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Influence of Temperature and Substrate Composition on Anaerobic Biogas Production in a Pilot-scale Reactor

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ABSTRACT

This research investigates how temperature and substrate composition impact anaerobic biogas production in a pilot-scale reactor. Anaerobic digestion is a vital process for converting organic substrates into biogas, mainly composed of methane and carbon dioxide. Temperature plays a crucial role in influencing this process. The experiment was conducted using leaf litter of ***Rcinus Communis*** *and* ***Datura Stramonium*** *mixed with cow dung under mesophilic (35°C), thermophilic (55°C), and hyper thermophilic (80°C) conditions, using a pilot-scale reactor with a capacity of 50 liters and an effective volume of 40 liters. Key parameters, including biogas production, carbon and nitrogen content, carbon-to-nitrogen (C:N) ratios, pH levels, and biomass concentrations, were monitored throughout a 60-day operational period. The highest biogas production, reaching 6398 ml/d, occurred under mesophilic conditions. Scanning Electron Microscopy (SEM) and phylogenetic analysis were performed, revealing the presence of* ***Methanococcus aeolicus*** *species in the treated sludge.*

Index-words: *Organic waste, Temperature, Biogas yield, Microbial identification.*

I. INTRODUCTION

The global community has increasingly focused on renewable resources in recent decades, which are crucial for current CO_2 reduction strategies. Biomass and waste-derived energy are considered popular renewable sources, providing a continuous electricity generation capability. Organic waste materials, owing to the rapid growth of energy crops, cost-effective cultivation, and the abundance of manure, play a particularly significant role (Roy et al., 2021; Aravani et al., 2022). Anaerobic digestion, a biotechnological process, transforms organic substrates into valuable biogas, mainly composed of methane and carbon dioxide. The temperature during anaerobic digestion is a pivotal factor influencing its performance. Various operational characteristics, including temperature, pH, reactor configuration, organic loading rate, and hydraulic retention time (HRT), have been investigated as contributing variables to biogas production (Tsigkou et al., 2019, 2020, 2021, 2022; Zakoura et al., 2022). pH, a parameter previously mentioned, significantly affects the distribution of acidogenic products (Tsigkou et al., 2020, 2022; Zagklis et al., 2021). HRT is crucial, impacting the microflora and features of continuous stirred-tank reactor (CSTR) systems, necessitating careful management. Batch test results can serve as a basis for methane productivity testing due to the complexity of continuous anaerobic digestion procedures (Antonopoulou and Lyberatos 2020). In recent years, there has been increased attention on assessing the anaerobic co-digestion of energy crops with other wastes. For example, Giuliano et al. (2013) found that co-digestion of manure, energy crops, and agro-wastes using pilot-scale CSTRs was feasible under various operating conditions. Manure, rich in volatile organic chemicals and alkalinity, is a favored substrate in anaerobic co-digestion situations (Dareioti et al., 2021). Specifically, Ricinus communis and Datura stramonium leaves are deemed suitable for biological processes due to their toxic nature and high organic matter concentration for biogas production. These leaves, rich in cellulose and other organic compounds, are ideal for biogas production. However, the literature on the anaerobic digestion (and co-digestion) of *Ricinus communis*, *Datura stramonium*, and sweet sorghum as substrates, particularly in two-stage systems for hydrogen and methane production, is limited (Antonopoulou et al., 2008; Matsakas et al., 2014; Ma et al., 2020; Nozari et al., 2008). This study aimed to investigate the impacts of mesophilic (35°C), thermophilic (55°C), and hyperthermophilic

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(80°C) conditions on anaerobic digestion using leaf litter of *Ricinus Communis* and *Datura Stramonium* mixed with cow dung in a pilot-scale reactor. The assessment included biogas production, carbon and nitrogen content, C: N ratios, pH levels, and biomass concentrations over a 60-day operational period.

II. MATERIALS AND METHODS

A. Reactor Setup

In this investigation, a pilot-scale anaerobic reactor was utilized, featuring a total volume of 50 liters and a working volume of 40 liters. The reactor was equipped with essential components such as an agitator, a pH sensor, and a temperature sensor to facilitate the monitoring and control of crucial parameters. The hydraulic retention time (HRT) was specifically set at 24 hours, ensuring optimal conditions for the anaerobic digestion process. Maintaining the influent pH within the range of 6 to 7 was a critical aspect of the experimental setup to create favorable conditions for microbial activity. The reactor operated under different temperature regimes, including mesophilic (35°C), thermophilic (55°C), and hyperthermophilic (80°C), with agitation maintained at 60 revolutions per minute (rpm). This controlled environment allowed for a systematic exploration of the impact of temperature on anaerobic biogas production, providing valuable insights into the process under varying thermal conditions.

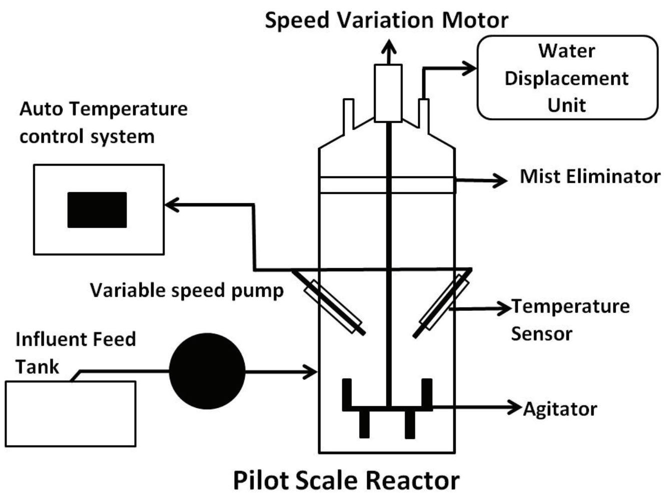

Fig. 1. Schematic representation of a pilot scale reactor

B. Data Collection

Throughout the 60-day operational period, a comprehensive set of parameters was monitored daily. Biogas production, carbon and nitrogen content, pH levels, and biomass concentrations were among the key variables investigated to assess the performance of the anaerobic reactor under different temperature conditions. Biogas production, expressed in milliliters per day (ml/day), served as a crucial metric reflecting the efficiency of the anaerobic digestion process in converting organic substrates into biogas. Total Organic Carbon (TOC): TOC is typically measured using a TOC analyzer, which oxidizes the organic carbon in the sample to CO_2, which is then quantified. Total Nitrogen (TN): TN is often measured using the Kjeldahl method, which involves digesting the sample in sulfuric acid, distilling the ammonia produced, and titrating it to determine nitrogen content. The carbon and nitrogen content, measured in milligrams per liter (mg/L), provided insights into the composition of the digestate and the nutrient dynamics within the reactor. Additionally, the carbon-to-nitrogen (C:N) ratio, a calculated parameter, offered valuable information about the substrate's suitability and its impact on the overall digestion process. pH levels were monitored to ensure the maintenance of optimal conditions for microbial activity. Biomass concentrations were also analyzed to understand the microbial population dynamics and their response to different temperature regimes. This comprehensive daily monitoring regimen allowed for a detailed assessment of the reactor's performance over the entire experimental duration.

III. RESULTS AND DISCUSSION

A. Biogas Production

The biogas production during the mesophilic (35°C) condition exhibited notable fluctuations throughout the 60-day observation period. It showcased a range from a minimum of 607.8 ml/day on the initial day to a peak of 6398.4 ml/day on the 37^{th} day. Similarly, the thermophilic (55°C) condition experienced variability, with a low of 835.7 ml/day at the startup and a high of 5,109 ml/day on the 35th day. In the hyperthermophilic (80°C) condition, biogas production ranged from 686.4 ml/day on the first day to 3,742.5 ml/day on the 35th day. Li et al. (2022) investigated the effects of different temperature regimes on biogas production from agricultural residues, emphasizing the role of thermophilic conditions. Additionally, it is important to note that the volatile solids (VS) reduction of dry cattle manure (DCM) in continuously stirred biogas digesters is approximately 27-33% due to its high fiber content. High fiber content poses a challenge for efficient VS reduction. The findings underscore the significant influence of temperature on biogas production, with the mesophilic condition demonstrating the highest overall production. A comprehensive review by Smith et al. (2021) discussed advancements in anaerobic digestion technologies and their implications for renewable energy production. The potential for enhancing methane production through anaerobic co-digestion, especially with substrates featuring higher volatile solids (VS) and nutrient concentrations than dry cattle manure. Studies, such as that by Gaballaha et al. (2020) suggest that anaerobic co-digestion of cattle manure (CM) with pre-treated rape straw can lead to a 59.0% increase in methane production, showcasing the potential benefits of combining different organic materials. However, it is crucial to consider hydraulic retention time (HRT) limitations in continuous feeding digesters, especially when dealing with high-strength substrates like Leaf litter mixed with cow dung, and slaughterhouse manure. Increased VS concentration may not guarantee optimal degradation of all organic materials, leading to lower digestion efficiency in digesters with a high substrate ratio. Therefore, post-digestion tests become essential to assess the residual biogas yield, particularly in digesters with a significant proportion of substrate in the combined substrate. Zhang et al. (2023) explored the co-digestion of food waste and manure, highlighting the enhanced biogas production and microbial dynamics. The aim is to maximize methane production through anaerobic co-digestion, aligning with the observed results and findings in Mathew et al.'s (2015) experiment.

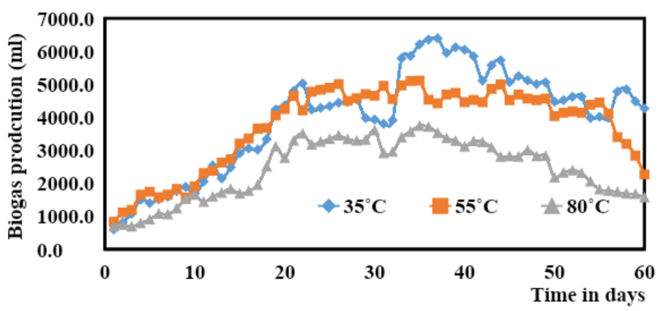

Fig. 2. Biogas production at 35°C, 55°C and 80°C in a pilot scale reactor

B. Carbon and Nitrogen Content

The carbon and nitrogen content within the reactor exhibited a parallel trend to biogas production. In the mesophilic condition, carbon content ranged from 3124 mg/L on the initial day to 3375 mg/L on the 37th day, while nitrogen content varied between 665 mg/L and 2474 mg/L. The C:N ratio in the mesophilic setting fluctuated from 19 on the initial day to 31.7 on the 37th day. Under thermophilic conditions, carbon content ranged from 1,831 mg/L to 3382 mg/L, and nitrogen content varied from 665 mg/L to 1031 mg/L. The C:N ratio in this condition spanned from 21 on the initial day to 32.3 on the 37th day. For the hyperthermophilic condition, carbon content ranged from 665 mg/L to 3343 mg/L, with nitrogen content fluctuating between 665 mg/L and 1,319 mg/L. The C:N ratio in this condition varied from 24.4 on the initial day to 31.7 on the 37th day. These outcomes underscore the influence of substrate composition and temperature on carbon and nitrogen content, with peak values observed in the mesophilic condition.

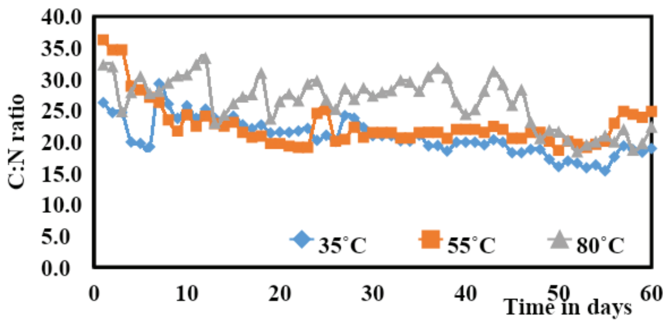

Fig. 3. Carbon to Nitrogen ratio at 35°C, 55°C and 80°C in a pilot scale reactor

C. pH Levels

The co-digestion of cow dung and mixed leaf litter of *Datura Stramonium, Ricinus Communis* demonstrated no significant effect ($p>0.05$) on the pH values of the digested slurries, maintaining a range of 6.93-6.99. This pH range aligns with the reported ideal stability range of 6.8 to 7.4 for anaerobic digestion processes, as highlighted by Mao et al. (2015). The volatile solids (VS) reduction fell within the range of 35-37%. Interestingly, the utilization of mixed leaf litter as a co-substrate for CD did not yield a positive effect on VS digestibility ($p>0.05$). Bruni et al. (2022) noted a biodegradation rate of approximately 40-50% of total solids (TS) for livestock manure, attributing the low rate to the significant fraction of lignocellulosic biofibers present in animal manure. Throughout

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the 60-day duration, the pH levels in the reactor consistently remained within the desired range of 6 to 7 for all temperature conditions, ensuring the stability of the anaerobic digestion process.

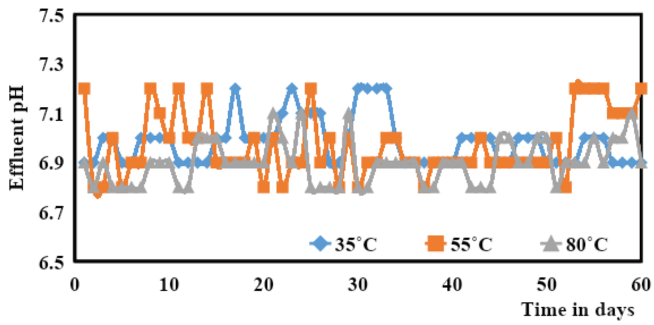

Fig. 4. Effluent pH at 35°C, 55°C and 80°C in a pilot scale reactor

D. *Biomass Concentration*

The biomass concentration within the reactor exhibited variations under different temperature conditions. In the mesophilic condition, the biomass concentration declined from 26.2 g/L on the initial day to 21 g/L on the 37th day. Similarly, in the thermophilic condition, there was a decrease from 36.3 g/L to 28.9 g/L, and in the hyperthermophilic condition, a decrease from 32.2 g/L to 27.1 g/L was observed.

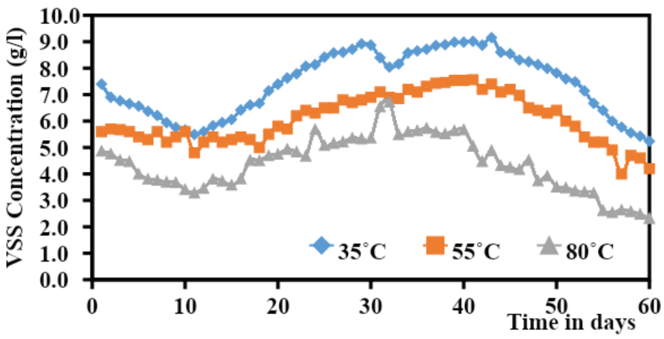

Fig. 5. Biomass concentration at 35°C, 55°C and 80°C in a pilot scale reactor

E. *Phylogenetic analysis*

The sequences of these 16S rRNA genes were compared using the BLASTN programme (Altschul et al., 1990) against the sequence available from Gen Bank and were aligned using CLUSTALW software (Thompson et al., 1994). According to Kimura (1980), two parameter correction distance were computed. Using the neighbor joining process, phylogenetic tree was constructed (Saitou and Nei, 1987). Based on 1000 replications bootstrap analysis was performed. The MEGA4 package (Tamura et al., 2007) was used for all analyses.

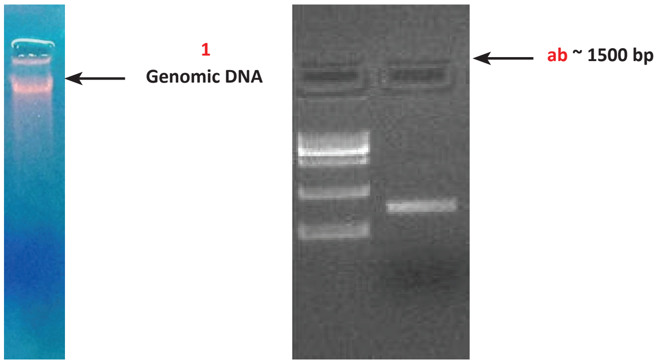

Fig. 6. Genomic DNA and PCR amplification methanogenic bacterial isolate

Conditions: 1.5% agarose gel electrophoresis
(Lane a: 1kb DNA Ladder; b: Sample)
1 KB DNA Ladder (bp):5000, 4000, 3000, 2000, 1000

1. Sequences of the methonogenic bacteria

>Seq_methonogenic Isolate

TTTCCGGTTGATCCCGCCGGAGGCTACTGC
TATTGGGATTCGACTAAGCCATGCGAGTCTATG
GACTTCGGTCCATGGCGGACGGCTCAGTAACAC
GTGGCTAACCTACCCTCAGGTGGGGCATAACCTC
GGGAAACTGAGGATAATACCCCATAGGAAAA
GAGGTTTGGAATAATCCTTTTCTGAAAGGA
TATCCGCCTGAGTATGGGGCTGCGTCCGATTAGG
TAGTTGGTGGGGTAATGGCCACCAAGCCTCGATC
GGTACGGGCCTTAGAGAGGGAGCCCGGAGAT
GGGGACTGAGACACGGCCCGAGGCCCTACGGG
GCGCAGCAGGCGCGAAACCTCCACAATGCAC
GAAAGTGCGATGGGGGGATCCCAAGTGCCTAT
GCACAGCATAGGCTTTTCCCAAGTCTAAA
CAACTTGGGGAATAAGGGCTGGGCAAGTCCGGT
GCCAGCAGCCGCGGTAACACCGGAGGCCCGAT
GGTAGCTACTCTTATTGGGCCTAAAGCGTCCG
TAGCCTGTTCAGTAAGTCTCTGTTTAAATCCTAC
GGCTTAACCGTAGACCTGGCAGAGATACTGCT
GGACTTGGACCGGGAGAGGAAGAGGGTACTTC
GGGGGTAGCGGTGAAATGCGTTGTCCCTGAGG
GACCACCTATGGCGAAGGCACTCTTCTGGAACG
GGTCCGACGGTGAGGGACAAAAGCCAGGGGAG
CGAACCGGATTAGATACCCGGGTAGTCCTGGC
CGTAAACTTTGCGAACTAGGTGTCATCTGGACTC
GGGTCCAGGTGGTGCCGAAGGGAAGCCATTA
AGTTCGCCGCCTGGGGAGTACGGTCGCAAGACT
GAAACTTAAAGGAATTGGCGGGGGAGCACCA
CAACGGGTGGAGCCTGCGGTTTAATTGGAATTA
ACGCCGGGAATCTCACCGGAGCGACAGCATGAT

GAAGGTCAGGTTGACGACCTTACCTGAAGCGCT GAGAGGTGGTGCATGGCCATCGTCAGCTCGTAC CGCGAGGCGTCCTGTTAAGTCAGGTAACGAGC GAGACCCGTGCCCTATGTTGCTACTTTCTTCTC CGGAGGAAAGGCACTCATAGGGGACCGCTG GTGTTAAACCAGAGGAAGGAGCGGGCAAC GATAGGTCCGCATGCCCCGAATCTCCTGGGC TACACGCGGGCTACAATGGTTAGGACAATGG GAAGCAACCCTGAGAAGGGAAGCAAATCTCT TAAACCTAATCGTAGTTCGGATCGTGGGCTG TAACTCCCCACGTGAAGCTGGATCCGTAGTA ATCGCAGTTCATAATACTGCGGTGAATGTGTC CCTGCTCCTTGCACACACCGCCCGTCACACCAC CCGAGTTGGGTTGAGGTGAGGCCCTAGCCTTTG GCTAAGGTCGAACCTCGGCTCAGCAAGGGCGGT

2. Phylogeny tree analysis of the methonogenic bacteria

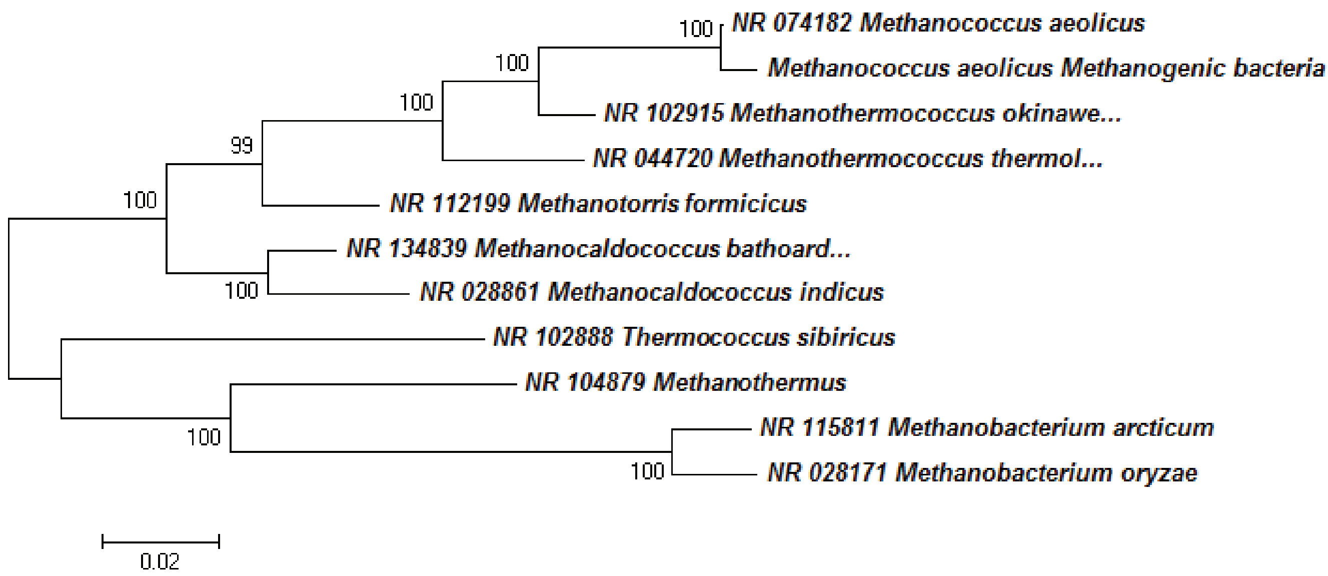

Fig. 7 Phylogeny Tree analysis of the methonogenic bacteria

Using the Neighbor-Joining method, evolutionary history was inferred (Saitou and Nei, 1987). The optimal tree with the length of the branch sum = 0.50428292 is illustrated. Next to the branches (Felsenstein, 1985), the percentage of replicate trees in which the associated taxa clustered together in the boostrap test (1000 replicates) is shown with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree, the tree is drawn to scale. The evolutionary distance was determined using the kimura 2-parameter model (kimura, 1980) and are in units of the number of base substitutions per location. The codon positions used are 1^{st} + 2^{nd} +3^{rd} + Noncoding. All locations containing alignment gaps and missing data have only been removed in pairwise sequence comparisons (pairwise deletion option). A total of 1409 positions were identified in the final dataset. Phylogenetic analyses were performed in MEGA4 (Tamura et al., 2007). Based on the BLAST analysis in the NCBI, RDB taxonomy analysis and phylogeny tree clearly revealed that that the given sample was belog to the taxa is *Methanococcus aeolicus*.

3. SEM image of the methonogenic effluent

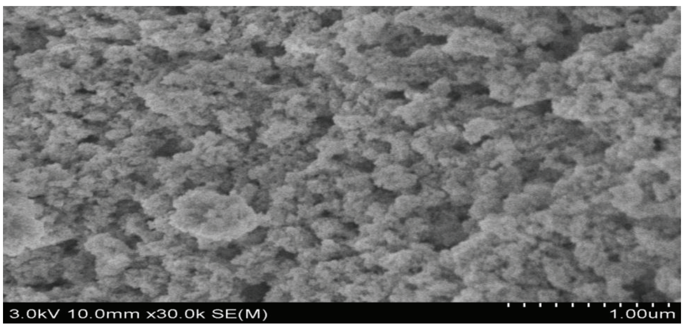

Fig. 8. SEM image of the methanogenic effluent

A scanning electron microscopy was used to observe the sample under different magnifications. Most of the studies were focused on the microbial population distribution in the ABR, and the results showed partly disparity of microbial population distribution under different experimental conditions (Sallis and Uyani, 2003). The sludge was taken in this report, for SEM examination.

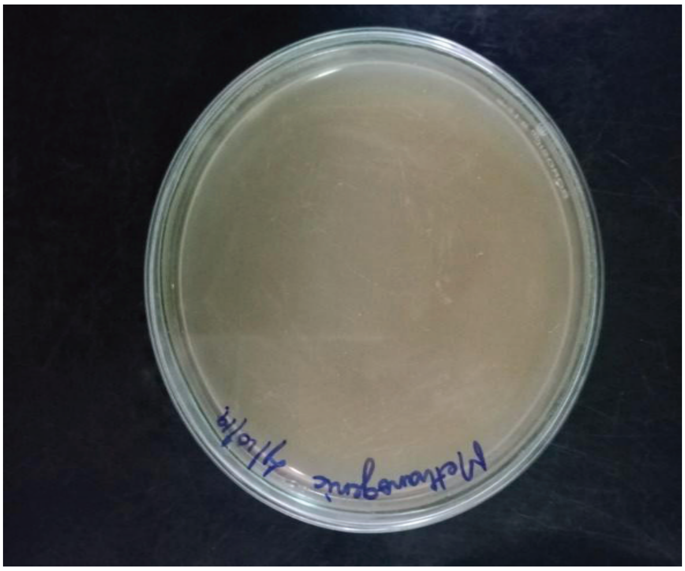

Fig .9 Isolated methanogenic bacteria on bacterial agar plate

4. Morphology and cell structure

Methanococcus aeolicus is a non-motile gram-negative, non-sporulating bacterium. In agar, the colonies vary from non-pigmented to brownish white, which is shown in figure 9.

IV. CONCLUSION

This investigation illustrates the mixed leaf litter of *Ricinus Communis* and *Datura Stramonium* with cow dung substantial influence of temperature on anaerobic biogas production in a pilot-scale reactor. Optimal biogas production, along with higher carbon and nitrogen content and favorable C:N ratios, was observed under mesophilic conditions (35°C). These results indicate that maintaining mesophilic conditions is conducive to effective biogas production. Additionally, pH levels remained within the desired range throughout the study. A comprehensive understanding of how temperature and substrate composition impact anaerobic digestion is crucial for refining biogas production processes and advancing the sustainability of renewable energy generation. Further exploration is warranted to assess the implications of these findings for the scalability and economic feasibility of large-scale biogas production systems.

Acknowledgement

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