

Evaluation of Biogas Production from Food Waste Under Thermophilic and Mesophilic Conditions under Swiss Conditions

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Abstract

Green fuel production from food waste can help manage waste disposal. A study was conducted to test the efficiency of biogas production under different temperature conditions in small-scale anaerobic digesters from canteen food waste and biogas slurry from waste water treatment plant as starter culture of Yverdon Les Bains, under Swiss conditions, which have potential for waste disposal and energy supply in urban areas. This anaerobic digester was operated first at thermophilic then mesophilic temperature ranges with specific bacteria active in each range. Efficiency of the biogas digester was checked with varying the temperature in the digester from thermophilic to mesophilic conditions, with and without stirring for a period of three months. Temperature, methane concentration and biogas flow rate were continuously acquired using a Labview® Data Acquisition System, developed in-house with a lab-scale digester. The digester was constructed of stainless steel with a water jacket heated by an electric water heater with a circulating pump and agitation provided by impellers and baffles. The study shows anaerobic digestion can recover from abrupt changes between thermophilic and mesophilic conditions without intervention or changing the feeding regime. ATP analysis showed that microorganisms stress was higher under thermophilic than under mesophilic conditions. We can conclude that for food waste and mixtures of food-waste and cow-dung under thermophilic conditions result in increased biogas flow and an 11% increase in methane concentration compared to mesophilic condition, without stirring. Results also show biogas production reached 66% of methane at pH of 7.48 and volume concentration within the first month under thermophilic condition (56-58°C). Later the same digester was set to mesophilic conditions, *i.e.* 30-40°C with an efficiency of 55%. Maximum and continuous biogas was recorded above 57°C until 59°C with better solid destruction and biogas yield.

Keywords: Biogas, Mesophilic, Thermophilic, Methane and Labview®

1. Introduction

Disposal of food waste in the right way keeps the environment clean. One such way of utilizing food waste is to produce biogas also called green fuel and future generation fuel. Developing countries face a lot of such problems in the disposal of combined wet and dry waste and way to manage the city's wet waste is biogas production *i.e.* energy production Jain et al. (2018). The waste generated in India was collected and collecting even separately is due to problems with their disposal into the landfill, even to date, full segregation has not been achieved (Dey and Thomson 2022). Food waste from houses can be utilized to produce CH₄ gas for cooking purposes (Srinvasa Reddy et al., 2017). While the need in the developed countries is towards studies to be conducted in the lab, for students and towards research related to checking the efficiency of the biogas production with the focus towards thermophilic and mesophilic conditions where external heating is supplied to have maximum gas efficiency, considering productive, microbiological and environmental criteria, an attempt is made in the present work. The temperature and substrate composition are among the main factors affecting performance and stability of anaerobic digestion process (Ziganshin et al., 2013; Labatut et al., 2014). The thermophilic conditions will be usually above 45°C and have

been reported as being superior to mesophilic conditions which is 25–40°C, due to a higher organic matter degradation rate (Nielsen and Petersen, 2000). The major microbial community in food waste and cow dung are Bacteroidetes, Methanosarcina, Methanosaeta, Clostridium, Formicates and Methanosarcinales, Methanobacteriales Methanomassiliicoccales respectively (Goberna et al., 2009). The microbial isolates from the digesters included Bacillus spp and Clostridium spp. and Bacillus pseudomonas species were responsible for biogas production in rumen content. (Mauerhofer et al., 2019). This study was carried in the laboratory of Business and Engineering Vaud (HEIG- VD), University of Applied Science Western Switzerland, Thermal Engineering Institute, Industrial Bioenergy System Unit, Avenue des Sports, 20 1401 Yverdon-les-Bains. The fabricated biogas digester was constructed by the Swiss University of Applied Sciences and one of the authors of this paper (Verma et al., 2013). Studies were conducted with the main objective to produce biogas much faster (in a short duration of time) and working under various temperature ranges like thermophilic and mesophilic conditions of the digester, were worked on. The produced biogas was studied under a lab scale, with changing thermophilic and mesophilic temperatures, to, study the efficiency of biogas production from the canteen food waste of the University of Western Switzerland, Yverdon-les Bains was carried out and the starter culture was taken from the wastewater treatment plant of Yverdon Les Bains, Switzerland.

1.1 Purpose of the work

Small-scale anaerobic digesters, containing a slurry volume between 200 and 2000 liters are widely employed to produce biogas from animal wastes, kitchen wastes and other organic residues. Food wastes are substances that were once nutritious and safe for human consumption but are no longer suitable for food applications after being discarded. According to a report by the Food and Agriculture Organization (FAO) in 2019, approximately 1.3 billion tons of food, accounting for about 33% of the global food supply, is wasted each year and it is estimated that the economic impact of food waste amounts to approximately 750 billion dollars per year (Mirmohamadsadeghi et al., 2019). The potential for further implementation of small-scale anaerobic digesters to solve waste disposal and energy supply problems in urban areas is very large and currently under development/not utilized by all (Jutta Gutberlet, 2018). Anaerobic digestion is a highly promising technology for the simultaneous recovery of resources from food wastes and their treatment. In recent years, significant efforts have been made to enhance biogas production from food waste. This technology has proven to have a lesser environmental impact when compared to other methods such as incineration and landfilling. Despite its immense potential to generate biogas and provide power to cities worldwide, only a limited number of large-scale plants have been established, primarily in developed nations (Xu et al., 2018; Campuzano and González-Martínez, 2016). In South India, the temperature of the digester slurry is typically between 28 and 35°C without external heating or temperature control and low-cost, widely available solar thermal water heaters could be used to heat the digester contents to increase the digestion rate and biogas production (Gaballah et al., 2020) and maintaining of thermophilic conditions for 10 hours would also result in a hygienic waste sludge product (Verma et al., 2013). To minimize the cost and the complexity of the system, digester

heating through immersed pipes and a hot water circulator with manual temperature regulation are proposed using advanced system to reduce the heat loss meanwhile, the temperature of the digester contents is expected to vary between 28°C and 70°C depending on such factors as the outside temperature, incident sunlight, feed temperature and rate, external hot water demands and the manual operator interventions to control the hot water circulator. The slurry content of most small-scale digesters is not continuously agitated and in some cases, the slurry is manually mixed intermittently, while in some instances the slurry is not mixed. The purpose of the work is to monitor and evaluate the effect of temperature and mixing on biogas and methane production, organic loading capacity, pH and digester stability.

2. Material and Method

Anaerobic digesters are normally operated in either the mesophilic temperature range (~37°C) or the thermophilic temperature range (~57°C). A specific set of bacteria is active in each of the two temperature ranges. The approach used in this study was to simulate possible conditions in a small-scale digester with manually controlled solar heating and mixing. Biogas flow rate, methane concentration, solids removal and pH were measured during 4 months' while varying the temperature between 28 and 70°C and varying the mixing regime. The lab- scale digester was operated in semi-continuous mode. (Manser et al., 2015) but the focal disadvantage of semi-continuous digester appears when the results are unstable and the study will not be possible on a site with continuous operational conditions as the impact of the different variables could not have been elucidated (Rocamora et al., 2022). Food waste was homogenized in a kitchen blender, stored at 4°C and used for daily digester feeding. This is a representation indicating the food waste which was available from the canteen waste, can be utilized in small digesters within the university itself if they wish to process it for the production of biogas at that level. Feeding was one time per day, sludge was removed from a side port and a mixture of the food waste and cow dung mixed in 1:1 ratio was well blended with adequate amount of water added via a top port. The slurry level was maintained between 34 liters and 38 liters by filling and the remaining digester volume was filled by the biogas produced. When the slurry was agitated, the impeller rotation speed was 105 rpm and the applied power was 40 Watts. The complete set up of the digester is described below.

2.2 Physical Characteristics of the Digester

The digester (Figure 1a & 1b) is a 70 liter continuously stirred tank reactor constructed of stainless steel. A water seal cup and sim-ring ensure an air tight seal of the impeller drive axel. A concentric water jacket is heated by a manually adjusted 3 kW electric water heater and circulating pump as we go for full scale digester. Larger compressors are required and often have several subsystems, which include jacket water heating and cooling, which utilizes a jacket water pump, as well as lube oil heating and cooling, which relies on a lube oil pump (Palmer et al., 2020). The temperature of the digester is determined by employing a thermocouple that is submerged in the liquid contents and to ensure optimal insulation, the sides and bottom surfaces of the digester are enveloped with a layer of polyurethane foam insulation measuring 15 mm in thickness. The process of agitation within the digester is

facilitated by the presence of two Rushton type impellers and four side wall baffles powered by a 150W, 24V DC motor. The rotation speed of the impellers is manually regulated by adjusting the current. The biogas produced within the digester exits through a 2 mm diameter port that is connected to a flexible hose measuring 12 mm in diameter. To ensure the proper functioning of the digester, a water seal check-valve is incorporated, which allows the biogas to exit while preventing the entry of air into the digester.

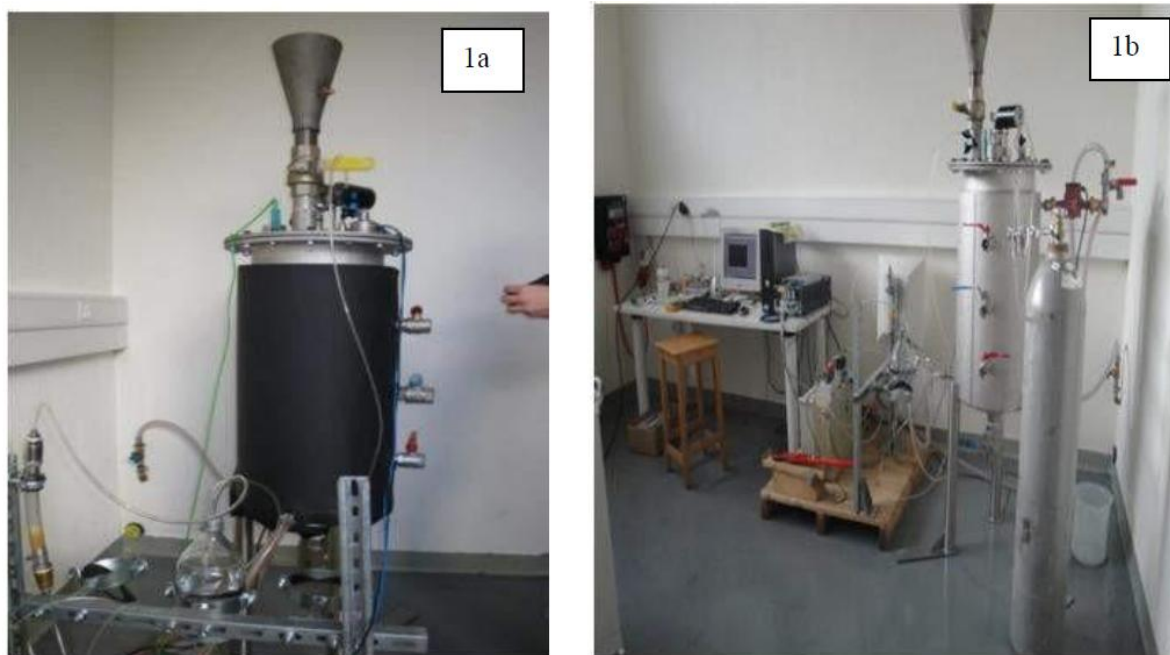


Figure 1a & 1b. Bio digester used for the lab study and its complete set up

2.2.1 Data Acquisition and Recording

The biogas flow rate is continuously measured by the thermal mass principle using a Red-y® flowmeter (Voegtlin, CH) calibrated with a mixture of 60% CH₄ and 40% CO₂. Methane concentration in the biogas is continuously measured by an IR measuring cell (Pewatron Smart Module®, CH) and biogas outlet pressure is estimated by measuring the liquid depth in the water check-valve at which bubbles are observed. Temperature, methane concentration and biogas flow rate were continuously acquired using a Labview® Data Acquisition system and recorded using Labview® program developed in-house. The flow rate and methane concentration were monitored 15 minutes every hour. Biomass stress was evaluated as the ratio of extracellular Adenosine triphosphate (ATP) to total ATP (tATP) and is noted as the BSI or Biomass Stress Index TM (Aquatools, F).

2.3 Digester Start-up and Maintenance

The digester was filled with 25.2 liters of slurry taken from an anaerobic digester of the local sewage treatment plant (having a hydraulic retention time of 25 days and a temperature of 37°C). Then 500ml of food waste slurry (250mL food waste mixed with 250mL water)

was added. Finally, 6.4L of cow dung in a 1:1 ratio with tap water was added. The slurry was removed and the feed added one time per day. The daily feed volume was usually 120 ml/day. About 3877.1 g / 3.877 kg waste was collected from the canteen at around 12.30-12.45 pm in a steel bucket. The waste (Fig. 2) collected comprised coffee powder, buns, lettuce, potato fingerlings, peas, a piece of meat, noodles, apple remains, oranges, tomato and kiwi. The amalgamation of an anaerobic digester with a decentralized municipal wastewater treatment plant serves as an effective method for on-site treatment of household waste and using the sludge from the wastewater treatment plant. This integration acts as an inoculum and stabilizer for the food waste substrate during the initial stage. The slurry from Yverdon WWTP had good amount of bacteria, as it was an older culture of waste, the fresh food waste would not contain large amount of microbes, while in the anaerobic digester constituents are already enriched with good bacterial culture. Food waste has also better potential in gas production, hence it was decided to take some amount of WWTP sludge along with fresh food waste into the digester for this study.

From the collected waste, citric waste weighing 557g was removed by manual inspection. Water was added to the waste in 1:1 proportion (3320.1 ml of water was added to 3320.1 gm of waste) and the mixture was added into a mixer (Fig.2) and a fine paste was made. This paste was stored in a refrigerator. The capacity of the digester was 70L, during the same evening at 4.30 pm 12.6 L of slurry was taken from the anaerobic digester of Yverdon, Switzerland's waste water treatment plant and was added to the digester with 500g of food waste which was then stored in the refrigerator and prepared as mentioned above. The food waste was added to the bio-digester. The rationale was as WWTP had already a well-established population of the bacterial culture, it would be useful to hasten the process of biogas production in the lab scale biogas digester. Therefore, the actual amount of waste slurry required to be filled inside the digester is outlined in Table 2. (Digester theoretical value calculation). The anaerobic digester at the wastewater treatment plant had a hydraulic retention time of 25 days. Since the digester was well mixed, one can say the sludge had a residence time of 25 days in the digester. Biogas slurry waste was 15/20 days old. On the 11th day 3.2 kg of cow dung was mixed with 3.2 L of water, the bacterial culture from the ruminants are rich source of bacteria and the results of this study indicate that the mixture of food waste and cattle manure is a highly desirable substrate for anaerobic digesters with regards to its high biodegradability and methane yield. (Wang et al., 2023) and made in the form of a fine slurry (indicated in Figure 4) Figure 5 shows the complete diagrammatic view of the bio digester with the dimensions. Table 1, gives the dimensions of the digester and the calculated volume. For a 4.3 cubic meter (m³) digester, 3 m³ of gas cap is required (Clinica & Orrore, 1980) Considering the biogas digester for 64L and 70L the following m³ of gas cap was provided for this volume of the digester. The gas cap is considered within the digester. Therefore, the volume of the digester, amount of gas produced per day (ADD) and m³ of space for gas (or gas cap requirement) was worked out. According to (Dhital 2018) the amount of waste added to the digester is a maximum of 3 kg and a minimum of 1.5 kg of food waste/ kitchen waste added inside a 1000L/1m³ digester. Considering the above, the amount of food waste to be added inside the digester has been worked on and the dimensions of the digester and the calculated volume is given in Table 3. Biogas digester conditions were

maintained at thermophilic conditions and operated under mesophilic conditions.



Figure 2, 3 & 4. Food waste taken from the canteen, made into a fine paste with biogas slurry from WWTP and cow dung slurry

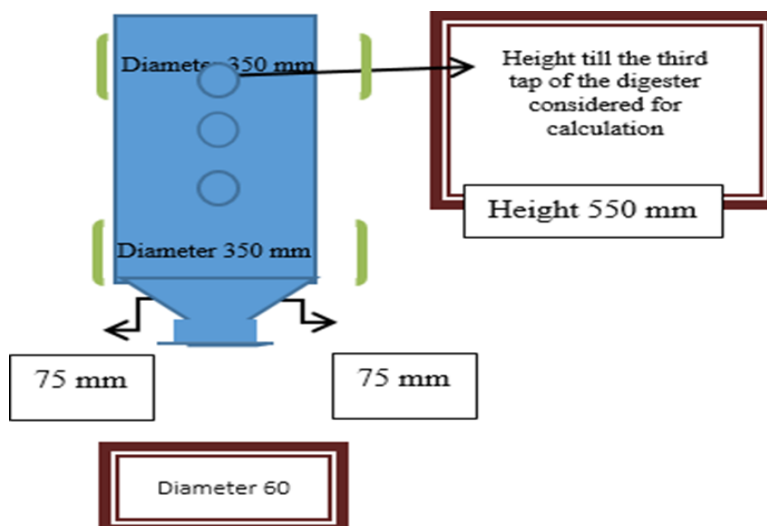


Fig 5. Diagrammatic representation of the Biogas digester represented above

The digester can thus be considered for two volumes, either 64 L capacity or considering the whole digester volume, that is up to 70L capacity.

For this experimental study 64L (up to the last outlet) capacity was considered operational under

the thermophilic conditions.

Reactor volume calculations:

$$(\text{Volume of the digester} = \pi r^2 h$$

$$\text{where } \pi = \frac{22}{7}$$

$$r^2 = \text{radius}$$

$$h = \text{height}$$

Di Dameter = 475mm/1000 = 0.475 m

Using the formula for the cone $V = \pi/12h (D^2 + Dd + d^2)$

$$V = 22/7/12 * 7.5 (35^2 + 35*0.5 + 0.5^2) = 18.26L$$

The total volume of the digester to be filled would be = 45.71+18.26 = 63.978 i.e. 64 L.

The biogas digester is a tank reactor, not an up flow Anaerobic Sludge Blanket (UASB) digester. In a UASB digester the wastewater enters at the bottom and overflows from the top. Organic pollutants in the presence of anaerobic microorganisms to give methane & carbon dioxide., UASB reactor is a form of anaerobic digester which used in wastewater treatment. UASB reactor is a methane-producing digester Spontaneous granulation of hydrogen-producing microbes can occur in a continuous stirred-tank reactor (CSTR) with a reduced hydraulic retention time (HRT). It was observed that the granulation of sludge significantly increased the cell inventory to 16.0 g-VSS/L. With this increased cell inventory, the CSTR can be operated at higher organic loading rates (OLRs) of up to 20 g-glucose/L·h, resulting in enhanced hydrogen production and it was also noted that granular sludge disintegrated within a period of less than 21 days when CSTRs were operated in a static condition instead of an agitated mode. Granulation of hydrogen-producing microbes can occur spontaneously with reduced HRT in CSTR found that granulation of sludge markedly increased the cells inventory to 16.0 g-VSS/L. With such cells inventory, the CSTR can be operated at higher OLRs (up to 20 g-glucose/L·h) along with enhanced hydrogen production and it was also reported that granular sludge disintegrated in less than 21 days when CSTRs were operated in static condition instead of agitated mode (Mainardis et al., 2020). The upward liquid flow rate is calculated so as to maintain the sludge in suspension without causing the sludge flow out of the top of the reactor. The digester was agitated intermittently with a continuously stirred tank reactor. When the digester is agitated it is a Continuously Stirred Tank Reactor (CSTR) The outer wall of the digester is a water jacket heated by an external source.

2.3.1 Daily Feeding Inside the Digester

If the volume of the digester (Table 2) fed is up to 41.05 liter the daily feed is 82g to 120 g (ranging from minimum to maximum feed). If the volume of the digester is fed up to 38 L the daily feed is 75g to 114 g (ranging from minimum to maximum feed)

3. Results and Discussion

The operating conditions and results are summarized in Table 3. Biogas production abruptly stopped when the temperature was increased from 42° to 50°C. Biogas production re-commenced after 5 days at 56°C. Biogas production abruptly stopped and methane content decreased to 5% volume when the temperature changed from 39 to 59°C from 54%. Biogas production recommenced and achieved steady production after 7 days under mesophilic conditions. The reason for the above was that, the temperature was self-operated to check difference towards high thermophilic and temperature change showing that it may be

due to the change in the microbial population and change in reaction when temperature was suddenly changed. In all conditions a stable temperature was maintained to achieve continuous biogas production and methane concentrations greater than 50% volume. The thermophilic process showed increases in terms of organic matter removal and biogas production compared to the mesophilic process as similar as in municipal sludge mentioned by (Wu et al., 2020); in particular, the volatile solids removal passed from 36% in mesophilic conditions to 48% in thermophilic conditions and then to 55%. As a consequence, the specific biogas production was $0.33 \text{ m}^3.\text{kg}^{-1}\text{VS}$ and $0.45 \text{ m}^3.\text{kg}^{-1}\text{VS}$. A test to find the limit of organic loading rate under mesophilic conditions with agitation showed that up to $2.1 \text{ kg VSS}.\text{m}^{-3}.\text{day}^{-1}$ could be loaded without causing a drop in pH. Biogas production increased to $1.440 \text{ m}^3.\text{day}^{-1}$ during this period while volatile solids also accumulated in the slurry. The Biomass Stress Index (BSI) was lowest under mesophilic conditions without stirring as also recommended by (Blasius et al. 2020). The highest BSI was measured at the start of thermophilic conditions when the temperature was increased from 35 to 60°C . The lowest BSI measured under thermophilic conditions (28%) was after 2 weeks of nearly constant temperature (57°C). A high BSI (48%) was measured under mesophilic conditions when the daily organic load was increasing rapidly. One potential reason may be that the reduced microbial stress caused by lower temperature and ammonia levels increases the vulnerability of the community to influences from other digester parameters. On the other hand, the differences observed between the mesophilic and thermophilic-to-mesophilic communities suggest that stochastic processes played a significant role in shaping the microbial community and consequently affecting the process performance during the investigation period (Westerholm et al., 2018)

3.1 Daily feeding the digester

If the volume of the digester fed was up to 41.05 liter, the daily feed is 82g to 120 g (ranging from minimum to maximum feed) while if the volume of liter is fed up to 38 L the daily feed is 5g to 114 g (ranging from minimum to maximum feed) can be considered. Feeding is often specified in terms of the Organic Loading Rate (OLR) as stated in literature (Musa et al. 2018). The OLR is the amount of volatile suspended solids (VSS)/volume of sludge in the digester. For a well-mixed digester, the OLR is in the range of 2 to 20 g VSS/l/day, however the similar values were observed by (Sun et al., 2017 and Hwang & Hansen, 1998). For 41 liters of sludge, the load of 82 g of VS. In the case of food waste, the VSS is approximately 90% of the dry solids. The dry solids are approximately 25% of total mass. In order to load 82 g of VSS, the requirement to be added is approximately 365 g of food waste (wet weight). $365 \times 25\% = 91 \text{ g dry matter}$. $91 \times 90\% = 82 \text{ g VSS}$. The current feed rate is probably correct for an unmixed digester. However, with rate can certainly be increased. Most of the time the digester was agitated. In order to compare biogas production from different volume of sludge, gas production can be expressed in terms of liters of biogas/liter of sludge/day. In the case of N°1: 28 liters' biogas/41.5 liters' slurry = 0.68. In the case of N°2: 26 liters' biogas/38 liters' slurry = 0.68. These results are in the range from 0.5 to 2. The digester was stirred and left unstirred during the weekend. After 2 days, *i.e.* on the 3rd day gas production commenced at 11 am. Initially the temperature was 42°C and later it was increased to 50 and

55°C and then to 58°C. Stirring was carried out initially for 1 hour in the morning and 1 hour in the evening. This was gradually increased up to 3 hours. During the maximum gas production, the stirrer operated continuously with only very small halts. The digester was insulated to avoid loss of heat from the digester during the nights. This shows higher gas production with respect to stirring. During the study, the digester was stirred and left unstirred during the weekend and the reason for the decrease in the biogas production during the weekend was due to no stirring which was carried out during day. This could be the reason. Sometimes, excess slurry was removed to see the changes happening. Once gas production started, it enters the water jar, where air bubbles were observed. The presence of the air bubbles is an indication of gas production. This is passed through silica gel to keep the gas pure as mentioned by (Al Mamun and Torii, 2017) the increase in gas concentration by removing contaminants from biogas mixtures using combined method of absorption and adsorption. The CH₄ sensor measures infrared absorbance and records the agitation, the feed percentage of methane in the biogas from the below Table 3, it can be inferred that there has been a reduction of volatile acids from 44.54% to 30.0% in the month of October, during the initial feed and at the end of October. On 30th October gas production reached 45-50%. The humidity decreased from 10.03 to 1.16%. pH decreased from 7.5 to 6.9 at the end of October., due to change in pH and temperature the humidity has decreased as similar results were revealed by (Yin et al., 2016) Methane production started to raise and was between 45-50% respectively. Brief description of the estimations: Gas production for the first time occurred in less than 2 and half days as the slurry was old when brought from the waste water treatment plant. With 7% of CH₄ production occurred between 11am – 2 pm. CH₄% production remained above 8% after 6pm and all through the night. Initial temperature was set to 42°C which was raised to 50°C within a short duration of time. The temperature was raised to check the efficiency of gas production at high temperature and how long it would take to derive at it. One of the main reasons was also to the thermophilic conditions.

3.2 Gas Production Details from the Graphs Obtained

It is observed that, when the temperature was risen from 42°C to 50°C the gas production abruptly stopped till the temperature was stabilized in about 4 hours (Fig 6). During the night of the third day the temperature was maintained at 55°C and the CH₄ production nearly reaching 12% on the fourth day, as there was drastic change in the temperature 49-60 °C on the fifth day the gas concentration was moving upwards. Sudden fluctuations in temperature led to drop off (activity at temperature above 45°C), as mesophilic bacteria become inhibited by heat. No gas production was recorded rather the gas production decreased on the 6th day the temperature was 52°C, CH₄ % came up to 4% and on the 7th day the temperature ranged between 49°C-51°C, CH₄ % raised up between 7-8%. (When temperature decreased to 45°C CH₄ percentage increased to 12%). Day 8 had a 12- 13% of CH₄, as temperature decreased to 42°C - 43°C gas production was seen to increase and remained at 20% CH₄ production. At almost 50°C the CH₄ concentration was almost nil. When temperature decreased to 42°C gas production was at 6% while at 50°C the CH₄ production remained at less than 4%. At 52°C-42°C gas production remained nil. At 55°C gas production started rising to 20-22% of CH₄. When the temperature was maintained between 55-58 °C, CH₄

risers above 30-33% when temperature was above 58°C. Gradually there was a rise to nearly 35% CH₄ when the temperature remained constant at 58°C. This shows that at 52°C gas production decreased and when the temperature brought down to 45 °C rise in gas production was seen gradually. At mesophilic temperature, the maximum gas production was, 55% and at thermophilic temperature maximum gas production was 66% both under non-stirring activity and with stirring (30 minutes/day), the temperature at mesophilic with 51 % and mesophilic (continuous) with 60 % gas production. During the month of October, the total amount of feed added into the biogas digester was 33,740 mL as compared to the initial start, which was about 13100 ml. During 15th October, it was noted that the decrease in biogas production was observed due to removal of 500mL of the digester slurry, followed by removal of 200mL of slurry on the 16th day. This was done deliberately for study purpose only. Therefore, by the end of October the amount of waste inside the digester was 33740ml. During November 1000mL of waste was removed, due to this there was a sudden drop in the biogas production, observed (Figure 7b). It nearly took 7 days for the gas production to get stabilized from October November months as temperature dropped by 10°C (Figure 7a & 7b).

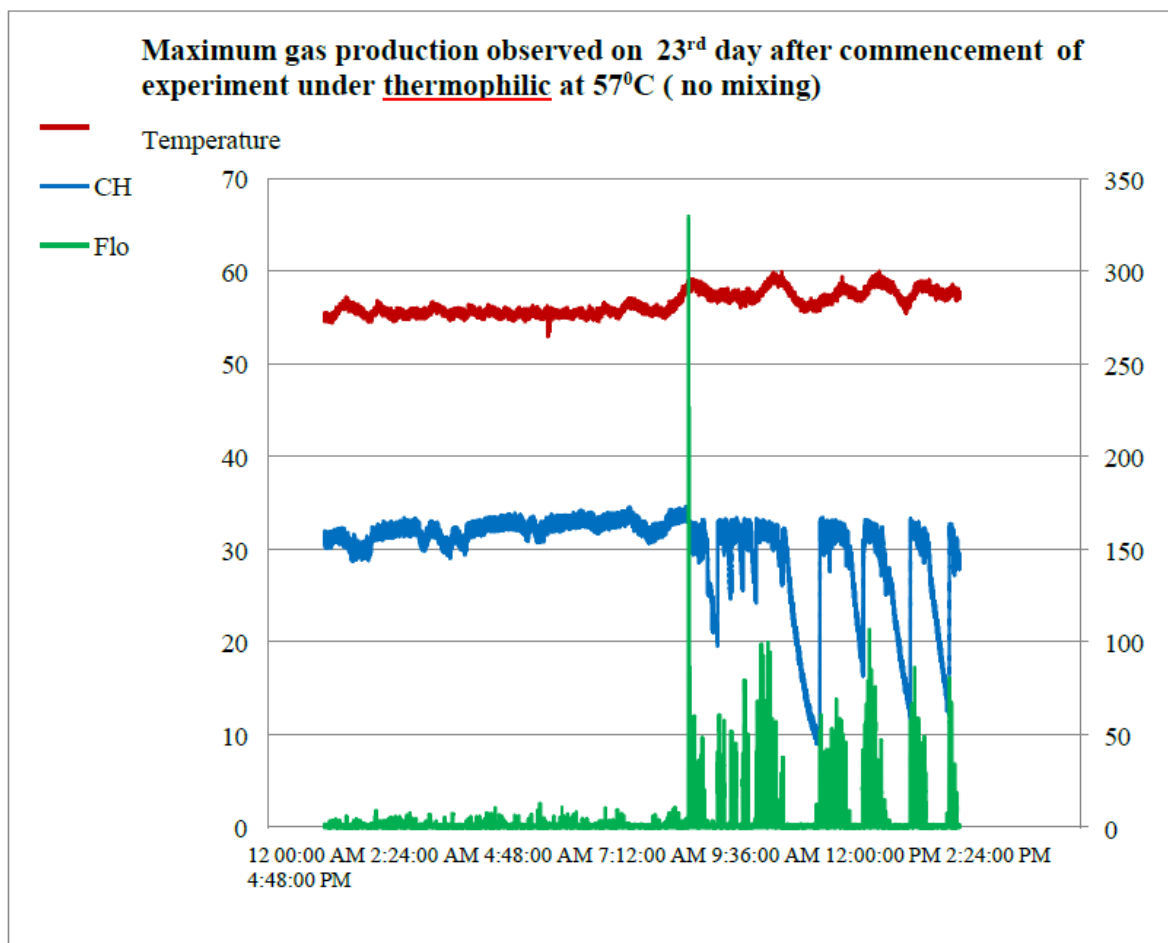


Figure 6. Gas production on the 23rd day after commencement of experiment (Thermophilic condition)

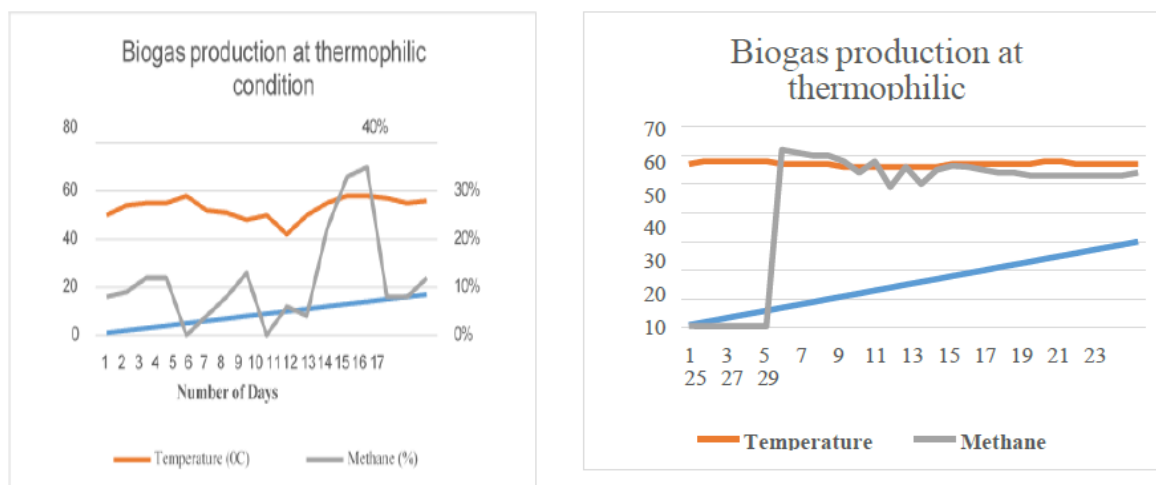


Figure 7a & 7b. Biogas production under thermophilic conditions

The amount of waste slurry during November was 32740mL. There was an increase in biogas production after 11 days, followed by drop of biogas production on 15th day with a gradual increase in biogas production. The methane production reached only up to 35% during this time. This was mainly because there was a sudden change in temperature from thermophilic condition to mesophilic condition (39-40⁰C) to 56⁰C. Further in November the methane production reached between 50-66% respectively. The temperature above 55⁰C -59⁰C, reported peak methane production. During December, the production of gas varied and fluctuated as the temperature was adjust to mesophilic conditions from thermophilic conditions. The biogas production ranged between 59⁰C to 30⁰C Reduction in temperature seen when temperature had changed to 39⁰C from 59⁰C, hence sudden fall in the values have been observed in figure 9a. As there was further reduction from 35⁰C to 31-32⁰C sudden increase to 46% methane was observed which further then reduced and dropped drastically between 30⁰C and 29⁰C with removal of sludge. The gas production severely dropped down as the temperature was maintained only at mesophilic conditions. At 40⁰C biogas production lowered.

The above four graphs plotted for October, November, December and January, point with rise up out gradual increase in gas production and sudden fall in gas when temperature changed drastically by 8 to 10⁰C. There was an increase in gas production in the month of November. In December, there were changes in biogas production as the bio digester worked only in thermophilic condition. Finally, in January there was a fall in the biogas production under mesophilic condition and feed inside the digester reduced drastically and finally stopped. On the 14th day, the amount of methane production was 35%, 44th day 66% under thermophilic conditions. At the starting 13100 mL of food waste and water was added by 18 days, 33740 mL was added. In the month of January 35858 ml of waste was present in the digester, where as in February the amount of waste was 35855 ml. During this time, the biogas production decreased. T-ATP, or transient adenosine triphosphate, represents a more

recently discovered form of ATP in biogas production. It is distinct from c-ATP and d-ATP and is believed to exist in a transient state during microbial metabolic processes. Although its precise role in biogas production is still being investigated, studies suggest that t-ATP may serve as an intermediary ATP pool for intercellular energy transfer, contributing to the overall efficiency of biogas production. The significance of the above analysis carried out included ATP analysis showed that microorganisms stress was higher under thermophilic than under mesophilic conditions, it can be concluded that for food waste and mixtures of food-waste and cow-dung, thermophilic conditions result in increased biogas flow and an 11% increase concentration compared to mesophilic conditions, without stirring. The comparative analysis of c-ATP, d-ATP, and t-ATP in biogas production highlights the significance of these adenosine triphosphates as vital energy carriers for microbial metabolic processes. C-ATP provides the necessary energy for various stages, including hydrolysis, acidogenesis, and methanogenesis. D-ATP, present in the liquid phase, acts as a readily available energy source for microbial uptake. While the role of t-ATP in biogas production requires further research, it is postulated to contribute to intercellular energy transfer (Whalen et al. 2014) From the above Fig it is observed that the digester was run first on thermophilic conditions until 65/77 days plus days and the remaining days were studied under mesophilic conditions. Initially the biogas digester ran up to 54% with maximum peak at 66% and then followed a trend towards 34% reaching 55% and then gradually dropping due to stopping entry of feed and some about of biogas slurry being removed. The whole experimentation lasting for a period of 109 days. According to the literature, the biogas plants with mesophilic digester are suggested to get upgraded with existing mesophilic digester's (35⁰C to thermophilic digester) (Vindis and Mursec 2009). 55⁰C is economically beneficial solution compared to construction of additional mesophilic digester in cold regions. On the 12th day gas production was stable and the temperature was maintained at 58⁰C. Food waste was added into the digester once a day and constant stirring was maintained. There was a good consistency in gas production with CH₄ concentration between 30- 35%, in comparison with the earlier the CH₄ concentration remained at 25% (*i.e.* on the 10th day). On the 13th day similar conditions were maintained and there was a good flow between 60-65ml/min following on the 14th day 106ml of food waste slurry was added into the digester and the biogas pipe connection was disconnected from the software and directly connected to the glass bottle containing water and the amount of water displaced by gas for three hours was about 215 mL and that from 8.58am-11.26am was about 200mL. The temperature was maintained at 58⁰C with constant stirring, after 50 days, there was a gradual reduction in the percentage of methane production to 54% - 55%. The temperature when altered from thermophilic condition to mesophilic conditions with gradually bringing the temperature to 37⁰C and further lowering the temperature between 32 and 35⁰C, with this the gas production dropped to 2%. On the 8th day gas production increased to 34% and on the 28th day the gas production increased to 46% at 31-32⁰C and from the 35th day there was a decrease in the biogas production with only 3-7% of biogas being produced (as there was removal of slurry) with this maximum efficiency in biogas production at 31-33⁰C with 55% on the 34th day. It took nearly 28 days for the methane production to reach 46% at 31-32⁰C and at 34th day the methane concentration rose to 55% and at 35th day there was a sudden decrease in the methane production. Fluctuations in temperature between

39-59°C reported 54% of biogas production and drop in temperature while setting the bio digester to mesophilic conditions showed immediate lowered gas production between 2-5% only between 35-37°C. Total thermophilic digester run time was about 77 days with maximum biogas production at 66% and total mesophilic digester run time is about 31 days with maximum biogas production at 55%. The calorific value of the digester was calculated at various methane percent (Table 3) The weight of the waste burned for energy is used to find the calorific value, or heat value produced. According to the literature, the biogas plants with mesophilic digester 35°C are suggested to get upgraded to thermophilic digester 55°C which is economically beneficial solution compared to construction of additional mesophilic digester. Reaction rates drop off considerably as temperature falls below 35°C and there is also a sharp drop off in activity at temperatures above 45°C, as mesophilic bacteria become inhibited by the heat. The above studies clearly point out that at 34°C methane production was 31-35% and at 46°C it was nearly the same but 54-56°C had the maximum gas production. Therefore, biogas production is higher on an average in thermophilic conditions compared to mesophilic conditions as stated in literature (Mursec et al., 2009). Gas production till the 22nd Oct was not continuous and it took place from the 23rd day. Gas production under thermophilic and mesophilic conditions has been indicated in the graphs (8a & 8b, 9a & 9b). The mesophilic bacteria will be killed after less than fifteen minutes at a temperature of 50°C or greater and various studies have reported that efficiency of methane production is equal to 57.7% and 62.8% respectively (Sathish and Vivekanandan 2016) and our study clearly points out maximum methane production at 66% at 56°C, while authors have mentioned good efficiency in the production biogas takes place at thermophilic conditions. Our operational efficiency may be attributed to the understanding that as this work was carried out in Switzerland, laboratory conditions, a layer of good insulation surrounding the digester, given for extra protection and for better performance. Thermophilic digestion offers the advantages of faster reaction rates compared to mesophilic digestion, leading to shorter retention times and thermophilic systems can be of benefit where high solid content feedstock with optimal C: N ratios are available consequently, temperature control for the anaerobic digestion process is considered as one of the main design parameters.

Mesophilic and thermophilic bacteria have high alkalinity, and digestion under thermophilic conditions showed a better solid destruction and biogas yield. On the other hand, Continuous-Feed Digesters have increments of charge added and subtracted on a daily basis to provide an ongoing replenishment of charge materials and water. It is obvious that the amounts withdrawn and replaced should be exactly the same or the digester may become either overloaded or under loaded. After feeding the digester with 300g of food waste on the 19th October the digester was operated at 54°C during the weekend. There was a fluctuation in the temperature and the temperature remained between 48- 49°C after changes were made. (This sudden changes in temperature causes drop in the biogas production). The temperature changes are due to external sources like the changing room temperature and the water heater and they are unavoidable if not the production cost may go up and the water heater maintains the temperature at give range with stable maintenance as similar type of setup with different objectives was studied by (Kabeyi and Olanrewaju, 2022). On the 11th day, the production of methane ranged between 30-35% after gas bubbles being produced and

values being recorded using the software. The 11th day had maximum gas production in comparison to the other days and after the addition of cow dung, gas production commenced within a period of 23 hours, at 58⁰C where the temperature was maintained stable, good gas production was recorded with continuous flow (Fig., 8) From this experimental study maximum and continuous gas production was recorded above 57⁰C until 59⁰C and addition of cow dung slurry has shown to increase the biogas production after 11 days period when gas production just commenced. On the 12th day gas production was stable and the temperature was maintained at 58⁰C and food waste was added into the digester once a day with constant stirring was maintained. There was a good consistency in gas production with CH₄ concentration between 30-35%, in comparison with the earlier the CH₄ concentration remained at 25%. On the 13th day similar conditions were maintained as on 12th day and there was a good flow between 60-65ml/min $60 \text{ ml/min} * 60 * 24/1000 = 86.4$ liters biogas/slurry volume = approximately and excellent result, the similar method and the quantification of flow rate was followed by (Okonkwo et al., 2018) however, these are the peak flows and the total flow is the area under the peaks. On the 14th day 106ml of food waste slurry was added into the digester and the biogas pipe connection was disconnected from the software and directly connected to the glass bottle containing water and the amount of water displaced by gas for three hours was about 215 mL, that from 8.58-11.26am was about 200mL with 58⁰C temperature maintenance and constant stirring. (24 hours * 0.2 liters' biogas/2,5 hours = 1.92 liters/day).

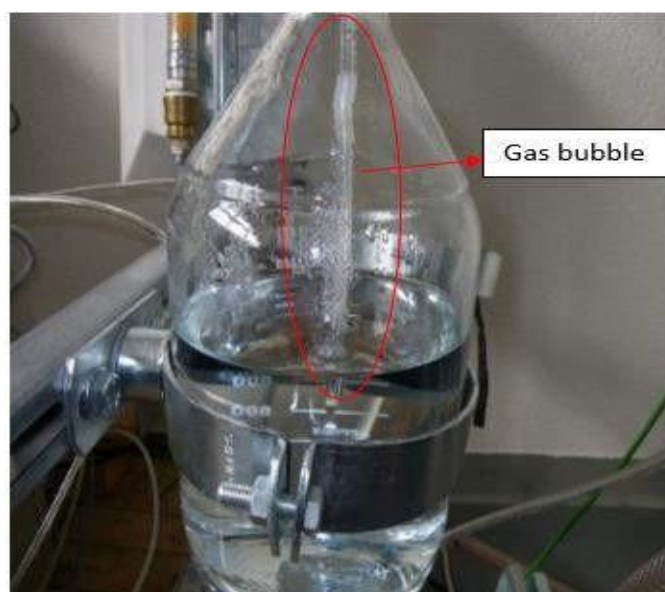


Figure 8. Gas bubbles seen when gas collected inside the jar containing water

Fluctuation in temperature between 39 and 59 degrees reported at 54% of biogas and drop in temperature between 35-37⁰ lowered gas production between 2-5%. It took nearly 25 days for the methane production to reach 46% at 31-32⁰C at 31st day the methane concentration rose to 55⁰C and at 32nd day there was a sudden decrease in the methane production, the reason for

this sudden decrease may be removal of the slurry. (91-92 days). On the 18th day when pH was 7.24 the methane concentration was at 35% and ATP was analyzed with drop in temp from 59% to 35-37 °C led to drastic reduction in biogas production with further decrease in temperature. Once temperature was increased again biogas production started to increase, the temperature increased too. The amount of waste in the digester was 33740 ml and 1000 ml was removed on the 22nd day later 1650 mL was added by two-month period along with periodical removal therefore, the total amount of waste present in the reactor at the end was 34390 mL. In the last month of the experiment 1923ml was added in total which accounts to 36313ml of waste in the biogas digester.

The biological activity in the digester by a method that quantifies ATP and the number of dead cells (BSI%) was very high, probably because the feeding of the digester has been carried out without wasting and after the start-up phase, excess sludge is to be removed daily. The ATP analysis results shows the following results: tATP: ATP (dead cells + live cells), dATP: ATP (dead cells only), cATP: ATP (live cells only) and the biological activity in the digester by a method that quantifies ATP (Mauerhofer et al., 2019). The number of dead cells BSI: Biomass stress index (BSI%) is very high, this is probably because the feeding of the digester has been carried out without wasting and after the start-up phase, excess sludge should be removed daily.

4. Conclusions

The study was focused to work on both thermophilic and mesophilic conditions using the anaerobic digester for both non stirring and stirring conditions. The study reveals that anaerobic digestion can recover from abrupt changes between thermophilic and mesophilic conditions without intervention or changing the feeding regime. The amount of biogas produced in thermophilic condition was at 66% at 7.48 pH and in mesophilic condition it was 55%. If biogas production is the objective, then the long time i.e., 7 days, is required to recover from an abrupt change from thermophilic to mesophilic conditions and consequent loss of biogas production suggests that the temperature should be regulated to maintain either in mesophilic or thermophilic conditions and solids loading rate at all times and the same in full scale digester as mentioned respectively also by (Andersson et al., 2020; Shin et al., 2022). The most stable and productive test condition was mesophilic, ~37°C with constant agitation. Methane production under mesophilic conditions with agitation one time per day was 20% greater than under mesophilic conditions with no agitation. In the case of a 1000-liter digester this difference is equivalent to approximately 400 liters/year of bottled propane at 10 bars pressure. Manual agitation one time per day and installation of a dual purpose solar water heater for domestic hot water supply and digester heating with a temperature sensor and an automatic valve to limit the digester temperature to 37°C are proposed. Finally, after feeding the digester with 300g of food waste the digester was operated at 54°C during the weekend and after 10 days no gas production was recorded in the morning because of fluctuation in the temperature but the temperature remained between 48-49°C, this clearly indicates that sudden changes in temperature indicates drop in biogas production as expected.

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