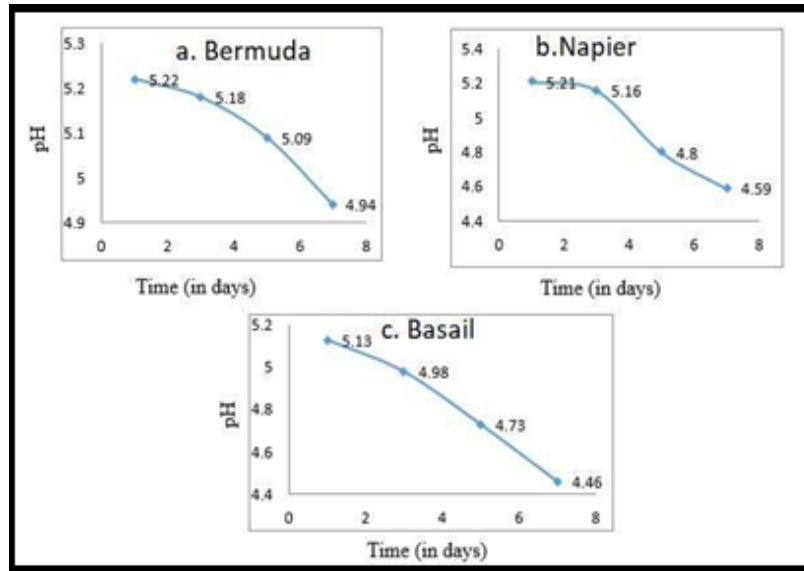
**2.2 Characterization**

To ease the hydrolysis process pretreatment was done by using Ca(OH)<sub>2</sub> and pH was raised to 8.5 [17]. Napier grass silage was kept for different durations in airtight containers i.e., 24 hours, 48 hours and 72 hours and properties such as pH and total solids were determined.

Fig. 3 contains the pH values of silage that was kept for different durations. It mainly showed that during hydrolysis the biomass becomes acidic. The decrease in pH has

been recognized as volatile fatty acid accumulation as a result of digester overloading [18].

Table 2 shows that the sample that was kept for 72 hours had the maximum content of total solids. BOD test was also conducted for the silage kept for 72 hours since it had the maximum content of total solids. The total average BOD value of the 72-hour mixture of silage and the bacteria obtained was 4847 mg/l. The BOD of the mixture has significantly increased due to the pretreatment which otherwise the silage had only 341.3mg/l without pretreatment. This indicates that the value of BOD is within the range of the that can produce significant quantities of biogas as shown in Table 3.



**Fig. 1. pH of three grass silages**

**Table 1. BOD using DO meter**

Sample Name	Vol. of sample (ml)	Consumed DO for 5 days (Initial - Final) DO	BOD (mg/L)
Napier	6	6.43	341.3
	15	6.04	
	30	6.97	
Bermuda	6	5.58	321.3
	15	6.48	
	30	7.34	
Basil	6	5.91	314.7
	15	5.79	
	30	6.08	

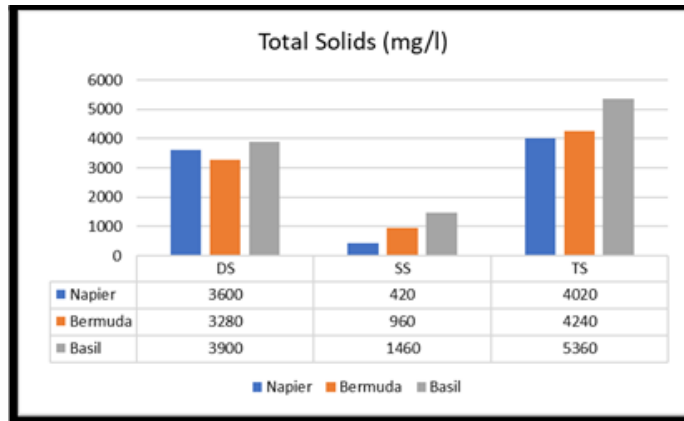


Fig. 2. Total Solids of three grass silages

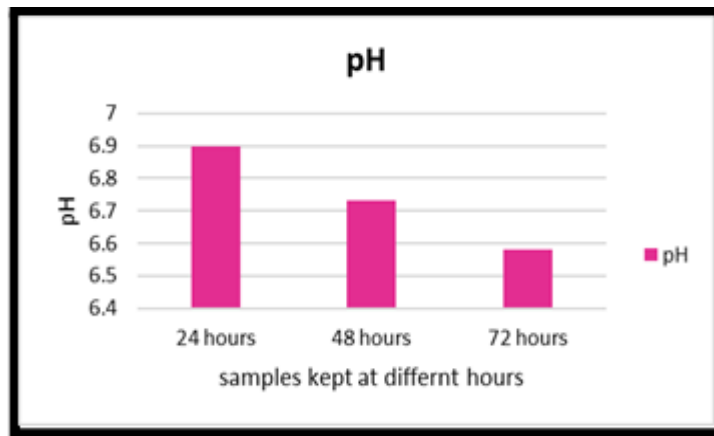


Fig. 3. pH of silage at different duration

Table 2. Total solids of silage at different duration

Samples kept for	TSS (total suspended solids) (mg/l)	Ts (total solids) (mg/l)	Dissolved solids (mg/l)
24 hours	600	2320	1720
48 hours	240	3600	3360
72 hours	680	4160	3480

Table 3. BOD results of Silage

Sample	BOD	Average BOD
1 ml Silage, 5ml Sludge	3874 mg/L	4847mg/L
5 ml Silage, 5ml Sludge	5820 L	

### 2.3 Bmp Tests

#### 2.3.1 Batch reactor

The BMP of Napier grass by batch reactor was done by using syringes. The test was

conducted in 4 samples including the control that is anaerobic sludge. Four syringes were used for batch fermentation, where each of the nipples were blocked tightly. After the process of anaerobic digestion, the gas that will push the plunger has been measured with the capacity of

syringe as 50ml each. The fermentation was conducted at about 42°C with the help of a water bath frequently. The room temperature at that time was approximately 25°C. The four samples were prepared were:

Sample 1: control. (sludge of 30ml)

Sample 2: sludge + substrate (Napier grass). (sludge of 25ml+substrate 5ml)

Sample 3: sludge + substrate. (sludge of 25ml +substrate 5ml)

Sample 4: sludge + substrate. (sludge of 25ml+substrate 5ml)

Three samples were prepared for the comparative analysis and one as a control. The substrate used was the Napier grass silage which was hydrolyzed for 72 hours and the initial pH was maintained at 8.5. In this method, the control used gave the production of biogas from the sewage sludge while the other syringes gave the gas production of Napier silage together with the sludge. Hence, from this arrangement, the difference between the control and the other three samples which gave a specific quantity of gas produced by Napier silage was observed. Also, the biogas generated was recorded using the piston displacement method where the piston in the syringe reactor moves up with daily biogas production the displacement volume is noted by reading off the progression lines existing in the syringes used as the BMP reactors [19]. BMP test using syringes has been considered as a batch reactor as shown in Fig. 4, since the feed was kept constant throughout the period. From the batch reactor, the HRT of the feed was known and the displacement shown by the syringes gave the production of gas for the given HRT [20].

### 2.3.2 Continuous reactor

A biogas reactor of 22.6 litres (0.0226 m<sup>3</sup>) was constructed (refer Fig. 5) and the freeboard maintained was half of the volume i.e., 11.3 litres (0.0113m<sup>3</sup>). Gas produced was measured using a manometer which was constructed using a plastic gas pipe of 3 meters and a diameter 10 mm. A one-meter metallic ruler was attached to the wooden plank for measuring the fluid level difference and to calibrate the pressure values. The fluid medium

used in this tube manometer was coke (Coca-Cola). The principle of the u-tube manometer was applied for analyzing the gas production.

### 2.3.4 Feed loading

The gases generated displaces the liquid inside the manometer and readings were recorded accordingly. Five litres of sludge were fed initially and a cumulative feed of Napier grass silage was added in a percentage of 5%, 10% to 20% for 16 days. This was solely done so that anaerobic digestion could take place effectively as bacteria inside the biogas reactor has to adapt with the feed provided. So, initially a 5% increase of feed was provided and then gradually increased till 20%. However, after the 16th day, less feed was provided and the gas produced was stored in a gas bag for 4 days.

## 3. RESULTS AND DISCUSSION

### 3.1 Continuous Reactor

The volume of sludge introduced was 5 litres which was maintained constant throughout the HRT of 20 days and the silage was gradually introduced. The displacement produced daily in the manometer due to the production of biogas was recorded constantly every day at 16:00 hours. The surrounding temperature was about 25 °C and a pH of 8.5 was maintained in the reactor. When the feed provided exceeded the freeboard limit, the same amount of effluent was removed from the outlet.

The daily gas production was quantified as given in Fig. 6, using the mathematical cylindrical formula, the diameter of the manometer tube was determined (i.e., 10mm) and the displacement of the fluid. Initially, the gas production decreased from the second day due to the following two reasons:

- i. Anaerobic bacteria kept feeding on the sludge and the nutrients available in it got exhausted.
- ii. The delay in the rate of hydrolysis due to the time required for the breakdown of proteins and lipids contained in the substrate.

The displacement was minimal during the fifth day and increased rapidly after the sixth day which indicated that the anaerobic bacteria got accustomed to the new substrate (i.e., Napier silage) and started feeding on it. The maximum

production was on the 10th day and the feed provided was 1020 ml.

The amount of biogas accumulated at the end of 16 days was 317.69ml as given in Fig. 7. The gas stored for four days was analyzed and the following gas compositions were found.

- i. Methane -30.8%
- ii. Co<sub>2</sub>-8%
- iii. Oxygen-2.3%
- iv. H<sub>2</sub>-176ppm
- v. H<sub>2</sub>S-54ppm
- vi. Balance gas-58.9%

The ratio CH<sub>4</sub>: CO<sub>2</sub> was found to be 3.81:1 which comes around 80-20% respectively. Since the percentage of CO<sub>2</sub> was very less compared to that of the CH<sub>4</sub>, the efficiency of combustion

and the consistency of flame could be improved significantly. In biogas production, it was generally found that the carbon dioxide composition was up to 50%. The research conducted on the effect of CO<sub>2</sub> on flame characteristics showed that the level of CO<sub>2</sub> content affects the flame angle and flame height. The increase in CO<sub>2</sub> level increased the flame angle but decreased the flame height correspondingly. Hence in this study, the CO<sub>2</sub> percentage was lower than what was produced in literature and even it can be assured that the flame quality would be a satisfactory one with respect to flame angle and height since 20% is much lesser than 50% [21]. Considering the average production of methane gas as 30.8% throughout the HRT of 20 days, the total gas was further quantified into daily methane production.

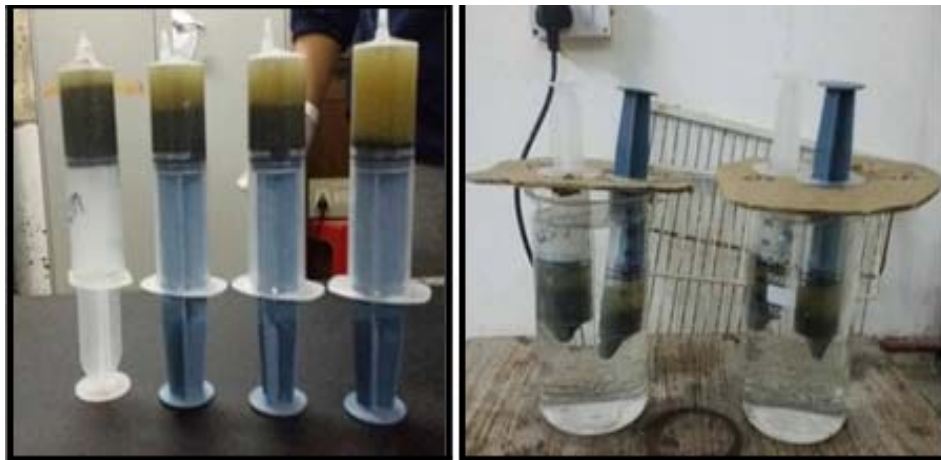


Fig. 4. BMP test using syringes



Fig. 5. BMP test using biogas reactor

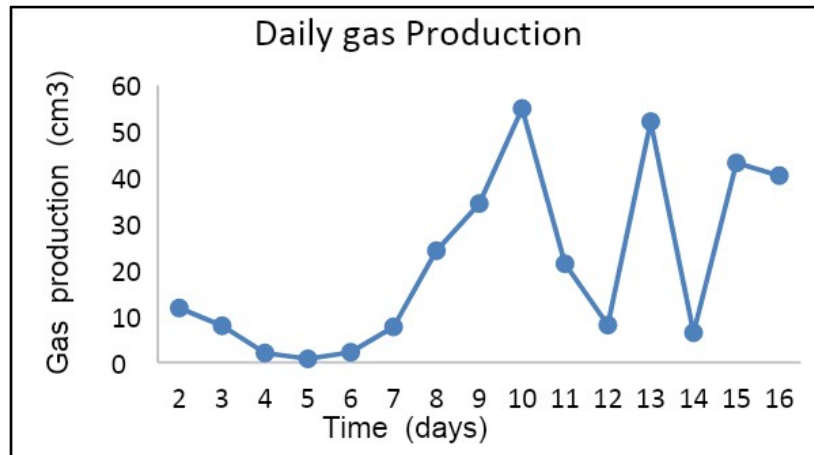


Fig. 6. Daily gas production

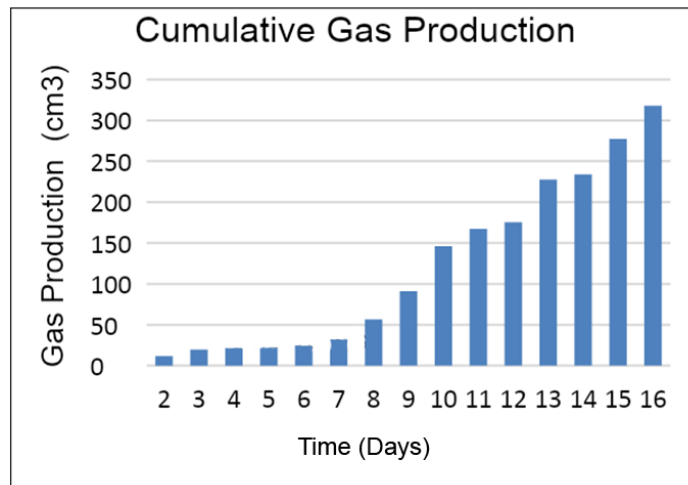


Fig. 7. Cumulative gas production

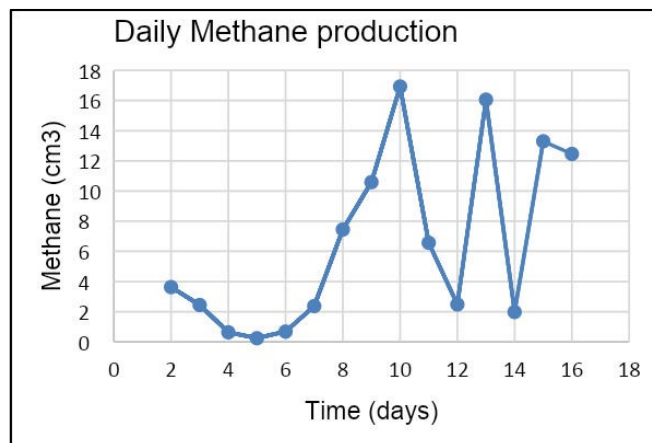
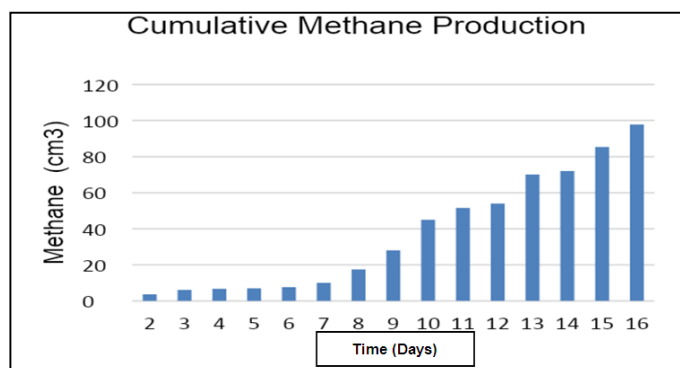


Fig. 8. Daily CH<sub>4</sub> production





**Fig. 9. Cumulative CH<sub>4</sub> production**

The variation of daily methane production was the same as that of Fig. 7 and Fig. 8, since it was the direct derivation based on percentage.

The CH<sub>4</sub> accumulated at the end of 16<sup>th</sup> day was 97.81ml (Fig. 9) and the average rate of CH<sub>4</sub> production was 6.1 ml/day.

### 3.2 Batch Reactor

The BOD<sub>5</sub> of Napier silage was determined to carry out the BMP analysis of the batch reactor. The BOD<sub>5</sub> of Napier silage was found as 20225 mg/L which was determined by subtracting the BOD<sub>5</sub> of control (10ml sludge and diluted to 100ml) from the BOD<sub>5</sub> of the sample (5ml silage and 5ml sludge diluted to 100ml). Approximately, after 25 days, the displacements were observed as 4mm and 3mm. These displacement values were subtracted by the control and the difference obtained was the biogas produced by the Napier silage containing certain amount of BOD<sub>5</sub>/L. Since the BOD content of the samples was known, it helped in relating the amount of biogas produced and the amount of BOD contained in it. After analysis, it was found that 1 mg BOD<sub>5</sub>/L of Napier silage was able to produce 0.0002ml of biogas. Therefore, one liter of Napier silage produced 809ml of biogas.

### 4. CONCLUSION

The Biochemical Methane Potential analysis was conducted in a continuous reactor and batch reactor, from which the continuous reactor, CH<sub>4</sub> yield of Napier grass was deduced and the batch reactor gave the HRT value of 20 days with 809mL of biogas from 1 liter of Napier silage. The mesophilic conditions along with neutral pH of substrate and

inoculum proved to produce a high CH<sub>4</sub> production. Based on current study some of the general observations include, if anaerobic digestion is to be conducted in cold places of Bhutan, the mesophilic temperature should be maintained using insulations in the digesters, whereas in general pH of the substrate has a significant effect in the biogas production so it should be checked and controlled to maintain a pH of 7 to 8. Also, Volatile solids are an organic fraction of total solids, the potential of biogas production can be estimated by the content of volatile solids so it is very important to know the concentration of VS of the substrate which should be measured accurately.

The biogas stored for 4 days of I/S ratio yielded 1:1.26 showed a significant CH<sub>4</sub> percentage of 30.8%. The CH<sub>4</sub>: CO<sub>2</sub> ratio was 80% to 20% which has a potential to produce flame with less flickering. From this study, it can be concluded that Napier grass silage has a high affinity to produce a high yield of Methane because hydraulic retention time maintained was as short as 20 days.

The prospected work deals with the Biochemical Methane Potential Assessment by Anaerobic Digestion of locally available Grasses of Phuentsholing, Bhutan but not limited to the following; BPM of grass substrate with different inoculum and its efficiency on biogas generation considering gas production for 30 to 40 days; Cost analysis of the proposed method of biogas production; Development of the best management plan of effluent from reactors; Green energy technology and reduction of firewood as a fuel source to address climate change effects at the regional level.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist

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