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### **Effect of Temperature on Biogas production using a variable ratio of Cow Dung**

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#### **ABSTRACT**

A study was undertaken to evaluate the effect of temperature on biogas production, using cow dung in lab-based proto-type digesters over a period of 50 days. Eight experimental conditions were set up, using different ratios of cow dung, in four experimental groups (T1, T2, T3 and T4) under ambient (20-23°C) and elevated (35-37°C) temperatures using a water displacement method to monitor biogas production in the proto-type digesters. Biogas production was measured every 5 days for each of the four treatments at two temperatures. The average biogas production under ambient temperature was recorded as 30.36±2.77ml/day, 51.5±2.13ml/day, 97.48±1.98ml/day and 59.28±2.29ml/day in the groups T1, T2, T3 and T4, respectively. The average composition of CH<sub>4</sub> and CO<sub>2</sub> in the biogas produced under ambient temperature was 51.54±1.81% and 11.18±1.28% in T1, 43.87±2.44% and 6.13±1.08% in T2, 47.88±2.43% and 7.56±0.86% in T3 and 36.38±2.41% and 5.96±1.26% in the T4 group, respectively. Contrarily, average biogas production in the same treatment groups under elevated temperature was 89.66±2.39ml/day, 66.64±2.73ml/day, 159.68±2.88ml/day and 79.78±3.19ml/day respectively. The average composition of CH<sub>4</sub> and CO<sub>2</sub> at the elevated temperature was 51.06±1.39% and 9.6±0.72% in T1, 46.47±1.15% and 6.43±0.41% in T2, 51.47±1.68% and 8.28±0.54% in T3 and 37.24±1.87% and 6.26±0.60% in the T4 group, respectively. The mean difference of biogas and CH<sub>4</sub> produced at ambient and elevated temperature among four treatment groups was found statistically significant ( $P<.05$ ). The findings of the present study could be useful for enhancing activities of any kind of domestic biogas digesters available in Bangladesh using cow dung-based biomass.

**KEYWORDS:** Biogas, cow dung, prototype digester, water displacement method, CH<sub>4</sub>

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## **INTRODUCTION**

Bangladesh is considered one of the fastest growing economies (mid economy) in Southern-Asia<sup>1</sup>. The main driving force of the country's development is energy. Proper use of energy is essential to meet the country's growing demands as well as to enable the progression from a mid-income country to a developed country. Energy plays a vital role in implementing Vision-2121, Vision-2041 and achieving Sustainable Development Goals<sup>2</sup>. Bangladesh is one of the lower energy consuming countries of the world. At present, the per capita energy consumption is 293 kilograms of oil equivalent (kgoe)<sup>2</sup>. A mere 6% of the entire population of Bangladesh has access to natural gas, primarily in urban areas. However, about 63% of the people of Bangladesh live in rural areas<sup>3</sup>. The main source of income of Bangladeshi rural people is agriculture. Agricultural waste constitutes a significant resource of biomass, in addition to animal waste and household waste, resulting in great potential for exploitation of biomass in the country<sup>4</sup>. The main agricultural residues available in Bangladesh are rice, maize, wheat, coconut, groundnut, bean, vegetables, jute and sugarcane. At present approximately 46% of Bangladesh's biomass energy comes from rice, rice straw, husk, jute stick, and sugarcane<sup>4</sup>. In Bangladesh, about 70% of energy demand is met from natural gas. The country has a very limited fossil fuel energy reserve<sup>5</sup>, hence when our gas reserves are exhausted there will be no electricity. To face this worsening situation, finding alternative or renewable sources of energy is the only option. Biogas technology may be one of the most promising and important resources of renewable energy for Bangladesh, using animal and municipal wastes<sup>6</sup>. Raw materials for biogas are easily and cheaply available everywhere in Bangladesh<sup>7</sup>. Cattle dung is available from 22 million cows and buffaloes, comprising approximately 0.22 million tons per annum. One ton of dung can produce 37 m<sup>3</sup> of biogas<sup>8</sup>. The urban area of Bangladesh generates approximately 16,015 tons of waste per day, over 5.84 million tons annually<sup>9</sup>. Biogas is a renewable gas fuel which can be produced by the anaerobic digestion of biodegradable material that takes place in a digester, by anaerobic organisms in the absence of oxygen. In its pure state it is odorless, tasteless, and colorless and burns with a clear blue flame without smoke<sup>10</sup>. The composition of biogas is about methane (50-75%), carbon dioxide (25-50%), nitrogen (<1-2%), hydrogen (0-1%), hydrogen sulfide (0-2%) and oxygen (<1%)<sup>11</sup>. It also contains several trace gases including ammonia (NH<sub>3</sub>) and carbon monoxide (CO)<sup>12</sup>. The production of biogas is enabled by consortia of microorganisms and influenced by factors such as temperature, pH, volatile fatty acid (VFA), hydraulic retention time (HRT), C/N ratio, condition of the input charges, nutrient concentration, organic loading rate and toxic compounds etc<sup>13,14,15</sup>.

Among all these, temperature is one of the most important factors for biogas production. The temperature range required for anaerobic digestion is 3°C-70°C. Temperatures between 22°C-37°C are considered optimal for biogas production. Three temperature ranges are common, the psychrophilic (below 20°C), the mesophilic (between 20°C-40°C) and the thermophilic (above 40°C) ranges<sup>16</sup>.

The aim of the present study was to evaluate the effect of temperature (ambient and elevated) on biogas production using a variable ratio of cow dung and inoculum in lab-based proto-type biogas digesters.

## **MATERIALS AND METHODS**

### ***Biomass source***

Fresh cow dung from cross-breed cows was used as the source of biomass, collected early morning from the Animal Research farm, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202. The samples were collected in sterile polyethylene bags adopting standard procedures<sup>17</sup> and transported immediately to the biogas research laboratory.

### ***Source of inoculum***

Cow dung slurry used as inoculum collected randomly from a PVC-type locally-made water tank domestic biogas digester (Gazi Co. Ltd., Bangladesh) of the Biogas Research Laboratory, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202.

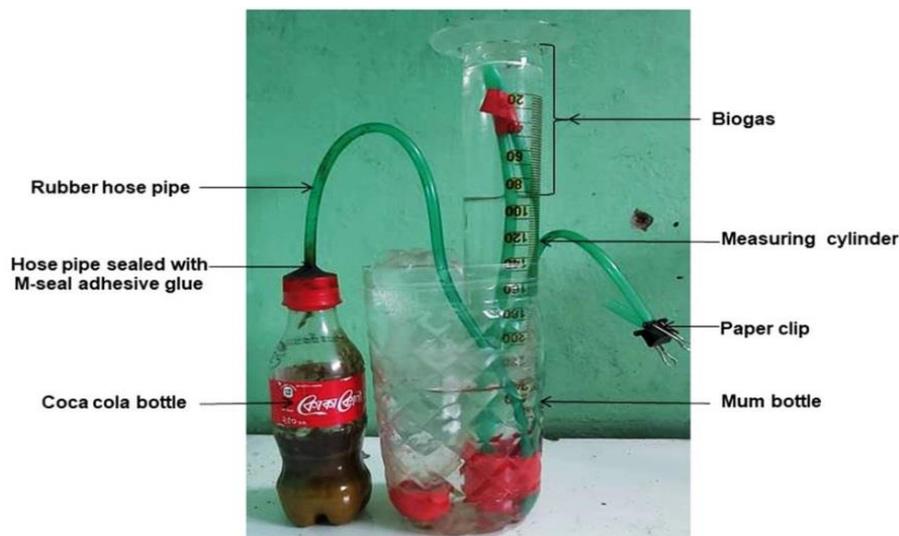
### ***Preparation of experimental/treatment groups (cow dung with water and inoculum in various ratio)***

Treatment group 1 (T1)-150 gm of fresh cow dung was mixed thoroughly with 150 ml of distilled water at a ratio of 1:1; Treatment group 2 (T2) - 150 gm of fresh cow dung was mixed thoroughly with 150 ml of inoculum at a ratio of 1:1; Treatment group 3 (T3) - 225 gm of fresh cow dung was mixed thoroughly with 75 ml of inoculum at a ratio of 3:1; Treatment group 4 (T4) - 75 gm of fresh cow dung was mixed thoroughly with 225 ml of inoculum at a ratio of 1:3. Fibers and large solid particles in the cow dung were removed. 300 ml of each mixture was transferred into each lab-based in-house proto-type digester.

### ***Set up of lab-based in-house proto-type anaerobic digester***

Laboratory based batch digesters were made using 400 ml Coca Cola plastic bottles. A hole (0.4 cm in diameter) was made in the center of the cap of each bottle. Rubber hose of length 2 feet and diameter 0.5 cm was inserted through the hole and sealed with M-seal adhesive glue<sup>18</sup>. The hose

served as the gas conduit leading into a measuring cylinder of 250 ml capacity, filled with water. The cylinder was kept inverted and immersed in water in a 1.5 litre plastic water bottle. The hose pipe was placed in such a way that its tip was in contact with the base of the cylinder. A second hose pipe was inserted into the cylinder for withdrawal of biogas, with the free end sealed with a clip. The biogas generated in the digesters displaced water from the graduated cylinders, and the volume of the headspace of the graduated cylinders represented the volume of biogas generated in the digester<sup>18</sup> (Fig. 1). The gas was collected from the cylinder at five (5) days intervals until day fifty (50) using 100 ml plastic syringes.



**Fig.1** Showing experimental set up of lab-based prototype in-house biogas digester by water displacement method with different parts

### ***Set-up of temperature for proto-type anaerobic digestion***

Experiments were conducted at ambient (20-23°C) and elevated (35-37°C) temperatures, respectively. The ambient temperature was recorded by using a laboratory thermometer and the elevated temperature was maintained using an incubator. Each batch experiment comprised five digesters. The experiment was run for 50 days. To ensure anaerobic conditions within the digesters, the air above the feedstock was removed by using gas tight plastic syringe. Each treatment group of experiment was conducted five replications in this study.

### ***Collection and measurement of biogas from each treatment group***

Biogas generated in digesters was transported by a rubber hose pipe into graduated cylinders and displaced the water out of the graduated cylinders. The volume of the headspace of the graduated cylinders represented the volume of biogas generated in the digester<sup>19</sup>. The biogas was collected

from the graduated cylinder using another hose pipe and a 100 ml gas tight plastic syringe. When not in use, this tube was sealed with a clip.

### ***Determination of composition of the biogas (CH<sub>4</sub> and CO<sub>2</sub>) of each treatment group***

The percentage of methane and carbon dioxide in biogas was determined using a portable biogas analyzer gas board-3200P (Made in China).

### ***Statistical analysis***

All the data obtained from the five replications of four treatment group in this study were analyzed by using standard deviation method. All the data were recorded in MS excel sheet (MS-2010) and imported to SPSS software (IBM SPSS-25.0). Data were analyzed by ANOVA test and significance differences among the different treatment groups were tested by multiple comparison tests namely Tukey's test.

## **RESULTS AND DISCUSSIONS**

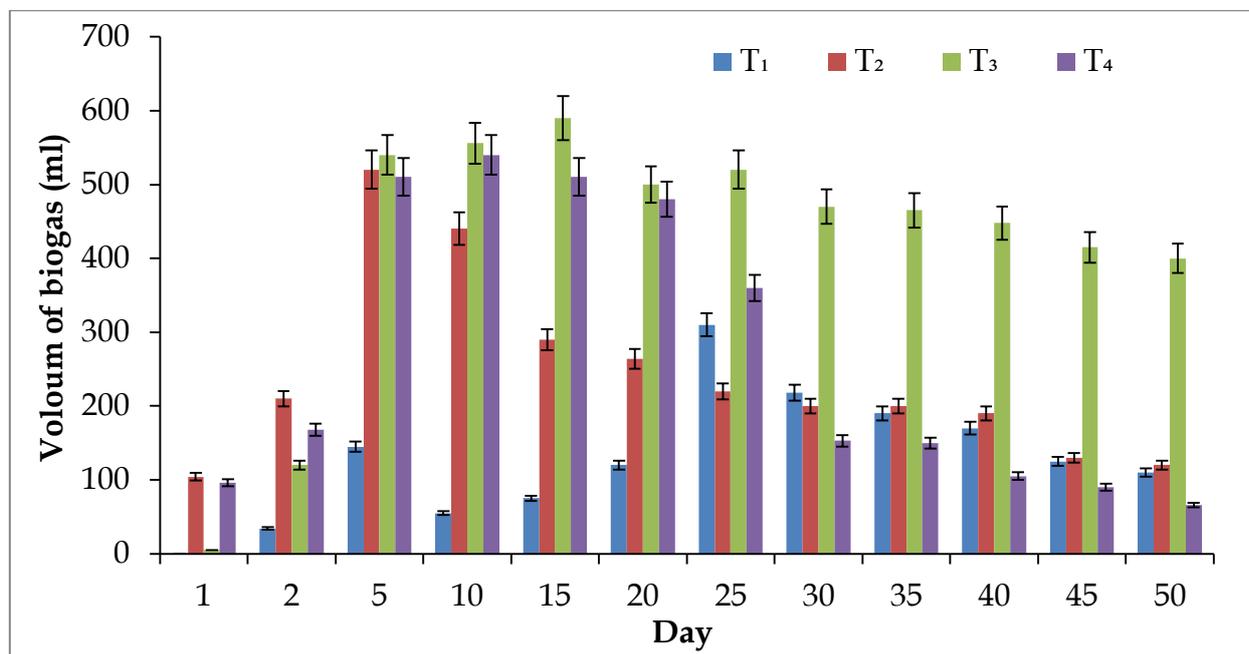
### ***Starting of biogas production***

Biogas production in the lab-based proto-type in-house anaerobic digesters started from day 1 in all the treatment groups (T2, T3 and T4), except in group T1, at both ambient and elevated temperatures (Fig. 2 & 5). This pattern of production of biogas indicates that there might be a higher concentration of anaerobic (methanogenic) microbes<sup>20</sup> and also trace amounts of ready to utilize monosaccharide (glucose) from the biological breakdown of cellulose and hemicellulose in the slurry that was used as an inoculum with cow dung in treatment groups T2, T3 and T4 at a ratio of 1:1, 3:1 and 1:3 respectively in this study<sup>21</sup>.

### ***Comparison of biogas production among different treatment groups under the ambient temperature***

Biogas production under ambient temperature was recorded on day 5 as 145±3.32 ml, 520±1.41 ml, 540±2.24 ml and 510±3.16 ml for groups T1, T2, T3 and T4 respectively. Biogas production per day increased to its highest level in treatment group T1 on day 25 (310±2.45 ml), in group T2 on day 5 (520±1.41 ml), in group T3 on day 15 (590±2.55 ml) and in group T4 on day 10 (540±2.24 ml). Subsequently, gas production declined gradually in group T1 from day 30 (218±2.45 ml), in group T2 from day 10 (440±3.16 ml), group T3 from day 20 (500±3.16 ml) and in group T4 from day 15 (510±1.22 ml) before reaching 110±2.00 ml, 120±1.58 ml, 400±3.74 ml and 66±3.16 ml at the end of the experiment (day 50) for groups T1, T2, T3 and T4 respectively. The mean difference of biogas production among four treatment groups at ambient temperature was found statistically significant

( $P < .05$ ). The average biogas production was  $30.36 \pm 2.77$  ml,  $51.5 \pm 2.13$  ml,  $97.48 \pm 1.98$  ml and  $59.28 \pm 2.29$  ml per day in groups T1, T2, T3 and T4, respectively (Fig. 2).



**Fig.2** Biogas production of different treatment groups under ambient temperature (20-23°C)

### ***Comparison in biogas production between T1 and T2 treatment groups under the ambient temperature***

The volume of biogas produced at ambient temperature on day 5 was more than three times higher in treatment group T2 than in group T1. The highest volume of biogas produced in group T1 was  $310 \pm 2.45$  ml on day 25 and in group T2  $520 \pm 1.41$  ml on day 5 and in general, the gas volume produced by group T2 from day 5 until day 50 was higher than that of group T1. Average gas production per day in treatment group T2 was also higher ( $51.5 \pm 2.13$  ml) compared to that of group T1 ( $30.36 \pm 2.77$  ml) (Fig. 2).

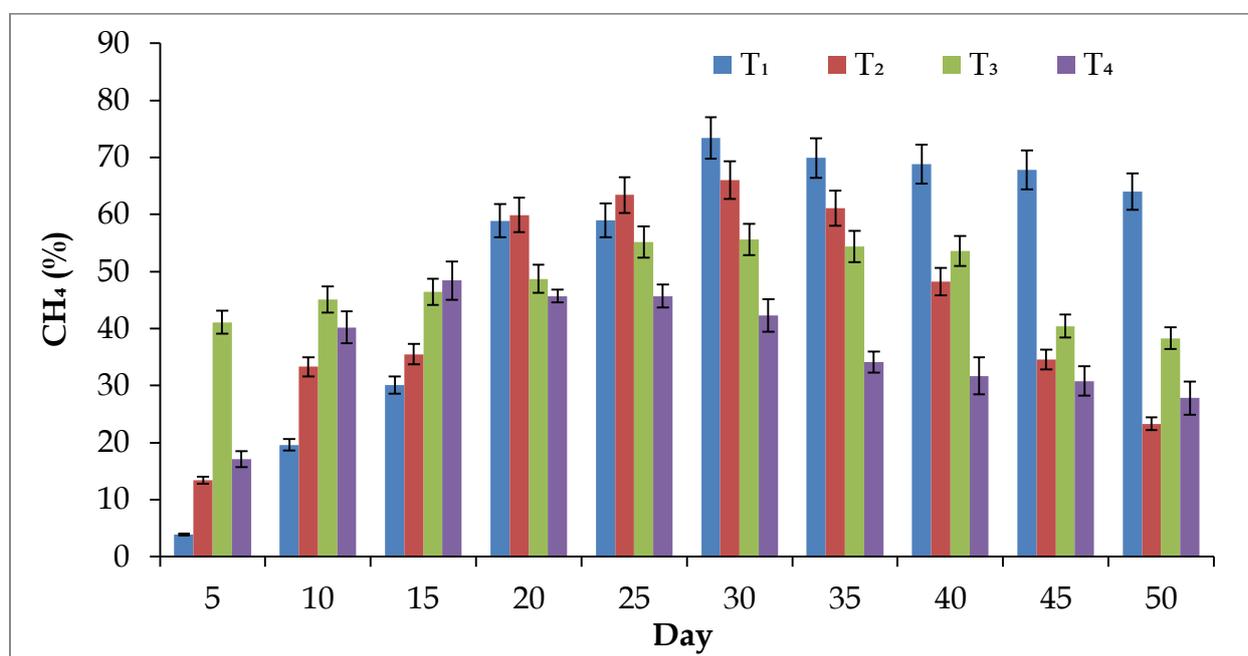
### ***Comparison in biogas production between T3 and T4 treatment groups under the ambient temperature***

The volume of biogas produced under ambient temperature for treatment group T3 on day 5 was higher than for group T4. The highest volume of biogas production for group T3 was  $590 \pm 2.55$  ml on day 15 compared with  $540 \pm 2.24$  ml for group T4 on day 10 although this difference was not significant. The gas volume of group T3 from day 5 until day 50 was consistently higher than that of group T4. In addition, following similar levels of gas production from day 5 until day 20, the volume produced by group T4 declined more rapidly from day 25 to day 50 than that of group T3. Average

gas production per day in treatment group T3 was also higher ( $97.48 \pm 1.98$  ml) than that of group T4 ( $59.28 \pm 2.29$  ml) in this study (Fig. 2).

### ***Biogas composition ( $CH_4$ and $CO_2$ ) of different treatment groups under ambient temperature***

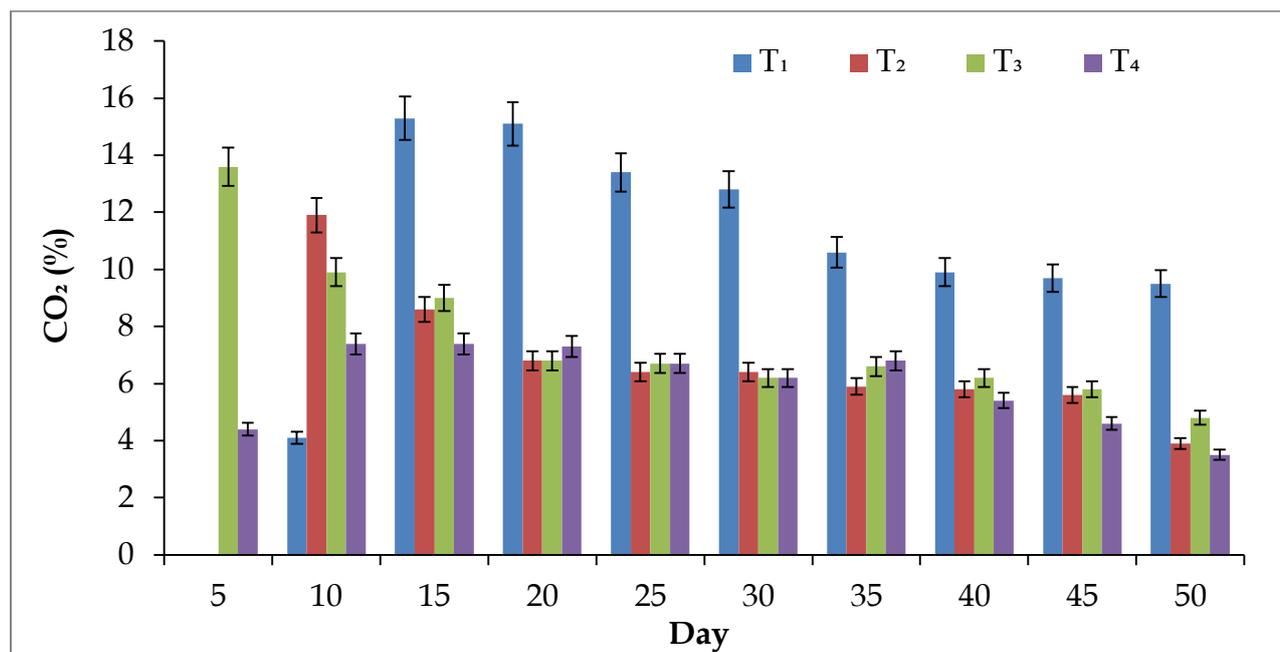
The concentration of  $CH_4$  in the biogas produced under ambient temperature was  $3.9 \pm 0.07\%$ ,  $13.4 \pm 1.90\%$ ,  $41.1 \pm 2.12\%$  and  $17.1 \pm 1.42\%$  (v/v) on day 5 in treatment groups T1, T2, T3 and T4 respectively (Fig. 3). The  $CH_4$  concentration increased to its highest level on day 30 in treatment groups T1 ( $73.4 \pm 1.99\%$ ), T2 ( $66 \pm 1.58\%$ ) and T3 ( $55.6 \pm 2.69\%$ ) but on day 15 in group T4 ( $48.4 \pm 3.37\%$ ). The  $CH_4$  concentration started declining gradually from day 20 ( $45.7 \pm 1.12\%$ ) in group T4 and in the other groups (T1, T2 and T3) from day 35 ( $69.9 \pm 1.91\%$ ,  $61.1 \pm 2.88\%$  and  $54.4 \pm 3.80\%$  respectively). At day 50, the final  $CH_4$  concentrations were  $64.0 \pm 3.54\%$ ,  $23.3 \pm 1.90\%$ ,  $38.3 \pm 2.62\%$  and  $27.8 \pm 2.95\%$ , and the average concentrations during the experiment were  $51.54 \pm 1.81\%$ ,  $43.87 \pm 2.44\%$ ,  $47.88 \pm 2.43\%$  and  $36.38 \pm 2.41\%$  in the biogas of groups T1, T2, T3 and T4, respectively (Fig. 3). The mean difference of  $CH_4$  produced at ambient temperature among four treatment groups was found statistically significant ( $P < 0.05$ ).



**Fig.3** Concentration of  $CH_4$  in the biogas of different treatment groups under ambient temperature

The concentration of  $CO_2$  in the biogas produced under ambient temperature of treatment groups T3 and T4 was  $13.6 \pm 1.08\%$  and  $4.4 \pm 0.68\%$  (v/v) on day 5, whereas  $CO_2$  was not detected in treatment groups T1 and T2 (Fig. 4). The  $CO_2$  concentration increased to its highest level on day 5 in

the biogas of treatment group T3 ( $13.6 \pm 1.08\%$ ), but on day 10 in groups T2 and T4 ( $11.9 \pm 1.74\%$  and  $7.4 \pm 1.47\%$  respectively) and on day 15 in group T1 ( $15.3 \pm 0.99\%$ ). The  $\text{CO}_2$  concentration declined gradually from day 10 in treatment group T3 ( $9.9 \pm 0.91\%$ ) but from day 15 in groups T2 and T4 ( $8.6 \pm 2.01\%$  and  $7.3 \pm 1.06\%$ ) and from day 20 in group T1 ( $15.1 \pm 1.79\%$ ). At day 50, the final  $\text{CO}_2$  concentrations were  $9.5 \pm 0.68\%$ ,  $3.9 \pm 0.67\%$ ,  $4.8 \pm 0.52\%$  and  $3.5 \pm 0.33\%$  and the average  $\text{CO}_2$  concentrations during the experiment were  $11.18 \pm 1.28\%$ ,  $6.13 \pm 1.08\%$ ,  $7.56 \pm 0.86\%$  and  $5.96 \pm 1.26\%$  in the biogas of the treatment groups T1, T2, T3 and T4, respectively (Fig. 4).



**Fig.4** Concentration of  $\text{CO}_2$  in the biogas of different treatment groups under ambient temperature

### ***Comparison of $\text{CH}_4$ concentration in biogas between T1 and T2 treatment groups under ambient temperature***

At ambient temperature, the  $\text{CH}_4$  concentration of the biogas of both the T1 and T2 groups increased from day 15 ( $30.1 \pm 1.4\%$  and  $35.5 \pm 2.89\%$  respectively), reaching its highest level on day 30 ( $73.4 \pm 1.99\%$  and  $66.0 \pm 1.58\%$  respectively). Interestingly, the  $\text{CH}_4$  concentration in the biogas declined gradually in group T1 from  $69.9 \pm 1.91\%$  to  $64.0 \pm 3.54\%$  and in group T2 from  $61.1 \pm 2.88\%$  to  $23.3 \pm 1.90\%$  from day 35 to day 50. The average concentration of  $\text{CH}_4$  was also higher in group T1 ( $51.54 \pm 1.81\%$ ) than in group T2 ( $43.87 \pm 2.44\%$ ) (Fig. 3). Although the reason for the increase and decrease in the biogas  $\text{CH}_4$  concentration of group T1 until day 50 was not clear, it may be speculated that there might be a sufficient concentration of monosaccharides, utilizable by the methanogenic consortium in group T1 compared to group T2, from day 40 to day 50. The rapid decline of  $\text{CH}_4$  in the biogas of treatment group T2 also indicates that the concentration of microbes

and utilizable monosaccharides might be higher in the reaction mixture of group T1 from day 35 to day 45, resulting maximum production of biogas.

### ***Comparison of CH<sub>4</sub> concentration in biogas between T3 and T4 treatment groups under ambient temperature***

At ambient temperature, the CH<sub>4</sub> concentration of the biogas of both the T3 and T4 groups increased from day 10 (45.1±2.27% and 40.2±2.78% respectively), reaching its highest level on day 30 and day 15 (55.6±2.69% and 48.4±3.37%) in groups T3 and T4 respectively. Interestingly, the CH<sub>4</sub> concentration in the biogas of group T3 declined gradually from day 35 to day 50 (54.4±3.80% to 38.3±2.62%), while that of group T4 declined dramatically from day 20 to day 50 (45.7±1.12% to 27.8±2.95%). The average concentration of CH<sub>4</sub> was also higher in group T3 (47.88 ±2.43%) compared with group T4 (36.38±2.41%) (Fig. 3). It may be speculated that there might be a higher concentration of monosaccharides, utilizable by the methanogenic consortium in group T3 compared to group T4, from day 35 to day 50. The rapid decline of CH<sub>4</sub> in the biogas of treatment group T4 also indicates that the concentration of microbes and utilizable monosaccharides might be higher in the reaction mixture from day 15 to day 35, resulting in maximum production of biogas.

### ***Comparison in biogas production among different treatment groups at the elevated temperature***

Biogas production at the elevated temperature was recorded on day 5 as 108±1.41 ml, 750±3.81 ml, 532±2.00 ml and 980±3.08 ml for groups T1, T2, T3 and T4 respectively. The biogas volume increased to its highest level in treatment group T2 on day 5 (750±3.81 ml), in group T4 on day 5 (980±3.08 ml), in group T3 on day 15 (1465±3.46 ml) and in group T1 on day 35 (725±2.74 ml). Subsequently, gas production declined gradually in group T1 from day 40 (718±2.55 ml) in group T2 from day 10 (620±2.55 ml), in group T3 from day 20 (1155±3.81 ml) and in group T4 from day 10 (720±3.16 ml) before reaching 320±2.92 ml, 72±1.41 ml, 305±3.61 ml and 77±3.85 ml at the end of the experiment, on day 50 for groups T1, T2, T3 and T4, respectively. The mean difference of biogas production among four treatment groups at elevated temperature was found statistically significant ( $P<0.05$ ). The average biogas production was 89.66±2.39 ml, 66.64±2.73 ml, 159.68±2.88 ml and 79.78±3.19 ml per day in groups T1, T2, T3 and T4 respectively (Fig. 5).

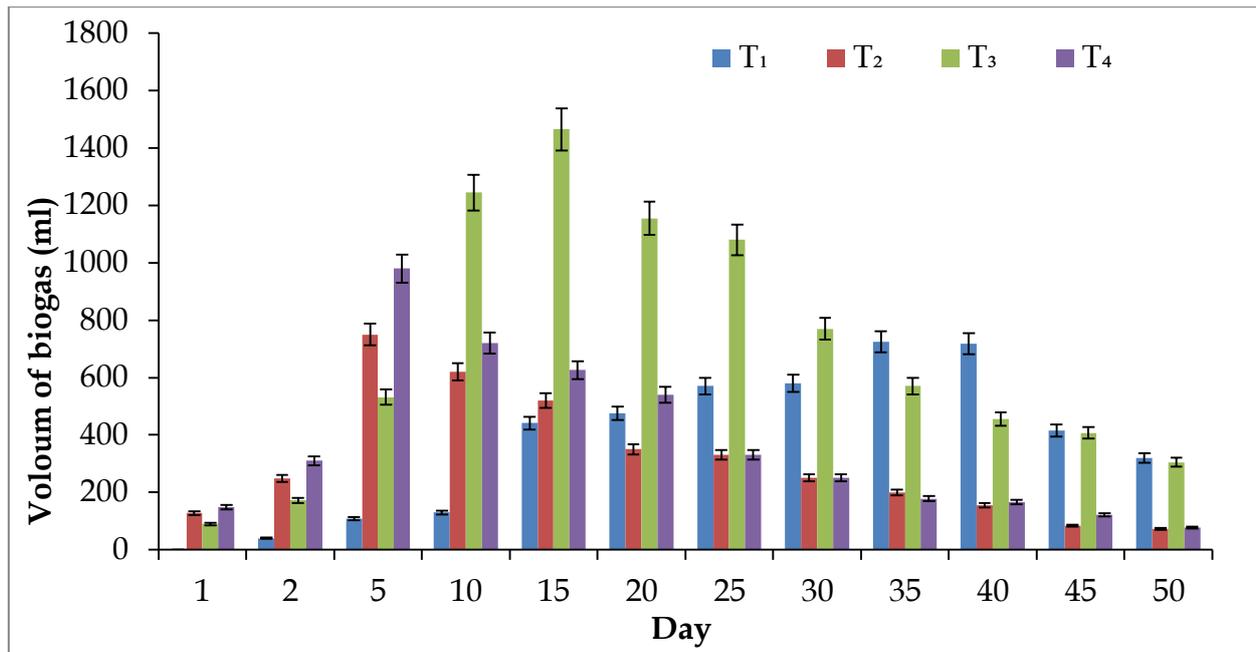


Fig.5 Biogas production of different treatment groups under elevated temperature (35-37°C)

### ***Comparison of biogas production between T1 and T2 treatment groups at the elevated temperature***

The volume of biogas produced at the elevated temperature on day 5 was six times higher in the T2 treatment group compared with the T1 treatment group. The highest volume of biogas produced was recorded in group T1 on day 35 ( $725 \pm 2.74$  ml) and in group T2 on day 5 ( $750 \pm 3.81$  ml). The gas volume of group T1 from day 20 until day 50 was consistently higher than that of group T2 under the elevated temperature. The average gas production per day in treatment group T1 was also higher ( $89.66 \pm 2.39$  ml) compared to that of group T2 ( $66.64 \pm 2.73$  ml) in the study (Fig. 5).

### ***Comparison in biogas production between T3 and T4 treatment groups at the elevated temperature***

The volume of biogas produced at the elevated temperature in treatment group T3 on day 5 was almost twice that of treatment group T4. The highest volume of biogas produced in group T3 was  $1465 \pm 3.46$  ml on day 15 and in group T4  $980 \pm 3.08$  ml on day 5. This variation of gas production between the two treatment groups was significant. Gas production in group T3 was consistently higher than that of group T4. The gas volume produced in group T4 from day 5 until day 25 was stable, but declined from day 30 to day 50 and was relatively lower than that of group T3. The average gas production per day in treatment group T3 was also higher ( $159.68 \pm 2.88$  ml) than that of group T4 ( $79.78 \pm 3.19$  ml) in this study (Fig. 5).

### Comparison of biogas composition ( $CH_4$ and $CO_2$ ) of different treatment groups at the elevated temperature

The concentration of  $CH_4$  in the biogas produced at the elevated temperature was  $6.8\pm 0.39\%$ ,  $47\pm 0.45\%$ ,  $34.5\pm 2.3\%$  and  $20.7\pm 1.42\%$  (v/v) on day 5 in treatment groups T1, T2, T3 and T4 groups respectively (Fig. 6). The  $CH_4$  concentration increased to its highest level on day 15 in treatment group T1 ( $74.7\pm 1.01\%$ ), on day 30 in group T2 ( $57.7\pm 1.56\%$ ), on day 30 in group T3 ( $69.5\pm 1.60\%$ ) and on day 15 in group T4 ( $49.9\pm 1.81\%$ ). The  $CH_4$  concentration declined gradually on day 20 in groups T1 and T4 ( $58.1\pm 1.77\%$  and  $46.8\pm 1.04\%$ , respectively) and on day 35 groups T2 and T3 ( $52.3\pm 1.26\%$  and  $63.5\pm 2.39\%$ , respectively). At day 50, the final  $CH_4$  concentrations were  $47.7\pm 1.53\%$ ,  $29.4\pm 0.95\%$ ,  $48.9\pm 1.42\%$  and  $30.3\pm 1.70\%$  and the average  $CH_4$  concentrations during the experiment were  $51.06\pm 1.39\%$ ,  $46.47\pm 1.15\%$ ,  $51.47\pm 1.68\%$  and  $37.24\pm 1.87\%$  for groups T1, T2, T3 and T4 respectively (Fig. 6). The mean difference of  $CH_4$  produced at elevated temperature among four treatment groups was found statistically significant ( $P < 0.05$ ).

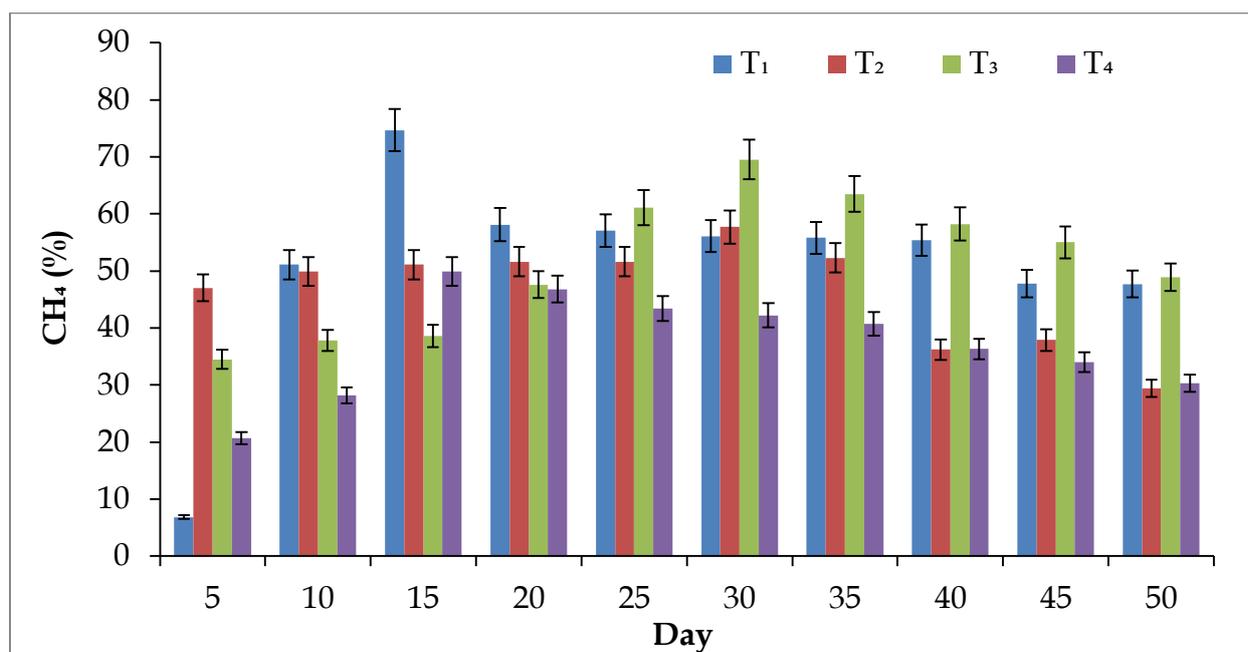


Fig.6 Concentration of  $CH_4$  in the biogas of different treatment groups under elevated temperature

The concentration of  $CO_2$  in the biogas produced at the elevated temperature on day 5 for treatment groups T2, T3 and T4 was  $10.3\pm 0.78\%$ ,  $9.6\pm 0.60\%$  and  $4.3\pm 0.16$ , respectively, whereas  $CO_2$  was not detected in treatment group T1 at this time point (Fig. 7). The  $CO_2$  concentration increased to its highest level on day 10 in the biogas of the treatment groups T2 and T3 ( $13.3\pm 0.73\%$  and  $14.4\pm 0.41\%$ ) and on day 20 in groups T1 and T4 ( $13.3\pm 0.69\%$  and  $6.9\pm 0.56\%$ ). The  $CO_2$  concentration declined gradually from day 15 in groups T2 and T3 ( $7.0\pm 0.27\%$  and  $13.7\pm 0.19\%$ ) and from day 25 in treatment groups T1 and T4 ( $11.4\pm 1.06\%$  and  $6.8\pm 0.60\%$ ). At day 50, the  $CO_2$

concentrations were  $6.2\pm 0.48\%$ ,  $1.8\pm 0.16\%$ ,  $5.8\pm 0.71\%$  and  $6.5\pm 0.46\%$  and the average  $\text{CO}_2$  concentrations during the experiment were  $9.6\pm 0.72\%$ ,  $6.43\pm 0.41\%$ ,  $8.28\pm 0.54\%$  and  $6.26\pm 0.60\%$  for groups T1, T2, T3 and T4, respectively (Fig. 7).

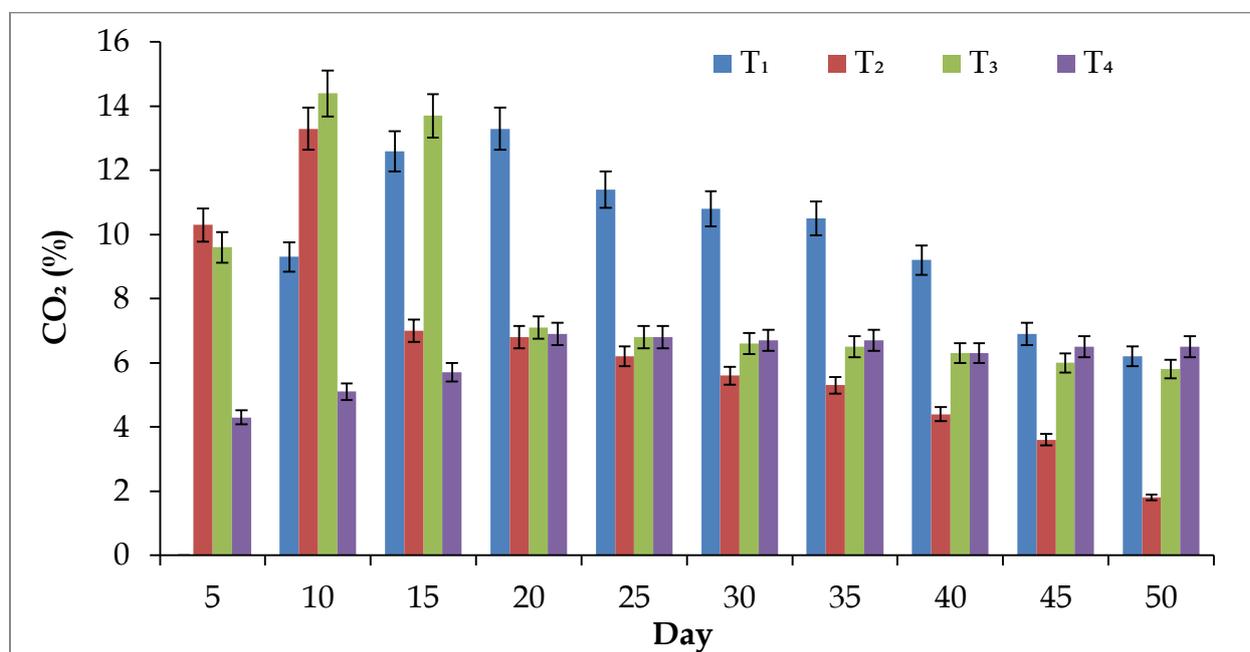


Fig.7 Concentration of  $\text{CO}_2$  in the biogas of different treatment groups under elevated temperature

### ***Comparison of $\text{CH}_4$ concentration in biogas between T1 and T2 treatment groups at the elevated temperature***

At the elevated temperature, the  $\text{CH}_4$  concentrations of the biogas of both the T1 and T2 groups increased from day 10 ( $51.1\pm 1.52\%$  and  $49.9\pm 0.87\%$  respectively), reaching its highest level on day 15 in group T1 ( $74.7\pm 1.01\%$ ) and on day 30 in group T2 ( $57.7\pm 1.56\%$ ). Interestingly, the  $\text{CH}_4$  concentration of the biogas declined gradually from day 20 to day 50 in group T1 ( $58.1\pm 1.77\%$  to  $47.7\pm 1.53\%$ ), whereas in group T2 the concentration declined dramatically from day 35 to day 50 ( $52.3\pm 1.26\%$  to  $29.4\pm 0.95\%$ ). The average concentration of  $\text{CH}_4$  was also higher in group T1 ( $51.06\pm 1.39\%$ ) than in group T2 ( $46.47\pm 1.15\%$ ) (Fig. 6). Although the reason for the increase and decrease in the concentration of  $\text{CH}_4$  in the biogas of the T1 group was not clear, it may be speculated that there might be a sufficient concentration of utilizable monosaccharides for the methanogenic consortium in the T1 group, compared to the T2 group, from day 40 to day 50. The rapid decline of  $\text{CH}_4$  in the biogas of treatment group T2 also indicates that the concentration of microbes and the utilization of monosaccharides might be higher in the reaction mixture from day 5 to day 30. The concentration of methanogens was also lowest from day 40 to day 50 in group T2

which might be due to trace amounts of nutrition in the digester which also reduced the production of biogas.

### ***Comparison of CH<sub>4</sub> concentrations in biogas between the T3 and T4 treatment groups at the elevated temperature***

At the elevated temperature, the CH<sub>4</sub> concentration of the biogas of both the T3 and T4 groups increased from day 10 (37.8±1.43% and 28.18±1.52% respectively) and reached its highest level on day 15 in group T4 (49.9±1.81%) and on day 30 in group T3 (69.5±1.60%). Interestingly, the CH<sub>4</sub> concentration of the biogas declined gradually from day 35 to day 50 in group T3 (63.5±2.39% to 48.9±1.42%), whereas the CH<sub>4</sub> concentration of group T4 declined dramatically from day 20 to day 50 (46.8±1.04% to 30.3±1.70%). The average concentration of CH<sub>4</sub> was also higher in group T3 (51.47±1.68%) than group T4 (37.24±1.87%) (Fig. 6). The reason for the increase and decrease in the CH<sub>4</sub> concentration in the biogas of the T3 group from day 5 until day 50 may be due higher concentration of monosaccharides, utilizable by the methanogenic consortium in the T3 group, compared to the T4 group, from day 35 to day 50. The rapid decline of CH<sub>4</sub> in the biogas of treatment group T4 also indicates that the microbial concentration and utilizable monosaccharides might be higher from day 15 to 30, resulting in the maximum production of biogas.

This study shows that temperature is one of the most important factors for maintaining optimum growth and productivity of mesophilic microorganisms in an anaerobic digestion process. Without a suitable environment for the methanogenic microbes (mesophilic in nature) in biomass (cow dung/slurry), the quantity (volume) and quality (concentration of CH<sub>4</sub>) of the biogas production in a digester is seriously compromised<sup>22</sup>. In this study it was found that the volume of biogas produced was lower and slower in all the treatment groups (T1, T2, T3 and T4) under ambient temperature (20-23°C) compared to elevated temperature (35-37°C). This suggests that reaction temperatures ranging from 20-23°C might have an influence on the optimum growth of the methanogens present in the different ratios of cow dung and slurry, since the volume and quality of biogas production in all the treatment groups was relatively lower at this temperature. Therefore, the biodegradation rate of the organic wastes was slower and the gas yield was poor under ambient temperature, compared to the elevated temperature (35-37°C). The results of the present study regarding the influence of temperature on the anaerobic digestion process in four different treatments groups clearly indicate that the volume of biogas produced was lower and the retention time was longer at the ambient temperature compared with at the elevated temperature. The findings of the study relating to the influence of temperature on the production of biogas and the retention time of anaerobic digestion in the in-house prototype lab-based digester agree with the findings of Uzodinma et al.<sup>23</sup>. In their study,

they also found that at the ambient temperatures, the methanogens failed to be activated sufficiently, and as a result the volume of biogas produced was lower and the retention time longer, compared to at elevated temperatures.

## **CONCLUSIONS**

Biogas is one of the most promising sources of renewable energy in Bangladesh. Biogas obtained by anaerobic digestion is the result of microbial degradation of organic matter that occurs in the absence of oxygen, and which produces primarily methane and carbon dioxide. The important findings of the present study are that the reaction mixture of biomass (T1, T2, T3 and T4 treatment groups) and the influence of temperature (ambient and elevated) have a direct influence on the production and concentration of CH<sub>4</sub> in the biogas of anaerobic prototype digester. From the findings of this study, it might be concluded that the reaction mixture of biomass with slurry in the treatment group T3 was found higher compared to that of others treatment groups. Of the two temperature levels, the elevated temperature (35-37°C) was found better in terms of biogas production compared to the ambient temperature.

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